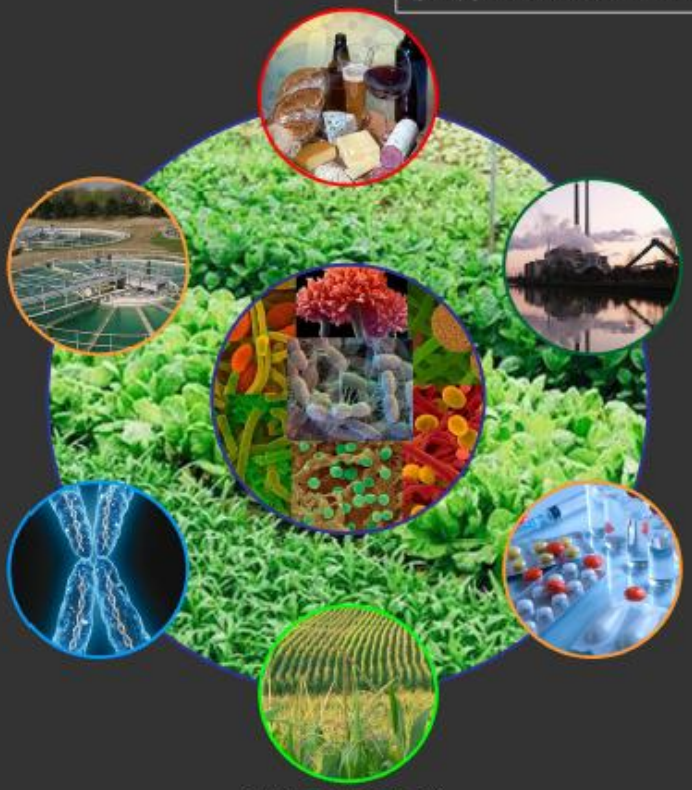
ESN PUBLICATIONS | ADVANCES IN APPLIED MICROBIOLOGY FOR SUSTAINABLE DEVELOPMENT

ADVANCES IN APPLIED MICROBIOLOGY FOR SUSTAINABLE DEVELOPMENT

ISBN : 978-81-950423-8-8



EDITORS

Prof. Neelam Jain • Prof. G. K. Aseri

ADVANCES IN APPLIED MICROBIOLOGY FOR SUSTAINABLE DEVELOPMENT

EDITORS

Prof. Neelam Jain
Secretary, IAC, Amity Institute of Biotechnology,
Amity University Rajasthan, Kant Kalwar, NH-11C, Jaipur-Delhi Highway, Jaipur, 303002 India

Prof. G. K. Aseri
Provost, Director IQAC & Amity Institute of Microbial Technology,
Amity University Rajasthan, Kant Kalwar, NH-11C, Jaipur-Delhi Highway, Jaipur, 303002 India

ESN PUBLICATIONS
INDIA

First Edition: 2021

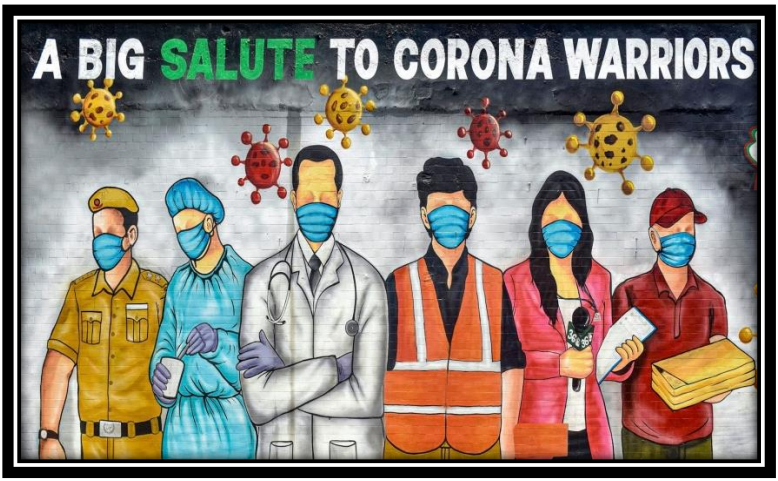
ISBN : 978-81-950423-8-8

Price : Rs 500

Published By:

ESN PUBLICATIONS,
3/151-A, Muthuramalingapuram, Kalloorani Post,
Aruppukottai Taluk, Virudhunagar District,
Tamilnadu, India,
Pincode-626105

Dedicated To
Our Mother (Late Smt. Seema Jain)
Family & Corona Warriors Around
The Globe



EDITORS

PREFACE

The book on ‘Advances in Applied Microbiology for Sustainable Development’ provides a comprehensive understanding on application oriented microbial concepts, giving readers insights into recent developments in Microbial biotechnology and Agricultural, Medical, Industrial, Food and Environmental Microbiology. It focuses on the use of microorganisms for sustainable development in field of crop improvement and plant protection, feremented food production, for environmental bioremediation purposes, and for the improvement of human health, disease diagnostics and biofuel generation for future generations. The book goes on to covers recent breakthroughs in microbial bioprocessing that can be employed in the Agri-food and health industry. This book not only highlights the most recent progress in the microbiology using innovative and sustainable microbiological approaches but also discusses actual challenges, and accomplishments of the latest technologies. Numerous applications of microorganisms are covered broadly in different chapters focusing on six major sections: Agricultural Microbiology, Environmental Microbiology, Food Microbiology, Medical Microbiology, Industrial Microbiology, and Microbial Biotechnology. This important resource provides an abundant information for a wide audience, including researchers, teachers, students, food, and health practitioners.

ABOUT EDITORS

Prof. G. K. Aseri is currently working as Provost, Dean Academics & Director, Amity Institute of Microbial Technology (AIMT) & Internal Quality Assurance Cell (IQAC) of Amity University Rajasthan. He is having 20 years of experience in academic administration in higher education. His research is well recognized by projects funded by DST, DBT-BIRAC, ICAR, ICMR, MoFPI, and Ministry of Agriculture–Govt. of India. His area of research includes wide spread area of Microbiology with more emphasis on soil microbial ecology, Microbial products, Bacteriocins, Biofertilizers and Biocontrol agents. He has supervised many PG & UG dissertations, 6 Ph.D. degrees and has published two patents. His research credentials include more than 70 research papers/presentations in International & National Journals and Conferences and book chapters. He has also organized International/National conferences/Symposia in his subject arena and DST-INSPIRE program for School students. Dr Aseri is member of Editorial Board and Reviewer of several Journals of International repute. He is member of many prestigious societies like International Society for Infectious Diseases (ISID), Epicore, ISCA (Indian Science Congress Association), Association of Microbiologists of India (AMI), Medicinal and Aromatic Plants Association of India (MAPAI). His work has been internationally recognized and has been invited to present his research work at Germany, Italy, Switzerland, Turkey, Egypt, Singapore & Nepal.

Prof. Neelam Jain is presently working as Secretary, Industry Advisory Council (IAC), Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur. She has 20 years of teaching experience in Microbiology and Biotechnology. Her research interest focuses on 'Health for all' as reflected from her publications in frontier areas of Agricultural and Environmental microbiology, Natural antimicrobials based on Herbal and Microbial products like Bacteriophages and Bacteriocins in combating AMR and treating Infectious diseases of Humans and animals. She has published more than 40 Research papers in Journals and Books of National and International repute and presented around 45 Papers at National and International forums through various Conferences and Symposia. She is actively engaged in teaching Advanced Microbial Technology, Food Microbiology & Biotechnology, Environmental Biotechnology, Bio fertilizers & Bio pesticides, Food & Nutrition to UG & PG students. Dr Neelam is member of many prestigious societies like Asian PGPR Society of Sustainable Agriculture, Alabama, USA, International Society for Infectious Diseases (ISID), ISCA (Indian Science Congress Association), Association of Microbiologists of India (AMI), AFSTI (Association of Food Scientists & Technologists India), Medicinal and Aromatic Plants Association of India (MAPAI) & The Science Advisory Board (SAB). She is an active member of Editorial Board and Reviewer of many National and International Journals. She has also organized International/ National Conferences, Symposia and DST -INSPIRE program for School students. Dr Neelam Jain has received TWAS Young Scientist Travel Award for Paper presentation in Alexandria, Egypt.

INDEX

Biofertilizer Technology: An Emerging Approach for Sustainable Agriculture in Environmentally Stressed Ecosystems

Kingsley Oyediran Oke[1*], Olubunmi Peter Ojo[2], Samuel Olusegun Oyewumi[3], Neelam Jain[4] and Gajender Kumar Aseri[5]

[1]Chief Lecturer, Department of Biology, Emmanuel Alayande College of Education, Oyo, Oyo State, Nigeria

[2] Assistant Lecturer, Department of Biology, Emmanuel Alayande College of Education, Oyo, Oyo State, Nigeria

[3] Lecturer II, Department of Agricultural Education, Emmanuel Alayande College of Education, Oyo, Oyo State, Nigeria

[4] Professor, Amity Institute of Biotechnology, Amity University, Jaipur, Rajasthan, India

[5] Professor, Amity Institute of Microbial Technology, Amity University, Jaipur, Rajasthan, India

[*]Corresponding Author: okeoyediran@gmail.com

Introduction

The concept of biofertilizer was first conceived by a French agriculturalist and chemist named J. B. Boussingault in 1834, following report of build-up of soil nitrogen after cultivation of legume (Bhattacharyya and Jha, 2014). However, the first scientifically produced bio-fertilizer (Nitragin) was generated from rhizobium by F. Noble and L. Hiltner in 1896. Since then, many scientists and researchers have made outstanding discoveries that resulted in the commercial production of various brands of biofertilizers for application in sustainable agriculture globally.

The term biofertilizers has been variously defined by many authors as ‘substances containing live microorganisms, which when applied

to roots, plant surfaces, seeds or soil colonize the rhizosphere or the interior of plants and help to improve soil fertility while also stimulating plant growth by increasing the availability of plant nutrients and growth substances to the host crops (Raimi et al., 2017). This term is often used interchangeably with bio-inoculant, inoculum, microbial inoculant, bio-formulation or effective microorganisms (Hassen, et al., 2016).

The global limitations posed by environmental stresses (such as drought, salinity, heat, cold and heavy metal, disease etc.) on crop growth and productivity is one of the major agricultural challenges that necessitated the rising agitation for adoption of more efficient and sustainable approach of curbing the growing menace of environmental stresses in most ecosystems particularly, the arid and semi-arid regions of the world (Kang et al., 2014). In fact, from agricultural point of view, there is no agro-ecosystem that is entirely free from environmental stress. However, the severity of biotic and abiotic stresses on agricultural development in a particular agro-ecosystem depends on the nature, number and intensity of the prevailing environmental stress.

The discovery of bio-fertilizer technology is a laudable scientific feat that has been studied to guarantee increased crop productivity, environmental safety, improved soil fertility and quality, enhanced nutrient use efficiency, cost effectiveness, increased crop resilience and tolerance as well as sustainability particularly in the most vulnerable agro-ecosystems (Lesueur et al., 2016).

Types of Biofertilizers

Biofertilizer microorganisms are commonly classified based on inoculant composition and functional interactions with plants as nitrogen fixing biofertilizers, solubilizing and mobilizing biofertilizers, micro-nutrient biofertilizers and growth promoting biofertilizers (Lesueur et al., 2016). Most commercial biofertilizers are transformed into bioformulations of individual or consortium of bacteria, fungi, blue-green algae (BGA) in solid, granular, powdered

or liquid carrier substances which ensure sustenance and longer storage life of the inoculants (Rashid et al., 2016).

Nitrogen fixing biofertilizers

Nitrogen fixing biofertilizers are collectively called diazotrophs and compose of microorganisms that are capable of transforming inert atmospheric nitrogen into compounds that can be assimilated by plants (Mahanty et al., 2016). Diazotrophs possess oxygen-sensitive enzyme complex called nitrogenase. Nitrogenase enzyme complex consists of two enzyme sub-units, dinitrogenase reductase and dinitrogenase which convert stable form of atmospheric nitrogen (N_2) to assimilable forms such as ammonia. Dinitrogenase reductase donates electrons for reducing atmospheric nitrogen (N_2) to ammonia (NH_3). The most potent inhibitor for the enzyme complex is oxygen which can bind with the enzyme complex easily to make it inactive. However, leg hemoglobin pigment present in diazotrophs has higher affinity for oxygen and can bind more easily with it thereby protecting the nitrogenase enzyme complex from binding with oxygen (Fig. 1).

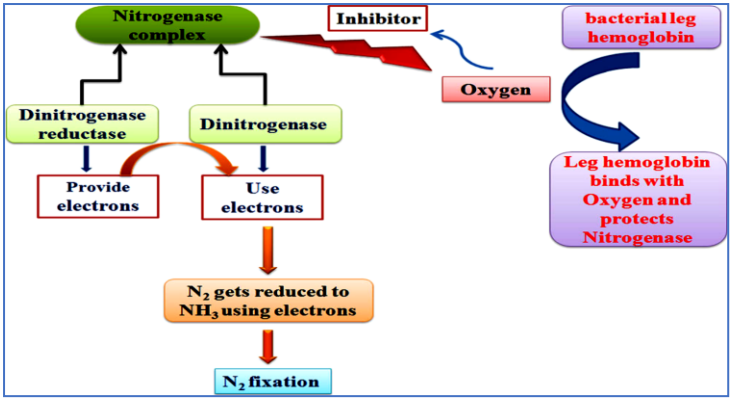


Fig. 1: Mechanism of molecular nitrogen fixation by diazotrophs. Adopted from: (Mahanty et al., 2016)

Diazotrophs exists in symbiotic and non-symbiotic relationships with plants. Symbiotic diazotrophs such as *Rhizobium sp, Sinorhizobium sp and, Bradyrhizobium sp* are common symbionts of root nodules of most leguminous crops. Non-symbiotic nitrogen-fixers are either free-living (*Azotobacter sp, Beijerinkia sp, clostridium sp, Nostoc Anabaena etc.*) or associative (*Azospirillum sp, Enterobacter sp etc.*)

Solubilizing and mobilizing biofertilizers

Despite the abundance of phosphorous in the soil, only about 0.1% is available for plant uptake; other form of phosphorous exist in insoluble and complex forms with many soil elements. The majority of phosphorus for plant growth is obtained from activities of phosphate solublizing and mobilizing microbes (Rawat, et al., 2020). These microbes help in releasing phosphorus to the soil for absorption by the plants in monobasic ($H_2PO_4^-$) and dibasic ($H_2PO_4^{2-}$) forms (Adeleke et al., 2017). Phosphorous solubilization and mobilization by microbes are mainly achieved by excretion of organic acids, ions (protons) and siderophores; exudation of extracellular enzymes (phosphatases and phytases) and; mineralization of substrates (Fig. 2).

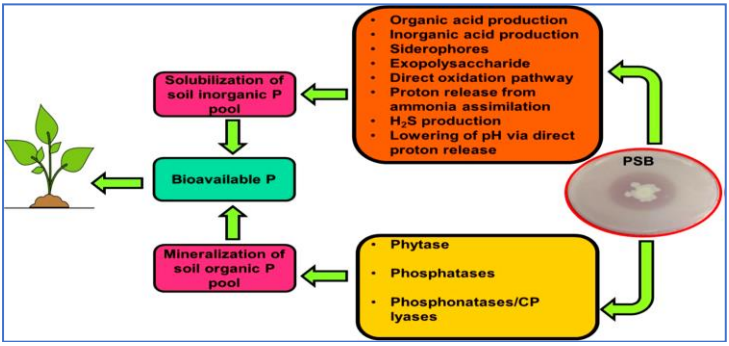


Fig. 2: Mechanism of Phosphate solubilization by Phosphate Solublizing Microbes. (Source: Rawat, et al., 2020)

Phosphate solubilizers are found among different groups of bacteria (*Pseudomonas sp, Bacillus sp),* fungi *(Glomus sp, Pisolithus tinctorius, Paxillus involutus, Phialocephala fortini, Suillus tomentosus Aspergillus sp, Penicillum sp, Trichoderma sp* etc.) and *Actinomycetes* (*Streptomyces* and *Nocardia sp*). Similarly, large proportion of potassium reserves in soil system has been reported to be in form of non-exchangeable minerals. However, efficient rhizospheric microbes (ERMs) have been studied to be capable of effectively dissolving and mobilizing potassium for plants uptake (Sattar et al., 2018). Various mechanisms utilized by potassium solubilizing microbes for K dissolution include acidolysis, organic acid production, lowering soil pH, exchange reactions, complexation and chelation (Ameen, et al., 2019). A diverse group of rhizobacteria (*Bacillus sp, Acidothiobacillus sp* and *Paenibacillus sp.*) and fungal strains (*Aspergillus sp, Glomus sp mosseae,* and *Penicillium sp*.) are capable of solubilizing potassium from minerals like feldspar, illite, orthoclase, muscovite, biotite, mica and mobilizing them for plant uptake (Fig. 3).

Micronutrient biofertilizer

Most essential micronutrient such as zinc (Zn), iron (Fe), manganese (Mn), copper (Cu) and Molybdenum (Mo) for plant growth are in insoluble complex forms in the soil and are thus not readily accessible by crops (Mahdi et al., 2010). However, biofertilizers of *Bacillus, Bradyrhizobium, Pseudomonas, Thiobacillus, Bacillus, Saccharomyces, Penicillum sps* have been studied by many authors to improve the availability and uptake of micronutrients in the soil (Adeleke, et al., 2017). Bacteria siderophore has also been reported to solubilize and chelate Fe into complexes that can be easily absorbed by the plant roots. Metallic Zn and MnO_2 have been found to be solubilized by fungal specie, *Trichoderma harzianum*, through chelation and reduction mechanisms. Pal et al. (2015) also reported the ability of vesicular arbuscular mycorrhiza (VAM) to solubilize Zn, Fe, Mn, and Cu in some agricultural soils.

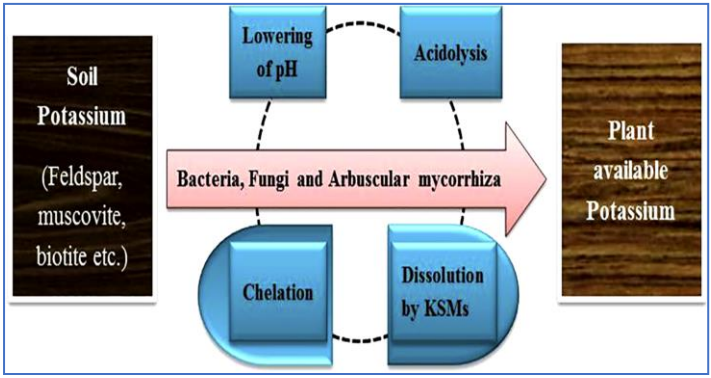


Fig. 3: Mechanism of Potassium solubilization
(Source: Sattar et al. 2018)

Growth-promoting biofertilizers

Growth-promoting biofertilizers enhances plant growth through production of variety of substances like phytohormones, enzymes, volatile organic compounds (VOCs) that acts as growth regulators; growth stimulators / inhibitors, stress modulators as well as bio-control, rhizo-remediating and phyto-pathogenic agents (Bhattacharyya and Jha, 2014). Several biofertilizers containing growth-promoting microbes such as *Azospirillum brasiliense, Rhizobium leguminosarum, Bacillus sp, Paenibacillus polymyxa, Enterobacter cloacae* and *Agrobacterium sp* have been reported to be efficient producers of plant growth promoting phytohormones like indole-3-acetic acid (IAA), cytokinin and gibberellin. Production of stress enzyme like 1-amino cyclopropane-1-carboxylic acid (ACC) deaminase by bacteria such as *Achromobacter, Azospirillum, Bacillus, Enterobacter, Pseudomonas* and *Rhizobium sps* has been reported by various authors (Bhattacharyya and Jha, 2014). Also, *Streptomyces sp, Pseudomonas fluorescens, Glomus fasciculatum, G. mossae* and *Gigaspora margarita* were studied for bio-control activities against a large number of phytopathogens like *Pseudomonas fluorescens,*

Pseudomonas fluorescens, Pseudomonas putida and Pseudomonas fluorescens, Bacillus subtilis and Bacillus cereus have been reported to exhibit increased efficiency of induced systemic resistance (ISR) against several pathogens (Ramamoorthy et al., 2001).

Effect of environmental stresses on sustainable agriculture

Abiotic and biotic stresses have been reported to increase crop yield losses and reduce cultivation quality of most arable lands on globally basis (Kang et al. 2014).

Effect of drought stress on sustainable crop production

Drought is a climatic term that describes long period of low rainfall and reduced soil water level. It is one of the most crucial environmental stress factors affecting plants in various regions of the world, particularly in arid and semi-arid ecosystems (Kang et al., 2014). Water is essentially required for most of the metabolic activities at all developmental stages in the plants. Hence, stress as a result of water deficit generally results in changes in the morphological, physiological, morphological, molecular, biochemical and ecological attributions of the plants (Salehi-Lisar, 2016). In addition, drought and water stresses reduces seed and seedling growth, availability of nutrient around the root zone, nutrient uptake by root hairs, water transpiration and nutrients translocation by the xylem and phloem vessels respectively, reduction of stomatal conductance and consequent impairment of metabolic processes in cells and tissues. Overall, effects of drought have been reported to trigger growth and yield reductions in many crop plants depending on their severity.

Effect of salinity stress on sustainable crop production

The excessive accumulation of soluble salts in the soil has not only reduced the quality of agricultural lands but has hindered various physiological and biochemical functions in many crops. The harmful effects of salinity stress on plants are observable by many

symptoms like shoot and root necrosis, reduced internode lengths, leaf abscission and reduced leaf area. Salt stress inhibits plant growth and induction of abscisic acid which results in stomata closure, oxidative stress and impairment of photosynthesis (Sayyad-Amin et al., 2016). Plant growth is generally reduced under salinity stress due to perturbation resulting from inaccessibility, immobility and separation of nutrients. Most often, high salinity results in nutrient deficiency as a result imbalanced sodium and chloride ions and/ or competition of these ions with other ions such as NO^{3-} , K^{+} and Ca^{2+} which ultimately leads to impairment of metabolic activities and reduction in plant growth (Xu and Mou, 2016).

Effect of heat stress on sustainable crop production

Heat shocks as a result of rising atmospheric temperatures are major factors that limit crop productivity on global basis. This unusual phenomenal temperature modification results in disturbance of plants growth phases and instability of agricultural plants. Elevated temperatures lead to denaturation of protein molecules, interruption of protein synthesis, inactivation of vital enzymes, and disruption of cell membranes (Huang et al., 2012). Also, the processes of cell division are significantly affected by excessively high temperature. Heat stress reduces the quantity, strength and length of plant roots resulting in reduced water and mineral uptake and eventually, lower growth and crop yield.

Effect of cold stress on sustainable crop production

Cold stress is one of the significant abiotic stress affecting growth and development of crops, mainly as a result of loss of strength due to alteration of the physical and chemical metabolic procedures (Barrero-Sicilia et al., 2017). Cold stress causes electrolytes leakage, decreases protoplasmic streaming and modification of cellular metabolism. Additionally, extremely low temperature regime alters reactions in protein and nucleic acid formation, nutrient and water equilibrium, affinity of enzyme and impairment of photosynthesis; importantly inhibition and down-regulation of photosystem II

(photo-inhibition) (Takahashi, et al., 2013). The processes of chilling and freezing characteristic of many cold environments lead to injury in crops. These alternating processes results in physical damage and disruption of biochemical and physiological activities which ultimately reduces growth rate. Cold stress is a major limiting factor of agricultural productivity in most low temperature eco-systems of the world.

Effect of heavy metal stress on sustainable crop production

The impacts of heavy metal stress are becoming serious and prevalent problem in many agro-environments. Heavy metals have different solubility and bio-availability because of their existence in different forms in the soil (Wuana and Okieimen, 2011). Excessive accumulation of heavy metals in plants tissue results in alteration of physiological, morphological and biochemical activities with overall harmful effects on crop productivity. Heavy metal stress reduce seed germination rate, re-mobilize seed reserves during active growth stage, and impairs photosynthetic and other physiological processes in plants. Heavy metal toxicity stress causes reactive oxygen species (ROS), modify redox reactions and causes oxidative stress in plants. Heavy metals such as zinc (Zn), arsenic (As), chromium (Cr), cadmium (Cd), mercury (Hg), copper (Cu), nickel (Ni), and lead (Pb) have been reported to cause mutagenic effects on crops by polluting the soil, irrigation systems, surface and underground waters (Wuana and Okieimen, 2011).

Effect of diseases stress on sustainable crop production

Diseases are major biotic stress that occurs as a result of harm caused directly or indirectly by living organisms to crops. Major agents of disease stress in crop plants include pathogens such as viruses, bacteria, fungi, invertebrates such as insects, nematodes, arachnids and plants weeds. Diseases occur in plants as a result of interaction among three major factors viz: plant, pathogen and environment. However, the impacts of disease stress vary from one agro-ecology to another depending on agricultural practices, type of

crop, crop resistance level and nature of cropping system. Insects (Dipterous, Lepidopterous, Spodoptera, etc.) and other invertebrates may cause no observable signs and symptoms at the initial stage of invasion but at later developmental stage, crop harm may appear as spots and stripes of different colors (white, yellow, brown or dark). Similarly, plant weeds appear small and difficult to recognize and differentiate from plant crops at early growth stage, but as growth progresses, their harmful effects on crops begin to manifest. Disease stress imparts both pre-harvest and postharvest losses on crops. The ultimate effects of disease stress is reduced vigor, blasts, wilt, streaks, necrosis and in extreme situations, death of the host plant (Youssef and Eissa, 2014).

Biofertilizers as alleviators of environmental stress

Biofertilizer microbes are endowed with different morphological, physiological, biochemical and molecular properties and mechanisms (Fig. 4) for alleviating effects of environmental stresses on crop plants.

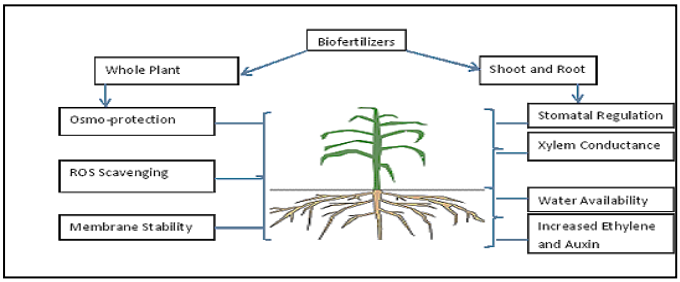


Fig. 4: Mechanisms of stress alleviation by bio-inoculants

Biofertilizers and drought stress

Many aspects of plant growth are affected by drought and water deficit stresses, with the most evident effects being reduction in growth, decrease in whole plant size and reduction in crop yield. Biofertilizer application is considered as a sustainable means of

conferring water stress tolerance on crops due to their unique ability to promote root elongation for exploring and capturing water from remote soil layers. They have been studied to be capable of creating excellent water potential gradient and favorable water uptake potentials for plants at low water situations. Application of biofertilizers has also been reported to stimulate rapid growth of root during seed germination, particularly under water deficit stress and low soil fertility conditions (Van Oosten et al., 2017). They enhance water availability by creating efficient absorption surfaces in the vicinity of the root system and encourage sequestration of soil water to the advantage of the plants (Van Oosten et al., 2017). These products generally increase crop growth and productivity by promoting resilience during prolonged water deficit conditions.

Biofertilizers and salt stress

Plants experiences multiple stress events inform of osmotic, ionic, oxidizing stresses and nutrient imbalance that results in alteration of different physiological and metabolic processes during exposure to high salts concentrations. However, inoculation of various important crops with active strains of biofertilizers has been documented by authors to assist in alleviation of effects of salinity stress in high saline agro-environments. Application of strains of *Azotobacter* biofertilizers on maize and wheat has been studied to show significant positive effects under salinity stress by enhancing uptake of potassium ion (K^+) and excluding sodium ions (Na^+). A number of biofertilzer strains are capable of producing 1-amino-cyclopropane-1-carboxylate (ACC) deaminase enzyme which has been reported to protect crops effectively against salinity stresses. Inoculation of lettuce (Fasciglione et al., 2015) chickpea and faba beans (Hamaoui et al. 2003), with *Azospirillum brasiliense* in salty environment showed positive results in form of increased germination percentage, fresh weight and dry weight, ascorbic acid content, and higher chlorophyll content levels (Bulgari et al., 2019. Also, potato, wheat, pea and cotton inoculated with *Rhizobium leguminosarum* and *Azotobacter chrococcum* biofertilizers produced increased root and shoot lengths and better growth and yield performances under salinity stress conditions (Van Oosten et al., 2017).

Biofertilizers and Temperature stress

The protections offered by microbial inoculants to field crops under extreme temperatures are of particular importance in ensuring increased productivity. Inoculation of wheat with strains of thermo-tolerant biofertilzer of *Pseudomonas putida* was reported to have increased seed size, root and shoot lengths and biomass. Generation of ROS and expression of ROS response genes such as ascorbate peroxidase, superoxide dismutase and catalase were also found to reduce significantly (Ali et al., 2011) with application of psychrophilic microorganisms. Similarly, cold-adapted strains of biofertilizers such as *Pantoea dispersa* have been studied to enhance nutrient uptake and plant growth due to production of IAA and solubilization of phosphorous under low temperature conditions (Selvakumar et al., 2008). *Burkholderia phytofirman* biofertilizer was reported to play crucial role in enhancing chilling tolerance in plants by increasing carbohydrate metabolism and photosynthetic processes during cold stress and acclimation. Inoculation of tomato plants (*Solanum lycopersicum* cv Mill) with cold-tolerant strains of *Pseudomonas vancouverensis*, and *Flavobacterium glaciei* isolated from agricultural fields during winter was reported to significantly increased growth due to reduced electrolyte leakage and reactive oxygen species (ROS) activity (Subramanian et al., 2016).

Biofertilizers and heavy metals toxicity stress

Although most heavy metals are micronutrient required by plant for enhanced growth, the incessant release of these elements in excessive quantities during anthropogenic, industrial and agricultural activities has resulted in the contamination and pollution of many agricultural soils. Heavy metal toxicity stresses mostly reduces soil quality and microbial community, alters vital metabolic processes, decreases growth rate, and most often lead to death of crop plants. However, diverse numbers of microbial biofertilizer strains have been examined to play vital role in alleviating the harmful effects of heavy metal stresses on crops in most heavy metal polluted environments (Dixit et al., 2015). Heavy metal

toxicity stress in plants results in the production of hormone ethylene that inhibits plant development when produced in elevated quantity. Biofertilizer inoculants are however capable of producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzymes with ability to reduce the effect of ethylene accumulation in plants (Singh et al., 2015). Some of the commonly used biofertilizer inoculants for bioremediation of heavy metal toxicity and stress include *Achromobacter xylosoxidans, Azotobacter chrocoocum, Bacilus subtilis, Bacilus megaterium, Pseudomonas putida, Pseudomonas aeruginosa, Brevibacillus sp., Ochrobactrum sp., Xanthomonas sp., Psycrobacter sp., Ralstonia metallidurans, Kluyvera ascorbata, Rhizobium sp., Bradyrhizobium, Mesorhizobium* and *Sinorhizobium sp.* (Shinwari et al., 2015).

Biofertilizers and disease stress

The prolonged use of chemicals in plant disease management has resulted into resistance by the various disease agents and has contributed immensely to environmental contamination (Hassen et al., 2016). Hence, microbial inoculants have been suggested as safe, effective and sustainable alternative for reducing the highly destructive effects of disease stresses on crop plants. Production of low molecular weight antibiotics is one of the major mechanisms by which biofertilizer microorganisms employ in combating phyto-pathogenicity. *Pseudomonas flourescens* and *Pseudomonas chlororaphis* have been reported to produced antibiotics pyrroltrin and pyoluteorin that efficiently suppressed take-all, *Pythium*, *Fusarium*, wilt and *Rhizoctonia* root rot diseases of wheat as well as black root rot disease in tobacco (Weller, 2012). Biofertilizer inoculants are also capable of triggering induced systemic resistance (ISR) in crop plants as a defensive mechanism against phytopathogens. In addition, certain *Pseudomonas sp* have been studied to possess unique ability for triggering systemic acquired resistance on plants through production of siderophore (Hassen et al., 2016). Furthermore, some bacteria strains are capable of releasing various compounds such as volatile compounds, hydrogen sulphide, enzymes, alcohol, phenols and hormones which are

inhibitory to the growth and proliferation of some invertebrate pest and vectors like insects, nematodes, etc. (Youssef and Eissa, 2014).

Conclusion

The negative impacts of environmental stresses and indiscriminate use of chemical inputs on crop productivity has actuated continuous call for sustainable approach to agricultural development globally. Interestingly, biofertilizer technology has proved to be significantly successful not only in gaining ground over the agrochemicals, but also in alleviating the severity of environmental stresses on crops. Therefore, appropriate adoption of this emerging technology is exigent for preventing and modulating abiotic and biotic stress effects on crops. This will go a long way in ensuring bumper crop harvests particularly in vulnerable agro-systems.

References

Adeleke, R., Nwangburuka, C., Oboirien, B., 2017. Origins, roles and fate of organic acids in soils: A review. *South African Journal of Botany*, *108*, 393–406. https://doi.org/10.1016/j.sajb.2016.09.002

Ali, S.K.Z., Sandhya, V., Grover, M., Linga, V.R., Bandi, V., 2011. Effect of inoculation with a thermotolerant plant growth promoting *Pseudomonas putida* strain AKMP7 on growth of wheat (Triticum spp.) under heat stress. J Plant Interact. 6 (4):239–46.

Ameen, F., AlYahya, S.A., AlNadhari, S., Alasmari, H., Alhoshani, W.M., 2019. Phosphate solubilizing bacteria and fungi in desert soils: species, limitations and mechanisms. Arch Agron Soil Sci 65: 1446–1459. https://doi.org/10.1080/03650340.2019.1566713

Barrero-Sicilia, C., Silvestre, S., Haslam, R.P., Michaelson, L.V., 2017. Lipid remodelling: Unravelling the response to cold stress in Arabidopsis and its extremophile relative Eutrema salsugineum. Plant Sci. 263:194-200.

Bhattacharyya, P., 2014. Biofertiliser use in organic farming: A practical and challenging approach. In Shetty, P. k. Alvares, C.

Yadav, A.K. (Eds.), *Organic Farming and Sustainability* (p. 157). Bangalore: National Institute of Advanced Studies.

Bulgari, R., Franzoni, G., Ferrante, A., 2019. Biostimulants Application in Horticultural Crops under Abiotic Stress Conditions Agron. 9, 306. doi:10.3390/agronomy9060306

Camejo, D., Rodríguez, P., Angeles, M., Dell'Amico, J., Torrecillas, A., Alarcón, J.J., 2005. High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. J. Plant Physiol. 162 (3), 281-289.

Crafts-Brandner, S.J., 2002. Sensitivity of photosynthesis in a C4 plant, maize, to heat stress. Plant Physiol. 129 (4), 1773-1780.

Dixit R, Malaviya D, Pandiyan K, Singh UB, Sahu A, Shukla R, Singh BP, Rai JP, Sharma PK, Lade H, Paul D (2015) Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. Sustainability. 7 (2):2189–2212

Du Jardin, P., 2012. The science of plant biostimulants—A bibliographic analysis, Ad hoc Study Report. Brussels: European Commission. Available from: http://hdl.handle.net/2268/169257 [Accessed: 18-12-2020]

Fasciglione, G., Casanovas, E.M., Quillehauquy, V., Yommi, A.K., Goñi, M.G. Roura, S.I., Barassi, C.A., 2015. Azospirillum inoculation effects on growth, product quality and storage life of lettuce plants grown under salt stress. Sci. Hortic. 195, 154–162.

Hamaoui, B., Abbadi, J.M., Burdman, S., Rashid, A, Sarig, S., Okon, Y., 2003. Effects of inoculation with Azospirillum brasilense on chickpeas (Cicer arietinum) and faba beans (Vicia faba) under different growth conditions. Agron. 21, 553–560.

Hassen, A. I., Bopape, F.L., Sanger, L.K., 2016. Microbial inoculants as agents of growth promotion and abiotic stress tolerance in plants. In Singh, D., Singh, H., Prabha, R. (Eds.), Microbial inoculants in sustainable agricultural productivity (pp. 23–36). New Delhi: Springer. https://doi.org/10.1007/978-81-322-2647-5

Kang, S.M., Khan, A.L., Waqs, M., You, Y.H., Kim, J.H., Kim, G.K., Hamayun, M., Lee, I.J. 2014. Plant growth promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in Cucumis sativus. J. Plant. Interact. 9, 673–682

Lesueur, D., Deaker, R., Herrmann, L., Bräu, L., Jansa, J., 2016. The production and potential of biofertilisers to improve crop yields. *Bioformulations: For Sustainable Agriculture*, 71–92.

Mahanty, T., Bhattacharjee, S., Goswami, M., Bhattacharyya, P., Das, B., Ghosh, A., Tribedi, P., 2015. Biofertilizers: a potential approach for sustainable agriculture development. Environ. Sci. Pollut. Res. 1-22. DOI 10.1007/s11356-016-8104-0

Rai, B.P., Sharma, .J.P., Lade, P.K., Paul, D., 2015. Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. Sustainability, 7 (2), 2189–2212.

Raimi, A., Adeleke, R., Roopnarain, A., 2017**.** Soil fertility challenges and Biofertiliser as a viable alternative for increasing smallholder farmer crop productivity in sub-Saharan Africa. Cog. Food Agricul. 3, 1400933.

Ramamoorthy, V., Viswanathan, R., Raguchander, T., Prakasam, V., Samiyappan R., 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. Crop Prot. 20, 1–11.

Rashid, M. I., Mujawar, L. H., Shahzad, T., Almeelbi, T., Ismail, I. M., Oves, M., 2016. Bacteria and fungi can contribute to nutrients bioavailability and aggregate formation in degraded soils. Microbiol. Res. 183, 26–41. https://doi.org/10.1016/j.micres.2015.11.007

Rawat, P., Das, S., Shankhdhar, D., Shankhdhar, S.C., 2020. Phosphate-Solubilizing Microorganisms: Mechanism and Their Role in Phosphate Solubilization and Uptake. J. Soil Sci. Plant Nutr. https://doi.org/10.1007/s42729-020-00342-7

Salehi-Lisar, S.Y., Bakhshayeshan-Agdam H., 2016. Drought stress in plants: Causes, consequences, and tolerance. In: Hossain, M.A., Wani, S.H., Bhattacharjee S., Burritt, D.J., Tran, L.P., editors. Drought Stress Tolerance in Plants: Physiology and

Biochemistry. Switzerland: Springer International Publishing. pp. 1-16. DOI: 10.1007/978-3-319-28899-4

Sathya, A., Vijayabharathi, R., Gopalakrishnan, S., 2016. Soil Microbes: The Invisible Managers of Soil Fertility. In Singh, D.P. et al. (eds.), Microbial Inoculants in Sustainable Agricultural Productivity. Springer India. DOI 10.1007/978-81-322-2644-4_1

Sattar, A., Muhammad Naveed, M., Ali, M., Zahir, A, Sajid M. Nadeem, M., Meena, V.S., Farooq M., Singh, R., Mahfuz Rahman, M., Meena, H.N., 2018. Perspectives of potassium solubilizing microbes in sustainable food production system: A review. Appl. Ecol. Article in press.

Sayyad-Amin, P., Jahansooz, M.R., Borzouei, A., Ajili, F., 2016. Changes in photosynthetic pigments and chlorophyll-a fluorescence attributes of sweet-forage and grain sorghum cultivars under salt stress. J. Biol. Phys. 42, 601–620.

Selvakumar, G., Kundu, S., Joshi, P., Nazim, S., Gupta, A.D., Mishra, P.K., 2008. Characterization of a cold-tolerant plant growth-promoting bacterium Pantoea ispersa 1A isolated from a sub-alpine soil in the North Western Indian Himalayas. World J. Microbiol. Biotechnol. 24 (7), 955–60.

Shinwari, K.I., Shah, A.U., Afridi, M.I., Zeeshan, M., Hussain, H., Hussain, J., Ahmad, O., 2015. Application of plant growth promoting rhizobacteria in bioremediation of heavy metal polluted soil. Asian J. Mult. Stud. 3 (4).

Singh, R.P., Shelke, G.M., Kumar, A., Jha, P.N., 2015. Biochemistry and genetics of ACC deaminase: a weapon to "stress ethylene" produced in plants. Front . Microbiol. 6, 1255.

Subramanian, P., Kim, K., Krishnamoorthy, R., Mageswari, A., Selvakumar, G., Sa, T., 2016. Cold stress tolerance in psychrotolerant soil bacteria and their conferred chilling resistance in tomato (Solanum lycopersicum Mill.) under low temperatures. PLoS ONE. 11(8):e0161592.

Takahashi, D., Li, B., Nakayama, T., Kawamura, Y., Uemura, M., 2013. Plant plasma membrane proteomics for improving cold tolerance. Front. Plant Sci. 4:90.

Van Oosten, M.J., Pepe, O., De Pascale, S., Silletti, S., Maggio, A., 2017. The role of biostimulants and bioe_ectors as alleviators of abiotic stress in crop plants. Chem. Biol. Technol. Agric. 4,1- 5.

Wahid, A., Gelani, S., Ashraf, M., Foolad, M., 2007. Heat tolerance in plants: An overview. Environ. Exp. Bot. 61(3):199-223.

Weller, D.M., Mavrodi, D.V., van Pelt, J.A., Pieterse, C.M., van Loon, L.C., Bakker, P.A., 2012. Induced systemic resistance in *Arabidopsis thaliana* against *Pseudomonas syringae* by 2,4-diacetylphloroglucinol producing *Pseudomonas fluorescens* . Phytopathol. 102:403–412.

Wuana, R.A., Okieimen, F.E., 2011. Heavy metals in contaminated soils: A review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecology. 2011:1-20.

Xu, C., Mou, B., 2016. Responses of Spinach to Salinity and Nutrient Deficiency in Growth, Physiology, and Nutritional Value. J. Am. Soc. Hortic. Sci. 141, 12–21.

Youssef, M.M., Eissa, M.F. 2014. Biofertilizers and their role in management of plant parasitic nematodes. A review. E3 Journal of Biotechnology and Pharmaceutical Research 5 (1), 1–6.

ROLE OF MICROORGANISMS IN SUSTAINABILITY OF AEROBIC RICE PRODUCTION SYSTEM

Ekta Narwal[1], Yudhvir Singh[2*]

[1] Research Scholar, Division of Microbiology, ICAR-Indian Agricultural Research Institute, New Delhi- 110012

[2]Principal Scientist, Division of Microbiology, ICAR-Indian Agricultural Research Institute, New Delhi- 110012

[*]Corresponding Author: yvsingh63@yahoo.co.in

Introduction

Rice is the staple food crop for more than half of the world's population. Because of the flexibility with which rice can be grown in different ecological conditions, rice is cultivated in 150 mha worldwide with a coverage of about 90% in different irrigated lowlands and only about 4% in upland conditions (Nascente et al.. 2019). In India, rice is grown in different ecosystems with around 10-15% grown in upland system. The sustainability of traditional method of rice cultivation now encounter issues like worldwide climate change and unpredictable availability of water which has threatened the food security of almost 60% population globally (Bouman et al., 2005).

Aerobic Rice Systems (ARS) aims at growing rice without puddling and flooding under nonsaturated soil conditions as other upland crops. Aerobic rice is responsive to high inputs, can be rainfed or irrigated, and tolerates occasional flooding. The driving force behind ARS is water economy and a saving of 73% in land preparation and 56% during crop growth (Rasul, 2016). ARS heavily relies on herbicides and other biocides, such as, nematicides and adequate supply of plant nutrients including N, P, Fe, Zn, and others that may become deficient under aerobic conditions. Excessive use of chemical fertilizers have resulted in higher emission of greenhouse

gases, altered biogeochemical cycles, declining soil organic matter and deprivation of biodiversity (Singh, 2018). The untoward effects of unrestricted use of fertilizers can be attenuated by switching to biofertilizers or microbial inoculants. There are numerous reports on the beneficial effects of microbial inoculation in traditional puddled rice cultivation system (Prasanna et al., 2012); however, in case of ARS, this literature is meagre.

Beneficial effect of microbial inoculants has been demonstrated in terms of greater biomass production, better nutrient uptake in plants and increase in grain N and P in rice grown under SRI practices and increased micronutrient uptake (Adak et al., 2016). Many authors have documented the beneficial effects of different rhizobacteria on rice. Cruz et al., 2014 have reported significant increase in P uptake and grain yield of upland rice inoculated with actinomycete under screenhouse conditions. *Bacillus* sp. (Tamreihao et al., 2018), *Pseudomonas* sp. (Gusain et al., 2015), *Burkholderia* sp. (Rego et al., 2014), *Lysinibacillus* sp. (Shabanamol et al., 2018) have been isolated from rhizosphere of upland rice varieties and have shown to be highly effective in growth promotion under greenhouse and field conditions. Inoculation of rice plants with *Pseudomonas putida*, *P. aeruginosa*, *P. fluorescens*, *Azotobacter chroococcum* and *Azospirillum brasilense* proved to produce higher grain yield and was economically cheaper than the rice plants fertilized with conventional N doses (Yadav et al., 2014). Banik et al., 2019 have demonstrated that utilization of an indigenous rice root endophyte *Azotobacter* strain was significantly effective in increasing plant height, root weight, leaf area and chlorophyll content. The yield parameters were similar compared with the recommended dose of fertilizers. Enterobacter sp. has been isolated from rice rhizosphere and shown to promote rice seedling growth under salt stress (Sarkar et al., 2018), tolerance of rice plants to salt or Cd2+ in soils (Liu et al., 2020), solubilize inorganic phosphorus in rice rhizosphere (Singh, 2018) *Stenotrophomonas maltophila* has been shown to promote growth of wheat plants under salt stress through the multiple plant growth promoting traits such as ACC deaminase (ACCD), gibberellic acid, indole acetic acid (IAA), siderophore, and

inorganic phosphate solubilization and protecting the plants from pathogens through the accumulation of defence enzymes like glucanases and peroxidases (Selvakumar et al., 2012).

PGPR in plant growth promotion

Plant growth-promoting rhizobacteria (PGPR) as bioinoculants are an important part of the sustainable plant nutrient management systems, particularly in upland/rainfed areas, because these are low-cost inputs that can be used by farmers. PGPR are microorganisms that can cultivate in, on, or around plant tissues and stimulate plant growth through various mechanisms including nitrogen fixation, phosphate solubilization, iron sequestration through siderophores, modulation of phytohormone levels, indole-3-acetic acid (IAA) and 1-aminocyclopropane-1- carboxylate (ACC)-deaminase, and siderophore production (Glick 2012). Due to their growth and productivity promoting potential in many other plants, extensive research is going on for their application in aerobic/upland rice. Many studies have shown the beneficial effects of PGPR inoculation in aerobic/upland rice (Table 1) such as:

Microorganisms display a variety of roles such as fixing atmospheric nitrogen, making it available to the rice plants and increasing uptake of nitrogen, thereby reducing dependency on chemical fertilizers upto 20-30% (Hala Y, 2020). Phosphate solubilizing microorganisms, viz. *Bacillus*, *Pseudomonas*, *Azotobacter*, *Aspergillus* spp. etc. can mineralize insoluble P thereby increasing P availability to plants. Therefore, efficient P-metabolizing bioinoculant application would promote plant growth, improve soil health and protect plants from different pathogens without disturbing the environment (Othman and Panhwar, 2014).

i. PGPR are known to be directly involved in increasing uptake of nitrogen, phosphorus, potassium and other nutrients, synthesis of phytohormones such as indole-3-acetic acid (IAA), cytokinins and gibberellins and solubilization of minerals such

as phosphorus, potassium, zinc and production of siderophores that chelate insoluble form of iron and make it available to the plant root (Glick, 2012).

ii. The activities of rhizospheric microorganisms helps to modulate root architecture affecting the rooting patterns, biochemistry of root exudates and the supply of available nutrients to plants, in a manner that modify the quality and quantity of root exudates (Selvakumar et al., 2012).

iii. PGPR play an important role in biological activities, nutrient cycling and accelerate mineralization and uptake of certain nutrients (Fe, P, Mn, Zn and Cu), helps in mineralization of organic substances, thus supplying pool of available nutrients to the plants and thereby increases crop yield by ~ 10–30% besides reducing the input cost for crop production (Parewa et al., 2018).

iv. PGPR are important components of sustainable agricultural production, because they characterize the attributes of ecosystems. These beneficial microorganisms that populate in the soil and actively participate in the interplay of nutritional dynamics of the soil (Selvakumar et al., 2012).

v. PGPR are capable of modifying the root system through changes in phytohormone signaling pathways such as auxins which increases of the surface area and the number of roots and root hairs, increase in mineral ion translocation via stimulation of ATPase proton pumps which are important for N uptake because in aerobic conditions, rice is less efficient in N uptake due to the higher levels of N available in the form of NO_3^- (Mantelin and Touraine, 2004).

PGPR in abiotic stress tolerance

Rice cultivation in aerobic conditions is affected by a battery of abiotic stresses which include extremes of temperature, both hot and cold, drought, soil salinity, and acidity, presence of toxic heavy metals / organic pollutants and low soil fertilityThese factors play a important role in the water holding and cation exchange capacity of

soil and the degree of root contact with the soil matrix and therefore, affect the plant growth and development

Table 1. PGPR reported and used as bioinoculant for growth promotion in aerobic/upland rice

PGPR used	Mechanism of action	Effect of inoculation	Design of Experiment	References
Pseudomonas sp. and Azospirillum sp.	Biological nitrogen fixation and phytohormones production	Higher grain yield with low fertilizer doses	Pot experiment	Yadav et al.,2014
Bacillus sp., Pseudomonas sp.	Solubilize P from insoluble P through the production of organic acid, phosphatases and phytases	Improved nutrient uptake and growth of aerobic rice	Pot experiment	Othman and Panhwar, 2014
Serratia sp.	Acidification of rhizospheric soil and solubilization of nutrients	Increase in stomatal conductance, nutrient uptake, dry matter accumulation and higher grain yields	Greenhouse experiment	Nascente et al., 2019
Actinomycetes	Indole-3-acetic acid (IAA)and ACC deaminase production	Improved dry weight and higher yields	Greenhouse experiment	Cruz et al., 2014
Klebsiella pneumonia and Azospirillum brasilense	Biological nitrogen fixation and production of plant growth regulators	Increase in tiller number, root and shoot weight and plant N content	Pot experiment	Hala Y. 2020
Nitrosomonas europaea,	IAA production,	Higher vigor index, root	Moist filter paper in	Supari et al.,

Rhodopseudomonas palustris, Acinetobacter sp.	biological N fixation	growth, shoot length	Petri dishes	2014
Pantoea agglomerans, Rahnella aquatilis, and Pseudomonas orientalis	Potassium solubilization	Enhanced chlorophyll content, stomatal conductance and photosynthetic efficiency	Pot and field experiment	Khangahi et al., 2019
Burkholderia pyrrocinia, Pseudomonas fluorescens, Trichoderma asperellum	Stimulation of root growth, expansion of root cortex, exodermis, aerenchyma lacunae and thicker pericycle	Efficient seed germination, lateral root formation, increase in dry matter accumulation	Glass tubes	Rego et al., 2014
Acinetbacter sp.	Zinc solubilization, phytohormones production	Solubilization of zinc carbonate and zinc oxides, increase in quantity and quality of plant productive factors	Pot experiment	Gandhi and Muralidharan, 2016

i. Drought is one of the major constraints aerobic rice production. Drought stress has several effects on plants; for example, ion stress and osmotic stress cause oxidative stress in plants, resulting in the production of reactive oxygen species (ROS), which are harmful to plants and thus reducing the productivity of aerobic rice. Water stress causes low initial vigor of rice seedling due to deficient nitrogen uptake. (Selvakumar et al, 2012).
ii. Due to increased salt concentration, plants become unable to draw water from soil. Entry of excess salts like Na and Cl into plant system can disturb plant's physiological processes. High

amounts of salt ions make many of the essential plant nutrients such as K, Mg, N or P unavailable (Selvakumar et al., 2012).

iii. The rhizosphere microbiome communities are mostly comprised of the Firmicutes phylum in the aerobic/upland rice roots as compared with that in the rhizosphere of irrigated rice. Firmicutes possess thick cell wall (monoderm and gram positive), can sporulate, are anaerobic bacteria, which are more tolerant to drying conditions than the diderms (gram negatives), commonly adapted to the aerobic and drought conditions of aerobic/upland rice roots (Xu and Coleman-Derr, 2019).

iv. The findings that monoderm abundance is greatest in the aerobic/upland root-associated fraction suggests that increased monoderm abundance in upland rice roots may promote increased drought tolerance of the upland rice. Like the bacterial community composition, the fungal community composition in the upland rice roots is different from that in the rhizosphere of irrigated rice, particularly no members of Chytridiomycota phylum are present in aerobic rice roots because they are aquatic fungi, so they can be well adapted to well-irrigated paddies (Xu and Coleman-Derr, 2019).

v. In plants, total soluble sugar and Proline contents are very important biochemical markers of abiotic tolerance. Soluble sugars, by maintaining osmotic turgor, play a role in drought tolerance. Under drought stress, the concentrations of soluble sugar in plant leaves increase sharply. Antioxidant levels are decreased in PGPR inoculated plants and the production of proline, free amino acids and soluble sugars increase under drought and salinity stress (Vardharajula et al., 2011).

vi. The production of exopolysaccharides (EPS) by some PGPR is very important in case of drought, salinity and heavy metal tolerance. The water-holding capacity of the soil can be ameliorated by the production of EPS, thus promoting the stability of soil aggregates (Naseem and Bano, 2014).

vii. ACC Deaminase activity is very important and is considered to be one of the major mechanisms performedby PGPR to improve plant growth under stress conditions by lowering ethylene levels. ACC Deaminase converts the ethylene precursor 1-

aminocyclopropane-1-carboxylate (ACC) into α-ketobutyrate and ammonia. High levels of are responsible for reduced plant growth, drooling and early senescence in plants under stress conditions. By lowering ethylene levels through its degradation within roots or in exudates, PGPR can stimulate root growth and improve plant tolerance to stress conditions (Bouffaud et al., 2018). Many studies have shown the role of various PGPR in growth promotion of rice under abiotic stress conditions (Table 2).

viii. The aerobic conditions in the rhizosphere of aerobic/upland rice roots induces the deposition of Fe and Mn oxides on the surface of roots and the formation of iron plaque. Fe plaques affect the bio-availability of heavy metals in the rhizosphere via adsorption or co-precipitation with Fe and Mn oxides. These Fe plaques show remarkable heavy metal removal and also act as nutrient reservoirs (Dong et al., 2016). Typical Fe-oxidizing bacteria (FOB) include *Acidithiobacillus ferrooxidans, Pseudomonas spp., Rhodopseudomonas palustris and Thiobacillus denitrificans* (Hedrich et al., 2011). Mn –oxidising bacteria (MOB) include species of *Erythrobacter, Leptothrix, Suberites, Gaeumannomyces* and *Bacillus* (Wang et al., 2011).

PGPR in biotic stress tolerance

Rice is an important staple food crop. Due to its wider adaptability, the crop is widespread all over the world under different ecological conditions. Rice is susceptible to various diseases such as leaf blight, sheath blight, spot, blast, etc. a lot of disease management strategies have been used to reduce the disease epidemics and crop yield losses such as use of chemicals like bactericides, fungicides, etc. but these remain unsuccessful due to (Bhattacharya and Jha, 2012):

a) Lack of specificity in the mode of action of chemicals used.
b) Variation in the sensitivity of pathogens.
c) Development of mutations in pathogens.
d) Leftover toxic residues which may enter food chain.

e) Use of host resistance genes for plant breeding become ineffective due to evolution of sub-populations that overcome these resistance genes.

Table 2. PGPR reported and used as bioinoculant for stress tolerance in rice.

PGPR used	Type of stress	Mechanism of action	Effect of PGPR inoculation	References
Enterobacter sp.	Salinity	ACC deaminase activity and IAA production	Promote rice seedling growth	Sarkar et al., 2018
Alcaligenes, Bacillus and Ochrobactrum	Salinity	ACC deaminase activity and IAA production	Higher root fresh weight and dry weight, better root system	Bal et al., 2013
Pseudomonas sp., Bacillus sp., Arthrobacter sp.	Drought	Higher proline content, higher activity of superoxide dismutase, catalase, ascorbate peroxidase and lower level of hydrogen peroxide, malondialdehyde in leaves	Enhanced chlorophyll content, higher fresh weights	Gusain et al., 2015
Aspergillus fumigatus	Drought	Higher levels of antioxidant molecules, regulation of NADPH oxidases and heat shock proteins	Higher root and shoot growth and dry matter accumulation	Qin et al., 2019

Bacillus megaterium and Neorhizobium huautlense	Cadmium	Immobilization, decreased available Cd content in the rhizosphere	Decreased Cd concentration in grains and plant	Li et al., 2017
Ochrobactrum sp.	Cadmium	ACC deaminase, siderphores and antioxidant molecules production	Enhanced germination percentage, relative root elongation and higher protease activity	Pandey et al., 2013
Bacillus sp.	Lead and Arsenic	ACC deaminase, siderphores and antioxidant molecules production	Enhanced germination percentage and protease activity, relative root elongation	Pandey et al., 2013
Burkholderia sp. and Bacillus sp.	Cadmium and Arsenic	Fe and Mn oxides and Fe plaque formation	Enhanced photosynthetic activity, improved plant growth	Dong et al., 2016

As a result, biological control using efficient microorganisms seems to be a cost effective and environmentally friendly way to manage different diseases in rice. PGPRs increase the availability of nutrient, synthesize siderophores which capture nutrients particularly iron, stimulate the growth of roots and shoots through the production of plant hormones such as auxin, cytokinins, and gibberellins and protect the plants against pathogen attack through various mechanisms (Table 3):

Production of antimicrobial compounds is the major mechanism by which inoculated biocontrol agent can avoid or inhibit the growth of

phytopathogens. e.g. surfactin, phenazine, pyochellin, phloroglucinol, rhamnolipis, cyclic lipopeptides, etc.

Certain pathogenic microorganisms are capable of producing various extracellular secondary metabolites, which possess a variety of attributes such as they can function as virulence factors, biosurfactants, high-affinity iron chelating siderophores and molecules in cell-to-cell signaling, thus, making them capable of adapting different environments, colonize various hosts and compete for space and nutrients (Glick , 2012).

Rhizobacteria activates the defense of the plant, known as induced systemic resistance (ISR). Reports show that during ISR various activities in plants are stimulated such as increased production of reactive oxygen species (ROS), activity of proteins related to pathogenesis (PRP), and accumulation of phytoalexins, as well as the deposition of callose and lignin into the plant cell wall (Bhattacharya and Jha 2012).

Many of ISR responses are regulated by cross communication between plant hormones, such as salicylic acid (SA), jasmonic (JA) acid, and their derivatives, and by the central roles, these hormones play in defense responses (Bhattacharya and Jha 2012).

The first reaction generally expressed as hypersensitivity reaction to the entry of phytopathogen into plant system is the physical and mechanical strength of the cell wall, which leads to histological changes, such as formation of papillae, lignification, deposition of callose, and thickening of the cell wall due to deposition of phenols and substances similar to hydrogen peroxide (Li et al., 2016).

Lignocellulosic biomass is composed of cellulose, hemicellulose, and lignin, and lignin is the most recalcitrant to biodegradation. The increase in the amount of these polymers may confer better organization of macrofibrils, which mediate the structural stability of the root cell wall (Pang et al., 2020).

Several PGPR are known to form biofilm formation which helps PGPR to attach to the roots of rice seedling and decreased the adhesion of pathogenic microbes at the root surface presented biofilm formation, which helps the bacteria attach to roots of rice seedlings (Pang et al., 2020).

Table 3. PGPR reported and used as bioinoculant for disease suppression in rice.

Disease	Causal organism	Biocontrol agent used	Mechanism of action reported	References
Rice blast	*Magnaporthe oryzae*	*Bacillus* sp. and *Serratia* sp.	Production of antimicrobial compounds, β-1,3-glucanase activity, lipoxygenase, phenylalanine ammonia lyase	Sperandio et al., 2017
Leaf blast	*Pyricularia oryzae*	*Pseudomonas aeruginosa, Corynebacterium agropyri, Bacillus amyloliquefaciens, Trichoderma harzianum*	Production of siderophore and chitinase, Polyphenol oxidase, phenylalanine ammonia lyase, Peroxidase	Ng et al., 2016
Panicle fungal disease	*Curvularia lunata, Fusarium semitectum Helminthosporium oryzae*	*Bacillus amyloliquefaciens*	Production of antimicrobial lipopeptides	Saechow et al., 2018
Bacterial leaf blight	*Xanthomonas oryzae*	*Bacillus pumilus* and *B. subtilis*	Induced systemic resistance, Production of Polyphenol oxidase, phenylalanine ammonia lyase,	Udayashankar et al., 2011

			Peroxidase	
Bacterial leaf blight	*Xanthomonas oryzae*	*Serratia sp., Bacillus sp.* and *Pseudomonas sp.*	Production of siderophores, Polyphenol oxidase, phenylalanine ammonia lyase, Peroxidase	Yasmin et al., 2016
Sheath blight	*Rhizoctonia solani*	*Paenibacillus polymyxa, P. jamilae*	Production of antimicrobial compounds	Holland, 2019
Blast	*Magnaporthe oryzae*	*Pseudomonas chlororaphis*	Production of antimicrobial compounds and HCN	Spence et al., 2014

Conclusion

Rice plant adaptations to aerobic conditions is necessity of the present world due to lack water availability in the future years as predicted. There are a few studies regarding the functionalities of root-microbiome communities associated with aerobic/upland rice. The survival of rice plants in aerobic conditions may be attributed to the large repertoire of beneficial microbes associated with their roots. A systematic study is required to analyze the diversity of culturable endophytic and rhizospheric microorganisms and establish their functional, ecological and nutritional roles in aerobic cultivation conditions of rice. Additionally, compound studies in field and greenhouse conditions are required to identify the most efficient PGPR inoculant which can competitively colonize the roots of rice plants and endow the plant with its beneficial aspects.

References

Adak, A., Prasanna, R., Babu, S., Bidyarani, N., Verma, S., Pal, M., Shivay, Y. S., and Nain, L. (2016). Micronutrient enrichment mediated by plant-microbe interactions and rice cultivation practices. *Journal of Plant Nutrition* **39**, 1216-1232.

Bal, H. B., Nayak, L., Das, S., & Adhya, T. K. (2013). Isolation of ACC deaminase producing PGPR from rice rhizosphere and evaluating their plant growth promoting activity under salt stress. *Plant and soil*, *366*(1-2), 93-105.

Banik, A., Dash, G. K., Swain, P., Kumar, U., Mukhopadhyay, S. K., & Dangar, T. K. (2019). Application of rice (Oryza sativa L.) root endophytic diazotrophic Azotobacter sp. strain Avi2 (MCC 3432) can increase rice yield under green house and field condition. *Microbiological research*, *219*, 56-65.

Bouffaud, M. L., Renoud, S., Dubost, A., Moënne-Loccoz, Y., & Muller, D. (2018). 1-Aminocyclopropane-1-carboxylate deaminase producers associated to maize and other Poaceae species. *Microbiome*, *6*(1), 114.

Bouman, B. A. M., Peng, S., Castaneda, A. R., & Visperas, R. M. (2005). Yield and water use of irrigated tropical aerobic rice systems. *Agricultural Water Management*, *74*(2), 87-105.

Cruz, J. A., Lantican, N. B., Delfin, E. F., & Paterno, E. S. (2014). Enhancement of growth and yield of upland rice (Oryza sativa L.) var. NSIC Rc 192 by actinomycetes. *Journal of Agricultural Technology*, *10*(4), 875-883.

Dong, M. F., Feng, R. W., Wang, R. G., Sun, Y., Ding, Y. Z., Xu, Y. M., ... & Guo, J. K. (2016). Inoculation of Fe/Mn-oxidizing bacteria enhances Fe/Mn plaque formation and reduces Cd and As accumulation in Rice Plant tissues. *Plant and soil*, *404*(1-2), 75-83.

Gandhi, A., & Muralidharan, G. (2016). Assessment of zinc solubilizing potentiality of Acinetobacter sp. isolated from rice rhizosphere. *European Journal of Soil Biology*, *76*, 1-8.

Glick, B. R. (2012). Plant growth-promoting bacteria: mechanisms and applications. *Scientifica*, *2012*.

Gusain, Y. S., Singh, U. S., & Sharma, A. K. (2015). Bacterial mediated amelioration of drought stress in drought tolerant and susceptible cultivars of rice (Oryza sativa L.). *African Journal of Biotechnology*, *14*(9), 764-773.

Hala, Y. (2020, April). The effect of nitrogen-fixing bacteria towards upland rice plant growth and nitrogen content. In *IOP*

Conference Series: Earth and Environmental Science (Vol. 484, No. 1, p. 012086). IOP Publishing.

Hedrich, S., Schlömann, M., & Johnson, D. B. (2011). The iron-oxidizing proteobacteria. *Microbiology*, *157*(6), 1551-1564.

Holland, A. (2019). Evaluation of Paenibacillus PGPR strains for growth promotion and biocontrol of rice sheath blight.

Khanghahi, M. Y., Pirdashti, H., Rahimian, H., Nematzadeh, G. H., Sepanlou, M. G., Salvatori, E., & Crecchio, C. (2019). Leaf photosynthetic characteristics and photosystem II photochemistry of rice (Oryza sativa L.) under potassium-solubilizing bacteria inoculation. *Photosynthetica*, *57*(2), 500-511.

Li, T., Sun, Y., Ruan, Y., Xu, L., Hu, Y., Hao, Z., Zhang, X., Li, H., Wang, Y., Yang, L., & Chen, B. (2016). Potential role of D-myo-inositol-3-phosphate synthase and 14-3-3 genes in the crosstalk between Zea mays and Rhizophagus intraradices under drought stress. *Mycorrhiza*, *26*(8), 879-893.

Li, Y., Pang, H. D., He, L. Y., Wang, Q., & Sheng, X. F. (2017). Cd immobilization and reduced tissue Cd accumulation of rice (Oryza sativa wuyun-23) in the presence of heavy metal-resistant bacteria. *Ecotoxicology and environmental safety*, *138*, 56-63.

Liu, Y., Tan, H., Cao, L., & Zhang, R. (2020). Rice sprout endophytic Enterobacter sp. SE-5 could improve tolerance of mature rice plants to salt or Cd2+ in soils. *Archives of Agronomy and Soil Science*, *66*(7), 873-883.

Mantelin, S., & Touraine, B. (2004). Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. *Journal of experimental Botany*, *55*(394), 27-34.

Nascente, A. S., Lanna, A. C., de Sousa, T. P., Chaibub, A. A., de Souza, A. C. A., & de Filippi, M. C. C. (2019). N fertilizer dose-dependent efficiency of Serratia spp. for improving growth and yield of upland rice (Oryza sativa L.). *International Journal of Plant Production*, *13*(3), 217-226.

Naseem, H., & Bano, A. (2014). Role of plant growth-promoting rhizobacteria and their exopolysaccharide in drought tolerance of maize. *Journal of Plant Interactions*, *9*(1), 689-701.

Ng, L. C., Sariah, M., Sariam, O., Radziah, O., & Abidin, M. Z. (2016). PGPM-induced defense-related enzymes in aerobic rice against rice leaf blast caused by Pyricularia oryzae. *European Journal of Plant Pathology*, *145*(1), 167-175.

Othman, R., & Panhwar, Q. A. (2014). Phosphate-solubilizing bacteria improves nutrient uptake in aerobic rice. In *Phosphate Solubilizing Microorganisms* (pp. 207-224). Springer, Cham.

Pandey, S., Ghosh, P. K., Ghosh, S., De, T. K., & Maiti, T. K. (2013). Role of heavy metal resistant Ochrobactrum sp. and Bacillus spp. strains in bioremediation of a rice cultivar and their PGPR like activities. *Journal of Microbiology*, *51*(1), 11-17.

Pang, Z., Zhao, Y., Xu, P., & Yu, D. (2020). Microbial Diversity of Upland Rice Roots and Their Influence on Rice Growth and Drought Tolerance. *Microorganisms*, *8*(9), 1329.

Parewa, H. P., Meena, V. S., Jain, L. K., & Choudhary, A. (2018). Sustainable crop production and soil health management through plant growth-promoting Rhizobacteria. In *Role of rhizospheric microbes in soil* (pp. 299-329). Springer, Singapore.

Prasanna, R., Joshi, M., Rana, A., Shivay, Y. S., & Nain, L. (2012). Influence of co-inoculation of bacteria-cyanobacteria on crop yield and C–N sequestration in soil under rice crop. *World Journal of Microbiology and Biotechnology*, *28*(3), 1223-1235.

Qin, W., Liu, C., Jiang, W., Xue, Y., Wang, G., & Liu, S. (2019). A coumarin analogue NFA from endophytic Aspergillus fumigatus improves drought resistance in rice as an antioxidant. *BMC microbiology*, *19*(1), 1-11.

Rasul, G. (2016). Managing the food, water, and energy nexus for achieving the Sustainable Development Goals in South Asia. *Environmental Development*, *18*, 14-25.

Rêgo, M. C. F., Ilkiu-Borges, F., de Filippi, M. C. C., Gonçalves, L. A., & da Silva, G. B. (2014). Morphoanatomical and biochemical changes in the roots of rice plants induced by plant

growth-promoting microorganisms. *Embrapa Arroz e Feijão-Artigo em periódico indexado (ALICE)*.

Saechow, S., Thammasittirong, A., Kittakoop, P., Prachya, S., & Thammasittirong, S. N. R. (2018). Antagonistic activity against dirty panicle rice fungal pathogens and plant growth-promoting activity of Bacillus amyloliquefaciens BAS23. *J Microbiol Biotechnol*, *28*(9), 1527-1535.

Sarkar, A., Ghosh, P. K., Pramanik, K., Mitra, S., Soren, T., Pandey, S., Mondal, M. H., & Maiti, T. K. (2018). A halotolerant Enterobacter sp. displaying ACC deaminase activity promotes rice seedling growth under salt stress. *Research in microbiology*, *169*(1), 20-32.

Selvakumar, G., Panneerselvam, P., & Ganeshamurthy, A. N. (2012). Bacterial mediated alleviation of abiotic stress in crops. In *Bacteria in agrobiology: Stress management* (pp. 205-224). Springer, Berlin, Heidelberg.

Shabanamol, S., Divya, K., George, T. K., Rishad, K. S., Sreekumar, T. S., & Jisha, M. S. (2018). Characterization and in planta nitrogen fixation of plant growth promoting endophytic diazotrophic Lysinibacillus sphaericus isolated from rice (Oryza sativa). *Physiological and Molecular Plant Pathology*, *102*, 46-54.

Singh, M. (2018). Isolation and characterization of insoluble inorganic phosphate solubilizer rice rhizosphere strain Enterobacter cloacae BAU3. *Journal of Applied and Natural Science*, *10*(4), 1204-1209.

Spence, C. A., Raman, V., Donofrio, N. M., & Bais, H. P. (2014). Global gene expression in rice blast pathogen Magnaporthe oryzae treated with a natural rice soil isolate. *Planta*, *239*(1), 171-185.

Sperandio, E. M., do Vale, H. M. M., de Souza Reis, M., Cortes, M. V. D. C. B., Lanna, A. C., & de Filippi, M. C. C. (2017). Evaluation of rhizobacteria in upland rice in Brazil: growth promotion and interaction of induced defense responses against leaf blast (Magnaporthe oryzae). *Acta Physiologiae Plantarum*, *39*(12), 259.

Supari, N., Alam, S. A. Z., Javed, M. A., Tin, L. C., & Sarmidi, M. R. (2014). Germination, seedling growth, amylase and protease activities in Malaysian upland rice seed under microbial inoculation condition. *Journal of Pure and Applied Microbiology*, *8*(4), 2627-2635.

Tamreihao, K., Nimaichand, S., & Ningthoujam, D. S. (2018). Antagonistic and plant growth promoting Bacillus sp. MBRL 576 enhances the growth and yield of rice (Oryza sativa L.). *ORYZA-An International Journal on Rice*, *55*(1), 202-213.

Udayashankar, A. C., Nayaka, S. C., Reddy, M. S., & Srinivas, C. (2011). Plant growth-promoting rhizobacteria mediate induced systemic resistance in rice against bacterial leaf blight caused by Xanthomonas oryzae pv. oryzae. *Biological Control*, *59*(2), 114-122.

Vardharajula, S., Zulfikar Ali, S., Grover, M., Reddy, G., & Bandi, V. (2011). Drought-tolerant plant growth promoting Bacillus spp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress. *Journal of Plant Interactions*, *6*(1), 1-14.

Wang, X., Wiens, M., Divekar, M., Grebenjuk, V. A., Schröder, H. C., Batel, R., & Müller, W. E. (2011). Isolation and characterization of a Mn (II)-oxidizing Bacillus strain from the demosponge Suberites domuncula. *Marine drugs*, *9*(1), 1-28.

Xu, L., & Coleman-Derr, D. (2019). Causes and consequences of a conserved bacterial root microbiome response to drought stress. *Current opinion in microbiology*, *49*, 1-6.

Yadav, J., Verma, J. P., Jaiswal, D. K., & Kumar, A. (2014). Evaluation of PGPR and different concentration of phosphorus level on plant growth, yield and nutrient content of rice (Oryza sativa). *Ecological engineering*, *62*, 123-128.

Yasmin, S., Zaka, A., Imran, A., Zahid, M. A., Yousaf, S., Rasul, G., Arif, M., & Mirza, M. S. (2016). Plant growth promotion and suppression of bacterial leaf blight in rice by inoculated bacteria. *PloS one*, *11*(8), e0160688.

MICROBIAL NITROGEN FIXATION ENHANCING SOIL FERTILITY IN ARID SOILS OF INDIAN THAR DESERT

Rajbala Junia[1], Vishakha Sharma[1], Neelam Jain[2] , G. K. Aseri[3*]

[1] Ph.D. Scholar, Amity Institute of Microbial Technology, Amity University Rajasthan
[2] Professor, Amity Institute of Biotechnology, Amity University, Jaipur, Rajasthan, India
[3] Professor, Amity Institute of Microbial Technology, Amity University, Jaipur, Rajasthan, India

[*]Corresponding Author: gkaseri@jpr.amity.edu

Introduction

The Thar Desert, or the Great Indian Sand Desert (285,000 km^2), is situated in the arid western part of Rajasthan state in India and includes the adjoining sandy terrain of Pakistan till the Indus River. It forms a distinctive, but integral part of the arid lands of western India that runs through the states of Punjab, Haryana, Rajasthan and Gujarat (Kar, 2014). The soils of this tract have been mapped in Entisols, Aridisols and Alfisols soil orders. The Entisols cover maximum 17134.26 thousand hectares (54.21%), followed by Aridisols and Alfisols. The area of latter two is 14254.32 and 213.10 thousand hectares, comprising 45.1 and 0.67% of hot arid India. Arid soil is one of the most prevalent soil orders of the world and it contains high level of calcium carbonates, gypsum as well as sodium (Kumar *et al.,* 2009).

Arid environments are characterized by a low amount of soil nutrients and organic matter, poor soil structure, high salinity, water deficiency, extreme temperature and desiccation, drought and physical instability caused by strong wind and UV radiation. Poor soil structure and low organic matter reduce the water-holding

capacity of the soil, which is also linked with loss of fertility and increased emission of greenhouse gases. These limitations cause loss of natural vegetation and crop yield, thus a reduction in CO2 uptake. The combined effects of some of these limitations, such as poor soil water quality because of high salinity, the application of pesticides and fertilizers to increase yield, and increased land use often lead to desertification, which also leads to land abandonment and displacement of people (Ayangbenro *et al.*,2020) .

Potential in Arid Soil

The Indian Desert is not an endless stretch of sand-dunes bereft of life or vegetation. To most of us, the word "desert" conjures up the vision of a vast, tree-less, undulating, buff expanse of sand, crisscrossed by caravans of heavily-robed nomads on camel-back. But in arid soils several biological phenomena including microbial population exist that are potentially used for restoring hot desert environments. Hence the arid wastes must be reclaimed for the future to feed our growing population. Although the rapid expansion of deserts in recent decades as a result of human actions combined with climate disasters has highlighted the necessity to understand biological processes in arid environment. Arid ecosystems have a highly heterogeneous distribution of resources with greater nutrient concentration & microbial densities occurring in vegetated than in bare soils. Sources of desert soil fertility include parent material weathering Aeolian deposition & on-site C & N biotic fixation. While parent materials provide many soil nutrients, aeolian deposition can provide up to 75% of plant, essential nutrients including N, P, K, Mg, Na, Mn, Cu & Fe. Soil surface biota is often sticky, & helps much of the N inputs. Most desert soils are protected by cyanobacteria-Lichen-moss soil crusts, chemical crusts (Gaskell *et al.*,2007).

Arid soils are very fertile as they include a living & biological crust which is formed by algae, moss, lichens in a gap, however it doesn’t have the sufficient rainfall to sustain life. When it rains dormant seeds wake up & form desert blooms. The permanent vegetation

(like cacti & shrubs) is very well adapted to living without moisture for long periods of time. The phosphate content of these soils is as high as in normal alluvial soils. Nitrogen is originally low but its deficiency is made up to some extent by the availability of nitrogen in the form of nitrates. Thus, the presence of phosphates and nitrates make them fertile soils wherever moisture is available (Kumar *et al.*,2009). There is, therefore, great possibility of reclaiming these soils if proper irrigation facilities area available. The changes in the cropping pattern in the Indira Gandhi Canal Command Area are a living example of the utility of the desert soils.

It has the potential to be extremely fertile if it is irrigated due to all the macro nutrients in the silt which is carried by Aeolians transport (wind). Crop or pasture production is possible with most efficient use of available water and required detailed study of plant climate relationships while selecting the best suitable crops or pasture species to be grown with occurrence of monsoon. History of arid land indicates that because of low precipitation soil is under-utilised for crop production and simultaneously not providing a good environment for microbial interactions throughout the year, desert soil activates its mineralization for one-fourth year only. Since it is kept untouched, its bound nutrients are available for future; this potential can be harness by adopting suitable technologies.

Soil Nitrogen Significance

After photosynthesis, nitrogen fixation is considered as the second most important process influencing primary productivity and is the basis of all life on earth. Annually, approximately 2.5×10^{11} kg NH_3 is fixed from the atmosphere through BNF (by legumes and cyanobacteria) and approximately 8×10^{10} kg are manufactured by ammonia industry (Cheng *et al.*,2008). Further, lightning may also contribute approximately 1×10^{10} kg NH_3/year worldwide. Currently, approximately 2 tonnes of industrially fixed nitrogen is needed as fertilizer for crop production to equal the effects of 1 tonne of nitrogen biologically fixed by legume crops. Therefore, biologically fixed nitrogen influences the global nitrogen cycle substantially less than industrially fixed nitrogen.

Table 1. Nitrogen forms available for plant uptake

Form of Nitrogen	Formula	Availability for plant uptake
Nitrogen gas	N_2	Although 78% of our atmosphere is nitrogen gas, this form of nitrogen must be transformed to usable forms before it is available for plant uptake.
Ammonia	NH_3	Ammonia is a gas. Ammonium can escape from the surface of the soil under certain conditions and is harmful to plants in high quantities. Ammonium is the basic building block of commercial nitrogen fertilizers.
Ammonium	NH_4^+	Soil particles attract and retain ammonium on cation exchange complexes. This form may be directly taken up by plants.
Nitrate	NO_3^-	Nitrate is the second form of nitrogen which is available for plant uptake. In most soils, nitrate is highly mobile. However, in the highly weathered soils of Hawaii, nitrate is stored in soils with 'anion exchange capacity' and becomes less mobile.
Nitrite	NO_2^-	Nitrite is an intermediate product in the conversion of ammonium to nitrate (nitrification). It is usually present in low quantities, but is toxic to plants.
Organic Nitrogen	Various compounds	Organic nitrogen must be converted to ammonium before it is used by plants. This conversion occurs with time and is known as mineralization.

Since 1970, world population has increased by 78% and reactive nitrogen creation has increased by 120%. Efforts for N budgeting for 2050 AD show that human activities are increasingly dominating the N budget at the global and regional scales. In addition, the terrestrial and open ocean N budgets are essentially disconnected, leading to the accumulation of fixed forms of N in most environmental reservoirs (Gallowy *et al.,* 2004). The largest uncertainties in our understanding of the N budget at most scales are the rates of natural biological nitrogen fixation, the amount of N storage in most environmental reservoirs and the production rates of N_2 by denitrification.

Nitrogen is essential for protein production in plants which is the direct or indirect source of protein for animals and human nutrition. It is the Key primary plant nutrient responsible for rapid growth of green vegetation in plants Nitrogen in soils is present in organic (95-99%) & inorganic (1-5%) forms. Depletion of soil organic nitrogen by synthetic nitrogenous fertilizers are disputed, there can be no disagreement about their conclusion that a transition may be required toward agricultural diversification using legume-based crop rotations, which provide a valuable means to reduce the intensity of ammoniacal fertilization with the input of less reactive organic N (Mulvaney *et al.*,2009).

Nitrogen fixation and Mobilization

The discovery of N-fixation was attributed to the German scientist, Hellrigel and Wilfarth in 1886, who reported that legumes bearing root nodules could be gaseous (molecular) nitrogen. In 1888, Brijerinck, Dutch microbiologist succeeded in isolating *Rhizobium leguminosarum* strain from root modular (Franc *et al.,* 2016). Nitrogen is fixed, or combined, in nature as nitric oxide by lightning and ultraviolet rays, but more significant amounts of nitrogen are fixed as ammonia, nitrites, and nitrates by soil microorganisms. More than 90 percent of all nitrogen fixation is affected by them. Two kinds of nitrogen-fixing microorganisms are recognized: free-living (non-symbiotic algae) *Anabaena* and *Nostoc* and genera such as *Azotobacter*, *Beijerinckia* and *Clostridium* and

mutualistic (symbiotic) bacteria such as *Rhizobium*, associated with leguminous plants, and various *Azospirillum* species, associated with cereal grasses (Franche *et al.*,2009).

The symbiotic nitrogen-fixing bacteria invade the root hairs of host plants, where they multiply and stimulate the formation of root nodules, enlargements of plant cells and bacteria in intimate association. Within the nodules, the bacteria convert free nitrogen to ammonia, which the host plant utilizes for its development (To ensure sufficient nodule formation and optimum growth of legumes(e.g., alfalfa, beans, clovers, peas, soybeans), seeds are usually inoculated with commercial cultures of appropriate *Rhizobium* species, especially in soils poor or lacking in the required bacterium (Zahren *et al.*,1999).

The biological fixation of nitrogen is an anaerobic process catalysed by the enzyme nitrogenase and requires a source of reductant, ATP and ammonia assimilating machinery. Enormous progress in almost all aspects of biological nitrogen fixation has been made in the past century, especially in the recent two decades in genetics and biochemistry (Swain *et al*.,2013). Nitrogenase is encoded by a set of operons, which includes regulatory genes such as nif LA, and structural genes such as nif HDK and other supplementary genes *Klebsiella pneumoniae* has been the *E. coli* of BNF and it is most well studied with respect to the regulation, synthesis and assembly of nitrogenase. A 24kb base pair DNA region contains the entire *K. pneumoniae nif* cluster, which contains 20 genes.

Nitrogen is an essential nutrient for plant growth and development but is unavailable in its most prevalent form as atmospheric nitrogen. Plants instead depend upon combined, or fixed, forms of nitrogen, such as ammonia and nitrate. Much of this nitrogen is provided to cropping systems in the form of industrially produced nitrogen fertilizers. Use of these fertilizers has led to worldwide, ecological problems, such as the formation of coastal dead zones. Biological nitrogen fixation, on the other hand, offers a natural means of providing nitrogen for plants. It is a critical component of

many aquatic, as well as terrestrial ecosystems across our biosphere (Hubdel *et al.,* 2009).

Microbes Mediated Mineralization

Microbe-mediated mineralization is ubiquitous in nature, involving bacteria, fungi, viruses, and algae. These mineralization processes comprise calcification, silicification, and iron mineralization. The mechanisms for mineral formation include extracellular and intracellular biomineralization. The mineral precipitating capability of microbes is often harnessed for green synthesis of metal nanoparticles, which are relatively less toxic compared with those synthesized through physical or chemical methods. Microbe-mediated mineralization has important applications ranging from pollutant removal and nonreactive carriers, to other industrial and biomedical applications. Microbe-mediated mineralization has important applications ranging from reclamation of degraded lands, pollutant removal and nonreactive carriers, to other industrial and biomedical applications. (Qin *et al.*,2020). The collective genome of rhizosphere microbial community enveloping plant roots is larger compared to that of plants which is referred as microbiome (Bulgarelli *et al.*,2013). A major focus in the coming decades would be on safe and eco-friendly methods by exploiting the beneficial microorganisms in sustainable crop production (Nina K *et al.*,2014). Such microorganisms in general, consists of diverse naturally occurring microbes whose inoculation to the soil ecosystem advances soil physicochemical properties, soil microbes biodiversity, soil fertility, plant growth and development and crop productivity (Sahoo *et al.*,2013). Mycorrhizal fungi as well as the bacteria present in nodulated legumes were both recognized as root symbionts from the second half of 19th century. Already in the 1950s, crop seeds were coated with bacterial cultures (*Azotobacter chroococcum* or *Bacillus megaterium*) to improve growth and yield. By the 1980s many different bacterial strains, mainly *Pseudomonas* but also *Azospirillum*, had been described as having plant growth promoting effects (Jacoby *et al.*,2017). In natural ecosystems, most nutrients such as N, P, and S are bound in organic molecules and are therefore minimally bioavailable for plants. To access these nutrients, plants are dependent on the growth of soil microbes such

as bacteria and fungi, which possess the metabolic machinery to depolymerize and mineralize organic forms of N, P, and S. The contents of these microbial cells are subsequently released, either through turnover and cell lysis, or via protozoic predation (Richardson *et al.*,2009) This liberates inorganic N, P, and S forms into the soil, including ionic species such as ammonium, nitrate, phosphate, and sulfate that are the preferred nutrient forms for plants (van der Heijden *et al.*,2008). In natural settings, these microbial nutrient transformations are key drivers of plant growth, and can sometimes be the rate-limiting step in ecosystem productivity (Schimel and Bennett, 2004). Different bacterial populations respond to the environmental modification, induced by the release of root exudates, modifying their own physiology. This leads to a selection favouring the bacterial populations best adapted to this new condition. This is at the base of the coevolution between the plant and its microbiota. As a result, the induced shift in the microbial community composition and activity leads to different effects on plant development. Microorganisms can stimulate rhizodeposition and establish relationships for nutrient acquisition. They can produce and consume trace gas, influence acidity, water availability, and hydrophobicity of soil. Moreover, they can degrade xenobiotic compounds and chelate metals (Gamalero *et al.,* 2020). Hence these microorganisms as bio-inoculants might have developed microclimate and initiate nutrient solubilization which has readily taken up by the plants and used in the metabolism and resulted in higher growth of the plants. (Junia *et al.*,2020). Dominant bacterial phyla typically found in different xeric soils include Actinobacteria, Bacteroidetes, Firmicutes, Proteobacetria and Cyanobacteria. Other less dominant phyla are the Chloroflexi, Deinococcus and Verrucomicrobiae. Archaea phyla found in dryland soils are Euryarchaeota, Crenarchaeota, Thaumarchaeota (Cowan *et al.*, 2019)

Similar microbial diversity associated with different drylands can be attributed to similar extreme abiotic conditions of these ecosystems, which affect microbial activities in these environments. Some free-living diazotrophs of the genera *Azoarcus* and *Azotobacter* are

present in the rhizosphere and bulk soil at comparable densities to fix atmospheric nitrogen, while the genera *Azospirillum* and *Herbaspirillum* only colonize the rhizosphere to fix nitrogen (Rashid *et al.*,2016). Examples of symbiotic nitrogen-fixing bacteria of the family Rhizobiaceae are *Azorhizobium*, *Allorhizobium*, *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium* and *Sinorhizobium*, which fix 80% of biological nitrogen. These organisms form a symbiotic relationship with leguminous plants such as *Arachis hypogaea*, *Cyamposis tetragonoloba*, *Medicago sativa* and *Melilotus* spp. that are well adapted to extreme abiotic conditions in arid and semi-arid lands. *Bradyrhizobium* and *Rhizobium* establish symbiotic relationships with roots of leguminous plants such as alfalfa, pea, peanut and soybean, transform atmospheric nitrogen to ammonia, which is accessible to plants in drylands. PGP bacteria found on the rhizoplane or within the apoplast of the root cortex such as *Arthrobacter*, *Azospirillum*, *Burkholderia*, *Bacillus*, *Chromobacterium*, *Azotobacter Caulobacter*, *Agrobacterium*, *Flavobacterium*, *Erwinia*, *Pseudomonas*, *Micrococcous*, and *Serratia* fix nitrogen biologically (Vimal *et al.*,2017). *Azotobacter*, *Azospirillum*, *Rhizobium*, *Cyanobacteria*, phosphorus and potassium solubilising microorganisms and Mycorrhizae are some of the PGPRs that were found to increase in the soil under no tillage or minimum tillage treatment (Aziz *et al.*,2012). Efficient strains of *Azotobacter*, *Azospirillum*, *Phosphobacter* and *Rhizobacter* can provide significant amount of nitrogen to increase the plant height, number of leaves, stem diameter percentage of seed filling and seed dry weight (Dhanasekar *et al.*,2012). Similarly, in addition of *Azotobacter*, *Azospirillum and Rhizobium* promotes the physiology and improves the root morphology (Choudhury and Kennedy, 2004). *Azotobacter* plays an important role in the nitrogen cycle in nature as it possesses a variety of metabolic functions. Besides playing role in nitrogen fixation, *Azotobacter* has the capacity to produce vitamins such as thiamine and riboflavin, and plant hormones viz., indole acetic acid (IAA), gibberellins (GA) and cytokinins (CK) (Abd *et al.*,2013). *A. chroococcum* improves the plant growth by enhancing seed germination and advancing the root architecture by inhibiting pathogenic microorganisms around the

root systems of crop plants. This genus includes diverse species, namely, *A. chroococcum, A.vinelandii, A. beijerinckii, A. nigricans, A. armeniacus* and *A. paspali.* It is used as a biofertilizer for different crops viz., wheat, oat, barley mustard, seasum, rice, linseeds, sunflower, castor, maize, sorghum, cotton, jute, sugar beets, tobacco, tea, coffee, rubber and coconuts (Wani *et al.*,2013). *Azospirillum* is another free-living, motile, gram variable and aerobic bacterium that can thrive in flooded conditions and promotes various aspects of plant growth and development (Bhattacharya *et al.,* 2012). *Azospirillum* was shown to exert beneficial effects on plant growth and crop yields both in greenhouse and in field trials. Diverse species of the genus *Azospirillum* including *A. lipoferum, A. brasilense, A. amazonense, A. halopraeferens* and *A. irakense* have been reported to improve productivity of various crops (Sahoo *et al.*,2014). Interestingly, it was observed that *Azospirillum* inoculation can change the root morphology via producing plant growth regulating substances via siderophore production. It also increases the number of lateral roots and enhances root hairs formation to provide more root surface area to absorb sufficient nutrients (Mehdipour *et al.*,2012). This improves the water status of plant and aids the nutrient profile in the advancement of plant growth and development. Thus microbial mediated mineralization not only enhances the mobilization of the nutrients to the plants but also builds the soil health and improves soil fertility in degraded and eroded arid soils.

Role of Microbial Nitrogen Fixers on Growth and Production of Arid Crops and improving the soil fertilityin Indian Thar Desert

Application of mineral fertilizers (N, P and K) is a common practice to improve soil fertility and fulfil demand for nitrogen (N) in cereal cropping systems in developed and developing countries but is of great concern now-a-days due its cost of production and also its negative impact on environment. Moreover the application of N-fertilizer requires the selection of Nitrate tolerant Nitrogen fixing legumes to enhance the crop yield. This has led to a growing interest in exploring other sources of N such as biological N_2 fixation.

Table 2: Role of Nitrogen Fixers on Growth and Production of Arid Crops

Crop	Family	Microbes Inoculated	Additional treatment	Effect on productivity and soil fertility	References
			Cereal Crops		
Zea mays (Corn)	Poaceae	*Azotobacter chroococcum* + *Bacillus megaterium* + *Pseudomonas fluorescence*; *Azotobacter chroococcum*	NPK + enriched compost; 150 kg N/ha with FYM @ 5 t/ha	Significant increase in plant height, total dry matter production, Weight of cob, grain yield, Test weight of seeds, maximum uptake of N,P,K and Protein content in maize grain	Umesha *et al.*,2014
Triticum aestivum (Wheat)	Poaceae	*Azospirillum* + PSB	-	Higher grain and straw yield and net returns over control	Kaushik *et al.*,2012
Pennisetum glaucum (Pearl millet)	Poaceae	*Azospirillum brasilense*	-	Enhancement in root growth and nitrogenase activity due to the production of indoles, gibberellins and cytokinins by Azospirillum, increase of grain yield and root dry weight	Joshi, N.L. and Singh, P., 1985
		Azospirillum lipoerum and *Azotobacter chroococcum*	-	Repeated inoculations increased mean grain yield, efficiency of N-assimiIation, Marginal increase in nitrogenase activity Increased leaf Nitrate Reductase Activi'ty (NRA)	Wani *et al.*,1988
Sorghum vulgare (Sorghum)	Poaceae	*Azospirillum brasilense* and *Azotobacter chroococcum*	-	Increase in yield and soil fertility	Tilak *et al.*,1982
			Fruit Crops		
Punica granatum (Pomegranate)	Lythraceae	*Azospirillum brasilense, Azotobacter chroococcum* + *Glomus mosseae*	-	Enhancement in the rhizosphere microbial activity, metabolites and nutrients, plant height, plant canopy, pruned material and	Mir *et al.*,2012

				fruit yield	
Ziziphus mauritiana (Ber)	Rhamnaceae	*Azotobacter chroococcum* + *Glomus mosseae*	-		Aseri and Rao, 2005
Emblica officinalis (Aonla)	Euphorbiaceae	*Azotobacter chroococcum* + *Glomus mosseae*; *Azospirillum brasilense*	-	Improvement in the rhizosphere microbial activity, metabolites and nutrients, plant height, plant canopy, leaf area, collar diameter and fruit yield	Aseri *et al*.,2009
Aegle marmelos (Bael)	Rutaceae	Arbuscular mycorrhizal (AM) fungi and *Azospirillum*	-	Production of maximum plant biomass, shoot phosphorus content and root colonization	Verma *et al*.,2015
			Spices Crops		
Capsicum annuum (Chilli)	Solanaceae	*Azospirillum; Glomusdeserticola* + *Azospirillum*	-	Significant increase in growth, increased plant height and dry weight, N & P content, Fruit dry weight	Vyas and Vyas, 2014
Cuminum cyminum (Cumin)	Apiaceae	*Azotobacter* and PSB	FYM	Increase in Plant height, Number of branches, Number of umbels, Number of umbellate per umbel, Number of seeds per umbellate, Test weight, Volatile Oil, seed yield, and Nutrient (N,P,K) uptake	Patel *et al*, 2013
		Azospirillum @ 1.5 kg/ha	100% inorganic N + 5 t FYM/ha	Maximum number of branches/plant, umbels/plant, umbellets/umbel, seeds/umbel, test weight of seeds,higher seed and straw yields and Nutrient (N,P,K) uptake	Choudhary *et al.*, 2006
Foeniculum vulgare (Fennel)	Apiaceae	-	50% recommended dose of nitrogen (RDN) through vermicompost	higher values of all the growth and yield attributes, viz. plant height, branches/plant, umbels/plant, umbellates/umbel,	Shivran and Jat, 2015

			(VC) + 50% RDN through fertilizers	seeds/umbellate and seed weight	
Pulse Crop (Grain Legumes)					
Vigna unguiculata (Cowpea)	Fabaceae	*Rhizobium* +PSB	-	Significant increase in the chlorophyll content, total root nodules, fresh weight and dry weight of nodules, leghaemoglobin content, number of pods/ plant, number of seeds/pod, seed and biological yield, plant height, number of branches/ plant, straw yield, N & P uptake and protein content	Meena, Verma and Pancholi, 2015
		Rhizobium + PSB	75 % of recommended dose of fertilizer i.e. 15 kg N + 30 kg P_2O_5 ha-^{1}	Increase in pods/ plant, seeds/pod, seed yield, straw yield, N & P uptake and protein content	Khandelwal *et al.*,2012
Cajanus cajan (Pigeon Pea)	Fabaceae	*Rhizobium* + PSB	50% RDF + 5 ton FYM/ha	Improvement in plant height, branches/plant, pods per plant and seed and stalk yield	Poonia, Raj and Pithia, 2014
Vigna radiata (Mung Bean/ Green gram)	Fabaceae	-	NPK 100 % of recommended dose + FYM 10 t ha-1 + vermicompost 5 t ha-1	Enhanced yield and uptake of N,P & K content in seed, straw, increase in residual soil NPK, zinc and iron	Meena *et al.*,2013
		Rhizobium +PSB	PM @ 5 t ha-1	Increase in pods per plant, number of seeds , grain yield, straw yield, N,P,K uptake, protein content,microbial biomass and C,N, P in soil	Mohammad, Yadav and Ahamad, 2017
		Rhizobium + PGPR+ PSB	-	Significant increase in numbers of nodules/plant, dry weight of nodules/plant and grain yield	Bansal, 2009

Cyamposis tetragonoloba (Cluster bean/ guar)	Fabaceae	Rhizobium	60 kg N/ha	Enhancement in seed yield, Nitrogen use efficiency (NUE) and nutrient recovery	Jatav *et al.*,2016
		Rhizobium+PSB	(+/-) 75% RDF (20-17.4 kg N-P/ha)	Increase in growth, yield attributes, and yield,number of effective nodules/plant, nodules dry weight/plant, clusters/plant, seed and stover yield, N and P uptake, higher protein content and Gum content	Singh *et al.*,2014
		Azotobacter	OM+ NPK 50%	Enhanced growth, height, dry weight, fruit weight and macro and micronutrients uptake	Junia *et al.*,2020 b
Cicer arietinum (Chick Pea)		*Rhizobium* + PSB	-	Enhanced growth, plant height, Root nodules/plant, pods/plant, Seeds/pod, Test weight, yield, protein content, Nutrient content and uptake	Das *et al.*,2013
Agro- Forestry Trees					
Acacia senegal (Gum Arabic/ Kher)	Fabaceae	*Bacillus licheniformis* + *Sinorhizobium kostiense*	-	Significant increase in seed germination, maximum root length, shoot length, seedling weight	Singh *et al.*,2011
Acacia nilotica (Babul/Kikar)	Fabaceae	*Azotobacter chroccocum* + *Azospirillum brasilense*+ *Glomus mosseae*	OM+ NPK 50%	Higher growth and improvement in nutrient uptake, maximum root length	Junia *et al.*,2020 a
Azadirachta indica (Neem)	Meliaceae	*Azospirillum brasilense* + *Azotobacter beijerinckii* + *Bacillus thurengensis* (PSB)+ consortia of	-	Increase in shoot length, root length, collar diameter, fresh weight and dry weight	Singh *et al.*,2016

		AMF (species of *Glomus*, *Gigaspora*, *Sclerocystis and Scutellospora*)			
Prosopis cineraria (Khejri)	Fabaceae	*Bacillus licheniformis* and *Sinorhizobium kostiense/Sinorhizobium saheli*	-	Maximum seedling germination, growth, root length, shoot length, seedling weight, length and vigour.	Singh *et al.*,2014
		Rhizobium (cowpea miscellany)+ VAM fungi	-		Niranjan *et al.*,2002.

Nitrogen fixation is a common event in most desert ecosystems, especially in association with leguminous plants or in bacterial populations residing on the surface. Thus Biological Nitrogen Fixation (BNF) mediated through symbiotic bacteria, heterotrophic bacteria, root-associated diazotrophs , AM fungi and legumes can serve as a key to introduce N in arid soils of Thar Desert and can replace nitrogen fertilization wholly or in part. Non-symbiotic N_2 fixation (by free-living bacteria in soils or associated with the rhizosphere) has the potential to meet some of this need especially in the lower input cropping systems worldwide (Roper and Gupta, 2017). The N_2 fixed by heterotrophic free-living bacteria is of minor importance as a mechanism for N input in arid soils. *Azotobacter*, *Azospirillum* and *Gluconacetobacter* are well-known free living aerobic diazotrophs associated with BNF in non-legumes in soil. *Azotobacter chrococcum* among various species is the most commonly occurring species in Indian soil and has the ability to use N_2 as the sole nitrogen source. The impact of these nitrogen fixers on the productivity and on the soil fertility of degraded and arid soils is presented in table 2.

Conclusion

Application of these potent Biological Nitrogen fixers and Bio-inoculants can replace agro-chemicals in the near future only if there is extensive search for effective microbial strains either single or in consortium with multiple traits. It can be concluded that adoption of a balanced fertilizer management approach will safeguard the plant economics and higher productivity of crops and soil fertility under arid conditions in Indian Thar Desert. Integrated Nutrient management can thus help in saving the fertilizer input but will also ensure better fertilizer use efficiency for the land of potential "Thar Desert".

References

Abd El-Fattah, D.A., Ewedab, W.E., Zayed, M.S. and Hassaneina, M.K. (2013). Effect of carrier materials, sterilization method, and storage temperature on survival and biological activities of Azotobacter chroococcum inoculants. *Ann Agric Sci*, 58:111–118.

Aseri, G.K. and Tarafdar, J.C. (2005). Fluorescein Diacetate: a potential biological indicator for arid soils. *Arid land and research management*, 20: 87-99

Aseri, G.K., Jain, N., Panwar, J., Rao, A.V. and Meghwal, P.R. (2008). Biofertilizers improve plant growth, fruit yield, nutrition, metabolism and rhizosphere enzyme activities of pomegranate (*Punica granatum* L.) in Indian Thar Desert. *Sci Hortic*, 117:130–135

Ayangbenro, A.S. and Babalola, O.O. (2020). Reclamation of arid and semi-arid soils: The role of plant growth-promoting archaea and bacteria Current Plant Biology, doi.org/10.1016/j.cpb.2020.100173

Aziz, G., Bajsa, N., Haghjo, T., Taule, C., Valverde, A., Mariano, J. and Arias, A. (2012). bundance, diversity and prospecting of culturable phosphate solubilizing bacteria on soils under crop–

pasture rotations in a no-tillage regime in Uruguay. *Appl Soil Ecol*, 61:320–326.

Bhattacharyya, P.N. and Jha, D.K. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World J Microbiol Biotechnol*, 28:1327–1350.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., Loren, V., van Themaat E. and Schulze-Lefert P. (2013). Structure and functions of the bacterial microbiota of plants. *Annu Rev Plant Biol*, 64:807–838.

Choudhary, G.R., Jain, N.K. and Jat, N.L. (2006). Response of cumin *(Cuminum cyminum)* to inorganic nitrogen, farmyard manure and biofertilizer. *Indian Journal of Agronomy*, 51(4): 334-336

Choudhury, M.A. and Kennedy, I.R. (2004). Prospects and potentials for system of biological nitrogen fixation in sustainable rice production. *Biol Fertil Soils*, 39:219–227.

Cowan, D.A., Hopkins, D.W., Jones, B.E., Maggs-Kölling, G., Majewska, R and Ramond J-B (2019). Microbiomics of Namib Desert habitats. *Extremophiles*, **24** 17-29

Das, S., Pareek, B.L., Kumawat, A. and Dhikwal, S. R.(2013). Effect of phosphorus and biofertilizers on productivity of chickpea (Cicer arietinum l.) In north western Rajasthan, India . *Legume Res*., 36 (6) : 511 – 514

Dhanasekar, R. and Dhandapani, R. (2012). Effect of biofertilizers on the growth of Helianthus annuus. *Int J plant, Ani Environ Sci*, 2:143–147.

Gamalero, E. Bona, E. Todeschini, V. and Lingua, G. (2020). Saline and Arid Soils: Impact on Bacteria, Plants, and their Interaction. Biology 2020, 9, 116; doi:10.3390/biology9060116 .

Franche, C., Lindström, K. and Elmerich, C. (2009). Nitrogen fixing bacteria associated with leguminous and nonleguminous plants. *Plant Soil*, 321:35–59

Frans, J. and Bruijn, D. (2016). Biological Nitrogen Fixation -Book Summary, *Advances in Microbiology*, 6, 407-411

Galloway, J.N., Dentener, F.J. and Capone, D.G. (2004). *Biogeochemistry*, 70:153-226

Gaskell, M., Smith, R., Mitchell, J., Koike, S.T., Fouche, C., Hartz, T., Horwath, W. and Jackson, L. (2007). Soil Fertility management for Organic crops, Division of Agriculture and Natural Resources, ISBN: 13, 9781879906969.

Hubbell, D. H. and Kidder, G. (2009). Biological Nitrogen Fixation. University of Florida *IFAS Extension Publication SL*, 16: 1-4

Jatav, M.K., Sharma, B.D., Samadia, D.K. and Meena, S.R. (2016). Efficacy of Rhizobium inoculation on graded N levels and net return from cluster bean seed production under hot arid regions. *Economic Affairs*, 61(3): 495-499

Joshi, N.L. and Singh, P. (1985). Additive and complementary effects of various agronomic inputs on the yield of pearl millet. *Ann. Arid Zone*, 24:218-222.

Junia, R., Kasana, R.C., Jain, N. and Aseri, G.K. (2020 a). Feldspar Mine Spoil Rehabilitation: Use of Legumes and Bio Inoculants to Establish Soil Fertility on Agro–Silviculture Basis. *Legume Research*. **doi:** 10.18805/LR-4320)

Junia, R., Kasana, R.C., Jain, N. and Aseri, G.K. (2020 b).Guar (Cyamopsis tetragonoloba L.): A Potential Candidate for the Rehabilitation of Feldspar Mine Spoil Amended with Bioinoculants. *Indian Journal of Agricultural Research.* doi**:** 10.18805/IJARe.A-5424

Kar, A. (2014). The Thar or the Great Indian Sand Desert. In, Landscapes and Landforms of India (Ed., V.S. Kale), pp. 79-90. World Geomorphological Landscapes Series, Springer, Dordrecht.).

Kaushik, M.K., Bishnoi, N.R. and Sumeriya, H.K. (2012). Productivity and economics of wheat as influenced by inorganic and organic sources of nutrients. *Ann. Plant & Soil Res.*,14(1): 61-64.

Khandelwal, R.., Choudhary, S.K., Khangarot, S.S., Jat , M.K. and Singh, P. (2012). Effect of inorganic and bio- effect of inorganic and bio-fertilizers on productivity and nutrients uptake in Cowpea [Vigna unguiculata (L.) Walp]. *Legume Res*, 35 (3): 235 – 238.

Rashid, M.I., Mujawar, L.H., Shahzad, T. , Almeelbi, T. , Ismail I.M.I. , Oves, M. (2016). Bacteria and fungi can contribute to nutrients bioavailability and aggregate formation in degraded soils. *Microbiol. Res*., 183, 26-41

Mathur, N., Singh, J., Bohra, S. and Vyas, A. (2004). "Relative efficiency of different AM fungi on nutrient uptake and productivity of moth bean". *J. Arid Legumes*, 1: 135-137.

Meena, J.S., Verma, H.P. and Pancholi, Pinki (2015). Effect of fertility levels and biofertilizers on growth and yield of cowpea on sandy loam soil of Rajasthan. *Asian J. Soil Sci.*, 10(1): 55-58.

Meena, M.D., Tiwari, D.D., Chaudhary, S.K., Biswas, D.R. and Narjary, B. (2013). Effect of Biofertilizer and Nutrient Levels on Yield and Nutrient Uptake by Maize (Zea mays L.). *Annals of Agribioresearch*, 18: 176-181.

Mehdipour-Moghaddam, M.J., Emtiazi, G. and Salehi, Z. (2012). Enhanced auxin production by Azospirillum pure cultures from plant root exudates. *J Agr Sci Tech*, 14:985–994.

Mir, M., Hassan, G.I., Sheikh, K. and Sharma, S.K. (2012) Impact of biofertilizers on growth, nutrient uptake, yield, metabolism and rhizosphere enzyme activities of pomegranate (*Punica granatum* L.) cv. 'Kandhari Kabuli'. *Applied Biological Research* 14(2): 168-175

Mohammad, I., Yadav, B.L. and Ahamad, A. (2017). Effect of Phosphorus and Bio-Organics on Yield and Soil Fertility Status of Mungbean [Vigna radiata (L.) Wilczek Under Semi- Arid Condition of Rajasthan, India. *Int. J. Curr. Microbiol. App. Sci*, 6(3): 1545-1553.

Mulvaney, R.L., Khan, S.A. and Ellsworth, T.R. (2009). *J Environ Quality*, 38 2295-2314.

Nina, K., Thomas, W.K. and Prem, S.B. (2014). Beneficial organisms for nutrient uptake. Virtual fertilizer research center. *Washington, DC: Wageningen Academic Publishers*, 63.

Niranjan, R., Shukla, R., Pareek, R. and Rao, V. M. (2002). Dual inoculation effect of Rhizobium (cowpea miscellany) and VAM fungi on growth, nodulation and nitrogen fixation in Prosopis cineraria. *J. Phytological Res*, 15: 149-153

Patel, S. G., Amin, A. U., Patel, S. P. , Agalodiya, A. V. and S. M. Patel (2013). Effect of different sources of organic manures with and without bio fertilizers in Cumin (Cuminum cyminum L.). *International J. Seed Spices* 3(2):54-58

Poonia, T. C., Raj, A. D. and Pithia, M. S. (2014). Effect of organic, inorganic and biofertilizers on productivity and economics of Groundnut-Pigeon pea relay intercropping system in vertisols of Gujarat. *Journal of Experimental Biology and Agricultural Sciences*, 2(6): 560-566

Kumar, P., Tarafdar, J.C., Painuli, D.K., Raina, P., Singh, M.P., Beniwal, R.K., Soni, M.L., Kumar, M., Santra, P. and Shamsuddin, M. (2009). Variability in Arid Soil Characteristics. Trends in Arid Zone Research in India(Eds. Amal Kar, B.K. Garg, M.P. Singh and S. Kathju),Central Arid Zone Research Institute, Jodhpur, 78-112 pp.

Qi Cheng, (2008). Perspectives in biological nitrogen fixation research. *J Integr Plant Biology*.50(7):786-98. doi: 10.1111/j.1744-7909.2008.00700.x.

Richard Jacoby, Manuela Peukert, Antonella Succurro, Anna Koprivova, and Stanislav Kopriva (2017). The Role of Soil Microorganisms in Plant Mineral Nutrition—Current Knowledge and Future Directions. Frontiers in Plant Sciences.8: 1617. doi: 10.3389/fpls.2017.01617.

Richardson A. E., Barea J. M., Mcneill A. M. and Prigent-Combaret C. (2009). Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil* 321 305–339. 10.1007/s11104-009-9895-2.

Roper, M. M. and Gupta, V.V.S.R. (2017). Enhancing Non-symbiotic N_2 Fixation in Agriculture. *The Open Agriculture Journal*, 11: 7-27

Vimal, S.R. , Singh, J.S., Arora, N.K. and Singh, S. (2017). Soil-plant-microbe interactions in stressed agriculture management: a review. *Pedosphere*, 27 , pp. 177-192

Sahoo, R.K., Ansari, M.W., Dangar, T.K., Mohanty, S. and Tuteja, N. (2013). Phenotypic and molecular characterization of

efficient nitrogen fixing Azotobacter strains of the rice fields. doi:10.1007/s00709-013-0547-2.

Sahoo, R.K., Ansari, M.W., Pradhan, M., Dangar, T.K., Mohanty, S. and Tuteja, N. (2014). Phenotypic and molecular characterization of efficient native Azospirillum strains from rice fields for crop improvement. *Protoplasma*, doi:10.1007/s00709-013-0607-7.

Schimel, J. P., Bennett, J. (2004). Nitrogen mineralization: challenges of a changing paradigm. *Ecology* 85 591–602. 10.1890/03-8002.

Shivran, A.C. and Jat, N.L. (2015). Integrated nutrient management influenced growth, yield and economics of fennel (*Foeniculum vulgare*) under semi arid conditions. *Indian Journal of Agronomy*, 60(2): 318-323

Singh, J.S., Pandey, V.C. and Singh, D.P. (2011). Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. *Agric Ecosyst Environ*, 140:339–353.

Singh, S., Nirwan, B., Sharma, K. and Choudhary, S. (2016). Development of microbial consortia for overall improvement of Azadirachta indica seedlings. *Plant Archives* ,16 (2): 918-924

Singh, S.K., Pancholy, A., Jindal, S.K. and Pathak, R. (2014). Effect of co-inoculations of native PGPR with nitrogen fixing bacteria on seedling traits in Prosopis cineraria. *Journal of Environmental Biology*, 35: 929-934

Swain, H. and Abhijita, S. (2013). Nitrogen fixation and its improvement through genetic engineering. *Journal of Global Biosciences*, 2(5): 98-112

Tilak K.V.B.R., C.S. Singh, N.K., Roy, N.S. Subba Rao, (1982). Azospirillum brasilense and Azotobacter inoculum effect of maize and sorghum. *Soil. Biol. Biochem.*, 14:419-750.

Umesha S, Divya M, Prasanna K. S, Lakshmipathi R. N, Sreeramulu K. R. (2014). Comparative effect of organics and biofertilizers on growth and yield of maize (Zea mays. L). *Curr Agri Res*, 2(1): 55-62

van der Heijden M. G. A., Bardgett R. D., Van Straalen N. M. (2008). The unseen majority: soil microbes as drivers of plant

diversity and productivity in terrestrial ecosystems. *Ecol. Lett.* 11 296–310. 10.1111/j.1461-0248.2007.01139.x)

Verma, R.K., Tripathee, R., Chourasiya , S. and Thakur, A.K. (2015). Effect of Plant Growth Promoting Microbes on Bael (Aegle marmelos) Seedlings in Nursery. *Indian Forester*, 141(1): 79-82

Vyas, M. and Vyas, A. (2014). Field Response of Capsicum Annuum Dually Inoculated with AM Fungi and PGPR in Western Rajasthan. *International Journal of Research Studies in Biosciences*, 2(3): 21-26

Wani, P., Chandrapalalh, S., Zambre, M. A. and Lee, K.K. (1988). Association between N2 -fixing bacteria and pearl millet plants: Responses, mechanisms and persistence. *Plant and Soil* ,110: 289-302

Wani, S.A., Chand, S. and Ali, T. (2013). Potential use of Azotobacter chroococcum in crop production: an overview. *Curr Agric Res J*, 1:35–38.

Wen Qin, Chen-yu Wang, Yu-xuan Ma, Min-juan Shen, Jing Li, Kai Jiao, Franklin R. Tay,and Li-na Niu, 2020. Advanced Materials. 321907833 DOI: 10.1002/adma.201907833.

Zahran, H.H. (1999). Rhizobium-Legume symbiosis and nitrogen fixation under severe condition and in an arid climate. *Microbiology and Molecular Biology Reviews*, 63 (4): 968-989.

ROLE OF PLANT-BASED MATRIX IN DELIVERING PROBIOTIC BENEFITS

Mehak Manzoor[1], Vikrant Sharma[2], Deepti Singh[3] and [*]Deepansh Sharma[3]

[1] Ph.D. Scholar, Amity Institute of Microbial Technology, Amity University Rajasthan
[1] Student, Amity Institute of Microbial Technology, Amity University Rajasthan
[3]Assistant Professor, Amity Institute of Microbial Technology Amity University Rajasthan 303002 INDIA

[*]Corresponding Author: deepanshsharma@gmail.com

Plant-based fermentation

Fermentation is known as ancient food processing and preservation methods in food history. Fermented foods and beverages have heterogeneity of customs and cultural inclination found in various topographical regions, from where they are being produced. Fermentation has allowed our lineages in temperate and cooler regions to survive during the winter season of the year and those in the tropics to endure dry spell periods. Fermentation can be defined as a slow breakdown of material brought about by bacteria, yeasts, and other microorganisms that convert starch either alcohols or into organic acids (FAO, 1998). Raw food materials particularly perishable ones such as vegetables and fruits are transformed through fermentation from nonfunctional forms to more stable forms like grape juice to wine, wheat to bread (Saa *et.al*.,2018), soybean to soy sauce (Wan *et.al*.,2013), etc. In most cases, manufacturing methods of various conventional fermented foods are ambiguous and pass to successive groups as family customs. World Health Organization (WHO) and Food and Agriculture Organization (FAO) suggested the consumption of a particular amount of vegetables and fruits in daily food to prevent various chronic pathologies which

include hypertension, coronary heart ailments, and risk of strokes (Swain *et.al*.,2014). Consumers are usually attracted towards the foods and beverages which are fresh, nutritious, health-promoting, and also ready to eat or ready to drink (Endrizzi *et.al* .,2009). Thus the question here arises why do we need to do fermentation? There are several benefits associated with fermentation which can be sum into the following points .1)Fermentation increases the shelf life of the product naturally, therefore, we don't need to add chemical preservatives like nitrates, sulfites, benzoates, and sorbates, etc which hurt our health resulting in several diseases like allergies, heart disease, obesity, cancer, urticaria, contact dermatitis, asthma, and skin rashes, etc. (Abdulmumeen *et.al*., 2012; Kinderlerer and Hatton, 1990). Even nuclear radiations when used for preservation of food results in unfavorable sensory changes, loss of fruit and vegetable firmness along with some vitamins (especially thiamine), and also less acceptance of irradiated food by consumers (Mostafavi *et.al*., 2012)2) The food becomes more nutrient-rich with the increase in mineral (Kim 2017) and vitamin contents(LeBlanc *et.al*.,2011) thus enhancing the micronutrient bioavailability 3) Fermentation increases the bio digestibility by breaking down the proteins to amino acids(Shurkhno *et. al.*, 2005), Starches are hydrolyzed into simpler carbohydrates such as glucose (Osman 2011) and fats into different fatty acids (Chen *et.al*.,2017). thus, making food easier to digest which is beneficial for the patients who have compromised digestion because of illness ,poor dietary choices or over reliance on antibiotics or any other medication 4) Fermented food or predigested food are better than medicines for those who are unwell regardless whether it is cancer or cold (Louis *et.al.*, 2014) 5) Antinutrients such as phytic acid, tannins, oxalates ,polyphenols ,saponins etc binds with proteins, calcium ,iron and other essential minerals hinders their bioavailability in the body, are broken down by fermentation this is important in case of grains and cereals which have high amount of antinutrients (Nkhata *et.al*.,2018) 6) Fermentation improves taste ,aroma and texture of food in such a way that before you know it your taste buds will be wanting it more(Marco *et.al*.,2017) and 7) Different methods of steaming, roasting, boiling, frying, sautéing, microwave & pressure cooking

etc (Andriana *et.al* ., 2016) results in the reduction of several key nutrients such as water soluble vitamins C&B ,fat soluble vitamins A,B,E &K and minerals primarily potassium, calcium (Otemuyiwa *et.al*.,2018). Some of the cooking methods especially grilling result in the production of potentially cancer-causing substances. Fermentation reduces the cooking time preventing the loss of valuable enzymes and water-soluble vitamins because of the excess heat. So the fermentation of fruits and vegetables is a common and reliable practice to preserve and enhance the sensory characteristics such as flavor, color, texture, and transformation of nutrients which includes improvement of digestibility and biofortification. (Cogan and Accolas 1996; Leroy and De Vuyst 2004, Leroy., *et.al* 2006).

ROLE OF FOOD MATRICES

Probiotic bacteria have been progressively combined in different food items which are expected to produce numerous health advantages in the human gut.LAB viability and their health effects depend on various important factors such as food matrix structure, composition, food manufacturing, storage, and digestion condition (Ranadheera et.al.,2010). Physico-chemical properties of food matrices which include buffering capacity, pH display a crucial part of the delivery of probiotics and they act as important factors that have an influence on the survival of probiotics and therefore potential probiotic properties throughout gastric transit. Food matrix can have an impact on the survival of probiotics, physiology, and potential efficacy but very fewer studies have been conducted as far as humans are concerned. To date, dairy products were considered as promising matrices for the delivery of probiotics and their health benefits.Fermented dairy products possess a range of products which are buttermilk, cheese, and yogurt drink (Gurakan & Altay 2010).Milk is considered as the only food which contains all important essential components which are necessary for human nutrition. However, in recent times researchers have found some potential or dangerous relationship between dairy products and cancer because of the presence of certain components like vitamin D, proteins, calcium, Conjugated linoleic acid (CLA), butyrate, saturated fatty acids, and also some pollutants which include

pesticides, estrogen, and insulin-like growth factor I (IGF-I) in milk (Davoodi *et.al*.,2013).Furthermore, Two major disadvantages associated with functional dairy products are lactose intolerance and cholesterol content (Prado *et.al*.,2008).In Europe, the frequency of lactose intolerance is about 5% among the British population and has raised to 17% in Finland and northern France (Lomer *et.al* .,2008). The frequency of lactose intolerance is above 50% in South America, Africa, and Asia, and almost 100% in some of the Asian countries (Zannini *et.al*., 2013). In India, 60-70% of people are lactose intolerant and the incidence of lactose intolerance is more common in south Indians (66.6%) rather than north Indians (27.4%) this may be due to the influence of central Asian origins of North Indians due to which they are more accustomed towards the dairy products (Tandon *et.al*., 1981).Babu *et. al* .,2010)reported that lactose malabsorption prevalence is higher among the southern population of India than that of the people belonging from North India this could be attributed to genetic differences prevailing in these populations which are reported by lactose tolerance test, lactose hydrogen breath test (HBT), and identification of lactase gene c/T-13910.There is growing interest in developing non-dairy products (Randheera *et .al.*,2010.Non-dairy products possessing probiotic strains have been launched, predominantly beverages which are fruits, vegetables, cereals, and soybeans (Saarela 2009; Soccol *et.al*., 2014).Plant-based food matrices can be considered as a major ingredient of choice because they offer several ingredients.They are a rich source of essential nutrients and in contrast to dairy products, they prevent the need for utilizing starter cultures, and thus there is no competition for nutrients with probiotic cultures. These juices remain for a very little time in the stomach and consequently, the probiotic species are exposed for a very short duration to the harsh acidic condition of the stomach (Kumar *et.al* ., 2015; Ding and shah, 2008). They can be considered as an ideal substrate for the growth of probiotics as they contain beneficial nutrients such as minerals, vitamins, antioxidants, and dietary fibers such as hemicellulose, pectin, gums, fructooligosaccharides(FOS), and non-carbohydrate component lignin that selectively stimulates the growth of beneficial bacteria (Yoon *et.al*., 2004; Dhingra

et.al.,2012; Holscher;2017). Unlike dairy, fermented products fermented plant products contain non-digestible fiber which possesses the activity of prebiotic and enhances the growth, particularly of *Lactobacillus* and *Bifidobacteria* by inhibiting the growth of other pathogenic bacteria present in the colon (Markowiak *et.al* ., 2017). Fermented soy products also have higher nutritional value, digestibility, and acceptability (Lakshmy *et.al*., 2016). Thus fruit and vegetable-based fermented products have higher health benefits and lesser side effects than dairy products whose consumption may lead to lactose intolerance, high cholesterol levels, presence of milk allergens, high amount of saturated fatty acids apart from these health concerns there are social, religion and economic concerns associated with them.(*Prado et.al*., 2008) reported that fruit and vegetable-based beverages can serve as food matrices to carry probiotic bacteria. The rate of survival was reported higher incase of *L. paracasei, L. Plantarum,* and of other probiotics within the table olives and artichokes on storage and under gastrointestinal conditions. These reports on the rate of survival were quite similar and in fact more than that of milk-based probiotic products (Lavermicocca, 2006; Lavermicocca *et. al*., 2005; Valerio *et. al*., 2006).This higher viability can be attributed to the micro-architecture of these vegetables, probiotics might be protected from the harsh acidic conditions because of the roughness and also because of the presence of prebiotic substances which positively influence the survival of probiotic bacteria (Valerio *et .al*., 2006). Non-dairy matrices can be fortified with acidulants such as ascorbic acid which can enhance the shelf-life by generating an anaerobic environment which is more ideal for the survival of probiotic cultures and is usually obtained by removing the oxygen. Fruit juices also contain sugars which help in supporting the growth of probiotics (Ding and Shah 2008). Probiotic encapsulation is one more advancement that is known to enhance stability, facilitate handling, and storage of probiotic cells from oxygen, freezing, and acidic conditions (Ningtyas *et.al*.,2019). Encapsulating is done with various readily available materials such as polysaccharides which include non-toxic alginates, plant/microbial gums, chitosan, starch, κ-carrageenan, cellulose acetate phthalate as well as proteins

(gelatin and milk proteins) and also fats which extend the cell viability during shelving (Cabuk *et.al*.,2015; Calinoiu *et.al*.,2019; de Araújo *et .al*.,2016; Singh *et.al*.,2017).Microencapsulation which utilizes an emulsion strategy is a delicate process that does not impose any harmful effect on the bacterial cells (Sultana *et.al*.,2000; Capela *et.al*.,2006). To improve the cell viability as well as functionality, various technological advances have been employed in modifying the matrix components of food in a controlled way and these modifications of the matrix make them suitable substrates for probiotics (Betoret *et. al*., 2003; Yoon *et. al*., 2004).

Challenges for plant-based matrix

Fortification of juices with probiotic is a challenging process and there are various parameters which could affect the survival rate and viability of probiotics in juices. The major influencing parameters are grouped into the following categories 1) intrinsic food parameters: which include titratable acidity, pH, molecular oxygen, water activity, presence of salt, sugar etc2) processing parameters- such as the extent of heat treatment, incubation temperature, packaging materials, and storage techniques;3) microbiological factor consisting of different type of probiotic strains, compatibility of different strains, inoculums proportion. Among the above-mentioned parameters, pH is the most influential factor which affects the probiotic viability. Consumers' acceptance of fortified juice is also one of the important challenges to overcome. Probiotic presence can be mask by the addition of certain pleasing fragrance and also with some volatile ingredients. Sugar-free juices have fewer acceptances than fermented juices containing sugar. Also, certain experiments have shown that the addition of sucrose during the beginning of fermentation has reduced the flavors due to which taste has become more acceptable (Sivudu *et. al*., 2014). Due to the presence of colored components such as flavonoids, lycopene, anthocyanin, β-carotene, and glucosinolates in fermented fruits ad vegetables, they act an antioxidant by scavenging dangerous free radicals within the body and thus protect from various degenerative diseases like cancer, arthritis, and aging (Kaur and Kapoor, 2001).

Suitable strains

The most commonly employed bacterial strains for the production of probiotics based fruit and vegetable products include different strains of Lactic acid bacteria such as *Lactobacillus acidophilus, Lb. helveticus, Lb. casei, Lb. paracasei, Lb. johnsonii, Lb. plantarum, Lb. gasseri, Lb. reuteri, Lb. delbrueckii subsp. bulgaricus, Lb. crispatus, Lb. fermentum,* and other species like *Escherichia coli Nissle, Streptococcus thermophilus, Weissella spp., Propionibacterium spp., Pediococcus spp., Enterococcus faecium, Leuconostoc spp. and Saccharomyces cerevisiae var. boulardii* (Nagpal *et .al*., 2012; Patel *et. al*., 2013)

Current trend and prospects

There are many non-dairy probiotic-based products available in the market that have the disadvantage of requiring a cold chain for storage and transportation, and thus prevent the addition of food supplements with probiotics to the general population particularly in poor countries. Hence the demand for probiotic-based food products which does not require any refrigeration is increasing among the consumers. As far as probiotics are concerned the development of different dried powder forms containing LAB can be produced which will minimize the requirement of the cold chain. These dried powder forms can also provide better viability and stability to cells. Techniques such as microencapsulation and freeze-drying can be used to obtain the probiotic powder. In various functional food models, the stability and viability of probiotics within the products have been improved even under adverse conditions by using the cell encapsulation technique. The transportation, as well as storage costs, can be dramatically decreased because of the production of stable probiotic powder and shelf-stable probiotic products, also the long-distance trade can be promoted. The viability and stability of probiotics in drying and storing are intrinsic to species or strains. The optimization of storage conditions is required for probiotic-based powder and products. As a result, screening of robust probiotic strains is still essential to get a suitable product probiotic pair. These studies, along with controlled human studies are

necessary for future research activities for advancing this field. Future studies are required to be done to assess probiotic adherence and viability in the human intestine using in vivo clinical trials. These studies would demonstrate the interaction of probiotics carried by-products of plant origin.

REFERENCES

Abdulmumeen, H. A., Risikat, A. N., & Sururah, A. R. (2012). Food: Its preservatives, additives and applications. International Journal of Chemical and Biochemical Sciences, 1(2012), 36-47.

Adriana, D.T., F. Guy and A.Crosby, 2016. A review of the impact of preparation and cooking on the nutritional quality of vegetables and legumes. Intern. J. Gastron. Food Sci., 3: 2-11.

Alzamora, S. M., Salvatori, D., Tapia, M. S., López-Malo, A., Welti-Chanes, J., & Fito, P. (2005). Novel functional foods from vegetable matrices impregnated with biologically active compounds. Journal of Food Engineering, 67(1-2), 205-214.

Babu, J., Kumar, S., Babu, P., Prasad, J. H., & Ghoshal, U. C. (2010). Frequency of lactose malabsorption among healthy southern and northern Indian populations by genetic analysis and lactose hydrogen breath and tolerance tests. The American journal of clinical nutrition, 91(1), 140-146.

Betoret, N., Puente, L., Dıaz, M. J., Pagan, M. J., Garcıa, M. J., Gras, M. L., ... & Fito, P. (2003). Development of probiotic-enriched dried fruits by vacuum impregnation. Journal of food Engineering, 56(2-3), 273-277.

Çabuk, B., & Harsa, Ş. T. (2015). Protection of Lactobacillus acidophilus NRRL-B 4495 under in vitro gastrointestinal conditions with whey protein/pullulan microcapsules. Journal of bioscience and bioengineering, 120(6), 650-656.

Călinoiu, L. F., Ștefănescu, B. E., Pop, I. D., Muntean, L., & Vodnar, D. C. (2019). Chitosan coating applications in probiotic microencapsulation. Coatings, 9(3), 194.

Capela, P., Hay, T. K. C., & Shah, N. P. (2006). Effect of cryoprotectants, prebiotics and microencapsulation on survival of probiotic organisms in yoghurt and freeze-dried yoghurt. Food Research International, 39(2), 203-211.

Chen, Q., Kong, B., Han, Q., Xia, X., & Xu, L. (2017). The role of bacterial fermentation in lipolysis and lipid oxidation in Harbin dry sausages and its flavour development. LWT, 77, 389-396.

Cogan, T. M., & Accolas, J. P. (Eds.) (1996). Dairy starter cultures (pp. 233}248). USA: VCH Publishers Inc. (Chapter 9).

Davoodi, H., Esmaeili, S., & Mortazavian, A. M. (2013). Effects of milk and milk products consumption on cancer: a review. Comprehensive Reviews in Food Science and Food Safety, 12(3), 249-264.

de Araújo Etchepare, M., Raddatz, G. C., de Moraes Flores, É. M., Zepka, L. Q., Jacob-Lopes, E., Barin, J. S., ... & de Menezes, C. R. (2016). Effect of resistant starch and chitosan on survival of Lactobacillus acidophilus microencapsulated with sodium alginate. LWT-Food Science and Technology, 65, 511-517.

Dhingra, K. (2012). Methodological issues in randomized trials assessing probiotics for periodontal treatment. Journal of periodontal research, 47(1), 15-26.

Ding, W. K., & Shah, N. P. (2008). Survival of free and microencapsulated probiotic bacteria in orange and apple juices. International Food Research Journal, 15(2), 219-232.

Endrizzi, I., Pirretti, G., Calò, D. G., & Gasperi, F. (2009). A consumer study of fresh juices containing berry fruits. Journal of the Science of Food and Agriculture, 89(7), 1227-1235.

Gurakan, G. C., & Altay, N. (2010). Yogurt microbiology and biochemistry. Development and Manufacture of Yogurt and Other Functional Dairy Product. F. Yildiz (Ed). CRC Press, Taylor & Francis Group. Boca Raton, USA, 98-116.

Holscher, H. D. (2017). Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut microbes, 8(2), 172-184.

Kailasapathy, K., & Chin, J. (2000). Survival and therapeutic potential of probiotic organisms with reference to Lactobacillus acidophilus and Bifidobacterium spp. Immunology and cell biology, 78(1), 80-88.

Kaur, S., Vohra, R. M., Kapoor, M., Beg, Q. K., & Hoondal, G. S. (2001). Enhanced production and characterization of a highly thermostable alkaline protease from Bacillus sp. P-2. World Journal of Microbiology and Biotechnology, 17(2), 125-129.

Kim, K. J., Im, S. B., Yun, K. W., Je, H. S., Ban, S. E., Jin, S. W., ... & Seo, K. S. (2017). Content of proximate compositions, free sugars, amino acids, and minerals in five Lentinula edodes cultivars collected in Korea. Journal of Mushroom, 15(4), 216-222.

Kinderlerer, J. L., & Hatton, P. V. (1990). Fungal metabolites of sorbic acid. Food Additives & Contaminants, 7(5), 657-669.

Kumar, B. V., Vijayendra, S. V. N., & Reddy, O. V. S. (2015). Trends in dairy and non-dairy probiotic products-a review. Journal of food science and technology, 52(10), 6112-6124.

Lakshmy, P. S., Usha, V., Sharon, C. L., & Aneena, E. R. (2016). Rice and green gram based 'Tempeh'-Development and in vitro mineral bioavailability. Journal of Tropical Agriculture, 53(2), 166-172.

Lavermicocca, P. (2006). Highlights on new food research. Digestive and Liver Disease, 38, S295-S299.

Lavermicocca, P., Valerio, F., Lonigro, S. L., De Angelis, M., Morelli, L., Callegari, M. L., ... & Visconti, A. (2005). Study of adhesion and survival of lactobacilli and bifidobacteria on table olives with the aim of formulating a new probiotic food. Applied and Environmental Microbiology, 71(8), 4233-4240.

LeBlanc, J. G., Laiño, J. E., del Valle, M. J., Vannini, V. V., van Sinderen, D., Taranto, M. P., ... & Sesma, F. (2011). B-Group vitamin production by lactic acid bacteria–current knowledge and potential applications. Journal of applied microbiology, 111(6), 1297-1309.

Leroy, F., & De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends in Food Science & Technology, 15(2), 67-78.

Leroy, F., Verluyten, J., & De Vuyst, L. (2006). Functional meat starter cultures for improved sausage fermentation. International journal of food microbiology, 106(3), 270-285.

Lomer, M. C., Parkes, G. C., & Sanderson, J. D. (2008). lactose intolerance in clinical practice–myths and realities. Alimentary pharmacology & therapeutics, 27(2), 93-103.

Louis, P., Hold, G. L., & Flint, H. J. (2014). The gut microbiota, bacterial metabolites and colorectal cancer. Nature reviews microbiology, 12(10), 661-672.

Marco, M. L., Heeney, D., Binda, S., Cifelli, C. J., Cotter, P. D., Foligné, B., ... & Smid, E. J. (2017). Health benefits of fermented foods: microbiota and beyond. Current opinion in biotechnology, 44, 94-102.

Markowiak, P., & Śliżewska, K. (2017). Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients, 9(9), 1021.

Mostafavi, H. A., Mirmajlessi, S. M., & Fathollahi, H. (2012). The potential of food irradiation: benefits and limitations. Trends in vital Food and control engineering, 5, 43-68.

Nagpal, R., Kumar, A., Kumar, M., Behare, P. V., Jain, S., & Yadav, H. (2012). Probiotics, their health benefits and applications for developing healthier foods: a review. FEMS microbiology letters, 334(1), 1-15.

Ningtyas, D. W., Bhandari, B., Bansal, N., & Prakash, S. (2019). The viability of probiotic Lactobacillus rhamnosus (non-encapsulated and encapsulated) in functional reduced-fat cream cheese and its textural properties during storage. Food Control, 100, 8-16.

Nkhata, S. G., Ayua, E., Kamau, E. H., & Shingiro, J. B. (2018). Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. Food Science & Nutrition, 6(8), 2446-2458.

Osman, M. A. (2011). Effect of traditional fermentation process on the nutrient and antinutrient contents of pearl millet during preparation of Lohoh. Journal of the Saudi Society of Agricultural Sciences, 10(1), 1-6.

Otemuyiwa, I. O., Falade, O. S., & Adewusi, S. R. A. (2018). Effect of various cooking methods on the proximate composition and nutrient contents of different rice varieties grown in Nigeria. International Food Research Journal, 25(2), 747-754.

Patel, A., & Prajapat, J. B. (2013). Food and health applications of exopolysaccharides produced by lactic acid bacteria. Advances in Dairy Research, 1-8.

Prado, F. C., Parada, J. L., Pandey, A., & Soccol, C. R. (2008). Trends in non-dairy probiotic beverages. Food Research International, 41(2), 111-123.

Ranadheera, R. D. C. S., Baines, S. K., & Adams, M. C. (2010). Importance of food in probiotic efficacy. Food research international, 43(1), 1-7.

Saa, D. T., Di Silvestro, R., Nissen, L., Dinelli, G., & Gianotti, A. (2018). Effect of sourdough fermentation and baking process severity on bioactive fiber compounds in immature and ripe wheat flour bread. LWT, 89, 322-328.

Saarela, M. (2009). Probiotics as ingredients in functional beverages. In Functional and speciality beverage technology (pp. 55-70). Woodhead Publishing.

Shurkhno, R. A., Gareev, R. G., Abul'Khanov, A. G., Validov, S. Z., Boronin, A. M., & Naumova, R. P. (2005). Fermentation of high-protein plant biomass by introduction of lactic acid bacteria. Applied Biochemistry and Microbiology, 41(1), 69-78.

Singh, P., Medronho, B., Alves, L., da Silva, G. J., Miguel, M. G., & Lindman, B. (2017). Development of carboxymethyl cellulose-chitosan hybrid micro-and macroparticles for encapsulation of probiotic bacteria. Carbohydrate Polymers, 175, 87-95.

Sivudu, S. N., Umamahesh, K., & Reddy, O. V. S. (2014). A Comparative study on Probiotication of mixed Watermelon and Tomato juice by using Probiotic strains of Lactobacilli. Int J Curr Microbiol Appl Sci, 3(11), 977-84.

Soccol, C. R., Prado, M. R. M., Garcia, L. M. B., Rodrigues, C., Medeiros, A. B. P., & Soccol, V. T. (2014). Current developments in probiotics. J. Microb. Biochem. Technol, 7, 11-20.

Soccol, C. R., Vandenberghe, L. P. D. S., Spier, M. R., Medeiros, A. B. P., Yamaguishi, C. T., Lindner, J. D. D., ... & Thomaz-Soccol, V. (2010). The potential of probiotics: a review. Food Technology and Biotechnology, 48(4), 413-434.

Sultana, K., Godward, G., Reynolds, N., Arumugaswamy, R., Peiris, P., & Kailasapathy, K. (2000). Encapsulation of probiotic bacteria with alginate–starch and evaluation of survival in

simulated gastrointestinal conditions and in yoghurt. International journal of food microbiology, 62(1-2), 47-55.

Swain, M. R., Anandharaj, M., Ray, R. C., & Rani, R. P. (2014). Fermented fruits and vegetables of Asia: a potential source of probiotics. Biotechnology research international, 2014.

Tandon, R. K., Joshi, Y. K., Singh, D. S., Narendranathan, M., Balakrishnan, V., & Lal, K. (1981). Lactose intolerance in North and South Indians. The American journal of clinical nutrition, 34(5), 943-946.

Tomar, B. S. (2014). Lactose intolerance and other disaccharidase deficiency. The Indian Journal of Pediatrics, 81(9), 876-880.

Valerio, F., De Bellis, P., Lonigro, S. L., Morelli, L., Visconti, A., & Lavermicocca, P. (2006). In vitro and in vivo survival and transit tolerance of potentially probiotic strains carried by artichokes in the gastrointestinal tract. Applied and environmental microbiology, 72(4), 3042-3045.

Wan, S., Wu, Y., Wang, C., Wang, C., & Hou, L. (2013). The development of soy sauce from organic soy bean. Agricultural Sciences, 4(5B), 116.

Yoon, K. Y., Woodams, E. E., & Hang, Y. D. (2004). Probiotication of tomato juice by lactic acid bacteria. The Journal of microbiology, 42(4), 315-318.

Zannini, E., Mauch, A., Galle, S., Gänzle, M., Coffey, A., Arendt, E. K., ... & Waters, D. M. (2013). Barley malt wort fermentation by exopolysaccharide-forming W eissella cibaria MG 1 for the production of a novel beverage. Journal of applied microbiology, 115(6), 1379-1387.

IMPACT OF CLIMATE CHANGE ON MICROBIAL COMMUNITIES IN SOIL

Gulshan Sharma[1], P. Chaitanya[1], Pooja Sharma[1] and Era Upadhyay[2]*

[1]Ph.D. Scholar, Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur, India
[2]Associate Professor, Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur, India

*Corresponding Author: era.upadhyay@gmail.com

Introduction

The climate change disrupts the natural resources mainly air, water and soil. Increasing trends in climate change directly influence the precipitation and temperature (IPCC, 2001). Mean temperatures have been increased from 1.4˚C to 5.8˚C and mean precipitation has also been increased with the rise in temperature. Evaporation increases with temperature, which results in more precipitation (IPCC, 2013). These changes influence the air, water and soil which form floods, cyclones and drought etc. (Singh et al., 2012). A massive number of microorganisms sustain in soil, which is a complex component of ecosystem. Soil microorganism is one of the largest components of biodiversity reservoirs on the earth (Calderón et al., 2017). These prokaryotic and eukaryotic microorganisms dwell the phases of their lives in the soil environment. Soil microbial communities are the variety of complex association comprised of genetic variability, mineral wealth and relative consistency in communities occur at the different levels of biological association (Islam, 2004).

The biological association of microbial communities in soil is affected by the change in climate which can modify distribution of

different species (Putten et al., 2012). Though soil community species have different physiology, temperature sensitivity, and growth rates; climatic change formulates a relative alteration in the function of soil communities (Whitaker et al., 2014). The climate change can affect directly or indirectly on soil microbes which plays a prominent role to providing nutrients, controlling plant population for terrestrial ecosystem sustainability (Classen et al., 2015). The moisture availability in soil was reported for alterations in fungal and bacterial communities (Bell et al., 2008; Briones et al., 2014). The main objective of this chapter is to understand the linkages between climate change and soil microbial communities based on responses of microbial communities in soil.

Linkages between climate change and soil microbial communities

Microorganisms decay organic matter and change mineral nutrients in terrestrial and aquatic ecosystems. Microorganisms are often considered as ubiquitous and allowed to respond rapidly towards environmental variations. However, the composition of microbial community in soil may alter with prevailing circumstances due to their responding tendency, along with edaphic or climatic circumstances, that regulates the potential outcomes (Balser et al., 2002). Soil microbial communities are associated with ecosystem functioning since they play main roles in Nitrogen (N) and Carbon (C) cycling (Philippot et al., 2013). The climate change may expand the frequency of heavy rainfall and drought, temperature elevation and increase litter inputs through increased CO_2 concentrations in atmosphere, consequently a significant effect on structure and functioning of soil microbial communities may occur (Vries and Shade, 2013). Underground responses towards climate change are more complicated as the plants' responses affect the concentration and type of carbon in the soil system. This has an indirect effect on soil microbial community biomass and composition. Though, their distinct metabolic activities in the microbial communities are key drivers in cycling of nutrients and their value in mediating ecosystem functioning and climate change should also be

considered. Microbial metabolic related activities can be changed by various ecosystem-scale elements such as nitrogen deposition, climate change, disturbance or elevated carbon dioxide (Balser et al., 2010).

Soil microorganisms are crucial element in the agricultural ecosystems towards climate change by their capability to process nutrients and cycle soil carbon (Balser et al., 2010). The crusts of biological communities in soil consist of various groups of cyanobacteria, bacteria, fungi, algae, mosses and lichens that cover arid land soils (Belnap et al., 2016). Some studies reported adverse effects of cool desert protozoa due to increased summer precipitation and immoderate summer temperatures, subsequently the desert protozoa can be adjusted in altered desert's precipitation regime and temperature. However, many protozoa found to be inactive for a long time in the soil but their reproduction and growth increase with rise in temperature when they become active (Darby et al., 2006; Schostag et al., 2019). Alpine grassland soils reserve nitrogen (N) in large amount by slow decomposition. Although, decomposition can be disturbed by climate change, and has a serious effect on soil N cycling (Lin et al., 2016). The physiological movement of soil microorganisms and plants control the flow of nitrogen and carbon in the terrestrial ecosystems. Ultimately, these CO_2-induced changes affect the soil microbial community according to resource availability. Enrichment of atmospheric nitrogen and CO_2 indirectly or directly affects the soil microbial activities, community structure and plant growth through altering nutrient, carbon and water availability. The figure 1 depicts the indirect and direct impacts of global change factors with underground soil communities along with possible feedbacks. Direct effects include increasing deposition of CO_2 and N with changing pattern of precipitation or temperature. On the other hand, indirect effects referred to increased deposition of CO_2 or N changes in precipitation or temperature (Balser et al., 2010) Temperature, water and nitrogen have a direct impact on the soil microbial community since the organisms react towards drought stress and temperature together with changed resource availability. Soil Microbial reaction towards

climatic changes, even if direct or indirect, results in a feedback that affects plants by pathogen productivity and nutrient availability (Fig. 1). Hence, the crucial step in the perspective of ecosystem reaction towards climate change must elevate the understanding of the soil microbial community response (Balser et al., 2010).

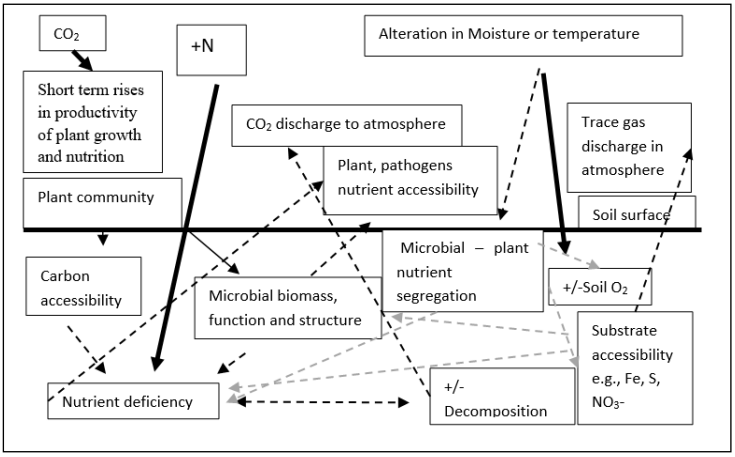


Fig. 1 Indirect and direct impacts of global change factors with underground soil communities, together with possible feedbacks.

Effects of climate change on carbon-nitrogen fixation and vice-versa

1. **Carbon fixation**

Increasing carbon dioxide (CO_2) concentration in atmosphere may modify the physiological movement of soil microorganisms and plants control the nitrogen flow and carbon flow in the terrestrial ecosystems. The CO_2 flux from soil signifies the combined feedback of soil microorganisms and plant roots towards increased atmospheric CO_2, and variation in the ground movement of carbon in soil may establish (Zak et al., 2000). Soil microbial communities

are important for the flow of carbon and cycling of nutrients in ecological community, and their movements are managed by biotic and abiotic components such as the temperature, quality and quantity of litter inputs, and moisture (Bardbellgett et al., 2008) Climatic and atmospheric changes influence both biotic and abiotic drivers in ecological community and the ecosystems feedback towards these changes. Such feedbacks from atmosphere to the ecosystem might also be managed by the soil microbial communities (Castro et al., 2010). However, high carbon availability cause increase in microbial biomass, and can initiate a consumption chain. The rising amount of microbial decomposition subsequent the increased microbial biomass which trigger microbial grazing through increasing nutrients proportion and experience the rapid cycling, resulting increase in plant growth (negatively feeding back towards atmospheric CO_2) (Zak et al., 1993). Rhizobia and mycorrhizal fungi develop symbiotic relationship with plants and directly depend on photosynthates through their hosts. However, these microbial groups might possibly be distressed primarily, and most vigorously, by any change induced by CO_2 in the carbon account of their host (Smith and Read, 2010).

2. Nitrogen fixation

The nitrogen cycle is directed by complicated microbial transformations, along with nitrogen fixation (Santos et al., 2014). Direct estimation of nitrogen fixation with a restricted plant species was observed with increased CO_2 level favors nitrogen fixation (West et al., 2005) For instance, nitrogen deposition is found to lessen mycorrhizal fungal biomass at the same time rising bacterial along with saprotrophic fungal biomass and having the capability to increase cycling of carbon by increasing the microbial enzymes activity related to carbon cycling (Balser et al., 2010). Nitrogen fixation could normally increase with rise in atmospheric CO_2 and decrease with increased nitrogen availability in soil. In this context, as symbiotic nitrogen fixation known as mutualistic exchange among bacteria and plants, it could possible that difference between plant species will vary in their capability to manage the result of the interaction (West et al., 2005). Additionally, in order to predict

ecosystem reaction towards environmental change, species identification could be a key driver in controlling the nitrogen fixation response towards global change (Doyle et al., 2003).

Soil respiration

High amount of CO_2 in soil was observed with faster microbial respiration rate consequent to increased plant growth (Nie et al., 2015). That means high CO_2 strengthen the carbon amount in the soil. Several studies reveal more speedy rates of soil respiration beneath high CO_2 levels (Camarda et al., 2019; Yiqi et al., 2010). Soil microorganisms also contribute to soil respiration which indicates that high CO_2 levels in soil differentially regulate the categories of plant-derived components and could be utilized for metabolism of microbes (Zak et al., 2000). Soil microbial com munities substantiate the decomposition weight and catalyze various important processes that run terrestrial nutrient and carbon cycling. These communities are commonly sensitive towards change and weaken after any natural calamity. However, studies evidenced that the climate change could immediately alter soil microbial communities (Sayer et al., 2017). In addition, the efforts to acknowledge the microbial response towards increased carbon dioxide, various other main drivers of global change, such as elevated temperature, nitrogen deposition and altered precipitation have been emphasized (Balser et al., 2010).

Tolerance of temperature & precipitation in soil and role of microbial communities

Several past studies have been emphasized on the direct and indirect effects of temperature and precipitation on plant growth due to consequently elevated CO_2 (Zak et al., 1996). Respiration rate influenced by increasing temperature shows increase in gram positive Phospholipid Fatty Acid (PLFAs) and decrease in gram negative PLFAs (Vries et al., 2018). After 2000's, the studies were more focused on the effect of global warming on microbial communities in which DNA based sequence methods permitted

various beneficial consequences (Wei et al., 2014). High temperature and precipitation change the soil microbial properties. The temperature increases the rate of soil respiration which contributes fungal and bacterial growth (Zhang et al., 2014). Elevated soil microbial communities contribute to significant characteristics in growing fungi and bacterial communities (Drigo et al., 2007). Some scientist found that elevated CO_2 increases microbial abundance and also fungal abundance in Mycorrhizal plants (Cheng et al., 2011; Guenet et al., 2012), which affects the quality and quantity of the plant carbon. Tu et al. observed that *Basidiomycota* may cause elevated CO_2, however, *Ascomycota* showed both type of responses viz. increases and decrease in CO_2 (Nguyen et al., 2011; Tu et al., 2015). Rising temperature in forest land ecosystem caused increased growth of *Actinobacteria* and *Alphaproteobacterial* in soil (Sheik et al., 2011). Similarly, elevated CO_2 increases the fungi abundance. In contrast, atmospheric CO_2 changes are occurred due to drought and affect the microbial communities (Richstein et al., 2013). Lower temperature due to flooding influence the microbial composition of soil as increased fungi growth was observed after heavy rainfall in drought prone prairie soil (Williams and Rice, 2007; Toberman et al., 2008). Whereas, decreased fungal biomass was observed in gram negative bacteria with low temperature (Wagner et al., 2015). A very few studies show decrease in fungal abundance in snow cover with high temperatures and precipitation effects (Robroek et al., 2013).

Grassland ecosystem changes as precipitation and temperature change due to increase in rainfall and drought. Permafrost soil bed of large regions of the Arctic has a large impact on soil microbes as this cold region shows low activity of carbon in microbes. When it warms up the microbes became active and release green house gases (Tas et al., 2018). Regional temperature increase shows increase in antimicrobial resistance which affect the common pathogens. Changes in precipitation cause coccidioidomycosis (valley fever) in California, by a soil pathogen *Cocccidiodes immitis* (Hutchins et al., 2019). Seasonal variability in precipitation patterns has a definite role in structure and functioning of microbes (Cregger et al., 2012).

Microbes synthesize polyols and amino acids by osmotic regulation in rainfalls and balance the osmotic pressure which become stress for soil microbes in biogeochemical cycling (Zhang et al., 2014) as they increase water potential after precipitation (Wood et al., 2001). So, rainfall affects the growth of both fungi and bacteria (Evans and Wallenstein, 2012). Fungi are the drought resistant and bacteria resists with the help of water filled micropores through which transfer of moisture occurs (Boer et al., 2015). For example, Junipers are drought resistant where precipitation plays a prominent role in its survival (Briones et al., 2009). Quantitative and qualitative alterations in soil microbes depend upon the changes in the climate either directly or indirectly. So, microbes play a key role in sinking carbon with release of greenhouse gases (Kannojia et al., 2019). Some examples of microbial traits which play a role in resistance of soil microbial communities against climate change are given in table 1. Soil decomposition also shows increasing impact on release of carbon emissions (55 millions/year) and green house gases (Zimmer, 2010).

Importance of soil microbial communities with reference to climate change

Microbes utilize trade-offs and adaptive strategies to survive with the response towards environmental change subsequent to the change in their community composition and biomass (Balser et al., 2002). Although, Global changes such as warming are precisely influence soil microbial respiration rates and soil microorganisms, along with processes they negotiate, are highly sensitive towards temperature. Microbial communities can affect major plant properties e.g. litter quality and productivity, properties that modulate variability in the carbon cycle (Izquierdo et al., 2019). Microbial activity plays a main part in terrestrial carbon feedbacks, despite that, our present understanding on climate impacts on plant-microbe and microbe-microbe associations remains unsettled. For instance, particular microbial groups control ecosystem functions such as nitrogen fixation, denitrification, nitrification, and methanogenesis. Although, some activities arise at a coarser scale, such as nitrogen mineralization, prone to be more compactly

associated with some abiotic factors like moisture and temperature than microbial community composition since a variety of organisms manage these processes (Classen et al., 2015).

Table 1. Examples of microbial traits which play a role in resistance of soil microbial communities against climate change.

Trait	Process	Climate Change Process	Reference
Heat resistance and desiccation	Trehalose synthesis Capsule	Drought Warming	Canovas et al., 2001 Zhang and Van, 2012 Vries and shade, 2013
Sporulation	>500 (number)	Broad Range of interruptions, under nutrient limitations	Higgins and Dworkin 2012 Vries and shade 2013
Use of distinct N forms	Nitric oxide reduction Nitrate reduction Nitrous oxide reduction Nitrite reduction Ammonia oxidation Nitrogen fixation	Rise in nitrogen availability through rewetting and warming behind drought, N changes by warming, Alteration in soil moisture, and soil C availability changes through increased CO_2	Lamb et al., 2011 Vries and shade 2013
Use of distinct C forms	Chitin degradation Methane oxidation Methanogenesis Citrate synthesis Cellulose degradation Glucose oxidation Lignin and Phenol oxidation	Alteration in soil C accessibility through rewetting behind drought, and increased CO_2	Theuerl and Buscot, 2010 Vries and shade 2013

Climatic change shifts the relative abundance and purpose of soil communities since soil community components vary in their physiology, growth rates and temperature sensitivity. For instance, in a temperate forest, warming beyond 58ºC change the relative abundances belonging to soil bacteria and elevate the fungal to bacterial ratio of this community. Microbial communities react to warming and other disturbances through protection or flexibility as the community recover to an initial formation after passing the stress (Allison and Martiny 2008). The intrinsic temperature susceptibility of microbial activity is represented as the factor through which microbial activity rise with a 108ºC increase in temperature. The microbial sensitivity is an important parameter (Zhou et al., 2009) generally used in models of climate change to narrate microbial temperature sensitivity; and the relationships of many linkages that impacts the temperature reactivity of microbial processes such as decomposition. In addition, soil respiration, organic matter decomposition, and growth of microbial biomass generally increase with temperature (Classen et al., 2015). Microorganisms in a very cold weather are capable to respond immediately towards a slight temperature change. However, microbial community in tropical forest was found with a different temperature response as compared with tundra community (Balser et al., 2002).

Increased soil moisture lead by greater precipitation may expand microbial predation. However, increased temperature might cause rise in metabolic activity of microbes in temperate ecosystems (Vinolas et al., 2001) and microbial community may die or acclimate. The efficiency of microbial community growth alters at greater temperatures (Balser et al., 2010). However, precipitation change alters the soil community composition along with its function (Classen et al., 2015). Climate change directly effects on structure of microbial community and activities through alteration in the physical and chemical environment of soil; and indirectly by land use changes. It is exclusively critical to consider that the specific feedback of a community towards environmental alteration rely on the disturbance, but also depends on the history of soil community (Balser et al., 2010).

Conclusion

Temperature and precipitation both are the part of environmental factors, which are very important for the growth of microbes. Soil microbes can change the productivity and growth mechanism. Carbon fixation and nitrogen fixation with soil respiration are mostly influenced by these microbes. They are also known as 'environmental modulators' (that impacts organism activity) and opposed towards resources which are used by life forms to reproduce and grow. Modulator changes affect the entire community, but frequently alter through their influence on resources. Microbial responses towards moisture and temperature stress could occur along with several mechanisms, which include physiological changes such as substrate preference changes, lipid membrane changes or the dormant structures formations. From the perspective of microbial physiology, microbial community indicates alterations in stress more than a community within ordinary conditions.

A microbial community can be more sensitive towards moisture and temperature changes if the new situation is aside from its normal climate range. In order to completely manage and understand the climate change impacts on soil communities, biomass and estimation of their composition must be evaluated. Different microbes such as algae, fungi, bacteria and pathogens in all types of soil ecosystems are influenced by climatic changes which play a key role in ecological balance. Although the studies on soil microbes related to biomass activity and process parameters indicate the requirement of implementation on genetic expressions of soil microbes. However, soil microbes are important natural sources in climate change and need to implement advance techniques to survive them. Culture and isolation techniques with microbial functional profiles should be implemented. Meta analysis of the studies could help in changing old process techniques for better benefits. Finally, the land use impacts on soil microbial communities are also recommended for further research.

References

Allison, S. D., & Martiny, J. B. (2008). Resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Sciences, 105(Supplement 1), 11512-11519.

Balser, T. C., Gutknecht, J. L., & Liang, C. (2010). How will climate change impact soil microbial communities?. In Soil microbiology and sustainable crop production (pp. 373-397). Springer, Dordrecht.

Balser, T. C., Kinzig, A. P., & Firestone, M. K. (2002). Linking soil microbial communities and ecosystem functioning. The functional consequences of biodiversity: empirical progress and theoretical extensions, 265-293.

Bardgett, R. D., Freeman, C., & Ostle, N. J. (2008). Microbial contributions to climate change through carbon cycle feedbacks. The ISME journal, 2(8), 805-814.

Bell, C., McIntyre, N., Cox, S., Tissue, D., & Zak, J. (2008). Soil microbial responses to temporal variations of moisture and temperature in a Chihuahuan Desert grassland. Microbial ecology, 56(1), 153-167.

Boer, W. D., Folman, L. B., Summerbell, R. C., & Boddy, L. (2005). Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS microbiology reviews, 29(4), 795-811.

Breshears, D. D., Myers, O. B., Meyer, C. W., Barnes, F. J., Zou, C. B., Allen, C. D., ... & Pockman, W. T. (2009). Tree die-off in response to global change-type drought: Mortality insights from a decade of plant water potential measurements. Frontiers in Ecology and the Environment, 7(4), 185-189.

Briones, M. J. I., McNamara, N. P., Poskitt, J., Crow, S. E., & Ostle, N. J. (2014). Interactive biotic and abiotic regulators of soil carbon cycling: evidence from controlled climate experiments on peatland and boreal soils. Global change biology, 20(9), 2971-2982.

Calderón, K., Spor, A., Breuil, M. C., Bru, D., Bizouard, F., Violle, C., ... & Philippot, L. (2017). Effectiveness of ecological rescue

for altered soil microbial communities and functions. The ISME journal, 11(1), 272-283.

Camarda, M., De Gregorio, S., Capasso, G., Di Martino, R. M., Gurrieri, S., & Prano, V. (2019). The monitoring of natural soil CO2 emissions: Issues and perspectives. *Earth-Science Reviews*, *198*, 102928.

Cánovas, D., Fletcher, S. A., Hayashi, M., & Csonka, L. N. (2001). Role of Trehalose in Growth at High Temperature ofSalmonella enterica Serovar Typhimurium. Journal of bacteriology, 183(11), 3365-3371

Castro, H. F., Classen, A. T., Austin, E. E., Norby, R. J., & Schadt, C. W. (2010). Soil microbial community responses to multiple experimental climate change drivers. Applied and environmental microbiology, 76(4), 999-1007.

Cheng, L., Booker, F. L., Burkey, K. O., Tu, C., Shew, H. D., Rufty, T. W., ... & Hu, S. (2011). Soil microbial responses to elevated CO 2 and O 3 in a nitrogen-aggrading agroecosystem. PLoS One, 6(6), e21377.

Classen, A. T., Sundqvist, M. K., Henning, J. A., Newman, G. S., Moore, J. A., Cregger, M. A., ... & Patterson, C. M. (2015). Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead?. Ecosphere, 6(8), 1-21.

Cregger, M. A., Schadt, C. W., McDowell, N. G., Pockman, W. T., & Classen, A. T. (2012). Response of the soil microbial community to changes in precipitation in a semiarid ecosystem. Applied and environmental Microbiology, 78(24), 8587-8594.

Darby, B. J., Housman, D. C., Zaki, A. M., Shamout, Y., Adl, S. M., Belnap, J., & Neher, D. A. (2006). Effects of altered temperature and precipitation on desert protozoa associated with biological soil crusts. Journal of Eukaryotic Microbiology, 53(6), 507-514.

De Vries, F. T., & Shade, A. (2013). Controls on soil microbial community stability under climate change. Frontiers in microbiology, 4, 265.

Doyle, J. J., & Luckow, M. A. (2003). The rest of the iceberg. Legume diversity and evolution in a phylogenetic context. Plant physiology, 131(3), 900-910.

Drigo, B., Kowalchuk, G. A., Yergeau, E., Bezemer, T. M., Boschker, H. T., & Van Veen, J. A. (2007). Impact of elevated carbon dioxide on the rhizosphere communities of Carex arenaria and Festuca rubra. *Global Change Biology*, *13*(11), 2396-2410.

Evans, S. E., & Wallenstein, M. D. (2012). Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter?. Biogeochemistry, 109(1-3), 101-116.

Guenet, B., Lenhart, K., Leloup, J., Giusti-Miller, S., Pouteau, V., Mora, P., ... & Abbadie, L. (2012). The impact of long-term CO2 enrichment and moisture levels on soil microbial community structure and enzyme activities. Geoderma, 170, 331-336.

Higgins, D., & Dworkin, J. (2012). Recent progress in Bacillus subtilis sporulation. FEMS microbiology reviews, 36(1), 131-148.

Hutchins, D. A., Jansson, J. K., Remais, J. V., Rich, V. I., Singh, B. K., & Trivedi, P. (2019). Climate change microbiology—problems and perspectives. Nature Reviews Microbiology, 17(6), 391-396.

Intergovernmental Panel on Climate Change. Climate Change 2001: The Scientific Basis: Contribution of Working Group I to the Third Assessment Report 1–-944 (Cambridge Univ. Press, Cambridge, UK, 2001).

IPCC (Intergovernmental Panel on Climate Change). 2013. Climate change 2013: The physical science basis. Working Group I contribution to the IPCC Fifth Assessment Report. Cambridge, United Kingdom: Cambridge University Press. www.ipcc.ch/report/ar5/wg1

Kannojia, P., Sharma, P. K., & Sharma, K. (2019). Climate Change and Soil Dynamics: Effects on Soil Microbes and Fertility of Soil. In Climate Change and Agricultural Ecosystems (pp. 43-64). Woodhead Publishing.

Lamb, E. G., Han, S., Lanoil, B. D., Henry, G. H., Brummell, M. E., Banerjee, S., & Siciliano, S. D. (2011). A High Arctic soil ecosystem resists long-term environmental manipulations. Global Change Biology, 17(10), 3187-3194.

Laurent Philippot , Ayme´ Spor , Catherine He´nault, David Bru , Florian Bizouard, Christopher M Jones, Amadou Sarr and Pierre-Alain Maron (2013) Loss in microbial diversity affects nitrogen cycling in soil. *The ISME journal*, *7*(8), 1609-1619.

Lin, L., Zhu, B., Chen, C., Zhang, Z., Wang, Q. B., & He, J. S. (2016). Precipitation overrides warming in mediating soil nitrogen pools in an alpine grassland ecosystem on the Tibetan Plateau. Scientific reports, 6(1), 1-9.

Nguyen, L. M., Buttner, M. P., Cruz, P., Smith, S. D., & Robleto, E. A. (2011). Effects of elevated atmospheric CO2 on rhizosphere soil microbial communities in a Mojave Desert ecosystem. *Journal of arid environments*, *75*(10), 917-925.

Nie, M., Bell, C., Wallenstein, M. D., & Pendall, E. (2015). Increased plant productivity and decreased microbial respiratory C loss by plant growth-promoting rhizobacteria under elevated CO 2. *Scientific Reports*, *5*, 9212.

Pérez-Izquierdo, L., Zabal-Aguirre, M., González-Martínez, S. C., Buée, M., Verdú, M., Rincón, A., & Goberna, M. (2019). Plant intraspecific variation modulates nutrient cycling through its below ground rhizospheric microbiome. Journal of Ecology, 107(4), 1594-1605.

Rafiq islam, (2004). Soil microbial communities. New York : Marcel Dekker

Reichstein, M., Bahn, M., Ciais, P., Frank, D., Mahecha, M. D., Seneviratne, S. I., ... & Papale, D. (2013). Climate extremes and the carbon cycle. Nature, 500(7462), 287-295.

Robroek, B. J., Heijboer, A., Jassey, V. E., Hefting, M. M., Rouwenhorst, T. G., Buttler, A., & Bragazza, L. (2013). Snow cover manipulation effects on microbial community structure and soil chemistry in a mountain bog. Plant and soil, 369(1-2), 151-164.

Santos, H. F., Carmo, F. L., Duarte, G., Dini-Andreote, F., Castro, C. B., Rosado, A. S., ... & Peixoto, R. S. (2014). Climate

change affects key nitrogen-fixing bacterial populations on coral reefs. The ISME Journal, 8(11), 2272-2279.

Sayer, E. J., Oliver, A. E., Fridley, J. D., Askew, A. P., Mills, R. T., & Grime, J. P. (2017). Links between soil microbial communities and plant traits in a species-rich grassland under long-term climate change. Ecology and Evolution, 7(3), 855-862.

Schostag, M., Priemé, A., Jacquiod, S., Russel, J., Ekelund, F., & Jacobsen, C. S. (2019). Bacterial and protozoan dynamics upon thawing and freezing of an active layer permafrost soil. *The ISME journal*, *13*(5), 1345-1359.

Sheik, C. S., Beasley, W. H., Elshahed, M. S., Zhou, X., Luo, Y., & Krumholz, L. R. (2011). Effect of warming and drought on grassland microbial communities. The ISME journal, 5(10), 1692-1700.

Singh, B. R., & Singh, O. (2012). Study of impacts of global warming on climate change: rise in sea level and disaster frequency. *Global warming—impacts and future perspective*.

Smith, S. E., & Read, D. J. (2010). Mycorrhizal symbiosis. Academic press.

Taş, N., Prestat, E., Wang, S., Wu, Y., Ulrich, C., Kneafsey, T., ... & Jansson, J. K. (2018). Landscape topography structures the soil microbiome in arctic polygonal tundra. Nature communications, 9(1), 1-13.

Theuerl, S., & Buscot, F. (2010). Laccases: toward disentangling their diversity and functions in relation to soil organic matter cycling. Biology and Fertility of Soils, 46(3), 215-225.

Toberman, H., Freeman, C., Evans, C., Fenner, N., & Artz, R. R. (2008). Summer drought decreases soil fungal diversity and associated phenol oxidase activity in upland Calluna heathland soil. FEMS Microbiology Ecology, 66(2), 426-436.

Tu, Q., Yuan, M., He, Z., Deng, Y., Xue, K., Wu, L., ... & Zhou, J. (2015). Fungal communities respond to long-term CO2 elevation by community reassembly. Applied and environmental microbiology, 81(7), 2445-2454.

Van der Putten, W. H. (2012). Climate change, aboveground-belowground interactions, and species' range shifts. Annual Review of Ecology, Evolution, and Systematics, 43.

Vinolas, L. C., Vallejo, V. R., & Jones, D. L. (2001). Control of amino acid mineralization and microbial metabolism by temperature. *Soil Biology and Biochemistry*, *33*

Wagner, D., Eisenhauer, N., & Cesarz, S. (2015). Plant species richness does not attenuate responses of soil microbial and nematode communities to a flood event. Soil Biology and Biochemistry, 89, 135-149.

Wei, H., Guenet, B., Vicca, S., Nunan, N., AbdElgawad, H., Pouteau, V., ... & Janssens, I. A. (2014). Thermal acclimation of organic matter decomposition in an artificial forest soil is related to shifts in microbial community structure. Soil Biology and Biochemistry, 71, 1-12.

West, J. B., HilleRisLambers, J., Lee, T. D., Hobbie, S. E., & Reich, P. B. (2005). Legume species identity and soil nitrogen supply determine symbiotic nitrogen-fixation responses to elevated atmospheric [CO2]. New Phytologist, 167(2), 523-530.

Whitaker, J., Ostle, N., Nottingham, A. T., Ccahuana, A., Salinas, N., Bardgett, R. D., ... & McNamara, N. P. (2014). Microbial community composition explains soil respiration responses to changing carbon inputs along an A ndes-to-A mazon elevation gradient. Journal of Ecology, 102(4), 1058-1071.

Williams, M. A., & Rice, C. W. (2007). Seven years of enhanced water availability influences the physiological, structural, and functional attributes of a soil microbial community. *Applied Soil Ecology*, *35*(3), 535-545.

Wood, J. M., Bremer, E., Csonka, L. N., Kraemer, R., Poolman, B., van der Heide, T., & Smith, L. T. (2001). Osmosensing and osmoregulatory compatible solute accumulation by bacteria. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 130(3), 437-460.

Yiqi, L., & Zhou, X. (2010). *Soil respiration and the environment*. Elsevier.

Zak, D. R., Pregitzer, K. S., Curtis, P. S., Teeri, J. A., Fogel, R., & Randlett, D. L. (1993). Elevated atmospheric CO 2 and

feedback between carbon and nitrogen cycles. Plant and soil, 151(1), 105-117.

Zak, D. R., Pregitzer, K. S., King, J. S., & Holmes, W. E. (2000). Elevated atmospheric CO2, fine roots and the response of soil microorganisms: a review and hypothesis. New Phytologist, 147(1), 201-222.

Zhang, C., Niu, D., Hall, S. J., Wen, H., Li, X., Fu, H., ... & Elser, J. J. (2014). Effects of simulated nitrogen deposition on soil respiration components and their temperature sensitivities in asemiarid grassland. *Soil Biology and Biochemistry*, *75*, 113-123.

Zhang, Q., & Yan, T. (2012). Correlation of intracellular trehalose concentration with desiccation resistance of soil Escherichia coli populations. Applied and environmental microbiology, 78(20), 7407-7413.

Zhou, T., Shi, P., Hui, D., & Luo, Y. (2009). Global pattern of temperature sensitivity of soil heterotrophic respiration (Q10) and its implications for carbon-climate feedback. Journal of Geophysical Research: Biogeosciences, 114(G2).

Zimmer, C. (2010). The microbe factor and its role in our climate future. *Retrieved December*, *15*, 2015.

BIOREMEDIATION OF TEXTILE AND FOOD INDUSTRIAL EFFLUENTS THROUGH MICROBIAL ACTIVITY

Muddapuram Deeksha Goud[1], Rishab Singh Jauhari[1], Bharti Singh Jadoun[1], Asmita Singh[1] and Neelam Jain*[2]

[1]Student, Amity Institute of Biotechnology, Kant-Kalwar, NH11C, RIICO Industrial Area, Jaipur, Rajasthan 303007, India

[1]Professor, Amity Institute of Biotechnology, Kant-Kalwar, NH11C, RIICO Industrial Area, Jaipur, Rajasthan 303007, India

*Corresponding Author: njain1@jpr.amity.edu

Introduction

Effluent and waste have an impact on the environment, escalating the pollution issue and creating a critical need for sustainable and eco-friendly remediation technology. Over recent years, there has been a significant rise in food waste generation. Studies are conducted in order to put an emphasis to employ biological treatments to manage food wastages to a minimum or to be converted into some valuable products. Utilization of microbes in various bioremediation processes is made in order to protect the environment from damage caused by industrial polluting effluents. A reliable treatment system is based on the characterization of the effluent to treat food wastes containing strong organic and fat-rich effluents. Biological treatment through microbes is an efficient method for removing these by degrading them into miscible molecules. Consortium of microbes is observed to be the one with the best biodegradable capability.

Waste materials and effluents from textile industry

The textile industry is a global market that incorporates industries from healthcare to high fashion to diapers and more. Nearly all

countries around the globe has their significant part in the textile industry, either in making of products, transporting of products, or in manufacture the technology that creates the textiles. This also means that there is an enormous amount of textile mills that create waste, use precious resources, and source pollution. (Jadhav, 2016).

The textile industries consume several hundred thousand gallons of water every day and proportionally produce an enormous volume of wastewater. Highly colored wastewater may be a major environmental barrier for the expansion of the textile and dye manufacturing industry, besides the conflicting minor issues like solid waste management.The textile industry produces about 10,000 commercially available dyes, and therefore the production rate of those dyes is bigger than 7×10^5 tons each year, with about 10- 15% of those dyes discharged as effluents during manufacturing and processing procedures. (Feng et al, 2012;). Most of those dyes in the effluents are predominantly azo dyes which are refractory and xenobiotic in nature, affecting both the aesthetic value of water and aquatic biota by decreasing light penetration for photosynthetic microbes and plants. They also aggravating the toxicity levels in the aquatic ecosystem through accumulation of aromatics, metals, and chlorides, alongside color. Textile and dye engineering industry wastewaters contain dyes, salts, surfactants, acids, binders, reducing agents, thickeners, etc.

Characterstics of textile industry effluents

In order to design a strong and effective treatment process, characterization of commercial effluent is that the most elementary and important step. The characteristics of this effluent are critical to grasp because the ensuing treatment alternatives are directly associated with these characteristics. The effluent characterization studies constitute common effluent treatment plants (CETPs). This wastewater is characterized in terms of salinity, temperature, pH, strong color, biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids, and nonbiodegradable organic compounds. It also contains trace heavy metals like chromium, arsenic, copper, and zinc. Additionally, COD and BOD

also very high for these effluents. Moreover their diverse molecular structures make them immune to bio-physico-chemical methods of degradation resulting into noxious and mutagenic metabolites. Owing to these deleterious attributes in textile effluents has led to significant attention from scientists within the past few decades with the aim of using ecofriendly approaches to maximise the removal efficiency with ameliorated energy regulation and low cost requirements (Ahmad et al., 2015).

Factors affecting biological removal of textile dyes

The factors affecting biological dye elimination from industrial effluents include temperature, dye concentration, molecular structure of dyes, pH, agitation, etc. Biological degradation of textile dyes using microbial cultures has been explained in Fig.1

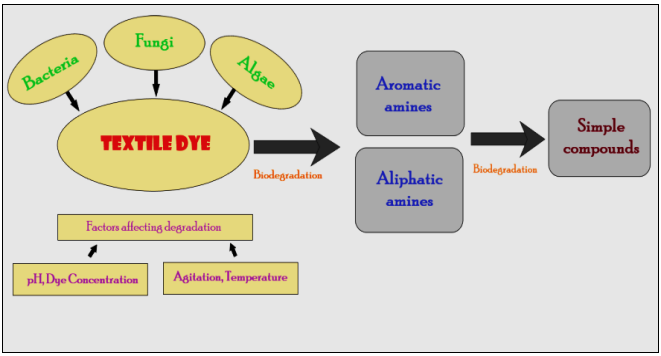


Figure 1. Mechanism for biological degradation of textile dyes.

The molecular configuration of dyes is found to possess an influence on the extent of dye bioremoval. The aromatic rings with hydroxyl, amino, or nitro functional groups are mineralized to a greater extent than unsubstituted rings in dye removal by *Phanerochaete chrysosporium*. Dye concentration additionally influences the colour removal efficiency. The pH impacts both the fungal biomass surface dye binding sites and therefore the dye chemistry within the

medium. At low pH, the fungal biomass will have a net charge. These sites become available for binding anionic groups of reactive dyes. Most textile and other colored effluents are generated at generally high temperatures and henceforth temperature is going to be a critical think about the important application of biosorption in future. Aksu and Cagatay (2006) found a rise in biosorption with increase in temperature up to 45°C for *Rhizopus arrhizus* culture. Dyeing processes consume tons of salt and consequently the dyeing wastewater contains high salt concentration. Consequently, the ionic strength is a vital factor.

Microorganisms and mechanism involved in bioremediation of textile dyes

Bioremediation of textile dyes can be attained through microorganisms and biocatalysts. Each has their own pros and cons in terms of decolorizing efficiency, aptness, and working ability (pH, temperature, and concentration of dye). Diverse strains of microbes are capable of removal or neutralization of harmful pollutants from contaminated sites through their enzymatic activities. It has been acknowledged that the color of the dyes reflects the presence of conjugated systems in their chromophores. However, some dyes possess auxochromes (such as sulfonic acid, hydroxyl, amino groups, and carboxylic acids) in addition to chromophores, which are not only responsible for the color shift, but also control their solubilities in different solvent systems. This decolorization of textile dyes is chemically accomplished by breaking electronic bonds within the chromophores. For an effective decolorization process, microbes alone or in consortia can be employed. Table 1 enlist the various microbes involved in the biodegradation of dyes.

Biodegradation of dyes via bacterial strains

Bacteria are a most studied organism for the remediation of textile dyes from industrial effluents. Most of the research involving bacterial strains has focused on studying the biodecolorization and degradation as compared with biosorption (application of dead bacterial biomass) of dyes.

Table 1. Biodegradation of Dyes through Microbial Activity

Microbial culture	Dye	Mechanism	References
Bacteria			
Lysinibacillus sp.	Remazol Red	Biodegradation	Saratale et al. (2013)
Pseudomonas desmolyticum	Direct Blue 6	Biodegradation	Kalme et al. (2007)
Micrococcus glutamicus	Reactive Green 19A	Biodegradation	Saratale et al. (2009)
Micrococcus Glutamicus NCIM 2168	Direct Red 81	Biodegradation	Sahasrabudh (2020)
Pseudomonas putida	Crystal violet	Biodegradation	Chen et al. (2009)
Pseudomonas pulmonicola	Malachite green	Biodegradation	Chen et al. (2009)
Brevibacillus parabrevis	Congo red	Biodegradation	Abu Talha et al. (2018)
Fungi			
Aspergillus bombycis	Reactive Red 31	Biodegradation	Khan and Fulekar (2017)
Penicillium ochrochloron	Cotton blue	Biodegradation	Shedbalkar et al. (2008)
Aspergillus sp.	Brilliant green	Biodegradation	Kumar et al. (2012)
Coriolopsis sp.	Crystal Violet Cotton Blue	Biodegradation	Munck et al. (2018)
Peyronellaea prosopidis	Scarlet RR	Biodegradation	Bankole et al. (2018)

Myceliophthora	Reactive Blue 220	Biodegradation	Patel et al. (2013)
Algae			
Cyanobacterium Phormidium	Indigo blue	Biodegradation and biosorption	Dellamatrice et al. (2017)
Chlorella vulgaris	Remazol Black B	Biosorption	Aksu and Tezer (2005)
Nostoc linckia	Methyl red	Biodegradation	El-Sheek et al., (2009)
Oscillatoria rubescens	Basic Fuchsin	Biodegradation	El-Sheek et al., (2009)

Biodegradation processes involving bacterial cells may be anaerobic, aerobic, or involve a combination of the two. The mode of dye uptake by dead/inactive cells in the biosorption process is extracellular; the chemically functional groups of the bacterial cell wall play vital roles in the process. Generally, decontamination of textile dyes in industrial effluent by bacterial culture involves breaking of azo bond with the help of different enzymes and electron donating systems (Brüschweiler et al., 2017).

Hence, application of a bacterial culture or bacterial consortium is extremely common. Recently, 93% of Reactive black 5 dye with 500 mg/L of dye concentration has been remediated using a *Pseudomonas entomophila* culture after 120 h of incubation time (Khan et al., 2015). A bacterial consortium of *Providencia rettgeri* and *Pseudomonas* sp. culture was reported to mineralize four textile azo dyes (Reactive Black 5, Direct Red 81, Reactive Orange 16, and Disperse Red 78) (Lade, H., et al 2015). Complete decolorization of those azo dyes was accomplished in microaerophilic, sequential microaerophilic/aerobic, and aerobic/microaerophilic environments with slight difference in incubation time, perhaps due to their difference in chemical structure. It is well-known that the subsequent breakdown products like aromatic amines from the degradation of dyes experience further mineralization by oxygenase

and hydroxylase enzymes secreted by bacterial culture (Xiang et al., 2016). However, structurally complex textile dyes and their degraded products minimize the application of bacterial cultures for bioremediation use. For instance, naphthylamine sulfonic acids are refractory as they're difficult to degrade using bacterial cultures due to the nonpermeability of a strongly charged anionic species, viz. sulfonyl group, through the bacterial membranes (Gao et al., 2014). *Pseudomonas pulmonicola* YC32 was able to initiate Malachite Green degradation over a stepwise demethylation process (Chen et al., 2009). Crystal Violet, a triphenylmethane dye is one of the most vital dyes used in textiles and acknowledged for its mutagenic and mitotic poisoning nature. Newly recognized bacteria *Enterobacter sp.* CVS1, was found as a probable bioremediation biocatalyst in the aerobic degradation/de-colorization of Crystal Violet dye (Roy et al., 2018).

Biodegradation of dyes through fungal strains

Fungi have emerged as effective biological tools for the degradation and mineralization of structurally rigid textile dyes owing to their strong enzymatic system and diverse metabolism (Ahmad et al., 2015; Rahimnejad et al., 2015). They possess the strong ability to degrade complex organic pollutants by producing extracellular ligninolytic enzymes including laccase, lignin peroxidase, and manganese peroxidase. Many fungi strains have been applied in either living or inactivated form. The use of white-rot fungi such as *P. chrysosporium* in textile wastewater remediation has been widely reported within the literature (Gomaa et al., 2008; Faraco et al., 2009). Apart from white-rot fungi, other fungi such as *Aspergillus niger*, *R. arrhizus*, and *Rhizopus oryzae* can also decolorize and biosorb diverse range of dyes. *Penicillium oxalicum* strain was able to biodegrade Acid Red 183, Direct Red 75, and Direct Blue 15 dyes (Saroj et al., 2014). Other fungal cultures, such as *Magnusiomyces* and *Candida* were also recognized for their capability to completely degrade textile dyes (Brüschweiler et al., 2017). Complete decolorization of a red azo dye (1000 mg/L dye concentration) was carried out using *A. niger* culture at 9 pH (Mahmoud et al, 2017). The removal of Azo dyes (Direct Red 23 and Direct Violet 51) by

biosorption was done using *Aspergillus oryzae* (Goud et al., 2020). The mechanism of fungal remediation involves adsorption, enzymatic degradation, or a combination of both. The main aim of fungal-based remediation is to decolorize and detoxify the dye-contamsinated industrial effluents.

Biodegradation of dyes via algae strains

Algal cultures have been proved to be potential candidates for the removal of dyestuff compounds from colored effluents. They are effective biosorbents and bio-coagulants because of their availability in both fresh and marine water. The dye remediation that specializes in algal applications could also be due to the buildup of dye ions on the surface of algal biopolymers and further to the diffusion of the dye particles from aqueous system onto the solid surface of the biopolymer. Diverse strains of algae (*Anabaena flos-aquae UTCC64, Cosmarium, 311 Chlorella vulgaris, Chlorella pyrenoidosa, Lyngbya lagerlerimi, Nostoc lincki, Oscillatori arubescens, Elkatothrix viridis* and *Volvox aureus Phormidium autumnale* UTEX1580, and Synechococcus sp. PCC7942) have been effectively employed for the degradation of different textile dyes (Daneshvar et al., 2007; Dellamatrice et al., 2017; El-Sheekh et al., 2009; Pathak et al 2015). Recently, three strains of cyanobacteria (*Anabaena flos-aquae* UTCC64, *Phormidium autumnale* UTEX1580, and *Synechococcus* sp. PCC7942) were assessed for degradation of indigo, RBBR, and sulfur black (Dellamatrice et al., 2017) out of which *Phormidium autumnale* could completely degrade indigo dye. Thus, algae can be used for tertiary treatment of effluents with refractory compounds.

Waste materials and effluents from food industry

World water bodies are increasingly polluted with food wastewaters, its effects can be irreversible for aquatic living organisms as it threatens their survival, and the consequences of these effects are transferred indirectly or directly to humans, as they are also involve in the food chain of the ecosystem. Wastewaters from food

industries are increasingly becoming more toxic in recent times (Qasim and Mane, 2013). The concentrations of pollutants like organic matters, fats, oil and grease (FOGS) in food wastewater have been observed to increase in wastewater bodies with increasing adverse effects on the environment due to indiscriminate discharge of wastewater into the water drains by food restaurants, industries and household. Fats, Oil and grease containing wastes generated from vegetable oil origin are generally classified as serious types of hazardous pollutants particularly when injected into the aquatic environments where they pose high toxicity to the aquatic organisms and other ecology damages to the water bodies. These dissolved organic components can seriously affect normal operations of ecosystems, flora and fauna (Xue Lin Gui, 2016).

Characterstics of food industry effluents

Food industry effluents contain hazardous substances such as heavy organic matter, fats, oil & grease, fatty acids, nitrogenous compounds, etc. The utilisation of cooking oil in food industries has lead to discharge of oil effluent and oily sludge into the environment without treatment or in a form that is below the standard discharge limits (Qasim and Mane, 2013). Recently, focus has shifted to microbial bioremediation due to its numerous advantages from the conventional physicochemical methods of wastewater remediation. Compared to mechanical and chemical method in carrying out remediation of food wastewater a lot of microbial bioremediation research has been conducted in view of finding lasting solution to this global menace.

Dairy industry involves processing of raw milk. The pasteurization, sterilization, separation, filtration and homogenization are several processing operations. Various products like market milk, condensed milk, milk powder, cream, butter, cheese, curd, yogurt and ice cream and many more products are being made. In manufacturing of all the products, numerous by-products such as, whey, butter milk, are produced in huge amount (Birwal et al., 2017). Rinsing water with addition of disinfectants which ranges

from 1 to 3 L for processing per liter of milk are generated in large quantity of effluent. The by-products contain high concentration of organic material such a protein, carbohydrates, fats, grease, minerals having high COD and BOD values. Therefore it needs proper treatment before its discharge from the factory premises. (Kavitha et al., 2013).

Microorganisms and mechanism involved in biodegradation of food industry effluents

Food effluent contain mostly organic waste therefore, biological degradation is the most promising options for the removal of organic material (Fig 2.). However, sludge formed, especially during the aerobic biodegradation processes, can lead to serious and costly disposal problems. This can be further influenced by the ability of sludge to adsorb specific organic compounds and even toxic heavy metals. However, biological treatment has the benefits of microbial transformations of complex organics compounds and possible adsorption of heavy metals by suitable microbes (Birwal et al., 2017).

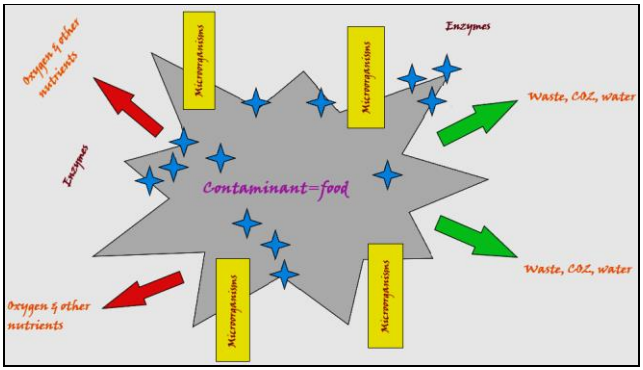


Figure 2. Mechanism for biological degradation of food effluents

Bioremediation of food industry effluents by bacteria

A major effluent in agro-industry is the presence of phenols which are degraded by laccases and tyrosinases use in wastewater treatment and bioremediation. It is found that for removing phenol from industrial waste water *Pseudomonas* spp.(isolated from active sludge) is more effective than nonaugmented one. *Pseudomonas aeruginosa* sp in waste water shows good potential for use in waste water treatment, biochemical oxygen demand (BOD) and lipid degradation(Kanu, Ijeoma, Achi, 2011). Certain types of bacteria like *Mycobacterium, Escherichia coli, Erwinia, Proteus vulgaris, Pseudomonas aeruginosa, Bacillus sp, Clostridium sp, Staphylococcus* are more efficient in food wastewater treatment (MupitDatusahlan et al., 2013). Due to constant aeration, agitation and recirculation of activated sludge involves microbes such as *Achromobacter, Alcaligenes, Arthrobacter, Citromonas, Flavobacterium, Pseudomonas, Zoogloe* and *Acinetobacter*. The microbes secrete wide varieties of enzymes such as cellulase, pectinase, lipase which assist in degrading organic matters in wastewater (P. Kanmani et al., 2016).

Oil rich wastewaters excreted from food processing contains lipid such as edible oil and long-chain fatty acids. Biological treatment is the most efficient method for removing fat oil and grease which is done by degrading them into miscible molecules (Odeyemi et al., 2010). Oil degrading microbes produce enzymes that tend to hydrolyze the oil content of wastewater. The lipid-rich wastewater decomposing organisms which produce highly efficient lipases have higher ability in both individually and when used as a consortium (Prasad and Manjunath, 2011). A few examples of lipolytic microorganisms that are significant in wastewater are bacteria of the genera: *Pseudomonas, Clostridium. Klebsiella and Staphylococcus spp* also exhibit potential in oil degradation process. In recent researches identified lipid-degrading bacterial strains with two groups: *Gammaproteobactera* and *Bacilli. Gammaproteobacteria* composed of *Acinetobacteria, Pseudomonas, Aeromonas* and *Stenotrophomonas* with high ratio in comparison to Bacilli with only two species- *Bacillus cereus* and *Bacillus pumilus* found to be highly efficient in oil and grease biodegradation (Ngo et al., 2014).

Biodegradation of FOG by fungi

The accumulation of fat, oil and grease (FOG) of food wastewater may rise up as 320 - 21,500 mg/L (Rashid et al., 2008). Number of problems arises due to deposition of FOG in sewerage systems as it accumulates inside pipes or completely blocking the pipelines. Formation of stable froth and scum may occur due to the existence of *filamentous actinomycetes*, which may in turn occur due negative affect in oxygen transfer because of the presence of high levels FOG in aerobic treatment processes. With the breakthrough in food processing biotechnology, have shown results in the production of enzymes with higher lipase activity produced by solid state fermentation (SSF) using fungi like *Penicillium, Aspergillus,* and *Fusarium* (Witharana et al., 2017) which catalyses FOG to long chain fatty acids lipase and other hydrolytic enzymes (Kumar et al., 2012). *Aspergillus terreus* is best for removal of nitrate and BOD while phosphate and COD removal ability from wastewater is best found in *Aspergillus niger* (Ayodhya Dattatray Kshirsaga, 2013).

Conclusion

Bioremediation provides a technique for cleaning up waster by enhancing the natural biodegradation processes. The destiny of synthetic chemicals reaching the environment from most parts depends on the microorganisms accessible for its degradation in the environment. The greatest potential of treatment systems incorporating whole bacterial cells to metabolise the azo dyes, present in the wastage of food has put a stress to employ innovative techniques for management of waste caused so that waste generation could be minimized or these wastes could be transformed into some valuable products. Food waste consists of high amounts of sodium and moisture and is generally mixed with other sorts of waste through its collection. Amount of waste generated is highly determined by two factors—population in a given area and its consumption patterns. In order to manage this huge waste production, progressive and effective waste management structures

are to be implemented that can be helpful to overcome the huge gap between production and management of waste disposal.

References

Abu Talha, M., Goswami, M., Giri, B. S., Sharma, A., Rai, B. N., & Singh, R. S. (2018). Bioremediation of Congo red dye in immobilized batch and continuous packed bed bioreactor by Brevibacillus parabrevis using coconut shell bio-char. *Bioresource technology*, *252*, 37–43. https://doi.org/10.1016/j.biortech.2017.12.081

Ahmad, A., Mohd-Setapar, S.H., Chuong, C.S., Khatoon, A., Wani, W.A., Kumar, R., Rafatullah, M. 2015. Recent advances in new generation dye removal technologies: novel search for approaches to reprocess wastewater. RSC Advances, 5(39): 30801-30818

Aksu, Z. and Çağatay, Ş. Ş., 2006. Investigation of biosorption of Gemazol Turquise Blue-G reactive dye by dried Rhizopus arrhizus in batch and continuous systems, Sep. Purif. Technol. 48: 24- 35

Aksu, Z. an Tezer, S., 2005. Biosorption of reactive dyes on the green alga *Chlorella vulgaris.*Proc. Biochem., 40: 1347-136

Ayodhya Dattatray Kshirsaga, 2013. Application of Bioremediation Process for Wastewater Treatment Using Aquatic Fungi. International Journal Of Current Research, 5:1737-1739

Bankole PO, Adekunle AA, Govindwar SP (2018) Enhanced decolorization and biodegradation of acid red 88 dye by newly isolated fungus, Achaetomium strumarium. J Environ Chem Eng 6(2):1589–1600. https://doi.org/10.1016/j.jece.2018.01.069

Birwal, P., Deshmukh G, Priyanka, et al. Advanced Technologies for Dairy Effluent Treatment. J Food Nutr Popul Health. 2017, 1:1.

Brüschweiler, B.J., Merlot, C., 2017. Azo dyes in clothing textiles can be cleaved into a series of mutagenic aromatic amines

which are not regulated yet. Regulatory Toxicology and Pharmacology 88, 214-226

Chen, C.Y., Kuo, J.T., Cheng, C.Y., Huang, Y.T., Ho, I.H., Chung, Y.C. 2009. Biological decolorization of dye solution containing malachite green by Pandoraeapulmonicola YC32 using a batch and continuous system. J Hazard Mater, 172(2-3), 1439-45

Daneshvar N, Ayazloo M, Khataee AR, Pourhassan M., 2007. Biological decolorization of dye solution containing Malachite green by microalgae Cosmarium sp. Bioresour Technol 98:1176–1182

Dellamatrice, P.M., Silva-Stenico, M.E., de Moraes, L.A.B., Fiore, M.F., Monteiro, R.T.R., 2017. Degradation of textile dyes by cyanobacteria. Brazilian Journal of Microbiology 48(1), 25-31

El-Sheekh, M.M., Gharieb, M.M., Abou-El-Souod, G.W., 2009. Biodegradation of dyes by some green algae and cyanobacteria. International Biodeterioration and Biodegradation 63(6), 699-704

Faraco, V., Pezzella, C., Miele, A., Giardina, P., Sannia, G., 2009. Bio-remediation of colored industrial wastewaters by the white-rot fungi Phanerochaetechrysosporium and Pleurotusostreatus and their enzymes. Biodegradation 20(2), 209-220

Feng, Y., Zhou, H., Liu, G., Qiao, J., Wang, J., Lu, H., Yang, L., Wu, Y., 2012. Methylene blue adsorption onto swede rape straw (Brassica napus L.) modified by tartaric acid: equilibrium, kinetic and adsorption mechanisms. Bioresource Technology 125, 138-144

Gao, D.W., Hu, Q., Yao, C., Ren, N.Q., 2014. Treatment of domestic wastewater by an integrated anaerobic fluidizedbed membrane bioreactor under moderate to low temperature conditions. Bioresource Technology 159, 193-198

Garcı´a-Montan˜o, J., Dome`nech, X., Garcı´a-Hortal, J.A., Torrades, F., Peral, J., 2008. The testing of several biological and chemical coupled treatments for Cibacron Red FN-R azo dye removal. Journal of Hazardous Materials 154(1), 484-490

Goud, B.S., Cha, H.L., Koyyada, G., Kim, J.H., 2020. Augmented Biodegradation of Textile Azo Dye Effluents by Plant

Endophytes: A Sustainable, Eco-Friendly Alternative. Current Microbiology, 77, 3240–3255

Jadhav N. Evaluating the scope of technical textile in Raymond. India: NIFT; 2016

Kanu, Ijeoma, Achi , O.K., 2011. Industrial Effluent and Their Impacts on Water Quality of Receiving River in Nigeria. Journal of Applied Technology in Environmental Sanitation, 75-86.

Kalme, S.D., Parshetti, G.K., Jadhav, S.U., Govindwar, S.P., 2007. Biodegradation of benzidine based dye Direct Blue-6 by Pseudomonas desmolyticum NCIM 2112. Bioresource Technology 98, 1405–1410

Kavitha RV, Kumar S, Suresh R, Krishnamurthy V (2013) Performance evaluation and biological treatment of dairy waste water treatment plant by upflow anaerobic sludge blanket reactor. Int J Chem Petrochem Technol. 3: 9-20.

Khan, R., & Fulekar, M. H. (2017). Mineralization of a sulfonated textile dye Reactive Red 31 from simulated wastewater using pellets of *Aspergillus bombycis*. *Bioresources and bioprocessing*, *4*(1), 23. https://doi.org/10.1186/s40643-017-0153-9

Khan, S., Malik, A., 2015. Degradation of Reactive Black 5 dye by a newly isolated bacterium Pseudomonas entomophila BS1. Canadian Journal of Microbiology 62(3), 220-232

Kumar A, Kanwar SS. Lipase production in solid-state fermentation (SSF): recent developments and biotechnological applications. Dynamic Biochemistry, Process Biotechnology and Molecular Biology. 2012;6(1):13-27.

Lade, H., Kadam, A., Paul, D., Govindwar, S., 2015. Biodegradation and detoxification of textile azo dyes by bacterial consortium under sequential microaerophilic/aerobic processes. EXCLI Journal 14, 158

Lingui Xue, Erhunmwunsee Famous, Jinrong Jiang, Hai Shang, Ping Ma , 2016. Experimental Survey on Microbial Bioremediation of Food Wastewaters. International Journal of Scientific and Research Publications (IJSRP), 6(9): 110-118

Mahmoud, M.S., Mostafa, M.K., Mohamed, S.A., Sobhy, N.A., Nasr, M., 2017. Bioremediation of red azo dye from aqueous solutions by Aspergillusniger strain isolated from textile wastewater. Journal of Environmental Chemical Engineering 5(1), 547-554

Munck C, Thierry E, Grassle S, Ting SH (2018) Biofilm formation of filamentous fungi *Coriolopsis* sp. on simple muslin cloth to enhance removal of triphenylmethane dyes. J Environ Manage 214:261–266. https://doi.org/10.1016/j.jenvman.2018.03.025

MupitDatusahlan, W. M. F. Wan Ishak, and Essam A. Makky, 2013. Biodegradation of Wastewater Oil Pollutants, Identification and Characterization: A Case Study – Galing River, Kuantan Pahang, Malaysia. International Journal of Bioscience, Biochemistry and Bioinformatics, 3(6): 2013

Ngo ThanhPhong,, Nguyen ThanhDuyen , Cao Ngoc Diep , 2014. Isolation And Characterization Of Lipid-Degrading Bacteria In Wastewater Of Food Processing Plants And Restaurants In Can Tho City, Vietnam. American Journal Of Life Sciences, 2(6): 382-388

Nouren, S., Bhatti, H.N., Iqbal, M., Bibi, I., Kamal, S., Sadaf, S., Sultan, M., Kausar, A., Safa, Y. 2017. By-product identification and phytotoxicity of biodegraded Direct Yellow 4 dye. Chemosphere, 169(Supplement C), 474-484

Odeyemi, A.T., Dada, A.C., Ogunbanjo, O.R. , Ojo, M.A., 2010. Bacteriological, Physicochemical and Mineral Studies on Awedele Spring Water and Soil Samples in Ado Ekiti, Nigeria. African Journal of Environmental Science and Technology. 4(6): 319-327.

Patel, V.R., Bhatt, N.S., Bhatt, H. B̀. 2013. Involvement of ligninolytic enzymes of *Myceliophthora vellerea* HQ871747 in decolorization and complete mineralization of Reactive Blue 220. Chem. Eng. J., 233: 98-108

Kanmani, P., Aravind, J., Kumaresan, K. (2016). Hydrolytic Enzyme Profiling of Bacillus Subtilis COM6B and Its Application in the Bioremediation of Groundnut Oil Mill Effluent, Integrated Waste Management in India. 179-189.

Pathak, V.V., Kothari, R., Chopra, A.K., Singh, D.P. 2015. Experimental and kinetic studies for phycoremediation and dye removal by Chlorella pyrenoidosa from textile wastewater. Journal of Environmental Management, 163(Supplement C), 270-277

Prasad MP, Manjunath K., 2011. Comparative Study on Bioremediation of Lipid-Rich Wastewater Using Lipase Producing Bacterial Species. Indian Journal Of Biotechnology, 10: 121-124

Qasim, W., Mane A.V., 2013. Characterization and Treatment of Selected Food Industrial Effluents by Coagulation and Adsorption Techniques. Water Resources And Industry, 4:1–12

Rahimnejad, M., Adhami, A., Darvari, S., Zirepour, A., Oh, S.E., 2015. Microbial fuel cell as new technology for bioelectricity generation: a review. Alexandria Engineering Journal 54(3), 745-756

Rashid N, Imanaka T. Efficient degradation of grease using microorganisms. J.Chem.Soc.Pak. 2008 Vol.30 No. 4.

Roy, DC, Biswas SK, Saha AK, Sikdar B, Rahman M, Roy AK, Prodhan ZH, Tang S. 2018. Biodegradation of Crystal Violet dye by bacteria isolated from textile industry effluents*PeerJ* 6:e5015

SaengsangaThanakorn, SiripornadulsilWilailak, SiripornadulsilSurasak.Molecular and enzymatic characterization of alkaline lipase from Bacillus amyloliquefaciens E1PA isolated from lipid-rich food waste, Enzyme and Microbial Technology. 82 (2016) 23-33.

Saroj, S., Kumar, K., Pareek, N., Prasad, R., Singh, R.P., 2014. Biodegradation of azo dyes acid red 183, direct blue 15 and direct red 75 by the isolate Penicilliumoxalicum SAR-3. Chemosphere 107, 240-248

Saratale, R. G., Saratale, G. D., Chang, J. S., & Govindwar, S. P. (2009). Ecofriendly degradation of sulfonated diazo dye C.I. Reactive Green 19A using Micrococcus glutamicus NCIM-2168. *Bioresource technology*, *100*(17), 3897–3905.

Saratale RG, Gandhi SS, Purankar MV, Kurade MB, Govindwar SP, Oh SE, Saratale GD. 2013. Decolorization and detoxification of

sulfonated azo dye C.I. Remazol Red and textile effluent by isolated *Lysinibacillus* sp. RGS J Biosci Bioeng. 115:658–667.

Sahasrabudhe, M. 2020. Enzyme Assisted Biodegradation of Direct Red 81 By Micrococcus Glutamicus NCIM 2168 . *To Chemistry Journal*, *6*, 16-22. Retrieved from https://purkh.com/index.php/tochem/article/view/862

Shedbalkar, U., Dhanve, R., & Jadhav, J. (2008). Biodegradation of triphenylmethane dye cotton blue by Penicillium ochrochloron MTCC 517. *Journal of hazardous materials*, *157*(2-3), 472–479.

Shen C, Xiong J, Zhang H, et al., 2013. Soil Ph Drives The Spatial Distribution Of Bacterial Communities Along Elevation On Changbai Mountain. Soil Biology & Biochemistry, 57: 204-211.

Talha Z, Hamid A, Guo D, Hassan M, Mehryar E, Okinda C, et al., 2018. Ultrasound assisted alkaline pre-treatment of sugarcane filter mud for performance enhancement in biogas production. Int J Agric & Biol Eng, 11(1): 226–231.

Vikrant, K., Giri, B.S., Raza, N., Roy, K., Kim, K-H., Rai, B.N., Singh, R.S., 2018. Recent advancements in bioremediation of dye: Current status and challenges, Bioresource Technology

Witharana, A., Manatunge, J., Ratnayake, N., Nanayakkara, C. M., & Jayaweera, M. 2018. Rapid degradation of FOG discharged from food industry wastewater by lipolytic fungi as a bioaugmentationapplication. *Environmental technology*, *39*(16), 2062–2072. https://doi.org/10.1080/09593330.2017.1349837

Xiang, D., Wang, X., Jia, C., Lee, T., Guo, X., 2016. Molecular-scale electronics: from concept to function. Chemical Reviews 116 (7), 4318-4440

HAIRY ROOTS: A PROMISING BIOTECHNOLOGICAL TOOL FOR BIOREMEDIATION

Smriti Yadav[1], Keya Patel[1] and Neeraj Khare[2]*

[1] Ph.D. Scholar, Amity Institute of Microbial Technology, Amity University Rajasthan
[2] Assistant Professor, Amity Institute of Microbial Technology, Amity University Rajasthan, NH-11C, Kant-Kalwar, Jaipur-303002, Rajasthan, India
*Corresponding Author: nkhare@jpr.amity.edu

Introduction

Pollutant has become a serious matter of concern worldwide due to its lethal effect on human and ecosystem. Various organic and inorganic pollutant are consistently accumulating in the soil, water, and atmosphere due to the increasing anthropogenic activities. Major source of organic pollutant in soil are the surplus use of herbicidesand pesticidessuch as triazines, chloroacetanilide, glycophosate isopropylammonium, 2,4-D, and manozeb etc for the agriculture purpose and its consistent accumulation in the soil severely deteriorated the soil quality and ultimately effects the human health. Other organic pollutants are falls into another category known as polycyclic aromatic hydrocarbons, which consist of biphenyls, anthracene, pyrene, triphenylene etc (Hooper et al. 2009). Pharmaceutically important biomolecules are also a major source of organic pollutant (Zhang et al., 2012). Lead, copper, mercury, etc are the most toxic inorganic pollutant.

The traditionally these pollutants are degraded by mainly physical and chemical process. Development of environment friendly and economically viable bioremediation techniques has opened a new era for the management of these toxic biomolecules. Bioremediation involves the involvement of microbes and plants

origin used for the biotransformation of pollutant in to nontoxic and biodegradable form (Doran et al. 2009). Phytoremediation is an emerging approach wherein plant and closely associated microbes have been utilized for the detoxification of pollutant (Chaudhry et al., 1998). Phytoremediation is economical due to its in-situ remediation process which does not requires the transport of contaminated soil to the treatment plant. The importance of phytoremediation is reflected by consistently increasing scientific reports in molecular biology, genetics and biochemistry of the molecular mechanism of the plant tolerance against these pollutants.

Induction of Hairy Root (crown gall) Culture System

Plants measure requisite supply for the manufacture of phytochemicals. Bacteria genus *Agrobacterium rhizogenes* (or *Rhizobium rhizogenes*) is a soil-born gram-negative microorganism that has the potential to introduce a region of its deoxyribonucleic acid polymer brought up as T-DNA into the plant cells ensuring into the manufacturing of neoplastic roots known as hairy roots. The T-DNA of *A. rhizogenes* is approximately twenty-four thousand base pair long and accommodate series of sequences or genes that code for enzymes synthesizing opines and phytohormones. On the sort of opines made, the strains of *A. rhizogenes* are often separated into 5 types:- Nopaline, Mannopine, Cucumopine (found in carrot hairy root culture) Octopine, Agropine (have strongest induction ability)(Zhou et al 1998).To induce hairy roots explants are individually wounded and co- cultivated or inoculated with *A. rhizogenes*. Sometimes, 2 to 3 days later the explants are often transferred into solid media with antibiotics to kill or eliminate redundant microorganisms. The hairy roots are going to be evoked at intervals within a brief amount of time which varies from one week to over a month relying upon the assorted plant species. Optimizing the composition of nutrients for crown gall cultures it is essential to obtain a high production of secondary metabolites. The ß-glucuronidase (GUS) factor is typically transferred into

hairy roots as a reporter gene and they are often analyzed simply by microscopy anatomy assay.

Table 1. Hairy root cultures of various plant species utilized for phytoremediation of assorted environmental pollutants

Pollutant	Hairy root culture of plant species	Reference
Cadmium	*Beta vulgaris*, *Nicotiana tabacum*	Metzger et al. (1992)
	Thlaspi caerulescens	Nedelkoska and Doran (2000)
	Adenophora lobophylla, A. potaninii	Wu et al. (2001)
Copper	*Rubia tinctorum*	Maitani (1996)
	Hyptis capitata	Nedelkoska and Doran (2000)
	Alyssum murale	Vinterhalter (2008)
Phenol	*Brassica juncea*	Singh (2006)
	B. napus	Coniglio (2008)
	Lycopersicon esculentum	Gonzalez (2006)
	Armoracia lapathifolia	Flocco and Giulietti (1998)
	Daucus carota	Santos et al. (2002)
RDX and HMX	*Catharanthus roseus*	Bhadra et al. (2001)
DDT	*Brassica juncea, Cichorium intybus*	Suresh et al. (2005)
TCE	*Atropa belladonna*	Bernejee (2002)
PCBs	*Solanum nigrum*	Mackova et al. (1997)

Advantages of Hairy Root Culture

Hairy roots give many edges benefits as in comparison to de-differentiated cells, tissues, callus and cell suspension cultures. Hairy roots represent phenotypic, genetic and biochemical stable system for an awfully long amount of time (Benyammi et al. 2016).

Harvesting or gathering roots for extraction of secondary metabolites can lead to destruction of the entire plant. Therefore, interest in production of secondary metabolites by developing crown gall culture has been elevated. Hairy roots doubtless grow quicker without having associate in nursing external offer of plant hormones. All thanks to their high genetic stability all hairy root cultures are stable in metabolic production. Cell immobilization, elicitation, stimulation and biotransformation of crown gall culture can upgrade secondary metabolite production. Hairy roots give an outsized expanse because of their quick growth and extremely branched nature and hence proximity among the contaminants and tissue in contrast to naturally growing roots so give the authentic and generative experimental system to grasp the pollutants and their response to noxious substances (Suza et al. 2008) These roots are useful to grasp the catalyst processes concerned in bioconversion of noxious pollutants to nontoxic compounds.

Disadvantages of hairy root culture

In a very number of plant species, the main accumulation site is the root system (Kabata-Pendias and Pendias 2001) As a new method in the study of plant genetic engineering hairy root culture shows many useful functions but at the same time it also has some potential problems that remain to be solved. It includes possible reduction of chromosomes number during subculturing. It is responsible for morphological alterations of regenerated plants.

Conclusion and future perspective

In theory, HRCs is iatrogenic from primarily basically all plant

species. Therefore, this technology may probably be enforced to rare, valuable, threatened, or endemic medicative species in order to protect multifariousness. Hairy root cultures have become necessary tools for learning synthesis of plant-derived molecules and there bioproduction systems with various benefits over cell suspensions. HRCs are terribly best model systems for recombinant protein/specialized matter production. Hairy root culture is capable for the conversion of polychlorinated biphenyls into hydroxyl derivates. Genetic manipulation of hairy roots with appropriate genes has enabled hairy roots to convert toxicant compounds and increase their rhizo/phytoremediation potential. Another advantage of using hairy roots is their high biomass production that leads to enlargement of surface area where contaminant and tissue come in contact with each other; their high rate that allows studies to be completed quickly; genetic and metabolic stability in the engineered hairy roots; their constant size regardless of the size of the whole plant, and their quality as a tool to screen hyperaccumulator plants. Further studies on hairy roots ought to stay centered on the target genes concerned during this method, and latest studies reveal the basic hairy root phytoremediation model to the environment.

References

Bemejee S, Shang TQ, Wilson AM , Moore AL, Strand SE, Gordon MP, Doty SL (2002) Expression of functional mammalian P450 2E1 in hairy root cultures. Biotechnol Bioeng 77(4):462—466

Benyammi R, Paris C, Khelifi-Slaoui M, Zaoui D, Belabbassi O, Bakiri N, Aci MM, Harfi B, Malik S, Makhzoum A, Desobry S, Khelifi L (2016) Screening and kinetic studies of catharanthine and ajmalicine accumulation and their correlation with growth biomass in Catharanthus roseus hairy roots. Pharm Biol 17:1—I1

Chaudhry T, Hayes W, Khan A and Khoo C (1998) Phytorcmediation–fo cusing on accumulator plants that remediate metal-contaminated soils. Aust I Ecotoiicol 4:37-51

Coniglio MS, Busio VD, Gormllez PS, Medina MI, Mikad S, Agostini H (2008) Application of Brassica napus hairy root cultures for phenol removal from aqueous mlutions. Chemosphere 72:1035-1042

Doran PM (2009) Application of plant tissue cultures in phytoremediation research: incentives and limitations. Biotechnol Bioeng 103:6O—76

Flocco CG, Giulietti AM (1998) In vitro hairy root cultures as a tool for phytoremediation research. In: Willey N (ed) Methods in biotechnology, vol. 23: phytoremediation: methods and reviews. Humana Press, Totowa

Gonzalez PS, Capozucca CE, Tigierm HA, Milrad SR, Agostini E (2006) Phytoremediation of phenol from waste water, by peroxidases of tomato hairy root cultures. Enzyme Microb Technol 39:647-653

Hooper SW, Pettigrew CA, Sayler GS (2009) Ecological fate, effects and prospects for the elimination of environmental polychlorinated biphenyls (PCBs). Environ Toiicol Chem 9:655-667

Kabata-Pendias A, Pendias H (2001) Trace elements in soils and plants, 3rd edn. CRC Press, Boca Raton. p 432

Macek T, Mackova M , Kas I (2000) Exploitation of plants for removal of organics in environmental remediation. Bioiechnol Adv 18:23-24

Maitani T, Kubota H, Sato K, Takeda M, Yoshikira K (1996) Induction of phytochelatin (class III metallothionein) and incorporation of copper in transformed hairy roots of *Rubia tinctorum* exposed to cadmium. J Plant Physiol 147:743–748

Metzger L, Pouchault I, Glad C, Prost R, Tepfer D (1992) Estimation of cadmium availability using transfo d roots. Plant Soil 143:249-257

Nedelkoska TV, Doran PM (2000) Hyperaccumulation of cadmium by hairy roots of Thlaspi caerulescens. Biotechnol Bioeng 67:607—615

Nedelkoska TV, Doran PM (2001) Hyperaccumulation of nickel by hairy roots of Alyssum species: comparimn with whole regenerated plants. Biotechnol Prog 17(4):752-759

Santos D, Charlwood BV, Pletsch M (2002) Tolerance and metabolism of phenol and chloroderivatives by hairy root cu ltures of Daucus carota L. Environ Pollut 117:329-335

Singh S, Melo JS, Eapen S, D" Souza SF (2006) Phenol removal using Brassica juncea hairy roots: role of inherent peroxidam and 1-1202. I Biotechnol 123:43M9

Suresh B, Ravishankar GA (2004) Phytoremediation — a novel and promising approach for environmental clean-up. Crit Rev Biotechnol 24:97-124

Suza W, Harris RS, Lorence A (2008) Hairy roots: from high-value metabolite production to phytoremediation. Electron I Integr Biosci 3(1):57—65

Vinterhalter B, Savié J, Plati& J, Raspor M, Ninkovié S, Mitié N, Vinterhalter D (2008) Nickel tolerance and hyperaccumulation in shoot cultures regenerated from hairy root cultures of Alyssum murale Waldst et Kit. Plant Cell Ties Org Cult 94:299-303

Wu SX, Zu YG, Wu M (2001) Cadmium respond of the hairy root culture of the endangered species Adenophora lobophylla. Plant Sci 160(3):551-562

Zhang DQ, Tan SK, Gersberg RM, Sadreddini S, Zhu I, Tuan NA (2012) Removal of pharmaceutical compounds in tropical constructed wetlands. Ecol Eng 37:460-A64

Zhou L, Wang I, Yang C (1998) Progress on plant hairy root culture and its chemistry. Induetim and culture of plant hairy roots. Nat Product Res Dev 10:87-95

MICROBIAL BIOREMEDIATION FOR REMOVAL OF HEAVY METALS

Ravneet Chug*[1] and Manishita Das Mukherji[2]

[1] Assitant Professor, Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur, India
[2] Associate Professor, Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur, India

*Corresponding Author: rchug@jpr.amity.edu

Introduction

Heavy metals are pervasive in nature and contamination of water with these toxic elements is a matter of grave concern as these pollutants can affect the biological systems leading to many health hazards and causing detrimental diseases (Yang et al., 2018; Zhang et al., 2019; Wang and Zhang, 2019; Yuan et al., 2020). Untreated/or improperly treated industrial effluents directly discharged in to the environment pose a serious threat to the aquatic organisms and also human beings. Owing to their toxicity (even at very low concentrations), the capacity of living cells to bio accumulate these substances and resistance to biodegradability the heavy metals have been classified as priority pollutants.

Various industries like electroplating, mining, batteries, fertilizers, oil refineries and nuclear power plants release effluents enriched with toxic heavy metals (He et al., 2016; Song et al., 2019). These substances are also emitted via natural sources such as weathering, natural forest fires, eruptions of volcanoes, air carrying heavy metal particles from soil, sea salt sprays, and, heavy metals from various living sources.

There are certain methods physico-chemical methods such as adsorption, chemical precipitation, coagulation, electrochemical treatment, evaporation, ion exchange, membrane filtration, and

reduction or oxidation, employed for heavy metal from water samples (Rajasulochana and Preethy, 2016). The salts of heavy metals get dissolved in water easily hence it's very difficult to remove these salts from aquatic medium. The aforementioned methodologies have drawbacks as these are not economic, efficacy is lesser and the final disposal has environmental constraints. As an alternative to the physicochemical removal methods, various methods including carbon based nanoadsorbents, nanotubes, nanoparticles, nanocomposites, photocatalytic and photoelectrocatalytic reduction have been employed for the combating heavy metal pollution (Huang et al., 2018; Zhao et al., 2019b; Ul-Islam et al., 2019; Li et al., 2019;Wei et al., 2020; Zhao et al., 2019) but modern researchers and scientists are focusing more towards biological weapons for bioremediation of heavy metals owing to their low cost, eco- friendly nature and reduction in the discharged biological or chemical sludge (Dixit et al., 2015; Men et al., 2018).

The microbes have the remarkable capability of heavy metal degradation. Pertaining to the same, various microorganisms like bacteria, fungi, yeast, algae which are capable of adsorbing these heavy metals on the surface have been used (Panwar, 2020; Jalilvand et al., 2020; Kalaimurugan et al., 2020; Mosai et al., 2020; Leong and Chang, 2020). These microbial species are able to accumulate these heavy metals within their microbial structures (Kiprono et al., 2018). Many bacterial species accumulate heavy metals via the metal resistant gene which is carried either in plasmid or in genome (Singh et al., 2020).

This review encompass the latest findings and new insights in bioremediation of heavy metals , so future evaluation of a natural biosorbent, which would be quite economical and effective will be helpful in treating contaminated site.

Types of Bioremediation

Bioremediation is an effective and ecofriendly technology for the removal and recovery of heavy metals from contaminated land and

water. Microorganisms are endowed with resistance and metabolic potential to tolerate heavy metals. Owing to this potential, different detoxifying mechanisms such as biosorption, bioaccumulation, biotransformation and biomineralization have been adopted by various microorganisms. Bioremediation is a general concept of biotransformation of contaminated environment to its original state.and is the safest method for sequestering pollutants (Garbisu and Alkorta, 2003; Adhikari et al. , 2004; Girma 2015).

Bioremediation may be categorized into two types- In-situ and Ex-situ bioremediation on the basis of removal and transportation of waste for treatment.

Insitu Bioremediation

It is an onsite treatment of the contaminants where there is no need to excavate or remove soils or water to complete the remediation process. Growth of naturally occurring microorganisms is being promoted by supplying proper oxygen and nutrients. It is a safe and cheap method as it uses harmless microbial organisms for degradation of harmful chemicals and compounds. It can further be of two types- intrinsic bioremediation and engineered in- situ bioremediation. Intrinsic bioremediation involves stimulation of indigenous microbial species while engineered insitu bioremediation comprises supplying certain microbes to the contaminated site. In engineered insitu bioremediation pollutant degradation may be increased or decreased by manipulating the physiochemical conditions which affects the growth of microorganisms ((Vidali 2001, Evans and Furlong 2003).

Ex-situ Bioremediation

The excavation of pollutants from the polluted sites and transferring these to another site for treating the pollutants is ex situ bioremediation. The ex situ treatment is advantageous as the time duration required to remediate is reduced and as the process is externally monitored it is easier to obtain treated waters with uniform pollutant load. This method is costlier owing to enhanced

costs involved in processes as pumping, cost of equipment, and, handling of material, which renders this method less preferable.

Factors Affecting Microbial Remediation

Redox potential, chemical form of the metal ion and total metal ion concentration are some of the deciding factors which affect the metal removal potential of the microorganism. Additionally, environmental factors like temperature, pH, low molecular weight organic acids, and humic acids can also affect the the bioavailability of heavy metals towards microorganisms. Due to the increased hydrogen ion concentrations at acidic pH, more protons are available to saturate metal binding site which reduces attraction between biosorbent and metal ion and in turn increases its toxicity. Temperature is an important parameter affecting heavy metal adsorption on the surface of adsorbent as increased temperature increases the bioavailability of metal ions. Also, the increase in temperature till a suitable range, accelerates metabolic and enzymatic activity of microorganisms. However, final sequestration of metal ion depends on the biosorbent, available sorption sites, cell wall structure of microbial cell and various physicochemical parameters (Bandowe etal., 2014; Igiri et al., 2018).

Mechanism of Microbial Detoxification

In response to survive in the harsh environment including metal toxicity, microorganisms have developed their own unique mechanisms of heavy metal resistance and detoxification. These include electrostatic interaction, ion exchange, precipitation, redox process, and surface complexation.

The anionic groups present on the surface of microorganisms are hydroxyl, alcohol, phosphoryl, amine, carboxyl, ester, sulfydryl, sulfonate, thioether, and thiol groups. These negatively charged functional groups endow the microorganism with the potential to bind metal cation and enable its detoxification. In addition, microorganisms can detoxify metal ions by valence conversion,

volatilization, or extracellular chemical precipitation (Gavrilescu et al., 2004; Dixit et al., 2015)

Conclusion

Environment contamination is one of the major global threats in the current era. Since, industrial effluent is a combination of many organic and inorganic components, so a variety of microorganisms, known as microbial consortia has to be utilized which may efficiently and effectively remediate waste water. Each of the participating microbial strain of microbial can remove specific metal ion, so concurrently many different contaminants may be removed in lesser time in an ecofriendly way. This review provides an overview to reveal the role of microorganisms in remediation of heavy metals. This would also help to identify the suitable microbial species with enhance potential for bioremediation.

References

Adhikari, T.; Manna, M. C.; Singh, M. V. and Wanjari, R.H. (2004). Bioremediation measure to minimize heavy metals accumulation in soils and crops irrigated with city effluent. Food Agri. Environ., 2: 266-270.

Bandowe, B. A. M., Bigalke, M., Boamah, L., Nyarko, E., Saalia, F. K., & Wilcke, W. (2014). Polycyclic aromatic compounds (PAHs and oxygenated PAHs) and trace metals in fish species from Ghana (West Africa): bioaccumulation and health risk assessment. Environ. Int., *65*, 135-146.

Dixit, R., Malaviya, D., Pandiyan, K., Singh, U.B., Sahu, A., Shukla, R., Singh, B.P., Rai, J.P., Sharma, P.K., Lade, H., 2015. Bioremediation of heavy metals from soil and aquatic environment: An overview of principles and criteria of fundamental processes. Sustainability 7, 2189–2212.

Dixit, R., Malaviya, D., Pandiyan, K., Singh, U.B., Sahu, A., Shukla, R., Singh, B.P., Rai, J.P., Sharma, P.K., Lade, H. and Paul, D., 2015. Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. Sustainability, 7(2), pp.2189-2212.

Evans, G.M. and Furlong, J.C. (2003). Environmental Biotechnology Theory and Application. John Wiley & Sons, West Sussex, England.

Garbisu, C. and Alkorta, I. (2003). Review basic concepts on heavy metal soil bioremediation. Eur. J. Min. Process Environ. Protec., 3: 58-66.

Gavrilescu, M. (2004). Removal of heavy metals from the environment by biosorption. Eng. Life Sci., *4*(3), 219-232.

Girma, G. (2015). Microbial bioremediation of some heavy metals in soils: an updated review. Egypt. Aca. J Bio. Sci., G. Micro., *7*(1), 29-45.

He, Z., Hu, Y., Yin, Z., 2016. Microbial diversity of chromium-contaminated soils and characterization of six chromium-removing bacteria. Environ.Manage. 57, 1319–1328.

Huang, J., Li, Y., Cao, Y., Peng, F., Cao, Y., Shao, Q., Liu, H., Guo, Z., 2018. Hexavalent chromium removal over magnetic carbon nanoadsorbents:synergistic effect of fluorine and nitrogen co-doping. J. Mater. Chem. A 6 (13062).

Igiri, B. E., Okoduwa, S. I., Idoko, G. O., Akabuogu, E. P., Adeyi, A. O., & Ejiogu, I. K. (2018). Toxicity and bioremediation of heavy metals contaminated ecosystem from tannery wastewater: a review. J. Toxicology., 2018.

Jalilvand, N., Akhgar, A., Alikhani, H.A., Rahmani, H.A., Rejali, F., 2020. Removal of heavy Metals Zinc, Lead, and Cadmium by Biomineralization of urease-producing Bacteria Isolated from Iranian mine Calcareous soils. J. Soil Sci. Plant Nutr. 20, 206–219.

Kalaimurugan, D., Balamuralikrishnan, B., Durairaj, K., Vasudhevan, P., Shivakumar, M.S., Kaul, T., Chang, S.W., Ravindran, B., Venkatesan, S., 2020.Isolation and characterization of heavy-metal-resistant bacteria and their applications in environmental bioremediation. Int. J. Environ. Sci. Technol.17, 1455–1462.

Kiprono, S.J., Ulla, M.W., Yang, G., 2018. Surface engineering of microbial cells: Strategies and applications. Eng. Sci. 1, 33–45.

Leong, Y.K., Chang, J.-S., 2020. Bioremediation of Heavy Metals using Microalgae: Recent Advances and Mechanisms, Vol. 303. 122886.

Li, S., Yang, P., Liu, X., Zhang, J., Xie, W., Wang, C., Liu, C., Guo, Z., 2019. Graphene oxide based dopamine mussel-like cross-linked polyethylene imine nanocomposite coating with enhanced hexavalent uranium adsorption. J. Mater. Chem. A 7, 16902–16911.

Men, C., Liu, R., Xu, F., 2018. Pollution characteristics, risk assessment, and source apportionment of heavy metals in road dust in Beijing, China. Sci.Total Environ. 612, 138–147.

Mosai, A.K., Chimuka, L., Cukrowska, E.M., Kotzé, I.A., Tutu, H., 2020. Removal of platinum (IV) from aqueous solutions with yeast-functionalised bentonite. Chemosphere 239, 124768.

Panwar, S., 2020. Microbial bioremediation of heavy metals: Emerging trends and recent advances. Res. J. Biotechnol. 15, 164–178.

Rajasulochana, P., Preethy, V., 2016. Comparison on efficiency of various techniques in treatment of waste and sewage water–A comprehensive review. Res. Eff. Technol. 2(4), 175- 184.

Singh R.P., Anwar M.N., Singh D., Bahuguna V., Manchanda G., Yang Y. (2020) Deciphering the Key Factors for Heavy Metal Resistance in Gram-Negative Bacteria. In: Singh R., Manchanda G., Maurya I., Wei Y. (eds) *Microbial Versatility in Varied Environments*. Springer, Singapore.

Song, T., Li, R., Li, N., Gao, Y., 2019. Research progress on the application of nanometer TiO2 photoelectrocatalysis technology in wastewater treatment. Sci. Adv. Mater. 11, 158–165.

Ul-Islam, M., Ali, J., Khan, W., Haider, A., Shah, N., Ahmad, Md.W., Ullah, M.W., Yang, G., 2019. Fast 4-nitrophenol Reduction using Gelatin Hydrogel Containing Silver Nanoparticles. Eng. Sci. 8, 19–24.

Vidali, M. (2001). Bioremediation: An overview. Pure Applied Chemistry, 73:1163-1172.

Wang, X.L., Zhang, Y., 2019. Synthesis and characterization of zeolite A obtained from coal gangue for the adsorption of F−in Wastewater. Sci. Adv.Mater. 11, 277–282.

Wei, H., Wang, H., Li, A., Cui, D., Zhao, Z., Chu, L., Wei, X., Wang, L., Pan, D., Fan, J., Li, Y., Zhang, J., Liu, C., Wei, S., Guo, Z., 2020. Multifunctions of polymer nanocomposites: Environmental remediation, electromagnetic interference shielding, and sensing applications. Chem. Nano. Mat. 6, 174–184.

Yang, Q.1., Li, Z., Lu, X., Duan, Q., Huang, L., Bi, J., 2018. A review of soil heavy metal pollution from industrial and agricultural regions in China: Pollution and risk assessment. Sci. Total Environ. 642, 690–700.

Yuan, Y., Niu, B., Yu, Q., Guo, X., Guo, Z., Wen, J., Liu, T., Zhang, H., Wang, N., 2020. Photoinduced multiple effects to enhance Uranium extraction from natural seawater by black phosphorus nanosheets. Angew. Chem. 132, 1236–1243.

Zhang, S.J., Wang, M.Y., Liu, B.B., Meng, J.S., Li, D.Y., Tong, W., 2019. Preparation of macroporous magnetic particles for the treatment of wastewatercontaining emulsified oil. Sci. Adv. Mater. 11, 1299–1305.

Zhao, S., Yuan, Y., Yu, Q., Niu, B., Liao, J., Guo, Z., Wang, N., 2019b. A dual-surface amidoximated halloysite nanotube for high-efficiency economical uranium extraction from seawater. Angew. Chem. Int. Ed. 58, 14979–14985.

Zhao, Z., An, H., Lin, J., Feng, M., Murugadoss, V., Ding, T., Liu, H., Shao, Q., Mai, X., Wang, N., Gu, H., Angaiah, S., Guo, Z., 2019a. Progress on the photocatalytic reduction removal of chromium contamination. Chem. Rec. 19, 873–882.

BENZOATE DEGRADING MICROORGANISMS IN INDUSTRIAL WASTE

Shweena Krishnani[1], Deepansh Sharma[3], *Deepti Singh[3]

[1]Student, Amity Institute of Microbial Technology, Amity University Rajasthan 303002 INDIA
[2]Research Scholar, Amity Institute of Microbial Technology, Amity University Rajasthan 303002 INDIA
[3]Assistant Professor, Amity Institute of Microbial Technology, Amity University Rajasthan 303002 INDIA

*Corresponding Author: deepti.micro@gmail.com

INTRODUCTION

Aromatic compounds such as benzoate, its salts and other derivatives are widely distributed in nature as a result of anthropogenic activities, their natural occurrence and intermediate/end products of several chemical reactions. Benzoate is a compound widely used as preservatives by industries since 1980s after FDA approves sodium benzoate as 1st chemical food preservative (Jay, 2000) because of its strong antimicrobial activity. Until 1990, benzoic acid was not introduced as food preservative because it was never observed to be produced synthetically in larger quantities (Lueck, 1980). Soon enough sodium benzoate takes over pharmaceutical, cosmetic and beverage industries in order to increase shelf life of products and to prevent its early decay. Sodium benzoate is one of the widely used food preservative around the globe because of its major advantages such as affordability, easy product incomparability, comparatively lesser toxic, colorless and odorless (Davidson et al., 2013).

Due to their toxicity at high concentration, poor disposal of food preservative waste can have severe consequences in animals and plants. Poor disposal systems may result in often detection of such chemicals in Waste Water Treatment Plants (WWTPs) and Effluent

Treatment Plant (ETP) of food, beverage, pharmaceutical and cosmetic industries, responsible for disturbing the biological processes of the treatment plants. Effluent Treatment Plant is a type of treatment plants which is designed particularly for purification of industrial water for reuse and water conservation (Figure 1). The aim behind designing effluent treatment plants is to treat water and release them safely to environment so the resultant harmful consequences of effluents can be reduced. The planning of common effluent treatment plants (CEPTs) is done centralized for easy collection and faster recycling of water in order to provide feasible solution for a small or medium sized industry.

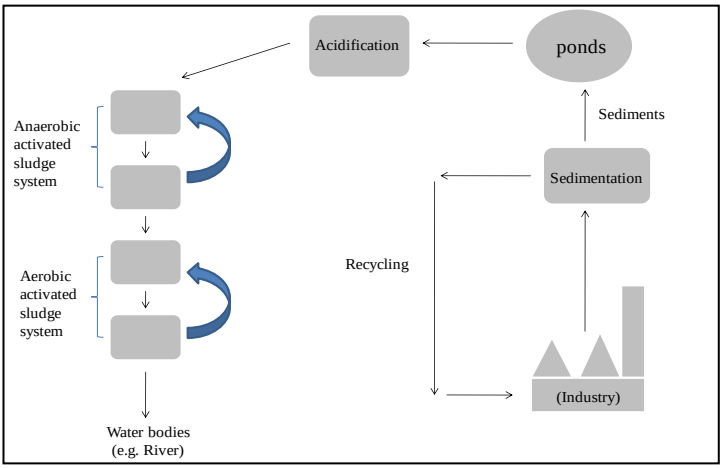


Figure 1. An industrial scale layout of an effluent treatment plant.

Microbes play a crucial role to combat the ETP pollution problems by their ability to utilizing harmful substances, compounds and convert them to lesser toxic substances. This protects the normal flora and fauna to function well in water bodies. These treatment plants are more efficient as compared to other types of treatment plants and maintain the chemical oxygen demand in the outlet by its under rated organics and hydrocarbons. In CEPTs when a biological treatment is performed, microbes oxidize the organic waste present

and form a community for efficient functioning of treatment plant. The system of the treatment plant is essentially open, where an organism is free to undergo oxygenic or an-oxygenic processes that rely on the absence or presence of oxygen and the presence of terminal oxygen acceptors in the microbes. Treatment with microbes is a part of biological processing that depends and varies from organism to organism and or species to species. These microbes adapt to grow together forming a consortia and acts upon the organic waste resulting in release of gases such as carbon dioxide and some lesser toxic end products (Ofiţeru et al. 2010).

However, the high concentration of chemicals, like benzoate, disturbs the native micro flora and inhibits the growth of indigenous susceptible microbes living inside treatment plants, crucial for its functioning. Accumulation of chemicals and preservatives also decrease the biological oxygen demand (BOD) due to which aerobic microbes are unable to survive. These factors enforce growing concern for future reuse of water which has a major application in industries. Disposal of the contaminated water of treatment plants to nearby water bodies are responsible for disturbing agriculture, aquatic, and terrestrial life. Thus, bioremediation of these chemical preservatives becomes necessary. Research focused on the biological methods based on functional and active microbial population from sampling through treatment plants which includes isolation of the organism and its functional applications in bioremediation and bioaccumulation is being practiced since a decade. So according to the most culturable microbe which is abundant in the environment can be isolated and screened according to the hydrocarbon it degrades. Such microbe is assumed to be most adapted to the environment. Thus, selecting the best microbe and checking its metabolic (catabolic) potentials is best fit for the field of bioremediation. Such non-indigenous microbes make it difficult for the survival of normal microflora that functions for treatment plants and hence disturbing the functioning and regulation of the treatment plants. The isolation of abundant microbe that is most culturable becomes crucial for bioremediation.

This chapter communicates such isolated microbes that can potentially degrade benzoate and its derivatives, their characteristics, molecular features, their functioning as consortia (community), future prospects and their potential functioning in bioremediation of such micro pollutants.

Benzoate as preservative, its continuous emerging market and impact on public health

Preservatives are a type of food additives that inhibit or kill the microbial growth and increase the shelf life of the product. Sodium benzoate was the first chemical to be approved as preservative by USFDA (Jay, 2000), due to its strong antimicrobial actions. Its antimicrobial actions were firstly observed in year 1875 due to its association between benzoic acid and phenol (Lueck, 1980) and its applications in increasing shelf life of products by inhibiting microbial growth in packaged food (Vogel, 1992; Giese, 1994), such as meat products (Gerhardt, 1995), beverage products (Giese, 1995) and the customers demand of such products and their reactions for the use of food preservatives (Jager, 1994). Sodium benzoate is one of the widely used food preservative around the globe because of its major advantages such as affordability, easy product incomparability, comparatively lesser toxic, colorless and odorless (Davidson et al., 2013). Benzoic acid was seen inhibiting the growth of *Saccharomyces bayanus* and *Pseudomonas fluorescens* extracted from cranberries (Marwan and Nagel, 1986).

Besides food industry, sodium benzoate is a widely accepted preservative in cosmetic, beverage and pharmaceuticals. This chemical when added to the product avoids prior decay of the product due to fluctuating temperature, pH and humidity by avoiding any bacterial or fungal growth that might affect the quality of the product. Low level of sodium benzoate is synthesized in apples, berries etc, in some species such as cloves cinnamon etc. When added to foods as a chemical preservative, about 75% of people can taste it. Since it is a sodium salt, it tastes salty, bitter, or sour for most people; but to others it may taste sweet. Permitted amount is 0.1% benzoate approved by FDA in foods (Jay, 2000).

Soft drinks are the number one source of sodium benzoate in the diet. On its own, it is not considered toxic, and studies show no adverse health effects in humans under normal conditions.

The demand of this chemical in industrial use is climbing by passing time. Effects of globalization and urbanization are positively impacting the economy. As per growing demands of consumer markets, the global brands face everyday target demands and work accordingly to meet consumer demand such as more shelf life of product. The financially empowered millennial population is developing new scopes for Sodium benzoate in the Asia Pacific market. Developing countries as India are reported to getting into new trends and change in their food habits, besides being number one in agricultural commodities, the country is growing demanding package food. Recent studies revealed that more than 60 percent of the population of India prefers to try packed food which is a favorable condition for the growth of sodium benzoate market to support their industries. The regions currently becoming potential hub of benzoate market are North America (U.S and Canada), Latin America (Brazil), Western Europe (Italy, Spain, U.K, Germany, and France), Asia Pacific Regions (China, India, Australia and New Zealand), Eastern Europe (Russia) and Middle East and Africa (S. Africa, N. Africa, GCC).

Although, in some industries, sodium benzoate is not only marked as a dangerous preservative but also has a much-reduced use of it in some regions. Some research suggests that overuse of this chemical increases the risk of cancer by affecting the oxygen uptake process by cells of human body. It becomes lethal after the permissible concentration in our body besides its natural presence in some fruits. Vinegar, juices, fruits, jams, soda etc., are the products of daily diet already possess some concentration of sodium benzoate. As the demand for the packed food that can be stored for several days increases, use of these chemicals also increases. The fast lifestyle and consumerism promote the growth of these industries which is aided by harmful preservatives such as sodium benzoate. The cosmetic consumer protection laws are facile. Thus, the opportunities for the preservative's makers are highest in this market

and are continuously growing since last few decades in many regions across the globe to promote packaged food.

Ecological importance of benzoate degraders

A daily residual discharge of sodium benzoate from cosmetic and food industry into water bodies such as ponds and rivers impose high risk to natural biodiversity of the aquatic ecosystem, that can somehow affect the terrestrial ecosystem too through plants to animals and from plants and animals to humans.

Demonstration of a food chain of an aquatic ecosystem as: the phytoplankton as the primary producers are consumed by zooplanktons (secondary producers and primary consumers) is then consumed by small fishes and small fishes are eaten up by large fishes. The fishes are also consumed by terrestrial carnivores. Energy flow occurs at the rate of 10% and at every tropic level remaining/extra energy is lost in the form of heat. All the tropic levels are available for decomposers to decompose, uptake of nutrients and energy transfer. Therefore, micro pollutants travel from aquatic ecosystem to terrestrial and vice versa (Figure 2).

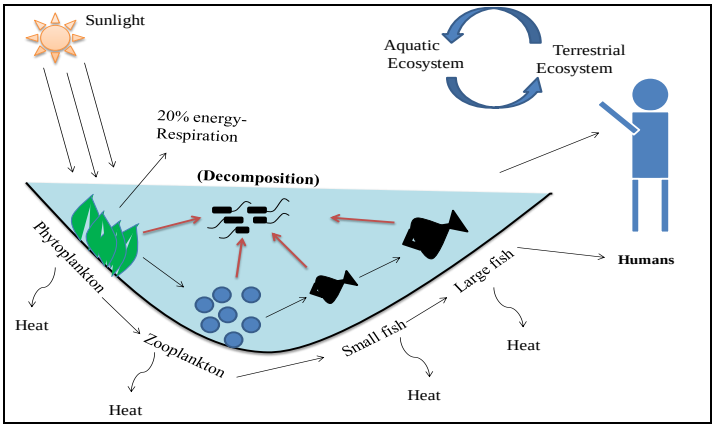


Figure 2. Aquatic food chain and its relationship with terrestrial food chain

Thus, due to high industrialization and mineralization, sodium benzoate is transferred to organism of every tropic level and gets transferred to humans and cause different type of diseases such as cancer and non-immunological pseudo-allergies. These conditions are deficient in healthy humans. An intake of 5 mg/kg of body weight every day is tolerable and permissible according to world health organization (Wibbertmann et al., 2000).

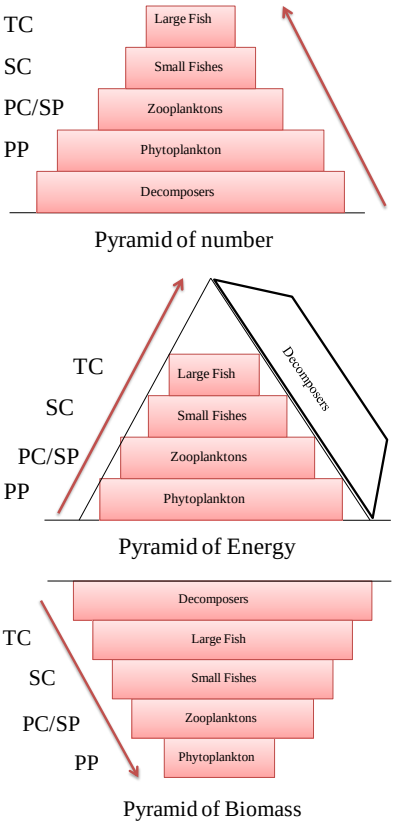


Figure-3. Ecological pyramids of number, biomass, and energy of an aquatic ecosystem

Ecological pyramids of an aquatic ecosystem

Nature, positioning and categorizing of decomposers (microbes) can be done using different types of ecological pyramids. Decomposers were placed at different positions within all three types of ecological pyramid of aquatic system i.e., pyramid of number, pyramid of energy and pyramid of biomass (Figure 3).

Pyramid of number is upright in aquatic ecosystem with the maximum number of decomposers in the ecosystem. Pyramid of biomass is based on total dry weight of organism at a tropic level where the pyramid is seen inverted and decomposers at top position because due to maximum number the overall biomass will be maximum for decomposers. Pyramid of energy is always upright, since decomposers can act at any tropic level for nutrients, energy and decomposition purposes, decomposers are placed which covers all tropic levels. Positioning of decomposers can be differing as per ecosystems but, its position within pyramid of energy of every ecosystem is always constant.

Isolation and techniques for detection of benzoate degraders from polluted sites and its characteristics

Mineralization of sodium benzoate by anaerobes present in sludge and treatment plants ranges from 50-96.5% up to 50-90 mg/L concentration in between 28-61 days (Birch et al., 1989). Whereas in other study 93% mineralization with concentration 50 mg/L was observed after a week of incubation in a sample of industrial and domestic wastewater collected from sludge (Battersby and Wilson, 1989). A concentration of up to 3000 mg/L of benzoate was observed to be completely degraded by microbes between 5 to 7 days period (Kobayashi et al., 1988).

The microorganisms reported to utilize and henceforth degrade benzoic acid can imply either oxygenic or an-oxygenic pathways for e.g. *Rhodotorula glutinis*, a fungi (Kocwa-Haluch and Lemek, 1995), *Penicillium frequentan*, mold (Hofrichter and Fritsche, 1996), and amongst bacteria are *Alcaligenes denitrificans* (Miguez et al., 1995), *Rhodopseudomonas palustris*, strains of denitrifying

pseudomonads (Fuchs et al., 1994; Elder and Kelly, 1994; Harwood and Gibson, 1997), and *Desulfomicrobium escambiense* (Genthner et al., 1997).

Different sludge samples, municipal wastewater treatment plant samples, soil (from gasoline station), sediment samples (freshwater and estuarine sediments) and methanogenic environment were analyzed by different researchers for isolation of benzoate degraders (Table 1). The anaerobic benzoate degraders were isolated using various techniques such as agar roll tube technique using basal media, enrichment culture techniques using minimal salt medium (MSM), agar shake dilution method and Hungate technique (Hungate, 1950). To determine the concentration and to differentiate between initial and final/threshold concentration, high pressure liquid chromatography, gas chromatography and photometry can be used.

Characteristics of benzoate degraders

The morphological, cellular and chemical characteristics about benzoate degrading bacteria such as final electron donor/acceptors, sole carbon compounds, shape of the cell, nature of the cell and the type of stain they take have been studied in detail by researchers. Electron donor/acceptor depends on the compounds utilized by the degraders. In most of the cases sulphate or nitrate acts as e^- acceptor/donor.

Sole carbon utilization

Various compounds such as crotonate, halo benzoates (2-fluorobenzoate, 3-fluorobenzoate, 4-fluorobenzoate, 2-chlorobenzoate, 3-chlorobenzoate, 4-chlorobenzoate, 2-bromobenzoate, 3-bromobenzoate, or 4-bromobenzoate), sodium benzoate, benzoic acid and non-benzoate compounds such as sodium acetate are used as sole carbon sources by benzoate degraders. These compounds are the primary source of energy and carbon. Sometimes benzoate itself can be used as sole carbon source. Various degraders can/cannot utilize these compounds according to their metabolism, electron acceptor/donors and

enzymes. In case of halobenzoate degradation, anaerobic utilization of halobenzoate depends on or coupled to denitrification, and denitrifying consortia with halobenzoate as the sole carbon source (Song et. al., 2000).

Table 1. List of benzoate degrading isolates their degradation concentrations and identification

Isolate name	Cocultured with	Degradation/ threshold concentration	References
Therminicola	Consortia	30 mM	Aromokeye et al., 2020
Dethiobacter	Consortia	30 mM	Aromokeye et al., 2020
Melioribacter	Consortia	30 mM	Aromokeye et al., 2020
Delta proteobacteria bacterium SG8_13	Consortia	30 mM	Aromokeye et al., 2020
Pseudomonas pseudoalcaligens YKJ	-	-	Safari et al., 2019
Sulfuritalea hydrogenivorans sk43H	-	25 mM	Sperfeld et al., 2019
Rhodopseudomonas palustris	-	2.0 mM	VanDrisse and Escalante-Semerena, 2018
Halopenitus	Consortia	2.5 mM	Dalvi et al., 2016
Ferroglobus placidus	-	1 mM	Holmes et al., 2012
Halomonas campisalis	-	380 mg/L	Oie et al., 2007
Sporotomaculum hydroxybenzoicum	*Methanospirillum hungatei*	2.5 mM	Qiu et al., 2003
Pseudomonas oleovorans	Consortia activity	100 mM	Song et. al., 2000
Azoarcus tolulyticus	Consortia activity	100 mM	Song et. al.,

			2000
Bradyrhizobium elkanii	Consortia activity	100 mM	Song et. al., 2000
Acidovorax avenae	Consortia activity	100 mM	Song et. al., 2000
Ensifer adhaerens	Consortia activity	100 mM	Song et. al., 2000
Pseudomonas stutzeri	Consortia activity	100 mM	Song et. al., 2000
Ochrobactrum anthropi	Consortia activity	100 mM	Song et. al., 2000
Thauera aromatic	Consortia activity	100 mM	Song et. al., 2000
Paracoccus denitrificans	Consortia activity	100 mM	Song et. al., 2000
Mesorhizobium loti	Consortia activity	100 mM	Song et. al., 2000
Thauera aromatic	Consortia activity	100 mM	Song et. al., 2000
Desulfotomaculum thermobenzoicum	-	10 mM	Tasaki et al., 1991
Desulfotomaculum sapomandens	-	8 mM	Cord-Ruwisc and Garcia, 1985
Desulfovibrio strain G-11	*Methanospirillum hungatei* strain JF-1	13.8 mM	Mountfort and Bryant, 1982
Methanobacterium formicicum	Consortia activity	0.3 µmoles	Ferry and Wolfe, 1976
Methanospirillum hungatii	Consortia activity	0.5 µmoles	Ferry and Wolfe, 1976

Mechanism of benzoate degradation

Absence of the terminal electron acceptor or hydrogen utilizing partners favors an-oxygenic pathways. In order to degrade Benzoate, anaerobes imply Reduction pathway (Dutton and Evans, 1969) and will undergo fermentation due to the absence of oxygen such that the Benzoate is converted into acetate, Carbon-dioxide and

Cyclohexane carboxyl-ate resulting in a thermodynamically stable reaction which in turn favors free energy (more positive energy) for substrate utilization (Elshahed and Mcinerney, 2001). About-face is the oxygenic route that follows Ortho-cleavage pathway where catechol and protocatechuate are the initial transitional compounds. Catechol and procatechuate are the substrates of an enzyme dioxygenases that directly attacks the aromatic ring between hydroxyl groups. This leads to the formation of 3-ketoadipate leading it to conversion of the final products i.e., acetyl-CoA and succinyl-CoA (Valderramaet al., 2012).

Benzoate degradation can be studied in two major ways:

(1) An-oxygenic bacterial degradation of benzoate
(2) Oxygenic pathways of bacterial degradation of benzoate

- **An-oxygenic pathways**

The metabolism of benzoate can be achieved by reduction pathway by anaerobes in the presence of sunlight by the reduction to cyclohex-1ene-1carboxylate followed by the hydration to 2-hydroxycyclohexanecarboxylate and dehydrogenation to 2-oxocyclohexanecarboxylate. Further hydration can result in ring-fission and the production of pimelate occurs (Dutton and Evans, 1969). The most common intermediate formed in aromatic metabolism of aromatic compounds is either benzoate or benzyl-CoA. Aromatic ring is reduced to ring cleavage if there is no molecular oxygen is present. Benzyl-CoA is activated by benzoate. De-aromatization of the benzene ring occurs upon a 2-electon reduction of benzoyl-CoA to cyclohex-diene-1-carboxyl-CoA. Two metabolic pathways are observed for oxidation of cyclohex-diene-1-carboxyl-CoA to 3-hydroxypimelyl-CoA; one which involves cyclohex-1-ene-1-carboxyl-CoA as intermediate and another which involves 6-hydroxy-cyclohex-1-ene-1-carboxyl-CoA (Harwood and Gibson, 1998). The left side of the reactions from 6-Hydroxycyclohex-1-ene-1-carboxyl-CoA to 3-Hydroxypimelyl-CoA is proposed and has not been identified experimentally. The Pimelyl-CoA degradation from Pimelyl-CoA to Acetyl-CoA is also

proposed and most of the enzymes of this sequence do not appear to have been directly assayed (Figure 4). Phototrophic bacterium e.g., *Rhodopseudomonas palustris*, *Thauera aromatic* K172 and *Azoarcus evansii* are known for an-oxygenic degradation.

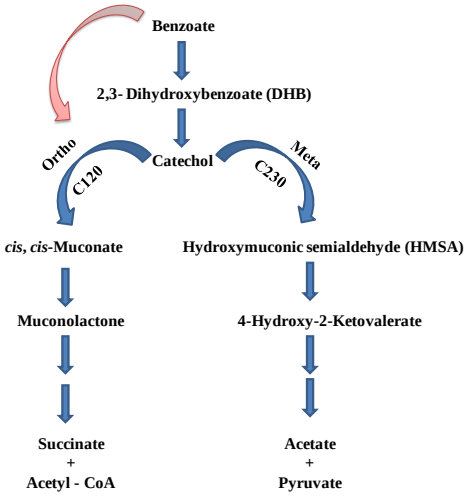


Figure-4. An-aerobic benzoate degradation pathway

- **Oxygenic pathways**

The biodegradation of aromatic compounds such as benzoate leads to the formation of the most commonly known intermediates that are; catechol, protocatechuic acid and gentisic acid (Figure 5). These intermediates further converted into pyruvic acid, succinic acid and acetyl-CoA implying ring fission mechanism to enter Krebs cycle. Of the three dihydroxy aromatic intermediates, the most frequently encountered metabolite before ring cleavage is catechol. The formation of catechol proceeds via the incorporation of molecular oxygen into its aromatic precursor, and the primary substrates that can be funnelled into catechol range from single ring benzene to three-ring phenanthrene. Once catechol is formed, it can be

degraded through either the meta or the ortho (also commonly known as β-ketoadipate) ring cleavage pathway by enzymes catechol 2, 3-dioxygenase and catechol 1, 2-dioxygenase, yielding 2-hydroxymuconic semialdehyde (HMSA) and cis, cis-muconate, respectively.

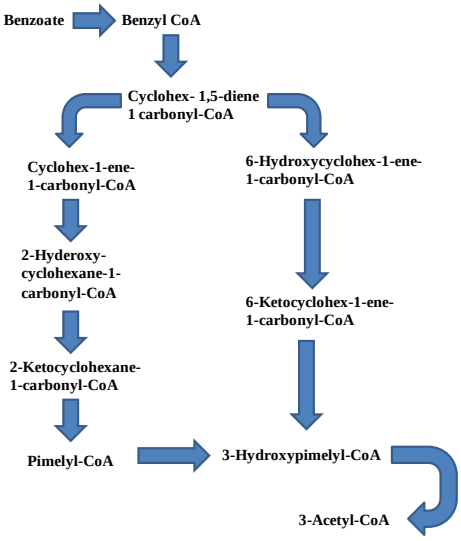


Figure-5. Aerobic benzoate degradation pathway

Conclusion

Organisms degrading benzoate are a crucial part of biological treatment of preservative pollution at various polluted sites. But a single organism is not enough to degrade a huge amount of SB from water bodies. Microbial consortia (group of bacteria living together having same functions, compete and adapt) function better than a single organism. That might also increase rate of degradation within same period of time as compared to single isolate which is ecologically very important. The methods to combat micro pollutants from such sites, finding a biological method to eradicate such highly toxic chemical preservatives from water bodies and

from treatment plants through microbes should be developed. Isolation, characterization and identification of an isolate is the primary step, modifying the isolate for an industrial use by innovating a technique that can be easy, affordable and a smarter way to use in an industrial scale. Further understanding the properties of isolates in sole-carbon utilization, adaptation to harsh conditions, electron acceptor/donors and spore formation can have an important role in industrial microbiology.

References

Aromokeye DA, Oni OE, Tebben J, Yin X, Richter-Heitmann T, Wendt J, Nimzyk R, Littmann S, Tienken D, Kulkarni AC, Henkel S. Crystalline iron oxides stimulate methanogenic benzoate degradation in marine sediment-derived enrichment cultures. The ISME Journal. 2020 Nov 5:1-6.

Battersby, N. S., & Wilson, V. (1989). Survey of the anaerobic biodegradation potential of organic chemicals in digesting sludge. *Appl. Environ. Microbiol, 55*(2), 433-439.

Birch, R. R., Biver, C., Campagna, R., Gledhill, W. E., Pagga, U., Steber, J., ... & Bontinck, W. J. (1989). Screening of chemicals for anaerobic biodegradability. *Chemosphere*, *19*(10-11), 1527-1550.

Dalvi S, Youssef NH, Fathepure BZ. Microbial community structure analysis of a benzoate-degrading halophilic archaeal enrichment. Extremophiles. 2016 May 1;20(3):311-21.

Davidson, P. M., Taylor, T. M., & Schmidt, S. E. (2013). Chemical preservatives and natural antimicrobial compounds. In *Food microbiology* (pp. 765-801). American Society of Microbiology.

Dutton, P. L., & Evans, W. C. (1969). The metabolism of aromatic compounds by *Rhodopseudomonas palustris*. A new, reductive, method of aromatic ring metabolism. *Biochemical Journal*, *113*(3), 525-536.

Elder, D. J., & Kelly, D. J. (1994). The bacterial degradation of benzoic acid and benzenoid compounds under anaerobic conditions: unifying trends and new perspectives. *FEMS microbiology reviews*, *13*(4), 441-468.

Elshahed, M. S., & McInerney, M. J. (2001). Benzoate Fermentation by the Anaerobic Bacterium *Syntrophus aciditrophicus* in the Absence of Hydrogen-Using Microorganisms. *Appl. Environ. Microbiol*, *67*(12), 5520-5525.

Fuchs, G., Mohamed, M. E. S., Altenschmidt, U., Koch, J., Lack, A., Brackmann, R.,& Oswald, B. (1994). Biochemistry of anaerobic biodegradation of aromatic compounds. In *Biochemistry of microbial degradation* (pp. 513-553). Springer, Dordrecht.

Genthner, B. R., Townsend, G. T., & Blattmann, B. O. (1997). Reduction of 3-chlorobenzoate, 3-bromobenzoate, and benzoate to corresponding alcohols by *Desulfomicrobium escambiense*, isolated from a 3-chlorobenzoate-dechlorinating coculture. *Appl. Environ. Microbiol.*, *63*(12), 4698-4703.

Gerhardt, U. (1995). Preserving agents in meat products-a real necessity? .*FLEISCHEREI*, *46*, VI-VI.

Giese, J. (1994). Antimicrobials: assuring food safety. *Food technology (Chicago)*, *48*(6), 102-110.

Giese, J. (1995). Developments in beverage additives. *Food Technology*.

Harwood, C. S., & Gibson, J. (1997). Shedding light on anaerobic benzene ring degradation: a process unique to prokaryotes? .*Journal of bacteriology*, *179*(2), 301.

Hofrichter, M., & Fritsche, W. (1996). Abbauaromatischer Kohlenwasserst offedurch den Schimmel pilz *Penicillium frequentans* Bi 7/2. *Gas-und Wasserfach. Wasser, Abwasser*, *137*(4), 199-204.

Holmes DE, Risso C, Smith JA, Lovley DR. Genome-scale analysis of anaerobic benzoate and phenol metabolism in the hyperthermophilic archaeon *Ferroglobus placidus*. The ISME journal. 2012 Jan;6(1):146-57.

Jager, M. (1994). Are preservatives still necessary. *Food Market. Technol*, *8*(4).

Jay, J. M. (2000). Modern Food Microbiology 6th Edition. Gaithersburg, Maryland (US).

Kobayashi, T. T. E. T., Hashinaga, T., Mikami, E., & Suzuki, T. (1988). Methanogenic degradation of phenol and benzoate in acclimated sludges. In *Water Pollution Research and Control Brighton* (pp. 55-65). Pergamon.

Kocwa-Haluch, R., & Lemek, M. (1995). Easy and inexpensive diffusion tests for detecting the assimilation of aromatic compounds by yeast-like fungi. Part II. Assimilation of aromatic acids. *Chemosphere*, *31*(11-12), 4333-4339.

Lueck, E. (1980). Antimicrobial food additives: Characteristics, uses, and effects Springer-Verlag. *Berlin, Heidelberg, Germany*.

Marwan, A. G., & Nagel, C. W. (1986). Microbial inhibitors of cranberries. *Journal of food science*, *51*(4), 1009-1013.

Miguez, C. B., Greer, C. W., Ingram, J. M., & MacLeod, R. A. (1995). Uptake of Benzoic Acid and Chloro-Substituted Benzoic Acids by *Alcaligenes denitrificans* BRI 3010 and BRI 6011. *Appl. Environ. Microbiol.*, *61*(12), 4152-4159.

Mountfort, D. O., and M. P. Bryant. 1982. Isolation and characterization of an anaerobic syntrophic benzoate-degrading bacterium from sewage sludge. Arch. Microbiol. 133:249-256.

Ofiţeru, I. D., Lunn, M., Curtis, T. P., Wells, G. F., Criddle, C. S., Francis, C. A., & Sloan, W. T. (2010). Combined niche and neutral effects in a microbial wastewater treatment

community. *Proceedings of the National Academy of Sciences*, *107*(35), 15345-15350.

Oie CS, Albaugh CE, Peyton BM. Benzoate and salicylate degradation by Halomonas campisalis, an alkaliphilic and moderately halophilic microorganism. Water research. 2007 Mar 1;41(6):1235-42.

Safari M, Yakhchali B. Comprehensive genomic analysis of an indigenous *Pseudomonas pseudoalcaligenes* degrading phenolic compounds. Scientific reports. 2019 Sep 4;9(1):1-4.

Song, B., Palleroni, N. J., & Häggblom, M. M. (2000). Isolation and characterization of diverse halobenzoate-degrading denitrifying bacteria from soils and sediments. *Appl. Environ. Microbiol.*, *66*(8), 3446-3453.

Sperfeld M, Diekert G, Studenik S. Anaerobic aromatic compound degradation in *Sulfuritalea hydrogenivorans* sk43H. FEMS microbiology ecology. 2019 Jan;95(1):fiy199.

Valderrama, J. A., Durante-Rodríguez, G., Blázquez, B., García, J. L., Carmona, M., & Díaz, E. (2012). Bacterial Degradation of Benzoate cross-regulation between aerobic and anaerobic pathways. *Journal of Biological Chemistry*, *287*(13), 10494-10508.

VanDrisse CM, Escalante-Semerena JC. Small-molecule acetylation controls the degradation of benzoate and photosynthesis in *Rhodopseudomonas palustris*. MBio. 2018 Nov 7;9(5).

Vogel, R. (1992). Nutritional and practical significance of food additives. *Food Market. Technol*, *6*(6).

Wibbertmann, A., Kielhorn, J., Koennecker, G., Mangelsdorf, I., & Melber, C. (2000). Concise International Chemical Assessment Document 26. Benzoic acid and sodium benzoate. *World Health Organisation Geneva*, *26*, 1-48.

DYE DEGRADATION USING MICROBES

Vigi Chaudhary[1] and Shweta Kulshreshtha[*2]

[1]Assistant Professor, Amity Institute of Biotechnology, Amity University Rajasthan
[2]Associate Professor, Amity Institute of Biotechnology, Amity University Rajasthan
[*]Corresponding Author: skulshrestha@jpr.amity.edu

Introduction

Dyes are substances which provide color to a substrate (Mansoor and Sharma, 2020). Perkin discovered the first synthetic dye, Mauvine, in 1856. Dyes are widely used in textile, pharmaceutical, food, cosmetic and paper industries. According to chemical structure of the chromophore, dyes are classified as azo dyes, anthraquinone dyes and phthalocyanine dyes. (Hunger, 2003; Sudha, et al., 2014) Dyes may also be classified on the basis of use or application method as reactive, disperse, acid, basic (cationic), direct, vat, sulphur, solvent, metal complex and mordant dyes. The complex structure of various dyes is shown in figure 1. Textile industry manufactures more than 10,000 dyes out of which 70% are azo dyes. (Hassaan and Nemr, 2017). The release of industrial effluents containing dyes causes environmental pollution and has become a serious concern as it adversely affects human health (Varjani, 2020; Mansoor and Sharma, 2020).

Adverse Effects of Dyes on Human Health and Environment

One of the major human health hazard imposed by the dyes are respiratory illnesses. Inhalation of dye particles may trigger the immune system to produce hypersensitivity reactions. Symptoms of respiratory sensitization may include watery eyes, sneezing, coughing and wheezing. Dyes may also irritate skin (Hassaan and Nemr, 2017). The long term effect of textile dyes is its potential for

genotoxicity. Dyes may inhibit plant growth, enter food chain and cause bioaccumulation leading to toxicity, mutagenicity and carcinogenicity (Lellis et al., 2019). Human bladder carcinoma and hepatocellular carcinoma have been attributed to some azo dyes (Saini, 2018).

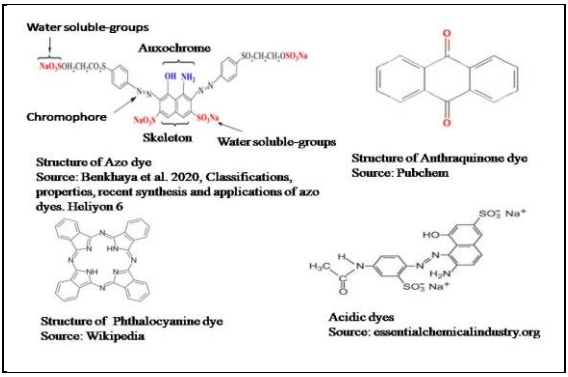


Figure 1. Structure of different dyes.

Environmental impacts of dyes include alteration of physical and chemical properties of soil as well as water causing harm to the flora and fauna. The agricultural productivity of the soil is also affected as these toxic chemicals kill soil microorganisms. Depending on the exposure time and concentration many azo dyes can produce acute or chronic effects on living beings and can result in mutations (Hassaan and Nemr, 2017). Textile industry effluents typically carry many azo dyes and have relatively high levels of chemical oxygen demand (COD) and biochemical oxygen demand (BOD). Textile dyes prevent penetration of light in water bodies and also cause aesthetic damage. Diminished availability of light results in reduced photosynthetic activity which in turn decreases the dissolved oxygen levels. This affects the entire aquatic life (Mansoor and Sharma, 2020). The highly toxic and potentially carcinogenic nature of the dyes and their degradation products causes various diseases in animals. Bioaccumulation and xenobiotic nature of dyes can

negatively impact the structure and function of ecosystems (Lellis, et al. 2019).

Degradation of Dyes

Owing to the hazardous nature of dyes posing serious threat to human health and environment, the industrial effluents containing dyes should be treated to remove or neutralize the harmful effects of dyes before its disposal to the environment (Saini, 2018). Dyes can be removed or degraded by physical, chemical and biological/microbial processes.

Physical Methods of Dye Removal

Adsorption, irradiation, and membrane filtration processes are the commonly employs physical methods for dye removal. Adsorption is one of the most effective, economical and established treatment methods for dye removal. Soluble organic dyes from industrial waste water are transferred to solid and highly porous surface of the adsorbent, eg. activated carbon. When the maximum adsorption capacity of the adsorbent has been reached, then the spent adsorbent is replaced by fresh ones. The spent adsorbents may be incinerated or regenerated. Attraction between dye and adsorbent, surface area and molecular size of adsorbent, pH, temperature and time duration of contact are the factors which affect the adsorption process. The radiations from a monochromatic UV lamp, is used for irradiation method of dye removal. A constant and adequate supply of oxygen is required for breaking down of organic dyes by irradiation. Titanium dioxide catalyst can increase the efficiency of irradiation treatment (Saini, 2018).

The common membrane filtration methods for dye removal include micro-filtration, ultra-filtration, nano-filtration and reverse osmosis. (Table 1) The nature of the industrial effluent determines the choice of the membrane filtration process. Expensive membranes, high maintenance costs and huge sludge production after the membrane separation process are the major difficulties associated with membrane filtration processes. These disadvantages make it more

suitable for industries which generate only small volumes of effluents (Singh and Singh, 2016: Saini, 2018).

Table 1. Membrane Filtration Processes

S.No.	Membrane filtration Type	Membrane material	Membrane Efficiency
1	Micro-filtration	Poly (Ether Sulfone), Poly Tetrafluoroethylene (PTFE), Poly (Vinylidiene Fluoride), Poly (Vinylidene Difluoride), Poly (Sulfone), Polypropylene, Polycarbonate, etc.	Removes 90% turbidity and may be used as pretreatment for nano-filtration or reverse osmosis
2	Ultra-filtration	Nylon-6, polytetrafluoroethylene (PTFE), polyvinyl chlorides (PVC), polysulfone, polypropylene, acrylic copolymer , etc.	Removes only 31-76% dyes and may be used as pretreatment for reverse osmosis
3	Nano-filtration	Cellulose acetate and aromatic polyamides, ceramics, zirconia , polyethersulphonate, etc.	Removes about 70% dyes and extremely sensitive to fouling by colloidal material and macromolecules
4	Reverse osmosis	Cellulose acetate and aromatic polyamides and of some inorganic materials.	Removes hydrolysed reactive dyes, most types of ionic compounds, chemical auxiliaries in a single step

Chemical Methods of Dye Removal

Oxidation, coagulation and precipitation are the chemical methods of dye degradation and removal. Oxidizing agents such as Fenton reagent, Ozone, Chlorine, Hydrogen peroxide etc, may be used to chemically oxidize the dyes to less harmful and biodegradable

organic compounds. Photochemical oxidation of dyes by the treatment of UV in the presence of H_2O_2 may even result in complete degradation of dyes to CO_2 and H_2O. The production of hydroxyl radicals initiates the decay of dye molecule (Sudha, 2014). Coagulation using Aluminum, Iron salts, Flocculants, organic polymers etc. helps in effective precipitation of otherwise colloidal dyes. Chemicals such as lime, ferrous sulfate, ferric sulfate, ferric chloride, aluminum sulfate, aluminum chloride, cationic organic polymers, etc neutralize the charge of small suspended particles and emulsions. This encourage the particles to come together and form clusters large enough to settle (coagulate) under gravity or become filterable. Coagulation coupled with electrolytic reaction at the electrodes result in electrocoagulation. The efficiency of this electrochemical reaction to remove dyes is depended on current intensity and the duration of the reaction (Saini, 2018). High chemical requirement and safe dumping of the large amounts of sludge are the disadvantages of chemical processes (Varjani, 2020).

Biological/Microbial Methods of Dye Removal

Biological degradation is considered as the most effective method of dye degradation. Physicochemical methods usually reduce the toxicity of the dyes rather than complete degradation of the chemical. Microbial degradation of the dyes is cost effective, eco-friendly and usually results in complete mineralization of organic dyes. For instance the azo dyes are reduced by microorganisms by secreting enzymes such as azoreductase, peroxidase, laccase and hydrogenase. Microbes further mineralize the reduced dyes for utilization as energy sources. Combinations of aerobic and anaerobic methods are often involved in the biological treatment of dyes (Sudha, et al., 2014). Biological dye degradation has been achieved using bacteria, fungi, yeast, actinomycetes, algae and certain plants (phytoremediation), of which bacterial, fungal and yeast degradation will be discussed further (Ajaz, et al., 2019).

Degradation of dyes by bacteria

Bacterial degradation of azo dyes are achieved by azo reductases which involves reductive cleavage of azo bonds (-N=N-) under anaerobic conditions. Further degradation of the intermediates like aromatic amines can be done both aerobically and anaerobically (Saratale, et al. 2011; Shah, 2014). Azo dye degraders include both aerobic and anaerobic bacteria such as *Bacilus subtilis, Bacillus stratosphericus, Pseudomonas* sp, *Escherichia coli, Rhabdobacter* sp, *Enterococcus* sp, *Enterobacter* sp, *Staphylococcus* sp, *Xenophilus* sp, *Corneybaterium* sp, *Clostridium* sp., *Micrococcus dermacoccus* sp, *Acinetobacter* sp, *Geobacillus, Lactobacillus, Rhizobium, Proteus* sp, *Morganella* sp, *Aeromonas* sp, *Alcaligenes ap*, and K*lebsiellla* sp. (Sudha, et al., 2014, Roy, et al., 2018, Akansha, et al., 2019, Bibi, et al, 2020). Bacterial degradation and decoloration is faster than fungal degradation. It has also been observed that mixed cultures are more efficient in biogradation of dyes as compared to pure cultures of bacteria. Collectively, microbial consortia can carry out a myriad of biodegradation tasks which will be impossible for individual pure cultures. However, reproducibility and interpretation of the results will be difficult as mixed cultures only provide an average macroscopic view of what is happening in the system (Saratale, et al., 2011; Joshi, et al., 2015).

Degradation of dyes by fungi

Filamentous fungi inhabit ecological niches such as soil, organic waste material and plants. Colored metallic effluents may be effectively degraded by fungal systems. Non-specific lignolytic enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP) and laccases are involved in degradation of colored substances. Bioprocesses for the mineralization of azo dyes have focused on fungal cultures from white rot fungi. *Phanerochaete chrysosporium, Neurospora crassa, Coriolus versicolar, Trametes versicolar, Trametes hirsute, Bjerkandera adusta, Fungalia trogii, Penicillium geastrivous, Rhizopus oryzar, Pleurotus ostreatus, Pleurotus sapidus, Pleurotus florida, Rigidoporus lignosus, Pycnoporus sanguineus, Aspergillus flavus*, and *Aspergillus niger* have been reported which are capable of degrading azo dyes

(Saratale, et al. 2011; Sen, et al., 2016; Ajaz, et al. 2019; Jamee and Siddique, 2019)

Degradation of dyes by yeast

Bioremediation of dyes by yeast focused on bio-sorption followed by enzymatic degradation. Azo dyes were degraded by *Candida tropicalis, C. zeylanoides Debaryomyces polymorphus, Issatchenkia occidentalis, S. cerevisiae MTCC-463, Galactomyces geotrichum MTCC 1360, Trichosporon beigelii NCIM-3326, Candida oleophil, Cyberlindnera samutprakarnensis* and *Sterigmatomyces halophilus SSA-1575.* (Bahia, et al., 2018, Ajaz, et al., 2019, Tohamy, et al., 2020)

Genetic Engineering Approaches for dye degradation

Each microorganism possesses a different set of enzymes and therefore has different capability of dye degradation, detoxification and decolorization. Significant revolution in the field of bioremediation has been made by genetic engineering which enables scientists to produce genetically modified organisms (GMOs) by transferring gene from one species to another species or by gene modification. Functional genes of various bacterial strains such as *Sphingomonas desiccabilis, Escherichia coli, Bacillus idriensis, Pseudomonas putida, Mycobacterium marinum, Ralstonia eutropha*, etc. has been transferred to other species for production of GMOs (Saxena et al., 2019; Varjani et al., 2020).

Conclusion

The release of industrial effluents containing synthetic dyes into the environment without proper treatment results in harmful impacts on soil and water. Bioremediation is a successful green technology option to tackle the menace of recalcitrant dyes. Pure culture or microbial consortia can be used for successful dye degradation. To enhance the process efficiency genetically engineered microorganisms may be utilized.

References

Ajaz, M., Shakeel, S., Rehman, A., 2019. Micobial use for azo dye degradation-a strategy for dye bioremediation. Int. Microbiol. 23(2) 149-159.

Akansha, K., Chakraborty D., Sachan, S.G., 2019. Decolorization and degradation of methyl orange by *Bacillus stratosphericus* SCA1007. Biocatalysis and Agricultural Biotechnology 18, 101044.

An Environmentally and Economically Sustainable Approach. European Journal of Microbiology and Immunology 9(4), 114–118. DOI: 10.1556/1886.2019.00018.

Bahia, M., Passos,.F., Adarme, O.F.H., Aquino,S.F., Silva, S.Q., 2018, Anaerobic-Aerobic Combined System for the Biological Treatment of Azo Dye Solution using Residual Yeast.Water Environment Reseasrch. doi:10.2175/106143017X15131012153167

Benkhaya, S., Mrabet S., Harfi, A.E. Classification, properties, recent synthesis and applications of Azo dyes. Heliyon 6, e03271.

Bibi, A., Zhu, H., Mahmood, Q., Wang, J., Li, X.D., Rehman, M., Hayat, T. Shaheen, N., Ali, A., 2020. Efficient bacterial isolate from roots of cactus degrading Reactive Black 5. Environmental Technology and Innovation 20, 101082.

Hassaan, M. A., Nemr, A.E., 2017. Health and Environmental Impacts of Dyes: Mini Review. Am. J. Env. Sci. Eng. 1(3), 64-67. doi: 10.11648/j.ajese.20170103.11

Hunger, K., 2003. Industrial Dyes: Chemistry, Properties, Applications. WILEY-VCH Verlag GmbH and Co. KGaA, Weinheim.

Jamee, R, Siddique, R., 2019. Biodegradation of Synthetic Dyes of Textile Effluent by Microorganisms:

Joshi, P.A. Jaybhaye, S. Mhatre, K., 2015. Biodegradation of dyes using consortium of bacterial strains isolated from textile effluent. Euro J Exp Biol 5(7), 36-40.

Kunjadia, P.D., Sanghvi, G.V., Kunjadia, A.P.,, Mukhopadhyay, P.N., Dave, G.S., 2016. Role of ligninolytic enzymes of white

rot fungi (*Pleurotus* spp.) grown with azo dyes. SpringerPlus, 5, 1487. DOI 10.1186/s40064-016-3156-7.

Lellis, B. Polonio, C.Z.F., Pamphile, J.A., Polonio, J. C., 2019. Effect of Textile dyes on the health and environment and bioremediation potential of living organisms. Biotechnology Research and Innovation. 3, 275-290.

Manzoor, J. and Sharma, M., 2020. Impact of Textile Dyes on Human Health and Environment. In Impact of Textile Dyes on Public Health and the Environment. 162-169. IGI Global.

Roy, D.C., Biswas, S.K., Saha, A.K., Sikdar, B., Rahman, M., Roy, A.K., Prodhan, H. Z., Tang, S.S., 2018. Biodegradation of Crystal Violet dye by bacteria isolated from textile industry effluents. PeerJ 6:e5015; DOI 10.7717/peerj.5015

Saini, R. D., 2018. Synthetic Textile Dyes: Constitution, Dying process and Environmental Impacts. Asian J. Research Chem. 11(1), 206-214. DOI:10.5958/0974-4150.2018.00040.8

Saratale, R.G., Saratale, G., D., Chang, J.S., Govindwar, S.P., 2011. Bacterial decolorization and degradation of azo dyes: A review. Journal of the Taiwan Institute of Chemical Engineers 42, 138–157.

Saxena, G., Kishor, R., Saratale, G.D., Bharagava, R.N., 2019. Genetically Modified Organisms (GMOs) and their Potential in Environmental Management: Constraints, Prospects, and Challenges. Bioremediation of Industrial Waste for Environmental Safety. In: Bharagava, R., Saxena, G. (Eds.), Bioremediation of Industrial Waste for Environmental Safety. Springer, Singapore, 1–19.

Sen, S.K., Raut, S., Bandyopadhyay, P., Raut, S., 2016. Fungal decolouration and degradation of azo dyes: A review. Fungal Biology Reviews 30, 112-133.

Shah K., 2014. Biodegradation of Azo-dye compounds. International Research Journal of Biochemistry and Biotechnology.1(2),005-013.

Singh, L., Singh, V.P., 2016. Textile Dyes Degradation: A Microbial Approach for Biodegradation of Pollutants. In Microbial Degradation of Synthetic Dyes in Wastewatres. Springer International Publishing. 187-204.

Sudha, M., Saranya, A., Selvakumar G., Sivakumar, N., 2014. Microbial degradation of Azo Dyes: A review. Int. J. Curr. Microbiol. App. Sci. 3(2), 670-690.

Tohamy, R.A., Kenawy, E.R., Sun, J., Ali, S.S., 2020. Performance of a newly isolated salt tolerant yeast strain Sterigmatomyces halophilus SSA-1575 for azo dye decolorization and detoxification. Front. Microbiol. 11.1163. doi: 10.3389/fmicb.2020.01163

Varjani, S., Rakholiya, P., Ng, H.Y., You, S., Teixeira J.A., 2020. Microbial degradation of dyes: An overview. Bioresource Technology. 314, 123728.

BIOSENSORS-BASED DIAGNOSTIC APPROACHES FOR DETECTION OF MICROBIAL FOOD SPOILAGE: A FOCUS ON SPOILAGE OF EDIBLE POULTRY MEAT

Manali Datta[1]*, Dignya Desai[2]

[1]Associate Professor, Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur, Rajasthan, India
[2]Project Engineer, Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur, Rajasthan, India

*Corresponding Author: mdatta@jpr.amity.edu

INTRODUCTION

Food spoilage is that process in which the condition of the food deteriorates in such a manner that the original nutriment value, composition, astringency of the food are damaged, the food become harmful to individuals and unsuitable to eat. According to a recent report "Food Micro—2008 to 2013" by the Strategic Consulting, Inc. (www.strategic-consulting.com), worldwide food industry microbiology testing includes 738.3 million tests in 2008, with a market value exceeding $2 billion. Routine tests account for 81.3% of all food microbiology testing at 600.2 million, a growth of 16.1% since 2005. Testing for food-borne pathogens has grown even faster at 25.6% and now represents 138.1 million tests. Conventional methods still account for approximately 60% of the microbiological food tests in 2008, although down from the 65% level in 2005. In contrast, rapid methods have increased by 36.8% from 2005, now accounting for 40% of all food microbial tests performed worldwide (Ge, 2009)

POULTRY: AN INTEGRAL PART OF FOOD INDUSTRY

Poultry, a thriving industry in India, is valued at Rs 80,000 Cr [2015-16] and constitutes a supplementary income and nutrition to some of the population living below poverty line. Processed chicken, whose consumption in India is rising at a rate of 15-20% per year. Indigenous poultry processing industry is expanding at a compound annual growth rate (CAGR) of ~12% between 2018 and 2023, expecting to reach an estimated value of INR 107.6 Bn by 2023. Indian processed chicken market is pegged at Rs 5,000 cr and it is predicted that transition from live bird market to a frozen market enhances value addition and finances from exports. Spoilage may shorten meat product shelf-life and thus the associated profits. Microbial based food spoilage is a huge economical problem and hence a timely detection method may save millions of money. Biosensors constitute the state of art technology currently being used for development of diagnostics. With advent of science, smart biosensors have become an integral part of the food industry in assessing the viability and sustainability of the products. In this chapter, we brief the about the biochemical indicators corresponding to food spoilage, biosensors and their uses in assessment of food quality and sustainability

MICROBIAL MEAT SPOILAGE SPOILAGE INDICATORS

The primary factors associated with food spoilage are changes in intrinsic food properties (e.g., endogenous enzymes, substrates, sensitivity for light, oxygen) and (cross) contamination during harvesting, slaughter and processing in combination with temperature abuse. For fresh foods, the primary quality changes may be categorized as bacterial growth and alteration in intrinsic metabolism resulting in possible pH fluctuations. A favorable pH for increase in growth of spoilage bacteria in meat is within the range of 5.5-7.0. As an aftermath, there is a progressive detection of slime, off-odours and appearance change with improper storage. Although classified as different causes, these two factors are strongly correlated and one might augment the outcome of other. The Table 1 enlists some microbes and their corresponding spoilage indicators (Balamatsia et al 2010).

Table 1: Meat spoilage microbes and their spoilage indicators

ORGANISM	MICROBIAL CANDIDATES AND THEIR SPOILAGE INDICATORS
Pseudomonas Sp.	*Pseudomonas fragi, Pseudomonas lundensis, Pseudomonas fluorescens*
	Cysteine, cystine, methionine, Hydrogen sulphide, methylsulphide, dimethylsulphide, Methylamine, dimethylamine, trimethylamine ethyl esters
Enterobacteriaceae Sp.	*Hafnia alvei, Hafnia paralvei,Rahnella, Yersinia, Buttiauxella, Serratia fonticola, Serratia grimesii, Serratia liquefaciens, Serratia proteamaculans, Serratia quinivorans*
	Hydrogen sulphide, Dimethylsulphide, hypoxanthine
Lactobacillus Sp.	*Enterococcus viikkiensis, Enterococcus saigonensis, Lactobacillus oligofermentans*
	Acetoindiacetyl and 3-methylbutanol
Brochothrix thermosphacta	*Acetoin and acetic acid*

Proteolytic enzymes cause protein degradation resulting in ageing and spoilage. Enzymes like endoproteases, proteinases, aminopeptidases and carboxypeptidases have been found to stimulate organoleptic alteration in meat. Although these enzymes are equally important in food processing like meat tenderization, excessive activity results in unpalatable food. It has been observed that decrease in level of glucose as an energy source induce the microbes to produce proteases and hence their dependency on amino acids (Ellis and Goodacre, 2001). The psychrotrophic flora predominately inducing proteolysis are the *Pseudomonas* Sp. and members of the Enterobacteriaceae. For example, in Mediterranean boque (*Boops boops*), some species identified were Pseudomonads, *Shewanella putrefaciens* followed by Enterobacteria and *Brochothrix thermosphacta* with an average density of 2-3 $\log_{10}$ CFU g^{-1} (Koutsoumanis and Nychas, 1999). Similarly, in turbot (Psetta maxima) massive proliferation of proteolytic strains like *Lactobacillus delbrueckii* subsp. *delbrueckii* and *Lactococcus lactis* subsp. *lactis* strains could be observed. Degradation characterized by proteolytic activity may be monitored by corresponding increase in N-acyl homoserine lactone [AHL] levels

(Liu et al 2006). Additionally, increase in confluence of bacteria on edibles results in increase of glucose, free amino acids and nitrogenous compounds affecting the organoleptic attributes of the meat.

Chicken meat contain approximately 14gm fat/ 100 gm of meat thus constituting a significant amount susceptible to hydrolytic and oxidative rancidity. *Micrococcus* and *Arthrobacter* Sp. can induce formation of metabolized lipid products like N-hydro-peroxy-eicosatetraenoic acid. Another fungi belonging to *Penicillium sp*, can use palmitate to form a toxic red pigment, 7-(2-hydroxyethyl)-monascorubramine (Pawar et al., 2011). The growths of lipolytic bacteria in meat and their products play a role in quality losses and render the foods unappetizing (Pereira and Vicente, 2013)

Biogenic amines and polyamines have been detected in fish, meat, cheese, vegetables, and wines, and are characterized as biological bases with aliphatic, aromatic, and heterocyclic moieties. The type of amine detected is a signature of the particular bacterial strain(s) present, the level of decarboxylase activity, and availability of amino acid substrate (Naila, 2010). Distinctive amines like tyramine (e.g. cheese), histamine (e.g. wine) , phenylethylamine (e.g. chocolate),agmatine, putrescine, cadaverine, spermidine (e.g. decomposing fish),tryptamine have been detected as an indicator of microbial presence. Biosensors designed to target specific molecules, microbial markers associated with bacterial spoilage have been designed and considered as most promising technologies for addressing instantaneous quantification of food spoilage. In the next section, biosensors specific for detection of microbial spoilage indicators have been discussed.

3. AN INTRODUCTION TO BIOSENSORS

An analytical device capable of eliciting perceptible signals upon binding to molecules of interest is defined as biosensor. Development of a blueprint for a functioning biosensor requires a pooled expertise of various fields of science and technology [**Fig 1**]. Research and development of biosensors has increased by leaps and

bounds over the years as it may be developed in easy point of care diagnostics [POCD]. Featured as easy, quick, low-cost, acutely sensitive, and high precision in target selection enables it to act as a next-generation ultrasensitive point-of-care detection platform.

The rapid progress in this field in past decade, has been possible mainly due to:

(i) Advance technologies supporting miniaturisation and microfabrication
(ii) Utilization of novel targets
(iii) Novel platforms
(iv) Increased collaborations amongst life scientists and engineering/physical scientists

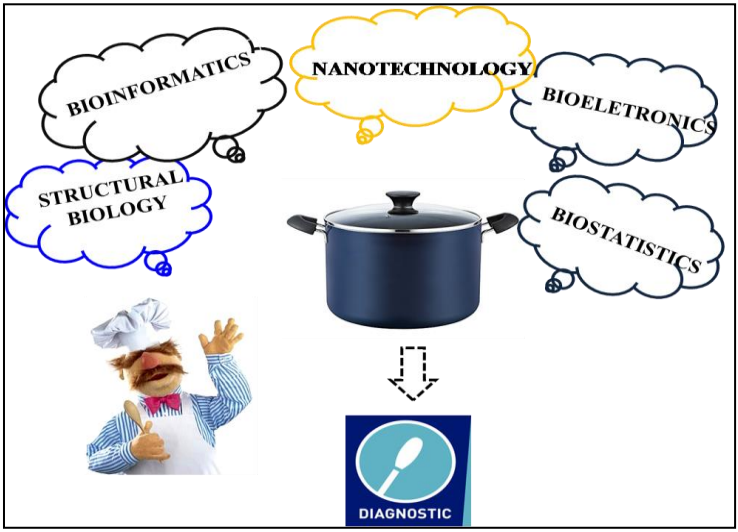


Fig 1. Fields of science and technology involved to design a functional biosensor

The evolution of biosensors can be categorized into three generations as per the grade of integration of the separate

components such as the technique of attachment of the bio-recognition or bio-receptor molecule to the base transducer division (Lee 2013) [**Fig 2**].

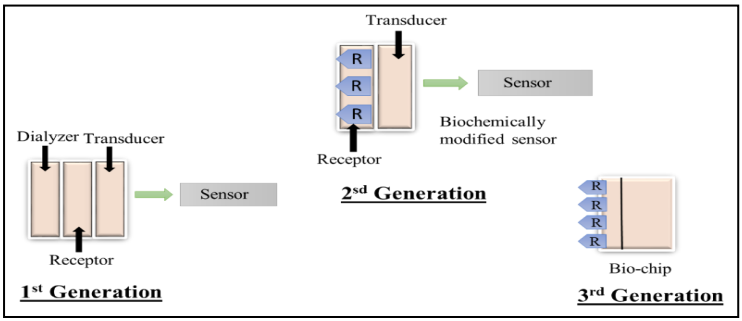


Fig 2. Representation demonstrates of three generations of biosensors, where R is various bio-receptor molecules In the 1st generation of biosensor the dialyzer, receptor and transducer are three distinct units which are performed synchronized way and produced signal could read by a sensor. While in the 2nd and 3rd generation dialyzer, receptor and transducer units are minimized in one chip set-up which can detect the signal.

Generations of biosensor

In the first generation, the bio- receptor is physically entrapped in the base sensor. In successive generations, immobilization is accomplished via covalent bonds at an appropriately modified transducer interface or by amalgamation technique into a polymer matrix at the surface. Furthermore first generation biosensors measure the concentration of analytes that diffuse to the transducer surface and generate an electrical response. e.g. 1st generation glucose biosensor in which the electrons are transferred to molecular oxygen resulting reduction in the oxygen concentration which is measured by sensor. In the second generation, the specific components remain fundamentally distinct like control electronics—electrode—biomolecule, while in the third generation the bio-

receptor molecule becomes an integral part of the base sensing element. In third-generation biosensors, e.g. 3rd generation glucose sensing mechanism, is independent of mediators or oxygen, the electrons are transferred straight from the enzyme to the electrode without any transitional stages. Although these classifications were perhaps proposed for enzyme electrode systems, comparable classifications suitable to biosensors in general can be made (Oliveira 2019).

Table 2: Classification of sensors and their corresponding transducers used

Biosensors	Bio-transducers used
Electrochemical Biosensors	Electrochemical biosensors counter with an analyte of interest to produce an electrical signal relative to the analyte concentration.
Optical Biosensors	Optical biosensors can be label-free or label-based biosensors. These biosensors measure the interface of an optical field with a bio-recognition sensing division.
Thermometric Biosensors	A number of biological reactions are accompanying the release of heat. Thermometric biosensors read the change in temperature of the solution containing the analyte produced by enzymatic reactions.
Piezoelectric biosensors	Piezoelectric biosensors also denoted to as acoustic biosensors, these sensors measure the change in the physical properties of an acoustic wave.
Magnetic Biosensors	Magnetic biosensors measure changes in magnetic properties or magnetically induced effects.

Likewise, Biosensors signify technology that can be useful to numerous areas of the food industry such as the storage of grains and raw supplies, food manufacture/processing, safety and defense and packaging of food. Various biosensors have developed in the last decade as a substitute for examining microorganisms and toxins

in food due to the ability for fast analysis, reproducibility, stability, and precision (Oliveira 2019). An extensive variety of transducers can be explored for toxin, spoilage and fungi detection. The biosensors are usually classified on base of transducers used and has been detailed in Table 2.

BIOSENSORS AS A TOOL FOR PROGNOSIS

Biosensors for assessing may be designed to detect whole cells, bio-indicators or a combination of both. Here we discuss the different discoveries and designs made to assess food quality.

Whole cell biosensors

Salmonella is a common pathogen associated with chickens and has been found to colonize on the surface of chicken meat; it has sometimes being found to percolate approximately 1 cm of the chicken meat depthwise. A magnetoelastic (ME) biosensor with phage C4-22 as a probe was used to detect all *Salmonella enterica* serotypes. C4-22 is a *Salmonella* specific phage, upon interacting with intact cells educes magnetic fluxes which may be detected by resonating frequency. The sensor could detect a concentration of 7.86 × 105 cfu /mm^2 on the surface of the meat and could perceive phage around 30% of Salmonella cells were present 0.1 cm inside the chicken meat surface. Moreover 23% percent of cells were present at the depth of 0.5 cm and 2.15% at a depth of 1.0 cm in the chicken meat (Chen, et al., 2017).

Another sensor detecting presence of cells based on the presence of cellular DNA was developed to perform point-of-care screening of chicken carcasses for *Campylobacter*. DNA probes specific for *Campylobacter* was immobilized on paper-based platform diagnostics and detection was mediated by well assessed biotin-streptavidin interaction and illuminated with horseradish peroxidase (HRP) in the presence of luminol and H_2O_2 . The technique called dot blot could successfully detect Campylobacter on contaminated chicken meat with a sensitivity of upto 3 pg/µL of DNA (Vizzini, et

al., 2020). A similar cellular DNA detecting multiplex fiber optic biosensor could detect *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella enterica* simultaneously with detection limit for each pathogen of 103 cfu/ml. The platform was coated with biotinylated polyclonal antibodies capable of conjugating to streptavidin optical waveguides could detect contamination in food samples within 2 h (Ohk and Bhunia, 2013)

Biogenic amine sensors

Chitosan-coated magnetic microparticles immobilized with diamine oxidase has been used in detection of biogenic amines. In addition to the magnetic template, an oxygen probe is incorporated in ORMOCER®. Enzyme catalyzed oxidation of amines resulted in consumption of oxygen, detected with resultant quenching of fluorescence of ruthenium complex (Pospiskova et al 2013). The main advantages of these biosensors with the enzyme on magnetic carrier and are ruggedness, the speed of preparation, on-line measurement, long term stability and the assay of analytes in broad concentration ranges.

Another amine, spermidine serves as a good biomarker for the determination of food quality. Diamine oxidase commonly used for the bio-recognition of amines is found to be inhibited in presence of spermidine and sometime its metabolite 1,3-diaminopropane. In order to overcome this setback, enzyme has been entrapped by glutaraldehyde onto an electrosynthesized bilayer film of Pt and Au. biosensor displayed a high response sensitivity in flow experiments, short response time, a good linear response and low detection limits (Carelli et al., 2007). A disposable biosensor using monoamine oxidase and diamine oxidase immobilized on screen printed carbon electrode was designed. Reduction of the hydroxymethylferrocene generated cathodic current in reference to screen-printed Ag/AgCl reference electrode.A linear response range from 0.2 up to 1.6 μM and from 0.4 to 2.4 μM of histamine was obtained for DAO/HRP and MAO/HRP based biosensors, respectively. The biosensor construction was highly reproducible, yielding relative standard

deviations of 10% and 11% in terms of sensitivity for DAO/HRP and MAO/HRP based biosensors, respectively (Lomillo et al 2010).

Comparison has been made with screen-printed electrodes (SPE) with array formed by classic carbon paste electrodes (CPE) fordetection of ammonia and 4 amines namely, dimethylamine, trimethylamine, cadaverine and histamine. It was demonstrated that CPE gave better sensitivity than SPE. However , mass-production of low-cost and miniaturization of SPE enabled good sensibility and repeatability (Rodríguez-Méndez et al., 2009). Another amperometric biosensor has been developed to identify putrescine using ceria nanoparticles modified glassy electrode. The sensor thus designed was used for determination of putrescine released by tiger prawn in its stages of deterioration (Gumpu et al, 2016). Another sensor designed to monitor the levels of histamine in fresh water fish samples. The screen printed carbon electrode is capable of sensing hydrogen peroxide released on catalysis by enzyme diamine oxidase. The novel biosensor shows high sensitivity (0.0631 μA·μM), low detection limit (2.54×10^{-8} M) and a broad linear domain from 0.1 to 300 μM (Apetrei and Apetrei, 2016). An upgraded version of biosensor developed could simultaneously detect spermine, spermidine, cadaverine and putrescine. This electrochemiluminescence (ECL) sensor consisted of Ru(bpy)32+-encapsulated silica nanoparticles (RuNP) immobilised on carbon electrode. The sensor exhibited high sensitivity and stability with a detection limit of 5 nM for spermine and spermidine, 90 nM for putrescine and 120 nM for cadaverine (Spehar-Délèze et al, 2015). This is an improvement for previously reported measurements reported where LOD for spermine and spermidine were reported to be 7.6 nM and for cadaverine and putrescine 170 nM (Liu et al., 2003)

In addition to enzyme based detection of amines, alternative mechanisms have been implemented in designing a piezoelectric quartz crystal microbalance (PQCM) modified with carbon nanotubes capable of detecting minute concentrations of amine in solution. Changes in conductivity of nanotubes was amplified to

attain vibrational frequency signal in the modified quartz crystal microbalance (Manoso et al., 2013). An investigation has revealed that perylene diimide (PDI) and perylene monoimide (PMI) may detect common amines in solution. Luminescence spectroscopy proved excited PDI and PMI interacts with the amine derivatives leading to photoinduced electron transfer and fluorescence quenching. PMI could effortlessly detect primary, secondary and tertiary amines whereas PDI demonstrated selectivity for bulky hydrophobic amines and hence these molecules may be used to propose perylene based sensors for amines (Sriramulu et al., 2016)

Smart packaging sensors

With the advent of newer technologies, the concept of smart packaging is being extensively used for point detection of food spoilage and has become convenient mechanism to monitor freshness. Researchers have developed a 13.56 MHz radio frequency identification (RFID) tag upgraded with nanofillers based sensing element capable of detect biogenic amine putrescine. As soon as that metabolite is detected, electrical circuit in the RFID tags is completed resulting in change in current. A smartphone subsequently interprets the current and generates a signal. The researchers envision their CARDs will become a part of "smart packaging" to detect food spoilage, monitor gas exposure levels in manufacturing plants, and help doctors diagnose diseases. (Tanguy, 2015).

One such application has been developed for real-time monitoring of the microbial breakdown products in the headspace of packaged fish. Polyaniline (PANI) film as a chemical sensor could change color in response to basic volatile amines, also referred as the total volatile basic nitrogen (TVBN)) released during fish spoilage. Additionally, PANI film may be recycled numerous times using acid solution, hence indicating it utility as a low-cost sensor (Kushwandi et al., 2012). Novel colorimetric sensor array using 15 chemically responsive dyes was designed for detection of meat spoilage bacteria like *Pseudomonas koreensis, Bacillus fusiformis,*

Acinetobacter guillouiae , and *Enterobacter cloacae* . Volatile organic compounds tends to elicit different profile upon interaction with the 9 metalloporphyrins and 6 pH indicators. A multivariate calibration followed by linear discriminant analysis (LDA) was followed to achieve classification of the four distinct microbes. Sensor based identification was found to be in agreement with the results using 16S rRNA sequences analysis (Chen et al., 2014).

FUTURE DIRECTIONS

Assessing food quality for microbial presence and thus its safety and palatability using standard microbiological methods had become a major hurdle for the food industry and public health. Biosensor technologies that target microbial biomarkers present themselves as promising alternatives for rapid screening of harmful bacteria in food samples. With time and Incorporation of nanomaterials with unique electrical and photonic properties, as well as biomaterials with high biocompatibility are the most effective strategies for developing biosensors with ultrafast response time and high stability.

REFERENCES

Alonso-Lomillo MA, Domínguez-Renedo O, Matos P, Arcos-Martínez MJ. Disposable biosensors for determination of biogenic amines. Analytica chimica acta. 2010 Apr 14;665(1):26-31.

Apetrei IM, Apetrei C. Amperometric Biosensor Based on Diamine Oxidase/Platinum Nanoparticles/Graphene/Chitosan Modified Screen-Printed Carbon Electrode for Histamine Detection. Sensors. 2016 Mar 24;16(4):422.

Balamatsia CC, Paleologos EK, Kontominas MG, Savvaidis IN. Correlation between microbial flora, sensory changes and biogenic amines formation in fresh chicken meat stored aerobically or under modified atmosphere packaging at 4 degrees C: possible role of biogenic amines as spoilage indicators.Antonie van Leeuwenhoek. 2006;89(1):9–17.

Carelli D, Centonze D, Palermo C, Quinto M, Rotunno T. An interference free amperometric biosensor for the detection of biogenic amines in food products. Biosensors and Bioelectronics. 2007 Dec 15;23(5):640-7.Kress-Rogers E, D'Costa EJ, Sollars JE, Gibbs PA, Turner AP. Measurement of meat freshness in situ with a biosensor array. Food Control. 1993 Dec 31;4(3):149-54.

Chen, I. H., Horikawa, S., Bryant, K., Riggs, R., Chin, B. A., & Barbaree, J. M. (2017). Bacterial assessment of phage magnetoelastic sensors for Salmonella enterica Typhimurium detection in chicken meat. Food control, 71, 273-278.

Chen Q, Li H, Ouyang Q, Zhao J. Identification of spoilage bacteria using a simple colorimetric sensor array. Sensors and Actuators B: Chemical. 2014 Dec 15;205:1-8.

Ellis DI, Goodacre R. Rapid and quantitative detection of the microbial spoilage of muscle foods: current status and future trends. Trends in Food Science & Technology. 2001 Nov 30;12(11):414-24.

Ge, B., & Meng, J. (2009). Advanced technologies for pathogen and toxin detection in foods: current applications and future directions. Journal of the Association for Laboratory Automation, 14(4), 235-241.

Gumpu MB, Nesakumar N, Sethuraman S, Krishnan UM, Rayappan JB. Determination of Putrescine in Tiger Prawn Using an Amperometric Biosensor Based on Immobilization of Diamine Oxidase onto Ceria Nanospheres. Food and Bioprocess Technology. 2016 Apr 1;9(4):717-24.

Koutsoumanis K, Nychas GJ. Chemical and sensory changes associated with microbial flora of Mediterranean boque (Boops boops) stored aerobically at 0, 3, 7, and 10 C. Applied and Environmental Microbiology. 1999 Feb 1;65(2):698-706.

Kuswandi B, Restyana A, Abdullah A, Heng LY, Ahmad M. A novel colorimetric food package label for fish spoilage based on polyaniline film. Food Control. 2012 May 31;25(1):184-9.

Liu, J.; Yang, X.; Wang, E. Direct tris(2,2'-bipyridyl)ruthenium(II) electrochemiluminescence detection of polyamines separated by capillary electrophoresis. Electrophoresis 2003, 24, 3131–3138.

Liu, M., Gray, J. M., & Griffiths, M. W. (2006). Occurrence of proteolytic activity and N-acyl-homoserine lactone signals in the spoilage of aerobically chill-stored proteinaceous raw foods. Journal of Food Protection®, 69(11), 2729-2737.

Manoso E.S., Herrera-Basurto R., Simonet B.M. Valcarcel M. A quartz crystal microbalance modified with carbon nanotubes as a sensor for volatile organic compounds (2013) Sensors and Actuators, B: Chemical, 186 , pp. 811-816.

Naila, A., Flint, S., Fletcher, G., Bremer, P., & Meerdink, G. (2010). Control of biogenic amines in food--existing and emerging approaches. Journal of food science, 75(7), 139–150

Ohk, S. H., & Bhunia, A. K. (2013). Multiplex fiber optic biosensor for detection of Listeria monocytogenes, Escherichia coli O157: H7 and Salmonella enterica from ready-to-eat meat samples. Food microbiology, 33(2), 166-171.

Pereira P and Vicente A. Meat nutritional composition and nutritive role in the human diet. Meat Science. 2013, 93: 586–592

Park YW, Kim SM, Lee JY, Jang W. Application of biosensors in smart packaging. Molecular & Cellular Toxicology. 2015 Sep 1;11(3):277-85.

Pospiskova K, Safarik I, Sebela M, Kuncova G. Magnetic particles–based biosensor for biogenic amines using an optical oxygen sensor as a transducer. Microchimica Acta. 2013 Feb 1;180(3-4):311-8.

Rodríguez-Méndez ML, Gay M, Apetrei C, De Saja JA. Biogenic amines and fish freshness assessment using a multisensor system based on voltammetric electrodes. Comparison between CPE and screen-printed electrodes. Electrochimica Acta. 2009 Nov 30;54(27):7033-41

Rouger, A., Tresse, O., & Zagorec, M. (2017). Bacterial contaminants of poultry meat: sources, species, and dynamics. Microorganisms, 5(3), 50

Sarika C. Pawar, Rahul M. Sarate and Jai S. Ghosh, 2011. Spoilage of Chilled Chicken Meat and Liver by Psychrophilic Lipase.American Journal of Food Technology, 6: 166-172.

Schmidt A, Standfuss-Gabisch C, Bilitewski U. Microbial biosensor for free fatty acids using an oxygen electrode based on thick

film technology. Biosensors and Bioelectronics. 1996 Dec 31;11(11):1139-45.

Spehar-Délèze AM, Almadaghi S, O'Sullivan CK. Development of Solid-State Electrochemiluminescence (ECL) Sensor Based on Ru (bpy) 32+-Encapsulated Silica Nanoparticles for the Detection of Biogenic Polyamines. Chemosensors. 2015 May 21;3(2):178-89.

Sriramulu D, Valiyaveettil S. Perylene derivatives as a fluorescent probe for sensing of amines in solution. Dyes and Pigments. 2016 Nov 30;134:306-14.

Tanguy NR, Fiddes LK, Yan N. Enhanced radio frequency biosensor for food quality detection using functionalized carbon nanofillers. ACS applied materials & interfaces. 2015 Jun 2;7(22):11939-47.

Vizzini, P., Manzano, M., Farre, C., Meylheuc, T., Chaix, C., Ramarao, N., & Vidic, J. (2020). Highly sensitive detection of Campylobacter spp. In chicken meat using a silica nanoparticle enhanced dot blot DNA biosensor. Biosensors and Bioelectronics, 171, 112689.

BACTERIOCIN: ITS EMERGING HORIZON FOR FOOD INDUSTRIES

Vishakha Sharma[1], Rahul C Ranveer[2], Neelam Jain[3] and Gajender Kumar Aseri[4*]

[1]Research Scholar, Amity Institute of Microbial technology, Amity University Rajasthan-India
[2] Associate Professor, PG Institute of Post Harvest Management, Raigad, Maharashtra- India
[3]Professor, Amity Institute of Biotechnology, Amity University Rajasthan-India
[4]Professor, Amity Institute of Microbial technology, Amity University Rajasthan-India

Corresponding Author: gkaseri@jpr.amity.edu

Introduction

Food safety and its quality have become the overriding preoccupation for food processing industries in the fast growing world. The statistical data of the Food and Agriculture Organization (FAO) indicates that approximately one-third of the total food produced is lost or wasted every year due to inadequate storage facilities, improper preservation, spoilage or deterioration caused by microbial activity (Motelica *et al*., 2020). Food contamination due to microbial growth is a global food safety and quality issue as it may arise in any part of the food chain. Both gram positive and gram negative concerns the food contamination. In order to reduce the food waste and prolong storage of fresh and minimally processed food, a number of physical, chemical and biological treatments have been employed. For example, fruits and vegetables were disinfected and washed with chlorinated water to reduce the growth of microorganisms but it attains only 1-2 log reduction in microbial growth (Siroli *et al*., 2015). The use of chlorine as disinfecting agent cause serious health problems to human because of the production of carcinogenic compounds (Ali *et al*., 2018). Moreover some other

ISBN: 978-81-950423-8-8

substances have also been used for the same aspire such as organic acid, peroxyacetic acid, ozone, electrolyzed water, acidified sodium chlorite, hydrogen peroxide etc (Sachadyn & Agriopoulou, 2020). Various physical treatments are used to control the microbial growth such as UV light, high pressure processing, low temperature storage etc (Leneveu- Jenvrin *et al.,* 2020). Also, food industries are using certain chemical preservatives (class II preservatives) to prevent the food from spoilage like benzoates, nitrates, nitrites, sulphites, propionic acid, sorbates, and aspartame but after certain limit these are hazardous to humans.

Food preservation by using natural antimicrobial compounds could be an alternative to chemical additives and can become a very promising and novel technique to maintain food safety and quality. Lactic acid bacteria play a major role in biopreservation. LAB posses very long history for their safer use in the fermentation of food and feed (Yepez *et al.*, 2017). They grant with a status of GRAS i.e. generally recognized as safe by U.S. FDA (Food and Drug Administration) and EFSA (European Food safety Authority) (Ramos *et al*., 2020). Lactic acid bacteria generally produces a number of antimicrobial compounds like organic acids (acetic, malic, lactic, fumaric and citric), carbon dioxide, reuterin, hydrogen peroxide, diacetyl, ammonia, acetaldehyde, ethanol, acetoin, bacteriocin and bacteriocin like inhibitory substances (Bartikiene *et al*., 2020). Among these compounds, bacteriocin dominates the literature to be used as natural preservative for its astonishing antimicrobial property against pathogenic microorganisms. A bacterium i.e. both gram positive and gram negative yield number of interesting substances of protein, having bactericidal/ bacteriostatic action is called bacteriocin. Bacteria used these substances as a natural defense mechanism. Bacteriocins are ribosomally synthesized small antimicrobial peptides that are extracellularly released by bacteria (Sharma *et al.*, 2019). They are contrasting from antibiotics as they are the product of log phase but antibiotics are secondary metabolites. Most of the bacteria whether gram positive or gram negative may produce at least one bacteriocin. The domain Archaea also produces some bacteriocin like antimicrobial

called archeocins. The concept of bacteriocin was mainly introduced in 1953. They are completely safe for human consumption due to their proteinaceous nature as they can be degraded in digestive system. They are also used as an effective biocontrol agent in food processing industries (Cotter *et al*., 2005).

Originally, the primary feature of bacteriocin was assumed to be their ability to signal and repel, rather than adverse bacteria inhibition. Bacteriocin exhibit significant strong antimicrobial activity at higher concentration than natural level and can cause pore formation and other membrane disruptions or even inhibits the process of cell division. Bacteriocin production form gram positive and gram negative bacteria build self-immunity mechanism for their protection from toxicity of their own peptides (Zgheib *et al*., 2020). Though there is no any universally accepted classification scheme of bacteriocin but most of the researchers follow the first classification scheme proposed by Klaenhammer. This scheme was based on the mode of action and structure of bacteriocin and this include four class. The genera that produce bacteriocin include *Lactococcus*, *Enterococcus*, *Lactobacillus*, *Streptococcus*, *Carnobacterium* etc. A variety of bacteriocins have been produced from various sources for industrial application. Organisms like *Lactobacillus paracasei* HD1.7, *Lactobacillus plantarum* ST16Pa, *Pediococcus pentosaceus* K23-2, *Bacillus subtilis*, *Leuconostoc pseudomesenteroides* 607, *Lactobacillus plantarum* 24 are previously isolated from Chinese cabbage sauerkraut, papaya, kimchi, persimmon fruit, marula fruit, baobab seed (Todorov *et al.*, 2012; Chen *et al.*, 2018). Till now around 230 bacteriocin from LAB have been discovered by researchers around the world but only three of them have been commercialized including nisin (produced by *Lactococcus lactis*), pediocin PA-1 (produced by *Pediococcus acidilactici*) and carnocyclin A (produced by *Carnobacterium maltaromaticum* UAL 307). Nisin is the only bacteriocin that is used in almost 50 countries around the world (Huang *et al*., 2016).

Guidelines for approval of bacteriocin in food preservation

Even after the GRAS status of high number of bacteriocin mentioned in literature, the application is very limited for food, medicinal or veterinary purpose. It should be stated that most existing application of bacteriocins are related to food safety, while in other industries only a few examples are published, which may be attributed to the lack of clear criteria for their legal acceptance (Soltani *et al*., 2020). Bacteriocins are generally deemed as technological agent or new food additive by the regulatory bodies as being active biological compounds. Technological agent is a compound used for producing a technical effect during manufacturing of food and prevents the production of residues in the finished product or by-product. Whereas food additive is a substance that is incorporated in food during its processing or prior to its storage to amend the food in order to reach or attain the required technical effect. The use of technological agent in foodstuff is usually not subjected to regulatory specifications but food additives must be licensed in advance by the regulatory bodies and should be authorized for marketing prior to their use in food industries. The evaluation steps required for the approval of a new bacteriocin as food additive is presented in Fig. 1. A bacteriocin should have following criteria for its approval as food preservative (Soltani *et al*., 2020).

i) Identification and molecular characterization of new bacteriocin. It should be purified and amino acid sequence must be known.
ii) The method of production and stabilization.
iii) The concentration of bacteriocin upto which it can be used and an accepted method of analysis which will determine the end concentration of bacteriocin in finished product.
iv) Detailed reports of its efficacy and safety under the recommended terms of use including the acute/sub-acute toxicity and the impact of long-term exposure.
v) Data on the appropriate residual concentration of bacteriocin in finished product as used in compliance with good manufacturing practice.

Application of bacteriocin in food industries

Large number of bacteriocin have been used as natural preservatives in number of food products i.e. dairy, meat and meat products, fruits and vegetables, seafood etc. Bacteriocin can be incorporated to food by three means i) as a pure bacteriocin culture ii) as bacteriocin-producing culture iii) as bacteriocin containing fermentates. Dairy industry is a dynamic leading industry that plays a significant role in the economies and growth of many countries around the world. The global demand for dairy product is expected to flourish at a substantial CAGR by 2027 (Sharma *et al*., 2019). The protection of dairy products is global public health's concern that needs new methods and technologies to manage the growth pathogenic microorganisms that are spread by food. *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* spp. and *Cronobacter sakazakii* are the main pathogens that are responsible for dairy product spoilage. In this industry, bacteriocins have extensive applications, primarily in the fermentation process. The FAO/WHO codex committee on milk and milk products permits the use of nisin as a food preservative in processed cheese at a concentration of 12.5mg/kg of product, while maximum permissible limit set by US FDA is upto 250mg/kg (Sobrino-Lopez & Martin-Belloso, 2008).

Nisin is used in many dairy products to ensure their protection, prolong shelf-life and maintain consistency, either directly added in the purified form or generated in situ by live bacteria (Silva *et al*., 2018). It has strong antimicrobial activity against both gram positive and gram negative bacteria including *Listeria* spp, *Staphylococcus aureus* and some spore forming bacteria like *Bacillus* and *Clostridium* (Chen & Hoover, 2003). The prevention of late blowing in cheese due to gas generating *Clostridium* spp. was one of the earliest uses of nisin in dairy products (Galvez *et al*., 2008). To substitute nitrate in food preservation for preventing the development of *clostridia* spores, nisin has been commonly used in cheese and cheese spreads (Chen & Hoover, 2003). Felicio *et al*., 2015 conducted a study where nisin was added in Minas Frescal cheese which reduced *Staphylococcus aureus* count by approx 1.5 log cycles. Pediocin produced by *Pediococcus* spp. shown to be more effective than nisin against food borne pathogens including

Listeria monocytogenes, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas* spp. (Jamuna and Jeevaratham, 2004). A study by Verma *et al*., 2017 found that the semi purified pediocin when added in fermented cheese whey; it was effective in controlling *Staphylococcus aureus* count, thus increasing the shelf-life of raw buffalo milk. Some other bacteriocins are also effective to control the spoilage of dairy products such as Lacticin 3147, Lacticin 481, enterocin AS-48, Lactococcin BZ, Aureocin A70 etc. Ribeiro *et al*., 2017 studied the effectiveness of semi purified enterocin, in order to reduce the contamination of *Listeria monocytogenes* in fresh cheese and this effect of enterocin was remained after 72 h of storage. The high cost of isolating and purifying bacteriocin also inhibits the commercial discovery of new bacteriocin. Furthermore, FDA and EFSA the health regulatory authority restricts the endorsement of new bacteriocin to be use as food preservative, as a result only nisin and pediocin are commercially accessible (Silva *et al*., 2018).

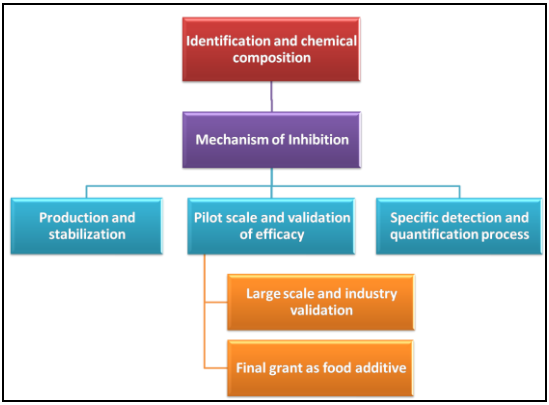


Figure 1: Steps in the evaluation for the approval of new bacteriocin as food additive.

The addition of bacteriocin producing strain during the manufacturing process of food products is an alternative method of using bacteriocin. In this context, the use of these bacteria in food provides a feasible method for preventing microbial contamination.

Table 1. Some suggested applications of bacteriocins

Bacteriocin	Producing strain	Application	Reference
Thermophilin 109	*Streptococcus thermophillus* ST 109	Used as probiotic	Renye *et al*., 2019
Plantaricin BM-1	*Lactobacillus plantarum* BM-1	Development of polyvinylidene chloride (PVDC) film to preserve pork meat	Xie *et al*., 2018
Bacteriocin[a]	*Enterococcus mundtii* QAUEM 2808	To be used in milk fermentation as culture	Nawaz *et al*., 2019
Bacteriocin[a]	*Lactobacillus plantarum*	High phenolic compound concentration, reduction of browing	Martínez-Castellanos *et al.*, 2011
Pediocin DT016	*Pediococcus acidilactici*	Reduction in count of *L. monocytogenes*	Ramos *et al*., 2020
Enterocins,	*Enterococcus faecalis* L3B1K3	Semi purified enterocin reduced *L. monocytogenes* in cheese within 6 h.	Ribeiro *et al*., 2017
Aureocin A70,	*Staphylococcus aureus* A70	Inhibited the growth of *L. monocytogenes* by 5.5 log after 7 days at 4°C.	Fagundes et al., 2016
Bacteriocin[a]	*Lactobacillus reuteri* INIA P572	Reduction in *Clostridium* count and prevented late blowing.	Gómez-Torres *et al*., 2014
Enterocin KT2W2G-cinnamon oil combination	*Enterococcus faecalis*	extented the shelf life of bananas	Ajingi *et al*., 2020

Benkerroum *et al*., 2002 conducted a study where the effect of bacteriocin producing strain *Lactococcus lactis* was tested. In this study the count of *Listeria monocytogenes* was reduced below the detectable level at 7°C storage for 24 h. A bacterium *Alicyclobacillus acidoterrestris* is the primary source of contamination in fruit juice industry. Carvalho *et al*., 2007 conducted a study where Bovicin HC5, a bacteriocin was produced from *Streptococcus bovis* HC5 added in mango pulp that inhibits the growth of *Alicyclobacillus acidoterrestris* and *Clostridium tyrobutyricum*.

Another method of using bacteriocin in food preservation is the addition of edible film or coating having bacteriocin (called antimicrobial film) is the most common technique for the foods that are consumed raw or with no cooking (Valdes *et al*., 2017). These antimicrobial films are primarily made up of thin layer of biopolymer that changes the existing atmosphere of food, creating a barrier between food and environment thus, improving the quality, safety and functionality of food without altering the nutritional and organoleptic properties (Valdes *et al*., 2017). This method of using antimicrobial film having purified form of bacteriocin or by adding bacteriocin-producing strain can be more effective to prevent the growth of pathogens. Nisin is a strongly surface-active molecule that can attach to various compounds, making it ideal for solid surface adsorption and destroying bacterial cells that then adhere to them. Various antimicrobial packaging systems by using nisin were able to decrease the growth of *Staphylococcus aureus*, *Micrococcus luteus*, *Listeria innocua* and *Bacillus cereus* in packaged milk product and cheese (Hanusova *et al*., 2010).

Combination of bacteriocin with other hurdles (Hurdle Technology)

Strategies to control the growth of pathogenic microorganisms and spoilage of food products are currently based on hurdle technologies such as the combination of other antimicrobial with bacteriocin to enhance food safety (David *et al*., 2013). In this approach bacteriocin is combined with some hurdle such as EDTA, potassium diacetate, sodium lactate, high-pressure treatments and other antimicrobial compounds. Pimentel *et al*., 2013 combined nisin with

bovicin HC5 which showed effective results in reducing the count of *Staphylococcus aureus* and *Listeria monocytogenes* in skim and whole milk. Similarly nisin was combined with reuterin and enterocin AS-48 to control food spoilage (Arques *et al*., 2011). The combination of bacteriocin and EDTA was found to be very effective to sensitize the gram negative bacteria because they become susceptible to bacteriocin if their outer membrane permeability is impaired by chelating agent like EDTA (Chen & Hoover, 2003). In addition, an enhanced antimicrobial effect may result from combining bacteriocin with other treatments. A common technique for the inactivation of microorganism at room temperature is high pressure processing, but this method does not provide the complete inhibition of microorganisms. Various studies have shown the synergistic impact of bacteriocin with high pressure processing like nisin to control food microorganisms.

Conclusion

In recent years, various innovations have been employed to improve the efficacy and ability of bacteriocins in food preservation. In this chapter, we have outlined the developments of bacteriocins as food additive. They can be successfully added in food products as purified or partially purified form to ensure their safety and quality. The knowledge obtained from research in this field would improve the awareness of their global effects on food environment and permit more stable approaches of application in food industries.

References

Ajingi, Y.S., Ruengvisesh, S., Khunrae, P., Rattanarojpong, T., Jongruja, N. (2020). The combined effect of formic acid and Nisin on potato spoilage. Biocatal. *Agric Biotechnol.* 24, 1015-23.

Ali, A., Yeoh,W.K., Forney, C., Siddiqui, M.W. (2018). Advances in postharvest technologies to extend the storage life of minimally processed fruits and vegetables. *Crit Rev Food Sci Nutr.* 58, 2632–2649.

Arques, J. L., E. Rodriguez, M. Nunez, and M. Medina. (2011). Combined effect of reuterin and lactic acid bacteria bacteriocins on the inactivation of food-borne pathogens in milk. *Food Control.* 22, 457–461.

Bartkiene, E., Lele, V., Ruzauskas, M., Domig, K.J., Starkute, V., Zavistanaviciute, P., Bartkevics, V., et al. (2020). Lactic acid bacteria isolation from spontaneous sourdough and their characterization including antimicrobial and antifungal properties evaluation. *Microorganisms.* 8, 64.

Benkerroum, N., Ghouati, Y., Ghalfi, H., Elmejdoub, T., Roblain, D., Jacques, P., et al. (2002). Biocontrol of *Listeria monocytogenes* in a model cultured milk (lben) by in situ bacteriocin production from *Lactococcus lactis* ssp. *lactis*. *Int J Dairy Technol.* 55, 145–151.

Carvalho, A.A.T., Costa, E.D., Mantovani, H.C., and Vanetti, M.C. (2007). Effect of bovicin HC5 on growth and spore germination of *Bacillus cereus* and *Bacillus thuringiensis* isolated from spoiled mango pulp. *J Appl Microbiol.* 102, 1000–1009.

Chen, H., and Hoover, D.G. (2003). Bacteriocins and their food application. *Compr Rev Food Sci Food Safety* 2, 82-100.

Chen, Y.S., Wu, H.C., Kuo, C.Y., Chen, Y.W., Ho, S., and Yanagida, F. (2018). Leucocin C-607, a novel bacteriocin from the multiple-bacteriocin-producing *Leuconostoc pseudomesenteroides* 607 isolated from Persimmon. *Probiotics Antimicrob Proteins*. 10, 148-156.

Cotter, P. D., Hill, C., and Ross, R. P. (2005). Bacteriocins: developing innate immunity for food. *Nat Rev Microbiol.* 3, 777–788.

David, J. R. D., L. R. Steenson, and P. M. Davidson. (2013). Expectations and applications of natural antimicrobials to foods: A guidance document for users, suppliers, research and development, and regulatory agencies. *Food Prot Trends.* 33, 238–247.

Fagundes, P. C., De Farias, F. M., Da Silva Santos, O. C., Da Paz, J. A. S., Ceotto Vigoder, H., Alviano, D. S., et al. (2016). The four-component aureocin A70 as a promising agent for food biopreservation. *Int J Food Microbiol.* 237, 39–46.

Felicio, B. A., Pinto, M. S., Oliveira, F. S., Lempk, M. W., Pires, A. C. S., and Lelis, C. A. (2015). Effects of nisin on *Staphylococcus aureus* count and physicochemical properties of Minas Frescal cheese. *J Dairy Sci.* 98, 4364–4369.

Galvez, A., Lopez, R.L., Abriouel, H., Valdivia, E., and Ben Omar, N. (2008). Application of bacteriocins in the control of foodborne pathogenic and spoilage bacteria. *Crit Rev Biotechnol.* 28, 125-152.

Gómez-Torres, N., Avila, M., Gaya, P., and Garde, S. (2014). Prevention of late blowing defect by reuterin produced in cheese by a *Lactobacillus reuteri* adjunct. *Food Microbiol.* 42, 82–88.

Hanušova, K., M. Šťastna, L. Votavova, K. Klaudisova, J. Dobiaš, M. Voldřich, and M. Marek. (2010). Polymer films releasing nisin and/ or natamycin from polyvinyldichloride lacquer coating: Nisin and natamycin migration, efficiency in cheese packaging. *J Food Eng*. 99, 491–496.

Huang, T., Zhang, X., Pan, J., Su, X., Jin, X., and Guan, X., (2016). Purification and characterization of a novel cold shock protein-like bacteriocin synthesized by *Bacillus thuringiensis*. *Sci reports.* 6, 35560. DOI: 10.1038/srep35560.

Jamuna, M., and Jeevaratnam, K. (2004). Isolation and partial characterization of bacteriocins from *Pediococcus* species. *Appl Microbiol Biotechnol.* 65, 433–439.

Leneveu-Jenvrin, Q., Quentin, B., Assemat, S., Hoarau, M., Meile, J.C., Remize, F. (2020). Changes of quality of minimally-processed pineapple (*Ananas comosus*, var. 'Queen Victoria') during cold storage: Fungi in the leading role. *Microorganisms.* 8, 185.

Martínez-Castellanos, G., Pelayo-Zaldívar, C., Pérez-Flores, L.J., López-Luna, A., Gimeno, M., Bárzana, E., Shirai, K. (2011). Postharvest litchi (*Litchi chinensis Sonn.*) quality preservation by *Lactobacillus plantarum*. *Post harvest Biol Technol.* 59, 172–178.

Motelica, L., Ficai, D., Ficai, A., Oprea, O.C., Durmu¸ Kaya, A. and Andronescu, E. (2020). Biodegradable Antimicrobial Food Packaging: Trends and Perspectives. *Foods*. 9, 1438.

Nawaz, F., Khan, M.N., Javed, A., Ahmed, I., Ali, N., Ali, M.I., Bakhtiar, S.M., and Imran, M. (2019). Genomic and Functional Characterization of *Enterococcus mundtii* QAUEM2808, Isolated From Artisanal Fermented Milk Product Dahi. *Front Microbiol.* doi: 10.3389/fmicb.2019.00434.

Pimentel-Filho, N. J., H. C. Mantovani, F. Diez-Gonzalez, and M. C. D. Vanetti. (2013). Inhibition of *Listeria* and *Staphylococcus aureus* by bovicin HC5 and nisin combination in milk. *J Agric Sci.* 5, 188–196.

Ramos, B., Brandão, T.R.S., Teixeira, P., Silva, C.L.M. (2020). Biopreservation approaches to reduce *Listeria monocytogenes* in fresh vegetables. *Food Microbiol.* 85, 103282.

Renye, J.A, Somkuti, G.A., and Steinberg, D.H. (2019). Thermophilin 109 is a naturally produced broad spectrum bacteriocin encoded within the blp gene cluster of *Streptococcus thermophilus*. *Biotechnol Lett.* 2, 283-292.

Ribeiro, S. C., Ross, R. P., Stanton, C., and Silva, C. C. (2017). Characterization and application of antilisterial enterocins on model fresh cheese. *J Food Prot*. 80, 1303–1316.

Sachadyn-król, M. and Agriopoulou, S. (2020). Ozonation as a method of abiotic elicitation improving the health-promoting properties of plant products—A review. *Molecules.* 25, 2416.

Sharma, V., Ranveer, R.C., Jain, N. and Aseri, G.K. (2019). Bacteriocin: production, different strategies of purification and applications. *Int J res pharma sci.* 10(3), 1808-1817.

Silva, C.C.G., Silva, S.P.M. and Ribeiro, S.C. (2018). Application of Bacteriocins and Protective Cultures in Dairy Food Preservation. *Front Microbiol.* 9, 594.

Siroli, L., Patrignani, F., Serrazanetti, D.I., Tabanelli, G., Montanari, C., Gardini, F. (2015). Lactic acid bacteria and natural antimicrobials to improve the safety and shelf life of minimally processed sliced apples and lamb's lettuce. *Food Microbiol.* 47, 74–84.

Sobrino-Lopez, A., and O. Martin-Belloso. (2008). Use of nisin and other bacteriocins for preservation of dairy products. *Int Dairy J.* 18, 329–343.

Soltani, S., Hammami, R., Cotter, P.D., Rebuffat, S. Said, L.B., Gaudreau, H., F., et al. (2020). Bacteriocins as a new generation of antimicrobials: toxicity aspects and regulations. *FEMS Microbiol Rev*. 39, 1–24.

Todorov, S.D., Leblanc, J.G., and Franco, B.D. (2012). Evaluation of the probiotic potential and effect of encapsulation on survival for *Lactobacillus plantarum* ST16Pa isolated from papaya. *World J Microbiol Biotechnol.* 28, 973-84.

Valdés, A., Ramos, M., Beltrán, A., Jiménez, A., and Garrigós, M. C. (2017). State of the art of antimicrobial edible coatings for food packaging applications. *Coatings*. 7:56. doi: 10.3390/ coatings7040056.

Verma, S. K., Sood, S. K., Saini, R. K., and Saini, N. (2017). Pediocin PA-1 containing fermented cheese whey reduces total viable count of raw buffalo (*Bubalis bubalus*) milk. *LWT Food Sci. Technol.* 83, 193–200.

Xie, Y., Zhang, M., Gao, X., Shao, Y., Liu, H., Jin, J., Yang, W., and Zhang, H. (2018). Development and antimicrobial application of plantaricin BM-1 incorporating a PVDC film on fresh pork meat during cold storage. *J Appl Microbiol.* 125(4), 1108-1116.

Yépez, A., Luz, C., Meca, G., Vignolo, G., Mañes, J., Aznar, R. (2017). Biopreservation potential of lactic acid bacteria from Andean fermented food of vegetal origin. *Food Control.* 78, 393–400.

Zgheib, H., Drider, D. and Belguesmia, Y. (2020). Broadening and Enhancing Bacteriocins Activities by Association with Bioactive Substances. *Int J Environ Res Public Health.* 17, 7835.

POTENTIAL OF YEAST IN FUNCTIONAL FOOD INDUSTRY

Akshata Mandloi[1] and Neelam Jain*[2]

[1] Student, Amity Institute of Biotechnology, Kant-Kalwar, NH11C, RIICO Industrial Area, Jaipur, Rajasthan 303007, India
[2] Professor, Amity Institute of Biotechnology, Kant-Kalwar, NH11C, RIICO Industrial Area, Jaipur, Rajasthan 303007, India

*Corresponding Author: njain1@jpr.amity.edu

Introduction

A boom in the demand for healthier baked food options evoked studies related to sourdough microbiota having beneficial metabolic traits which are utilized as a potential source in the industry of functional food. Yeasts have several health benefits including antioxidant activities. Yeasts having a great history of its utilization in fermentation of food has evidenced its significance and potential in industry of functional food. Isolation of various yeast species from foods (that are fermented) have led to its characterization and application in function food industries as starters or co-starters. In recent decade, modern research based on functional foods and nutraceuticals development with the help of yeast have shown robust potential in the branch of food biotechnology.

Potential of Yeast in Functional food Industry

Eukaryotic microorganisms, yeast having potential in the functional food industry comes under phyla Basidiomycetes and Ascomycetes. Yeast is utilized in the production of different typed of fermented products (Chandra, Sharma, & Arora, 2020). During the natural fermentation process, growth of different types of yeast or mixed cultured fermentation supported by filamentous fungi and LAB

decides the final product development (Palla et al., 2020). Yeast plays vital roles in fermentation process of food including reduction of toxins and anti-nutritional factors, production of alcohol, enhancing nutritional properties, production of organic acids & improvement of texture, flavor, & aroma (Rai, Pandey, & Sahoo, 2019). Isolation of various yeast strains from food products which are fermented have led to its characterization and application in industries of functional foods as starters or co-starters. In nutraceutical production, yeasts play the key role and are also responsible for the production of different types of functional foods (Corbo, et al., 2014). Functional foods can be termed as conventional food product which are included in normal diet that are beneficial for health apart from their nutritional properties. Nutraceuticals are termed as purified food components that are scientifically proven to have several health benefits. In food products that are fermented, the derivation of metabolites, enzymes or synergistically with other groups of microbes enhances bioactive components which improve functional properties (Gobbetti, Cagno, & Angelis, 2010).

In the industry of functional food, yeasts have several applications (Fig.1) such as (i) producing great value nutraceuticals, (ii) as probiotics, and (iii) extraction of bioactive metabolites (Giavasis, 2014) (Table 1). Yeasts producing specific enzyme that help in bioactive compounds that are substrate derived like free polyphenols, peptides and oligosaccharides (Johnson, & Echavarri-Erasun, 2011). In recent times, for the development of oligosaccharides, folates, Epolyphenols, and bioactive compounds potential strains of yeast have been developed by metabolic engineering (Rai, Pandey, & Sahoo, 2019).

Yeast as probiotic agent

For the wellness and improvement of the host's health probiotic microorganism are used in the form of supplements. To include a microorganism as probiotic it should have characteristics such as auto-aggregation, low pH, hydrophobicity of cell surface and

tolerance to bile (Lourens-Hattingh, & Viljoen, 2001). Yeast is also a potential probiotic. The species of yeast which include properties of probiotic are *Pichia kudriavzeii, Saccharomyces cerevisiae, Pichia fermentase, Kulyveromyces marxianus, Yarrowia lipolytica, Torulaspora delbrueckii, Kluyveromyces lactis, Saccharomyces boulardii, and Debaryomyces hansenii* (Rai, Pandey, & Sahoo, 2019). *Saccharomyces boulardii* have various probiotic properties and is used as a preventive and therapeutic agent. Furthermore, it is also efficient and economical probiotic yeast for treating variety of diarrhoea in animals & humans. As a probiotic agent it can effectively prevent diarrhoea associated with antibiotics in children and also adults (Kumura, et al., 2004). Probiotic yeast, *Kulyveromyces marxianus* CIDCA 8154, reduce oxidative stress and also possess anti-inflammatory effect (Fleet, & Balia, 2006). *Saccharomyces cerevisiae* ARDMC1, a yeast strain, which is isolated from starter cake of traditional rice beer, reflected hypocholesterolemic activity in the rat model apart from possessing probiotic effect (Kumura, et al., 2004). *Pichia kudriavzeii* AS-12 secrete probiotic metabolites exhibiting anticancer effect in cells causing colon cancer (Caco-2 and HT-29) (Saber, et al., 2017). Similarly, *Kulyveromyces marxianus* AS41which is isolated from dairy origin, produce metabolites which have probiotic properties shows proapoptotic action on epithelial cancer cells (Czerucka, Piche, & Rampal, 2007).

Yeast as functional food ingredient

Yeast cells are a source of β-glucan (Fig.1), fiber which are known to exhibit anti-inflammatory activity, improve immune system and lower blood cholesterol levels (Li, Karboune, & Asehraou, 2020). In different genus of yeast the bio-chemical composition of cell wall is different, it is primarily dependent on conditions of growth, that have an effect on the functional attributes of polysaccharide present in the cell wall of yeast (Shurson, 2018). Cell wall of *S. cerevisiae* comprises of chitin (2%), mannoprotein complex (35-40%), and β(1 → 3)-D-glucan (50–55%), β(1 → 6)-D-glucan (5–10%). Good antioxidant activity is shown by (1 → 3), (1 → 6)–D-glucan which

is isolated from cell wall fraction of *Saccharomyces cerevisiae.* In the cell walls of *S. cerevisiae,* the glucan content is affected by variation in growth medium (Querol, & Fleet,2006). The constituents of yeast cell wall used in fermented food are a significant bioactive molecules source that provide functional properties to the products (Bacchetti, et al., 2020).

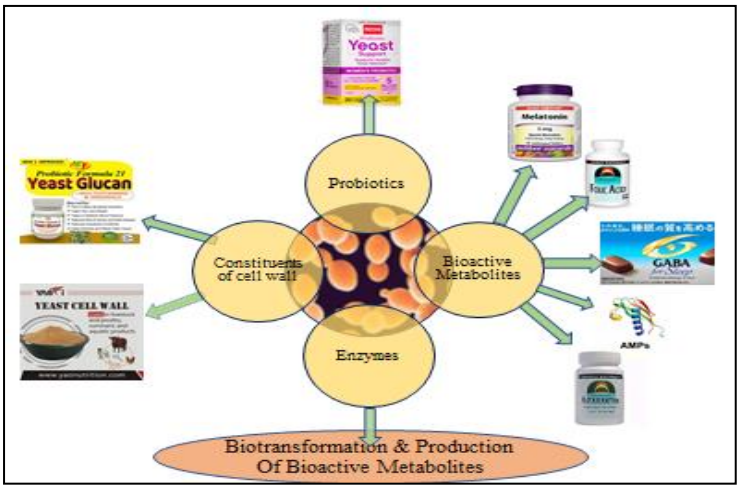


Figure 1: Potential of Yeast in Functional food Industry

Nutraceutical potential of yeast

a) Production of Bioactive metabolites by Yeast

Yeast species have both therapeutic properties as well as probiotic potential (Table 1). Several bioactive components are produced by yeast, which form an essential part of functional food industry including carotenoids, γ-aminobutyric acid (GABA), and folate (Rahmat, & Kang, 2020). Reportedly, GABA is produced via decarboxylation reaction which occurs by the enzyme glutamate decarboxylase (Xu, et al., 2020). One of the important cofactors in various biochemical reactions is folate and the deficiency of folate is

becoming a global problem. Folate rich fermented beverages and foods are the outcome of fermentation done using yeast for the evolution of functional foods. Current researches show that the strain of *Pichia kudriavzevii* isolated from the traditional cereal-based food of West Africa can potentially produce folate (Gmelch, et al.,2020). The naturally pigmented bioactive compounds known as carotenoids are potent ingredient in the industry of functional food as they have the ability to prevent oxidative stress and diseases related to it. *Sporobolomyces spp, Phaffia rhodozyma, Sporidiobolus spp., Rhodotorula spp.,* and *Rhodosporidium spp.* are commonly known species of yeasts as they produce variety of carotenoids. Torularhodin, astaxanthin, torulene, β and γ-carotene are the important carotenoids produced by yeast (Vargas-Sinisterra, & Ramírez-Castrillón, 2020). A non-protein thiol peptide, glutathione, can reduce the unfavourable effects of reactive oxygen species, hence it is one of the potential ingredients for application in nutraceuticals. Glutathione is majorly produced by the *Candida utilis and S. cerevisiae.* Strains of yeast & their bioactive metabolites in fermented products have good influence on consumers (Rai, Pandey, & Sahoo, 2019).

b) Yeast in biotransformation

During the process of fermentation, enzymes are produced which result in a variety of biochemical changes that are dependent on the specificity to the substrate. The changes occurring due to involvement of enzymes are accountable for (i)biomolecule transformation into its highly bioactive form, and (ii)complex substrate hydrolyzing to simple form. The outcome of the biochemical change is dependent on the substrate's biochemical composition and the microbes utilized in fermentation at the level of strain (Bianco, et al., 2020). The outcome of interaction between food components and yeast is release of various types of metabolites that are beneficial to health. The health benefits are dependent on the biochemical composition of the product. Bioactive oligosaccharides, biologically active peptides and free polyphenols

are the metabolites found in yeast fermented foods and are produced by bioprocess dependent on yeast (Rai, Pandey, & Sahoo, 2019).

c) Organically bound selenium yeast

Selenium or 'Se' is a significant micronutrient with anti-ageing properties and also plays a major role in prevention of cancer. To produce functional foods rich in selenium yeast cells can be useful because they can accumulate as well as also help in transformation of inorganic selenium to its organic form. In rats, selenium enriched *Candida utilis* has improved antioxidant capacity, growth performance and immunity factor after the intake of optimum dietary supplement (Liu, et al., 2020).

Table 1. Functional attributes and Nutraceutical production by yeast

Yeast Species	Functional Properties
S. cerevisiae CBS7764, *Candida glabrata* TY26 *and S. cerevisiae* TY08, *Pichia kudriavzevii, S. cerevisiae* ALKO 743, *S. cerevisiae* TS 146, *Candida milleri* CBS.	Folate content enhancement
Pichia kudriavzevii, M. caribbica, Pichia kudriavzevii OG32.	Probiotic properties
S.cerevisiae, Hanseniaspora sp., Pichia kudriavzevii OG32, baker's yeast.	Antioxidant properties
Pichia kudriavzevii OG32	Isoflavone and hypolipidaemic content
S. cerevisiae, Saccharomyces bayanus and Saccharomyces uvarum	Selenomethionine increasing
Baker's yeast	Phenolics increasing
S. cerevisiae	Prebiotic properties

Yeast Species	Nutraceuticals
Yarrowia lipolytica strain Enop56	Fructooligosaccharides
S. cerevisiae CEN.PK2	Lycopene
Pichia pastoris X-33	Lycopene
Y. lipolytica	Lycopene
S. cerevisiae	Lycopene
S. cerevisiae	Naringenin
S. cerevisiae	Resveratrol, naringenin, genistein, kaempferol and quercetin
S. cerevisiae	Six Flavanoids
S. cerevisiae	Kaempferol
S. cerevisiae	Astaxanthin
Y. lipolytica	γ-linolenic acid

Genetic engineering in yeast and functional food industry

Genetic engineering is a promising tool used for the production of high value additives for functional food (Table 1). It improves the efficiency of yeast strains that in turn produce particular enzymes. Recombinant yeast species *Saccharomyces cerevisiae* JZH expressed with heterologous endo-inulinase gene is a one-step bioprocess that results in high yield of fructo-oligosaccharides (Lajus, et al., 2020). To increase the folate content in wine, bioengineering of wine is also done. It is done by overexpressing the FOL2 (folate biosynthesis) gene. For those yeast cells which do not have any components of carotenoid biosynthesis pathway, metabolic engineering is done to produce carotenoids. Important nutraceutical for functional food industry such as DHA (docosahexaenoic acid), PUFA (polyunsaturated fatty acids), EPA (eicosatetraenoic acid) and linolenic acid are beneficial for health and are also produced by

yeasts. In the current scenerio, application of host yeast cell for the production of polyunsaturated fatty acids (PUFA) by genetic engineering. In the near future, it is expected that the approach genetic engineering will create significant bioprocess for nutraceutical using yeasts (Ibrahim, et al., 2020).

Conclusion

Based on the past studies and the recent researches, Yeast play a vital role in food biotechnology for the production of nutraceuticals and functional food. Yeasts have also been used in the development of substrate derived bioprocess for the production of nutraceuticals and bioactive metabolites like polyphenols, β- glucan, prebiotic oligosaccharides, carotenoids, organic selenium, glutathione, γ-aminobutyric acid, and bioactive peptides. As the awareness regarding the role of yeast in production has been increasing, to improve the functional properties of the product it is important to select the potential strains of yeast. For the production of nutraceuticals, modern biotechnological tools have been used which have resulted in development of recombinant yeasts with better and improved features. However, there are still a variety of yeasts from the traditional fermented food that remain unexploited for their technological properties.

References

Bacchetti, T., Annibaldi, A., Comitini, F., Ciani, M., Damiani, E., Norici, A., ... & Olivotto, I. (2020). Alternative Ingredients for Feed and Food. In *The First Outstanding 50 Years of "Università Politecnica delle Marche"* (pp. 529-545).

Bianco, A., Budroni, M., Zara, S., Mannazzu, I., Fancello, F., & Zara, G. (2020). The role of microorganisms on biotransformation of brewers' spent grain. *Applied microbiology and biotechnology*, *104*(20), 8661–8678.

Chandra, P., Sharma, R. K., & Arora, D. S. (2020). Antioxidant compounds from microbial sources: A review. *Food research international (Ottawa, Ont.)*, *129*, 108849.

Corbo, M. R., Bevilacqua, A., Petruzzi, L., Casanova, F. P., & Sinigaglia, M. (2014). Functional beverages: the emerging side of functional foods: commercial trends, research, and health implications. *Comprehensive Reviews in Food Science and Food Safety*, *13*(6), 1192-1206.

Czerucka, D., Piche, T., & Rampal, P. (2007). Review article: yeast as probiotics -- Saccharomyces boulardii. *Alimentary pharmacology & therapeutics*, *26*(6), 767–778.

Fleet, G., & Balia, R. (2006). The public health and probiotic significance of yeasts in foods and beverages. In *Yeasts in food and beverages* (pp. 381-397).

Giavasis I. (2014). Bioactive fungal polysaccharides as potential functional ingredients in food and nutraceuticals. *Current opinion in biotechnology*, *26*, 162–173.

Gmelch, L., Wirtz, D., Witting, M., Weber, N., Striegel, L., Schmitt-Kopplin, P., & Rychlik, M. (2020). Comprehensive Vitamer Profiling of Folate Mono- and Polyglutamates in Baker's Yeast (*Saccharomyces cerevisiae*) as a Function of Different Sample Preparation Procedures. *Metabolites*, *10*(8), 301.

Gobbetti, M., Cagno, R. D., & De Angelis, M. (2010). Functional microorganisms for functional food quality. *Critical reviews in food science and nutrition*, *50*(8), 716-727.

Guo, L., Xu, D., Fang, F., Jin, Z., & Xu, X. (2020). Effect of glutathione on wheat dough properties and bread quality. *Journal of Cereal Science*, *96*, 103116.

Hilmi Ibrahim, Z., Bae, J. H., Lee, S. H., Sung, B. H., Ab Rashid, A. H., & Sohn, J. H. (2020). Genetic Manipulation of a Lipolytic

Yeast *Candida aaseri* SH14 Using CRISPR-Cas9 System. *Microorganisms*, *8*(4), 526.

Johnson, E. A., & Echavarri-Erasun, C. (2011). Yeast biotechnology. In *The yeasts* (pp. 21-44).

Kumura, H., Tanoue, Y., Tsukahara, M., Tanaka, T., & Shimazaki, K. (2004). Screening of dairy yeast strains for probiotic applications. *Journal of dairy science*, *87*(12), 4050–4056.

Lajus, S., Dusséaux, S., Verbeke, J., Rigouin, C., Guo, Z., Fatarova, M., Bellvert, F., Borsenberger, V., Bressy, M., Nicaud, J. M., Marty, A., & Bordes, F. (2020). Engineering the Yeast *Yarrowia lipolytica* for Production of Polylactic Acid Homopolymer. *Frontiers in bioengineering and biotechnology*, *8*, 954.

Li, J., Karboune, S., & Asehraou, A. (2020). Mannoproteins from inactivated whole cells of baker's and brewer's yeasts as functional food ingredients: Isolation and optimization. *Journal of food science*, *85*(5), 1438–1449.

Liu, B., Xiong, Y. L., Jiang, J., Yu, D., & Lin, G. (2020). Cellular antioxidant mechanism of selenium-enriched yeast diets in the protection of meat quality of heat-stressed hens. *Food Bioscience*, 100798.

Lourens-Hattingh, A., & Viljoen, B. C. (2001). Growth and survival of a probiotic yeast in dairy products. *Food Research International*, *34*(9), 791-796.

Palla, M., Blandino, M., Grassi, A., Giordano, D., Sgherri, C., Quartacci, M. F., Reyneri, A., Agnolucci, M., & Giovannetti, M. (2020). Characterization and selection of functional yeast strains during sourdough fermentation of different cereal wholegrain flours. *Scientific reports*, *10*(1), 12856.

Querol, A., & Fleet, G. H. (Eds.). (2006). Yeasts in food and beverages.

Rahmat, E., & Kang, Y. (2020). Yeast metabolic engineering for the production of pharmaceutically important secondary metabolites. *Applied microbiology and biotechnology*, *104*(11), 4659–4674.

Rai, A. K., Pandey, A., & Sahoo, D. (2019). Biotechnological potential of yeasts in functional food industry. *Trends in Food Science & Technology*, *83*, 129-137.

Saber, A., Alipour, B., Faghfoori, Z., & Yari Khosroushahi, A. (2017). Cellular and molecular effects of yeast probiotics on cancer. *Critical reviews in microbiology*, *43*(1), 96-115.

Shurson, G. C. (2018). Yeast and yeast derivatives in feed additives and ingredients: Sources, characteristics, animal responses, and quantification methods. *Animal feed science and technology*, *235*, 60-76.

Vargas-Sinisterra, A. F., & Ramírez-Castrillón, M. (2020). Yeast carotenoids: production and activity as antimicrobial biomolecule. *Archives of microbiology*, 10.1007/s00203-020-02111-7. Advance online publication.

Xu, L., Zhu, L., Dai, Y., Gao, S., Wang, Q., Wang, X., & Chen, X. (2020). Impact of yeast fermentation on nutritional and biological properties of defatted adlay (Coix lachryma-jobi L.). *LWT*, 110396.

PROBIOTICS: A BOON TO OUR LIVES

Mohit Thorecha[1] and Neelam Jain*[2]

[1] Student, Amity Institute of Biotechnology, Kant-Kalwar, NH11C, RIICO Industrial Area, Jaipur, Rajasthan 303007, India
[2] Professor, Amity Institute of Biotechnology, Kant-Kalwar, NH11C, RIICO Industrial Area, Jaipur, Rajasthan 303007, India

*Corresponding Author: njain1@jpr.amity.edu

Introduction

Probiotics are like "tiny magic bullets" which when hits in adequate doses into the live tissues explores their broad spectrum bioactivity. These are viable microorganism that promotes or support a beneficial balance of the autochthonous microbial population of the gastrointestinal tract. Probiotics are a group of live, non pathogenic microbes that are consumed for the proper functioning of our immunity system by maintaining microbial balance. These microbes can reside in many parts of our body such as lungs, Gastro-Intestinal Tract, urinary tract, buccal cavity. Unlike that of antibiotics, probiotics have no side-effects as they don't kill the healthy bacteria and they contribute to heathier microbiome of our body (Williams, 2010). The word 'Probiotic' is also applicable for certain yeasts as it is often mistaken as a term that strictly abides by the 'good bacteria'. Extensive research and great results in the field of probiotics have led to the eradication of the fact that the bacteria are still considered pathogenic and harmful. In addition to this, this group of microbes are mistaken as 'Prebiotics', which is also wrong as prebiotics are special plant fibres which add to the count of beneficial microbes in our gut by stimulating the growth of pre-existing microorganisms (Grandy et al., 2010).

There are many ways to consume probiotics in our everyday lives such as lozenges, capsules, fermented beverages, powders, some

yogurts, etc. For children, probiotics can be inculcated in various chocolates to enhance the flavor. In this world of urbanization, where health has become such a great matter of concern, there is an increase in consumption of probiotics as many individuals lack essential nutrients which eventually lead to a weak immune system. In such cases, probiotics have proved to be useful in treating diseases such as lozenges of *Lactobacillus reuteri* is used in the treatment of oral wounds (Twetman et al., 2018). In the case of bacteria, genus like *Lactobacillus* and *Bifidobacterium* are commonly used and *Saccharomyces* is the common genus in the case of fungi.

Mechanism of action of probiotics

Probiotics promote the proper functioning of the immune system by increasing healthy bacterial count. These bacteria usually reside in our guts. They maintain an equilibrium between microbial count as increase in any type of bacteria (beyond the desired count) can lead to certain health issues. In order words, they help in establishing a neutral point. These microbes (both fungi and bacteria) fight off the disease by increasing their count and hence, decreasing the inflammation due to certain metabolic reactions that occur in our body. Besides this, one must keep in mind that probiotics can't be considered as an obligate method of disease's cure. The terms 'Probiotics' and 'Anti-biotics' are not the same as one promotes the growth of good bacteria and the other kills bad bacteria as well as some beneficial bacteria too, thus disrupting the microbe's complex community. The mechanism of probiotic action starts with the consumption and after the consumption of probiotics, they compete with pathogenic bacteria and this process is termed Antagonism. As mentioned in Figure 1, once the bacterium is done with antagonism, they block the active sites present in the epithelium and now the pathogenic bacteria are well – exposed to the threats. By getting attached to the epithelium, the probiotic bacteria increase their number. Now, they generate an immune stimulation that acts against

pathogenic bacteria and this process is termed as Immunomodulation. After increasing their population and immunomodulation, the probiotic bacteria produce toxins like Bacteriocins (peptide toxins) which further kills pathogenic bacteria (Stavropoulou et al., 2020)

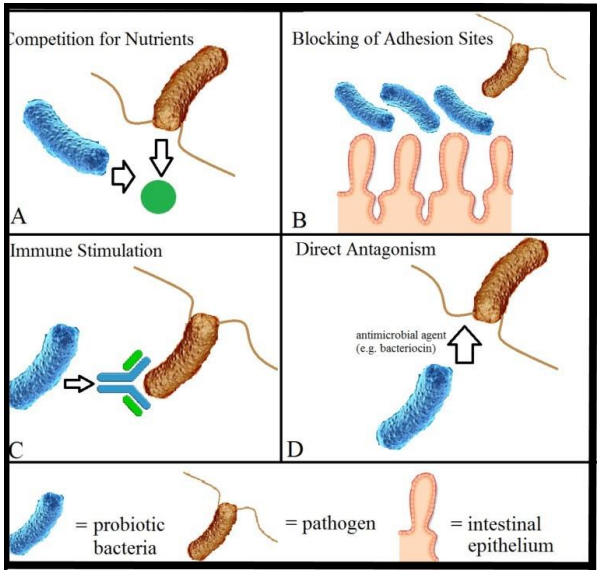


Figure 1. Mechanism of action of probiotics (Source: Fop Map Every day, 2019)

Forms of probiotics

There are many food products available in the market such as kefir, kombucha, pickles, yogurt sauerkraut, kimchi, tempeh, etc. From the above given examples, yogurt is the most commonly used as an exceptional source of good bacteria. Some bacteria like *Lactobacillus reuteri* are used to treat oral infections in the form of lozenges (Twetman S. et al, 2018). Probiotics capsules of Florajen are being used to reduce the risk of antibiotic-associated diarrhea in the age group of 18-65 years (Jafarnejad, S., et al, 2016). Companies

like Herbalife has introduced probiotics in the form of powder containing *Bacillus coagulans.* This product named 'Simply Probiotic' helps in curing irritable bowel movement, airway infections (Mu, Y. et al, 2019). Besides this, probiotics drops are introduced in market by Mommy's Bliss. It includes *Lactobacillus rhamnosus* as the main constituent which aids in proper digestion and proper immune function in infants (Markowiak, P. et al, 2017). The visualisation of above – mentioned content has been illustrated in Figure 2.

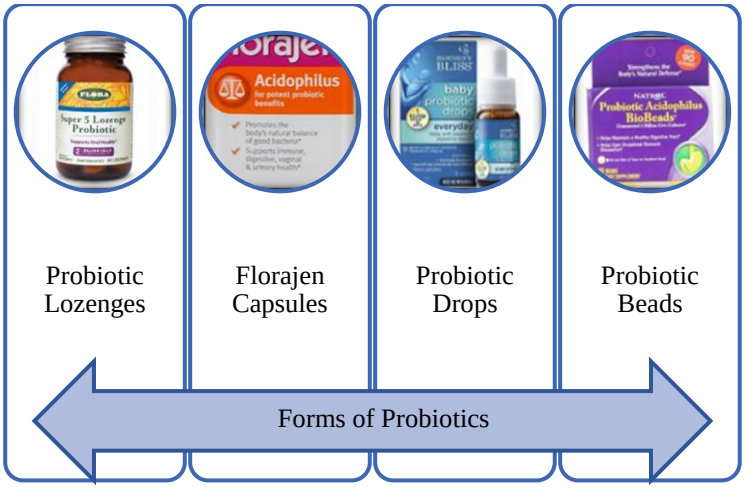


Figure 2. Forms of probiotics

Sources of Probiotics

As shown in the Figure 3, there are numerous sources of probiotics available in the form of dairy products, fermented foods and beverages such as tempeh, kefir, yogurt, etc. In the case of a child, we can enhance the nutritional value of chocolate and can make the probiotic healthier and tastier. Companies like Yakult are providing probiotic supplements which are easily available in market. Ginger

ale or ginger beer is commonly used ingredient in fish batter which contributes to fried fish's unique texture. The name of Yakult in current trend has rendered us a ray of hope for a great future in the field of probiotics.

Figure 3. Sources of Probiotics

Health Benefits of Probiotics

There are numerous researches going on probiotics and their health impacts (Table 1). However, some research concluded that microbes like *Lactobacillus acidophilus* have anti-carcinogenic properties and their use in diet helped in preventing colon and rectal cancer (Burns

et al., 2000). It has been found that *Lactobacillus* bacteria produce hydrogen peroxide, diacetyl, and bacteriocin which proved to be antimicrobial in nature. Hence, it forfeits pathogenic to prevail inside the gastro-intestinal tract (Helander, 1997). Also, Consumption of probiotics helps in the prevention of atherosclerosis and Coronary Heart diseases by lowering down the cholesterol level. It interferes with the level of LDL cholesterol and triacylglycerol (Cavallini et al., 2009). Some probiotics also help in prevention of diabetes and obesity as the gut flora suppresses insulin resistance, energy harvest, and blood LPS levels (Kobyliak et al., 2016). Also, the growth of probiotic bacteria leads to the improved synthesis of SCFA (short-chain fatty acids) which have proved to be useful for the prevention of type 2 diabetes, obesity (Peng et al., 2018). Many of the probiotic products are available as organic probiotics, wide-spectrum antibiotic, capsules, pickles, etc. Probiotic microbes increase the nutritional value of the product and hence, they play a vital role in prophylaxis.

Challenges ahead in the field of probiotics

The probiotics may have many benefits, but there are many challenges also that need to be tackled. Firstly, bacteria that reside in the gastro-intestinal tract doesn't last forever. It has a limited life span and that is why, we have to consume probiotics on regular basis. Also, during culture preparation, there is a possibility of culture contamination and obtaining pure culture is the very first step of manufacturing and keeping in a frozen state requires quite a lot of resources and transportation is a cumbersome process. In addition to this, we have to ensure that the bacteria must survive the acidic pH of the stomach. If we talk about market challenges, the main challenge being faced by a seller is storing probiotics at the ideal temperature as even a slight increment in temperature leads to the death of microbes and the consumers are still far away from accepting the healthy aspects of bacteria and fungi. At last, the use

of probiotics is still not considered safe for immunosuppressed individuals (Stadlbauer, 2015).

Table 1. Health impacts of probiotics

Probiotic strain	Products	Health impact	Reference
Lactobacillus GG	Wide–spectrum antibiotic	Anti-tumour	Di Cerbo, 2016
Lactobacillus acidophilus	Curae Health Gut Biotix capsule	Anti-microbial action	Anjum et al., 2014
Lactobacillus delbrueckii ssp. bulgaricus	Yogurt	Yogurt fermentation	Anbukkarasi et a.l, 2014
Lactobacillus cremoris	Himalayan organic probiotics	Anti-diabetic	Sasikumar et al., 2017
Lactobacillus brevis	Pickles	Supplement strains in yogurt	Rönkä, 2003
Lactobacillus reuteri	Swanson probiotic plus	Fights food – borne pathogen	Arqués, 2011
Lactobacillus paracasei	Used as supplement	Kills pathogenic bacteria	Chuang L.C. et al., 2010
Bifidobacterium longum	Soy yogurt	Maintain gut microbiome	Wong et al., 2019
Bifidobacterium animalis	Danon yogurt	Impact on gingival health	Kuru et al., 2017
Lactococcus lactis	Purayati complete probiotic capsules	Improvement in vaginal health	Gao et al., 2011
Streptococcus oralis	Nasal sprays	Heals nasal infections	Bidossi et al., 2018

Conclusion

The global forecast of probiotic consumption (Mamtani, 2019) shows a consistent increment in probiotic market from 2015-2026

with an annual increment of 7.3% with the market value of 2 billion USD in 2018. Accelerating demands and alarming needs are urging for extensive research in the field of probiotics. There is a possibility of using probiotics as a cure rather than just limiting their use to prophylaxis. There are many challenges and many more will come, which can be easily dealt with the advancement in technology. Even in the gruesome COVID -19 pandemic, immunity has played a crucial role in fighting against the infection. Nature has always favored individuals with better abilities. There are still many microbes to be discovered and explored and we can expect unbelievable wonders from the field of probiotics.

References

Anbukkarasi, K., UmaMaheswari, T., Hemalatha, T., Nanda, D. K., Singh, P., & Singh, R. (2014). Preparation of low galactose yogurt using cultures of Gal+ Streptococcus thermophilus in combination with Lactobacillus delbrueckii ssp. bulgaricus. *Journal of food science and technology*, *51*(9), 2183-2189.

Anjum, N., Maqsood, S., Masud, T., Ahmad, A., Sohail, A., & Momin, A. (2014). Lactobacillus acidophilus: characterization of the species and application in food production. *Critical reviews in food science and nutrition*, *54*(9), 1241-1251.

Arqués, J. L., Rodríguez, E., Nuñez, M., & Medina, M. (2011). Combined effect of reuterin and lactic acid bacteria bacteriocins on the inactivation of food-borne pathogens in milk. *Food Control*, *22*(3-4), 457-461.

Bidossi, A., De Grandi, R., Toscano, M., Bottagisio, M., De Vecchi, E., Gelardi, M., & Drago, L. (2018). Probiotics Streptococcus salivarius 24SMB and Streptococcus oralis 89a interfere with biofilm formation of pathogens of the upper respiratory tract. *BMC infectious diseases*, *18*(1), 653.

Burns, A. J., & Rowland, I. R. (2000). Anti-carcinogenicity of probiotics and prebiotics. *Current issues in intestinal microbiology*, *1*(1), 13–24.

Cavallini, D. C., Bedani, R., Bomdespacho, L. Q., Vendramini, R. C., & Rossi, E. A. (2009). Effects of probiotic bacteria, isoflavones and simvastatin on lipid profile and atherosclerosis in cholesterol-fed rabbits: a randomized double-blind study. *Lipids in health and disease*, *8*(1), 1.

Chuang, L. C., Huang, C. S., Ou-Yang, L. W., & Lin, S. Y. (2011). Probiotic Lactobacillus paracasei effect on cariogenic bacterial flora. *Clinical oral investigations*, *15*(4), 471-476.

Di Cerbo, A., Palmieri, B., Aponte, M., Morales-Medina, J. C., & Iannitti, T. (2016). Mechanisms and therapeutic effectiveness of lactobacilli. *Journal of Clinical Pathology*, *69*(3), 187-203.

Gao, Y., Lu, Y., Teng, K. L., Chen, M. L., Zheng, H. J., Zhu, Y. Q., & Zhong, J. (2011). Complete genome sequence of Lactococcus lactis subsp. lactis CV56, a probiotic strain isolated from the vaginas of healthy women.

Grandy, G., Medina, M., Soria, R., Terán, C. G., & Araya, M. (2010). Probiotics in the treatment of acute rotavirus diarrhoea. A randomized, double-blind, controlled trial using two different probiotic preparations in Bolivian children. *BMC infectious diseases*, *10*, 253. https://doi.org/10.1186/1471-2334-10-253

Helander, I., von Wright, A., & Mattila-Sandholm, T. M. (1997). Potential of lactic acid bacteria and novel antimicrobials against Gram-negative bacteria. *Trends in Food Science & Technology*, *8*(5), 146-150.

Jafarnejad, S., Shab-Bidar, S., Speakman, J. R., Parastui, K., Daneshi-Maskooni, M., & Djafarian, K. (2016). Probiotics Reduce the Risk of Antibiotic-Associated Diarrhea in Adults

(18-64 Years) but Not the Elderly (>65 Years): A Meta-Analysis. *Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition*, *31*(4), 502–513. https://doi.org/10.1177/0884533616639399

Kobyliak, N., Conte, C., Cammarota, G., Haley, A. P., Styriak, I., Gaspar, L., ... & Kruzliak, P. (2016). Probiotics in prevention and treatment of obesity: a critical view. *Nutrition & metabolism*, *13*(1), 14.

Kuru, B. E., Laleman, I., Yalnızoğlu, T., Kuru, L., & Teughels, W. (2017). The influence of a Bifidobacterium animalis probiotic on gingival health: a randomized controlled clinical trial. *Journal of periodontology*, *88*(11), 1115-1123.

Mamtani, K. A. (2019, 12 2). *Probiotics Market size to exceed $3.5 bn by 2026*. Retrieved 12 15, 2020, from Global Market Insights: https://www.gminsights.com/pressrelease/probiotics-market

Markowiak, P., & Śliżewska, K. (2017). Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. *Nutrients*, *9*(9), 1021. https://doi.org/10.3390/nu9091021

Mu, Y., & Cong, Y. (2019). *Bacillus coagulans* and its applications in medicine. *Beneficial microbes*, 1–10. Advance online publication. https://doi.org/10.3920/BM2018.0016

Peng, J., Xiao, X., Hu, M., & Zhang, X. (2018). Interaction between gut microbiome and cardiovascular disease. *Life sciences*, *214*, 153–157. https://doi.org/10.1016/j.lfs.2018.10.063

Rönkä, E., Malinen, E., Saarela, M., Rinta-Koski, M., Aarnikunnas, J., & Palva, A. (2003). Probiotic and milk technological properties of Lactobacillus brevis. *International journal of food microbiology*, *83*(1), 63-74.

Sasikumar, K., Vaikkath, D. K., Devendra, L., & Nampoothiri, K. M. (2017). An exopolysaccharide (EPS) from a Lactobacillus plantarum BR2 with potential benefits for making functional foods. *Bioresource technology*, *241*, 1152-1156.

Stadlbauer V. (2015). Immunosuppression and probiotics: are they effective and safe?. *Beneficial microbes*, *6*(6), 823–828. https://doi.org/10.3920/BM2015.0065

Stavropoulou, E., & Bezirtzoglou, E. (2020). Probiotics in Medicine: A Long Debate. *Frontiers in immunology*, *11*, 2192. https://doi.org/10.3389/fimmu.2020.02192

Twetman, S., Keller, M. K., Lee, L., Yucel-Lindberg, T., & Pedersen, A. (2018). Effect of probiotic lozenges containing Lactobacillus reuteri on oral wound healing: a pilot study. *Beneficial microbes*, *9*(5), 691–696. https://doi.org/10.3920/BM2018.0003

Williams N. T. (2010). Probiotics. *American journal of health-system pharmacy : AJHP : official journal of the American Society of Health-System Pharmacists*, *67*(6), 449–458. https://doi.org/10.2146/ajhp090168

Wong, C. B., Odamaki, T., & Xiao, J. Z. (2019). Beneficial effects of Bifidobacterium longum subsp. longum BB536 on human health: Modulation of gut microbiome as the principal action. *Journal of Functional Foods*, *54*, 506-519.

ANTIBIOTIC RESISTANCE IN BACTERIA

Umesh Chandra Pachouri[1*], Simranjeet Singh[2], Manoj Kumar[3] and Joginder Singh[4*]

[1]Associate Professor, Department of Biotechnology, Lovely Professional University, Phagwara, Punjab, India
[2]Post-Docotoral Researcher, Interdisciplinary Centre for Water Research (ICWaR), Indian Institute of Sciences, Bangalore, India
[3]Associate Professor, Department of Life Sciences, Central University Jharkhand, Brambe, Ranchi, Jharkhand, India
[4]Professor, Department of Biotechnology, Lovely Professional University, Phagwara, Punjab, India

[*]Corresponding Authors: pachouriu@gmail.com; joginder.15005@lpu.co.in

Introduction

Bacteria are amid the most malleable organisms on the Earth. The widespread use of antibiotics both inside and outside of medicine is playing a significant role in the appearance of resistant bacteria (Li and Webster, 2018**).** Resistance is defined as bacteria that are not inhibited by usually achievable systemic concentration of an agent with normal dosage schedule and/or fall in the minimum inhibitory concentration ranges. Likewise, the multiple drug resistance is defined as the resistance to two or more drugs or drug classes (Chang et al. 2015). Acquisition of resistance to one antibiotic conferring resistance to another antibiotic, to which the organism has not been exposed, is called cross resistance (Ghodousi et al. 2019).

The four main mechanisms by which microorganisms exhibit resistance to antibiotics are:

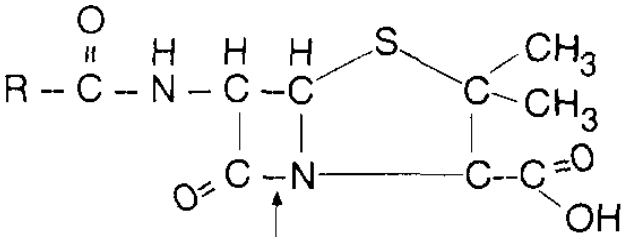
1. Drug inactivation or modification: e.g. enzymatic deactivation of *Penicillin* G in some penicillin-resistant bacteria through the production of β-lactamases.

2. Alteration of target site: e.g. alteration of PBP (Penicillin binding protein)—the binding target site of penicillins—in MRSA (Methicillin resistant *Staphylococcus aureus*) and other penicillin-resistant bacteria.
3. Alteration of metabolic pathway: e.g. some sulfonamide-resistant bacteria do not require para-aminobenzoic acid (PABA), an important precursor for the synthesis of folic acid and nucleic acids in bacteria inhibited by sulfonamides. Instead, like mammalian cells, they turn to utilizing preformed folic acid.
4. Reduced drug accumulation: by decreasing drug permeability and increasing active efflux (pumping out) of the drugs across the cell surface.

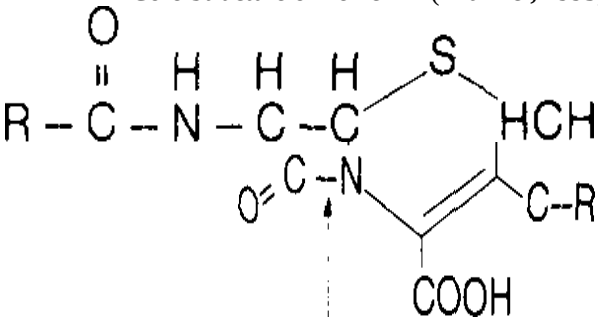
Watanabe discovered MDR in enteric bacteria brought about by the resistance transfer factors (RTFs) which are autonomous, extra-chromosomal and often self-transmissible plasmids (Watanabe et al.,1963). The number of antibiotics belonging to various families, their varied modes of action and the number of bacteria in which antibiotic resistance has been documented suggest that, in principle, any microbe could develop resistance to any antibiotic. Some modern approaches to the prediction of antibiotic resistance have been suggested (Van Camp et al., 2020). The classical example of this mode of resistance is the action of β - lactamase enzymes which cleave the β -lactam ring of penicillin, cephalosporin, etc. Penicillin is a unique molecule having a fused *b*-lactam– thiazolidine ring system, wherein the strained *b*-lactam ring is susceptible to cleavage by a variety of reagents as well as some enzymes (Tooke et al. 2019). The labile *b*-lactam ring of penicillins and other *b*-lactam antibiotics is characterized by its pronounced susceptibility to various nucleophiles, acid-base reagents, metal ions, oxidizing agents or even solvents like water and alcohol. The number of β-lactamases identified so far runs into hundreds. They have been classified into a number of groups and subgroups based on structure and function (Philippon et al. 2016). The enzymes discovered early (the TEM-1, TEM-2 and SHV-1 β -lactamases) were capable of inactivating penicillin but not cephalosporin. But subsequent

variants with a variety of amino acid substitutions in and around their active sites were identified in many resistant organisms. These have been collectively called 'extended spectrum β -lactamases (ESBLs)' and act on later generation β -lactam antibiotics also (Ventola, 2015). The early β -lactamases were sensitive to inhibitors such as clavulanic acid and sublactam. These compounds were incorporated in therapeutic formulations to inhibit β -lactamase activity and restore penicillin sensitivity (as a precaution in case the infection happens due to a resistant organism). However, some of the ESBLs are insensitive to these inhibitors. However, they are sensitive to another inhibitor called tazobactam. Newer families of ESBLs have been discovered recently and are causing much concern. Notable among these are cefotaximases (CTM-X enzymes) (Soeung et al., 2020). The most potent among the members of the β -lactam family are the carbapenems (imipenem, meropenem, panipenem, ertapenem, etc.), which have a broad antibacterial spectrum, including ESBL-producing pathogens, and are used in the therapy of infections that are not controlled by other members of the family (Shah *et al* 2008). Recently, carbapenemases, contributing to carbapenem resistance, have been discovered (Meletis, 2016). The CTM-X genes are believed to have descended from progenitor genes present in *Klyuvera* spp. (Decousser *et al* 2001). β -Lactam resistance contiues to be a problem which has not yet been conquered. In a recent report, Lloyd *et al* (2008) have described differences in the cell walls of penicillin-sensitive and resistant strains of *Streptococcus pneumoniae*, which could be exploited in future to tackle penicillin resistance. Another important source of resistance to β-lactam antibiotics is the family of penicillin-binding proteins (PBPs).

Like the β -lactam antibiotics, chloramphenicol is also inactivated by an enzymatic mechanism, namely acetylation (Schwarz *et al* 2004). This is the most common mechanism by which pathogens acquire resistance to chloramphenicol. Jayaraman (2009) have shown that O-phosphorylation of chlorophenicol affords resistance in *Streptomyces venezuelae* ISP 5230, which is a chloramphenicol-producing organism.



Core Structure of Penicillin (**Frankel, 1995)**



Core Structure of Cephalosporin **(Frankel, 1995)**

Spread of Antibiotic Resistance

The most prevalent Gram-negative pathogens, such as *Escherichia coli*, *Salmonella enterica*, and *Klebsiella pneumoniae*, cause a variety of diseases in humans and animals, and a strong correlation between antibiotic use in the treatment of these diseases and antibiotic resistance development has been observed over the past half-century. This is especially apparent with the β-lactam class of antibiotics and their related inactivating enzymes, the β-lactamases. At this time, several groups and classes have been identified, comprising up to 1,000 resistance- related β-lactamases. These include novel classes of genes and their mutant radiations (Bush *et al* 2010). Scientists confirmed the role of plasmids and conjugation in spreading antibiotic resistance during a dysentery epidemic in Japan in the late 1950s (Watanabe, 1963). The epidemic was

characterized by increasing numbers of *Shigella dysenteriae* strains that were resistant to as many as four antibiotics simultaneously. Such bacteria became so frequent that health officials concluded that their emergence could not be attributed to repeated mutations arising in one bacterium after another because mutations occur too rarely. Scientists showed that conjugational transfer of multiple-resistant plasmids accounted for the epidemic and established plasmids as major agents in the spread of antibiotic-resistant genes. Hughes *et al* (1983), who examined preserved bacterial strains from the pre-antibiotic era, showed that plasmids were present in many of the bacteria and that 24 percent of the plasmids were able to be transferred by conjugation between bacteria. However, very few of the preserved bacteria were resistant to antibiotics and those few were resistant to only one antibiotic. This indicates that multi resistance plasmids must have been created in the decades following the discovery of penicillin, when the use of antibiotics became extensive. Importantly, however, the pre existing transferable plasmids in bacteria became the vehicle for transfer of multiple antibiotic- resistant genes. Resistance genes can also travel on transposons, small pieces of DNA that can transfer to different sites on bacterial chromosomes and plasmids in the same bacterial cell or in different bacterial cells. Partridge and coworkers (2018) have been studying the structure of some transposons called integrons that carry antibiotic-resistance genes.

Conclusions

Antibiotic resistance is currently the greatest challenge to the effective treatment of infections globally. Resistance adversely affects both clinical and financial therapeutic outcomes, with effects ranging from the failure of an individual patient to respond to therapy and the need for expensive and/or toxic alternative drugs to the social cost of higher morbidity and mortality rates, longer duration of hospitalization, and the need for changes in empirical therapy. Thus the astonishing effects of antibiotics, the occurrence of resistance and the considerable resources spent on antibiotics globally are convincing reasons for concern about ensuring adequate and proper use of these powerful agents**.**

References

Bush, K, Jacoby G (2010). Updated functional classification of β-lactamases. Antimicrob. Agents Chemother. 54:969–976.

Chang, H. H., Cohen, T., Grad, Y. H., Hanage, W. P., O'Brien, T. F., and Lipsitch, M. (2015). Origin and proliferation of multiple-drug resistance in bacterial pathogens. Microbiology and Molecular Biology Reviews, 79(1), 101-116.

Decousser JW, Poirel L, Nordman, P (2001). Characterisation of chromosomally encoded, extended spectrum class 4, β-lactamase from *Kluyvera cryocrescens.* Antimicrob. Agents Chemother.45. 3595–3598.

Frankel DH (1995). Designing New Antibiotics. Contractor report to the Office of Technology Assessment. Photocopied typescript. January.

Ghodousi, A., Rizvi, A. H., Baloch, A. Q., Ghafoor, A., Khanzada, F. M., Qadir, M., ... and Cirillo, D. M. (2019). Acquisition of cross-resistance to Bedaquiline and Clofazimine following treatment for Tuberculosis in Pakistan. Antimicrobial agents and chemotherapy, 63(9), e00915-19.

Hughes VM, Datta N (1983). Conjugative plasmids in bacteria of the "pre-antibiotic" era. Nature. 302:725–726.

Jayaraman, R. (2009). Antibiotic resistance: an overview of mechanisms and a paradigm shift. Current science, 1475-1484.

Li, B., and Webster, T. J. (2018). Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopedic infections. Journal of Orthopaedic Research®, 36(1), 22-32.

Lloyd AJ. (2008). Characterisation of a t-RNA dependent peptidebond formation by Mur M in the synthesis of *S. pneumoniae* peptidoglycan. J. Biol. Chem.283, 6402–6417.

Meletis, G. (2016). Carbapenem resistance: overview of the problem and future perspectives. Therapeutic advances in infectious disease, 3(1), 15-21.

Partridge, S. R., Kwong, S. M., Firth, N., and Jensen, S. O. (2018). Mobile genetic elements associated with antimicrobial resistance. Clinical microbiology reviews, 31(4).

Philippon, A., Slama, P., Dény, P., and Labia, R. (2016). A structure-based classification of class A β-lactamases, a broadly

diverse family of enzymes. Clinical microbiology reviews, 29(1), 29-57.

Schwarz S, Kehrenberg C, Doublet B. (2004) "Molecular basis of bacterial resistance to chloramphenicol and florphenicol. FEMS Microbiol. Rev, 28, 519–542.

Shah PM. (2008). Parenteral carbapenems. Clin. Microbiol. Infect.14.175–180.

Soeung, V., Lu, S., Hu, L., Judge, A., Sankaran, B., Prasad, B. V., and Palzkill, T. (2020). A Drug-Resistant β-lactamase Variant Changes the Conformation of Its Active Site Proton Shuttle to Alter Substrate Specificity and Inhibitor Potency. Journal of Biological Chemistry, jbc-RA120.

Tooke, C. L., Hinchliffe, P., Bragginton, E. C., Colenso, C. K., Hirvonen, V. H., Takebayashi, Y., and Spencer, J. (2019). β-Lactamases and β-lactamase inhibitors in the 21st century. Journal of molecular biology, 431(18), 3472-3500.

Van Camp, P. J., Haslam, D. B., and Porollo, A. (2020). Prediction of Antimicrobial Resistance in Gram-Negative Bacteria from Whole-Genome Sequencing Data. Frontiers in Microbiology, 11, 1013.

Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. Pharmacy and therapeutics, 40(4), 277.

Watanabe T (1963). Infective heredity of multidrug resistance in bacteria. Bacteriol Rev. 27:87–115.

RECENT ADVANCES ON DIAGNOSTICS FOR HEPATITIS B INFECTION

Pallavi kachhawah[1], Dr. Vijay Upadhye[2*], Dr. A N Pathak[3]

[1]Student, Parul Institute of Applied Science (PIAS)
[2]Associate Professor, Parul Institute of Applied Science at Parul University
[3]Director, Centre of Research for Development (CR4D), Parul Institute of Applied Science, Parul University, Dist. Vadodara, Gujarat (India)

[*]Corresponding Author: vijay.upadhye82074@paruluniversity.ac.in

Introduction

Hepatitis B virus can cause scarring of the organ, inflammation of the liver, liver failure and liver cancer. HBV infection is caused by a virus that shows a diameter of 42 nm and comprises an icosahedral capsid surrounded by a lipid envelope (Babak et al., 2019) containing hepatitis B surface antigen (HBsAg) (Fig.1). Globally, in 2019-2020 an estimated 257 million people were living with chronic HBV infection and an estimated 2.7 million had chronic HBV infection. About 15-25% HBs Ag carriers are likely to suffer from cirrhosis and liver cancer and may die prematurely.

In 2016, the world health assembly endorsed the first global hepatitis elimination target which called for a 65% reduction in hepatitis related mortality and 90% reduction in incidence of hepatitis by 2030, an impact which was considered to represent elimination of viral hepatitis as a public health threat (Shipeng et al., 2011). Fig. 2 depicts global distribution of Hepatitis B (WHO Global hepatitis report, 2017). India has one fifth of the world's population, it accounts for a large proportion of the world wide HBV burden (Bhaumik, 2015). India harbors 10-15% of the entire pool of HBV carriers of the world. It has been estimated that India

has around 40 million HBV carriers. India is in the intermediate zone of pathogenesis of hepatitis B (2-5%) .The prevalence of HBV is higher among tribal populations than non tribal. Very limited tests are available due to which early diagnosis of HBV infection is delayed and causes an increase in mortality rate. Hence in the given study, efforts have been made to review current available diagnostic biomarkers and tools for detection of HBV Infection.

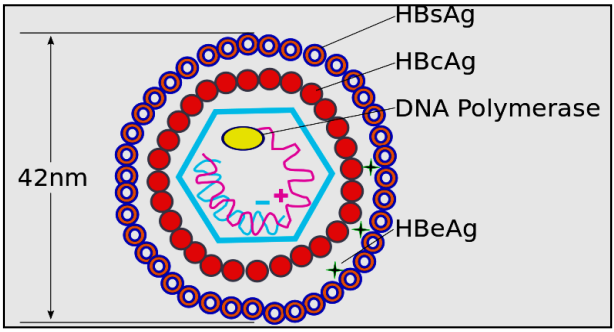


Fig. 1: Hepatitis B virus (HBV) Surface antigens (Adapted from Singh and Sinha, 2015)

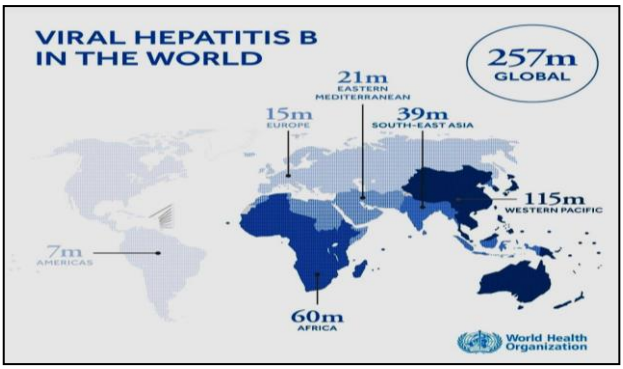


Fig. 2: Global distribution of Hepatitis B (Source: WHO Global hepatitis report, 2017)

Serological markers available:

- HBs Ag is a well established biomarker of HBV infection and is qualitatively used for the diagnosis of HBV infection in our daily practice. In the natural course of chronic hepatitis B, HBV DNA is only from mature infections particles and its level reflects viral replication. Accordingly a decline of HBV DNA means the reduction of HBV replication. In addition, HBs Ag can also be derived from defective subviral particles.
- Anti-HBs: Anti-HBs are a neutralizing antibody and its presence indicates immunity to HBV infection (Table 1). The presence of anti-HBs and HBs Ag has been documented in HBs Ag positive patients. Probably due to the incompatibility of antibodies to neutralize the circulating virions.

Table 1. Detection of HBV marker (Bonino et al., 2010)

HBV Marker	Diagnostic Category
Anti-HBs	Immunity
Anti-Hob	Exposure
HBs Ag and/or HBV DNA^{o}	Infection
HBe Ag and/or HBV DNA^{o}	Replication
Anti HBc and/or HBV DNA^{o}	Disease

- HBe Ag: HBe Ag is a surrogate marker of HBV replication with high HBV DNA levels and high infectivity. Approximately 90% of HBe Ag positive mothers transmit HBV to their babies compared with 10-20% of HBe Ag negative mothers. Spontaneous HBe Ag seroconversion is associated with low HBV DNA levels and clinical remission of liver disease in the majority of the patients. Earlier HBe Ag seroconversion is associated with a higher rate of sustained remission and slower progression of liver disease. In addition, HBs Ag clearance is more commonly seen in patients with treatment induced HBe

Ag seroconversion than those without HBe Ag seroconversion (Bonino et al., 2010)

- HBc Ag – HBc Ag (core antigen) is intracellular (Singh and Sinha, 2015) and for this reason is not detected in the serum of infected individuals. Figure 2 shows surface antigens of Hepatitis B virus (HBV). Antibodies against core protein (anti-HBc) appear shortly after HBs Ag in acute infection and persist after acute phase indicating previous exposure. Anti –Hob is the most useful and inexpensive diagnostic marker for the identification of occult HBV infection in HBs Ag- negative individuals. Quantification of anti-HBs provides a useful means of monitoring post liver transplant therapy with human anti-HBs immune gamma globulin in HBV positive patients.

Current Serological Methods Available:

- ELISA

The enzyme linked immunosorbent assay technique has been chosen by the world health organization as the preferred method for the detection of HBs Ag in developing countries of the world (Jokelainen et al., 1970). Infection with Hepatitis B virus is associated with the accumulation of viral envelope proteins in the blood. These proteins can be detected by ELISA methods (Fig.3). HBV consists of a nucleocapsid containing DNA associated to core proteins and a capsid whose main component is a protein known as surface antigen(HBs Ag). HBs Ag usually appears 6 weeks after exposure to HBV and persists for 4-14 weeks. It is present during the incubation period, before the onset of the clinical disease. It can be detected in blood 2 to 8 weeks before the onset of jaundice or biochemical evidence of liver dysfunction. Thus HBs Ag is the first indicator of HBV infection. Chronic hepatitis B is defined as the presence of HBs Ag in blood for more than 6 months. HBs Ag detection is important for the diagnosis of acute and chronic hepatitis, carrier control in blood banks, dialysis and transplanted patients units, pregnant women and for control of blood preparation and derivatives intended for transfusion.

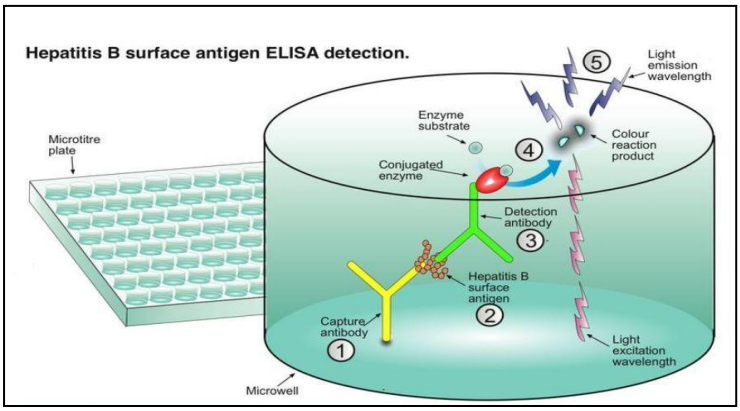


Fig. 3. Detection of Hepatitis B virus by ELISA (1) Micro wells are coated with capture antibodies that recognize Hepatitis B surface antigen. (2) Blood plasma is added to allow antigen to be bound. (3) A detection antibody conjugated to an enzyme is added which binds captured antigen. (4) An enzyme substrate is added and a color reaction is catalyzed. (5) Emitted light is measured as an indicator of Hepatitis B surface antigen concentration in blood. (Source: Immunopaedia.org)

- **Western Blot**

Western blot is taken as a confirmatory test for hepatitis B infection. The genome of hepatitis B virus (HBV) is a partially double stranded DNA molecule within virus particles that is approx 3.2 kbp long. It has four open reading frames, all on one strand, that encode the surface antigen or envelope polymerase and the so called X protein (HBx Ag). HBx Ag is a common marker in the livers of carriers. The ability to detect HBx Ag may depend on the type of cell line used for expression. HBx Ag expression has been documented by immunofluorescence or western blot (Fig. 4) among cells in which the protein was introduced by scrape loading or transient transfection where drug resistance was not used for the selection of HBxAg (Nazir et al., 2019).

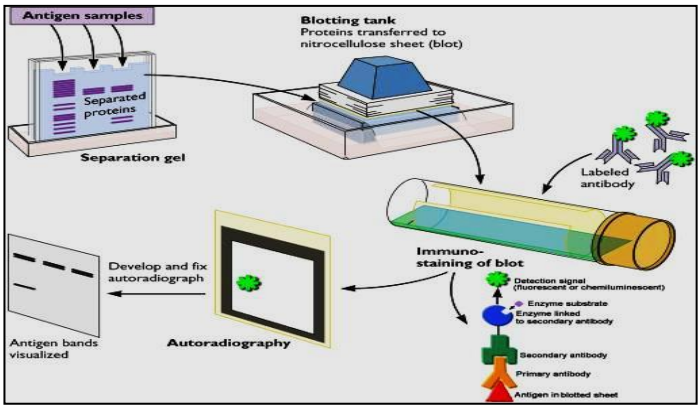


Fig.4. Detection of hepatitis B by Western blot (Source: This figure is adapted from MyBiosource, Inc)

- Rapid Immunochromatographic test

Commercially developed Rapid Immunochromatographic Test for detection of HBs Ag and HBe Ag. It was first developed by AMRAD ICT (French forest, NSW, Australia) in Australia. The test utilizes two sets of antibodies specific to HBs Ag and HBe Ag and captures the antigens in a standard sandwich format. The first set of antibodies is labeled with colloidal gold and is impregnated in a test pad. The second set is immobilized as two separate lines on a cellulose membrane that oppose the test pad in a bi-folded card (Ali et al., 2017)

Current Molecular Techniques Available:

- Polymerase chain reaction (PCR)

PCR is a highly sensitive technique for the detection of hepatitis B virus DNA (Sun et al., 2011). In chronic hepatitis B, it is particularly useful for identification of infectious subjects who are hepatitis B surface antigen positive and anti-HBe antigen antibody positive and for follow up of hepatitis B virus infection in liver transplantation

programmes (Datta et al., 2014). Polymerase chain reaction developed by is the amplification technique that has entirely transformed the field of biological research as well as clinical diagnostics. The discovery of PCR, identification and availability of thermostable taq polymerase and reverse transcriptase enzymes have enabled scientists and researchers to adapt and modify the basic PCR technology for automated amplification and detection of DNA and RNA. For HBV diagnosis through PCR, covalently closed circular DNA of HBV (cccDNA) level monitoring in the hepatocytes is the most precise way of assessing the number of infected hepatocytes (Garibyan and Avashia, 2013). The main advantage of PCR are its extreme sensitivity and the possibility to develop rapid assay using non radioactive probe.

- Immunofluorescence technique

Immunofluorescence is an antigen detection test that is used primarily on frozen tissue sections, cell smears or cultured cells. Antigen is detected through the binding to the sample matrix of specially modified, agent specific antibodies. The modification is the tagging of the antibody with a fluorochrome that absorbs ultraviolet light of a defined wavelength, but emits light at a higher wavelength. The emitted light is detected optically with a special microscope equipped with filters specific for the emission wavelength of the fluorochrome (David et al., 1986). The fluorochrome can be bound directly to the agent specific antibody (direct immunofluorescence) or it can be attached to an anti-immunoglobulin molecule that recognizes the agent specific antibody (indirect immunofluorescence). Lau et al (2003) developed a rapid immunochromatographic assay for hepatitis B virus screening.

- Biosensor

A biosensor is an analytical device used for the detection of analytes, which combines a biological component with a physicochemical detector. Recently, an increasing number of biosensors have been used in clinical research, for example, the

blood glucose biosensor. Biosensor research with respect to efficient, specific and rapid detection of hepatitis B virus (Yao and Fu, 2014) are mentioned in table 2 (Negahdari et al., 2019). The evolution of development of nanoscience and nanotechnology has increased the development of immunosensors for HBV diagnosis. Immunosensors are solid state affinity ligand based biosensing apparatus that combine immunochemical reactions to proper transducers. The sensing element is composed by means of the immobilization of antigens or antibodies and the binding event is transformed into a measurable signal by the transducer. The goal of immunosensor is to produce a signal proportional to the concentration of analyte. Tam et al (2017) studied on wide dynamic range of surface-plasmon-resonance-based assay for hepatitis b surface antigen antibody optimal detection in comparison with ELISA. Wang et al (2010) developed a gold nanorod based localized surface Plasmon resonance biosensor (Fig.5) that quantifies HBs Ag until 0.01 IU/mL. To develop a high sensitivity, low cost screening system for HBV detection. This limit of detection is about 40 times lower than the limit of detection of the EIA method. Another immunosensor for HBs Ag detection uses magnetic nanoparticle and three dimensional carbon nanotube-conducting polymer network detecting 0.001 to 0.015ng/mL of HBs Ag.

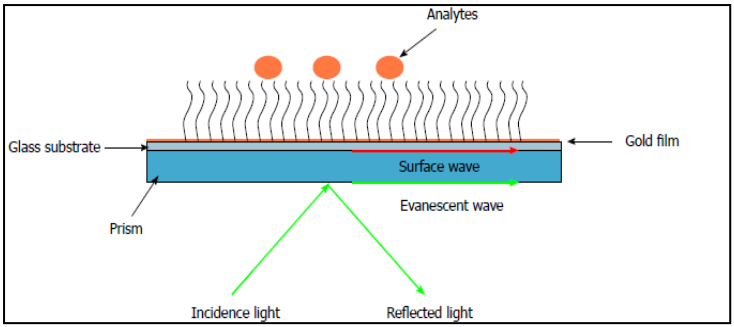


Fig.5. Schematic representation of surface plasma resonance biosensor (Adapted from Wang et al., 2010)

Table 2. Application of Biosensor technique in detecting HBV (Source: Negahdari et al., 2019)

Biosensor Features	Detecting Target
Nanorods Biosensor	HBsAg
Quartz Crystal Microbalance	HBs Ab, HBV DNA
Surface Acoustic Wave	HBs Ab
Surface Plasmon Resonance	HBs Ab, HBV DNA
Electrochemical Biosensor	HBV DNA
Microcantilever Biosensor	HBV DNA

Table 3. Advantages and Disadvantages of Diagnostic Techniques

Techniques	Advantages	Disadvantages
ELISA	Automation/manual, Highly Reproducible Results, Low Cost, highly sensitive, easy to perform, User friendly. Results can be qualitative and quantitative.	Time consuming, need plate reader and results are objective
Western Blotting	High Specificity	Low sensitivity, time consuming, Only detects 85% of RO positive sera, costly. Results are qualitative.

ISBN: 978-81-950423-8-8

Rapid Immunochromatic-graphic Test	Performing fast, rapidly and simply without requiring special instruments, easy to perform, User friendly, results within 20 minutes.	Less sensitive or less accurate compared to existing tests, problem of false positive result more, Result are qualitative.
Polymerase Chain Reaction	Extreme sensitive, highly specific,	Costly, need expertise, lab set up needed, problem of mutation will be there, time taking, specific primer designing is needed.
Immunoflorescence	Used assay in the determination of ANAs(Antinuclear Antibodies), highly specific, subjective type, qualitative results	Time consuming, Pattern sometimes difficult to interpret (e.g. cross reactive antibodies), subjective test, cannot be automated (observation under microscope to identify the pattern), expertise and lab set up is needed, IFA microscope is must. Costly and time taking.

Discussion:

In this review, we attempt to give information on Diagnosis of HBV infection to identify acute and chronic cases of infection. Each detection technique presents advantages and disadvantages. Table 3 represents a comparative account of advantages and disadvantages of Diagnostic Techniques.

Past/Present Available Methods-

- In earlier days, infection with hepatitis B virus (HBV) was detected by demonstration of antibody titer by complement fixation test.

- The first solid phase sandwich radioimmunoassay (RIA) was being utilized to improve the detection of HBs Ag. This highly sensitive detection method became a major discovery in the diagnosis of viral transfusion hepatitis. Counter electrophoresis is a widely accepted method that gained popularity due to its better sensitivity in detecting HBs Ag. RIA methods were shown to have sensitivity at least 10 to 200 times better than the counter electrophoresis, where the detection is able to detect three to eight times more HBs Ag carriers compared to the previous method.

- Later another method called the gel diffusion technique, this detection method was less sensitive for the blood screening procedure and the main disadvantages required a higher amount of blood from the donor. This had encouraged other methods being developed to increase the sensitivity of the assay. Since then serological and molecular methods have been developed for diagnosing HBV.

Complement fixation test was not specific. RIA is having problems with hazards of radioactivity exposure. Ethical approval is needed to conduct RIA. Disposal of radioactive waste is a major concern. RIA is presently not widely used and discontinued from many labs.

Present available method-

- In this review, we have studied present serological and molecular methods, in the last few years, the field of HBV molecular diagnostics has evolved rapidly with advancements in the molecular biology tools, such as Polymerase Chain Reaction (PCR) and real time PCR. Recently, apart from PCR based amplification methods, a number of isothermal

amplification assays, such as loop mediated isothermal amplification, transcription mediated amplification, ligase chain reaction, and rolling circle amplification have been utilized for HBV diagnosis. These assays also offer options for real time detection and integration into biosensing devices. A number of biosensors based on different principles have been developed recently for the detection of HBV DNA, or its antigen or anti-HBV human antibodies.

- Enzyme immunoassay offers excellent precision and reliability, high-speed throughput, random access, and the technical simplicity of full automation for high-volume clinical laboratories. Rapid diagnostic tests (RDTs) provide improved access to testing as they are simple, low cost, and can use serum and plasma. They can also be performed by trained lay providers or health-care workers in resource-limited settings without the need for specialized equipment or venepuncture.

Future possibilities and Conclusion

In this review, we attempt to give information regarding HBV diagnostic methods available in clinical areas. We needed the following tests: ELISA, Western Blot Method, Rapid Immunochromatographic Test, Polymerase Chain Reaction, Immunofluorescence Technique and Biosensor. Diagnosis of HBV infection is a key tool to identify acute and chronic cases of infection. First step of HBV diagnosis is achieved by using a serological marker for detecting antigens and antibodies against this virus. In order to verify the first step of diagnosis, to quantify viral load and qualitative or quantitative molecular tests are used. Diagnosis of HBV infection is important for determining acute, chronic and occult cases of infection in order to establish preventive remedies (Nayagam & Thursz. 2019).

Finally, good quality diagnosis has a cost that only developed countries can afford in routine practice so far, and this is delaying the implementation of new methods in the developing world and the endemic areas. However, there is hope that efforts will continue

towards developing new good quality tests affordable in low income countries, which would substantially strengthen disease control strategies for their populations.

The virus is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes present on its envelope proteins. These serotypes are based on a common determinant (a) and two mutually exclusive determinant pairs (d/y and w/r). Genotype D has 10 subgenotypes. There are mutant strains also for HBV. All these genotypes and serotypes cross react with pathogenic HBV strain during diagnosis. To avoid cross-reactivity from such epitopes, highly specific antigenic determinants, which are markers for specific stage of the disease (acute, chronic, convalescent and relapse form of infection) - are needed for effective and early diagnosis of HBV infection. RNA Microarray and gene expression studies on HBV can come out with RNA Signatures giving reliable biomarkers for HBV infection. We look forward more research outcomes from global scientific fraternity to come out with new reliable biomarkers soon, not only, to fight with HBV but also, for early diagnosis of HBV Infection.

References:

Ali Amini,Olivia Varsaneux,Helen Kelly,Weiming Tang, Wen Chen,Debrah I. Boeras,Jane Falconer,Joseph D. Tucker, Roger Chou, Azumi Ishizaki, Philippa Easterbrook,and Rosanna W. Peeling. Diagnostic accuracy of tests to detect hepatitis B surface antigen: a systematic review of the literature and meta-analysis. BMC Infect Dis. 2017; 17(Suppl 1): 698. https://dx.doi.org/10.1186%2Fs12879-017-2772-3

Babak Negahdari, Mohammad Darvishi & Ali Asghar Saeedi (2019) Gold nanoparticles and hepatitis B virus, Artificial Cells, Nanomedicine, and Biotechnology, 47:1, 455-461

Choi, Jin-Ha, Lee, Jin-Ho, Son J, and Choi Jeong-Woo (2020), Noble Metal-Assisted Surface Plasmon Resonance Immunosensors. Sensors, 20, 1003

Chun-Yan Yao, Wei-Ling Fu. Biosensors for hepatitis B virus detection. Review World J Gastroenterol . 2014 Sep 21;20(35):12485-92. https://doi.org/10.3748/wjg.v20.i35.12485

D. T.-Y. Lau H. Ma S. M. Lemon E. Doo M. G. Ghany E. Miskovsky G. L. Woods Y. Park J. H. Hoofnagle. A rapid immunochromatographic assay for hepatitis B virus screening. Journal of Viral Hepatitis. 23 June 2003

D.S. Dane , C.H. Cameron , Moya Briggs (1970). "Virus-Like Particles in Serum of Patients with Australia-Antigen-Associated Hepatitis". The Lancet 295: 695–698. https://doi.org/10.1016/S0140-6736(70)90926-8

Datta S, Chatterjee S, Veer V. Recent advances in molecular diagnostics of hepatitis B virus. World J Gastroenterol 2014; 20(40): 14615-14625. http://dx.doi.org/10.3748/wjg.v20.i40.14615

David A Anderson, Anthony G Coulepis, Maureen P Chenoweth and Ian D. Indirect Immunofluorescence Assay for the Detection of Hepatitis A Virus-Specific Serum Immunoglobulins. Journal of Clinical Microbiology 1986; 24(1):163-165

Ferruccio Bonino, Teerha Piratvisuth, Maurizia R Brunetto, Yun-Fan Liaw. Diagnostic markers of chronic hepatitis B infection and disease. Antivir. Ther. 2010;15 (3):35-44. https://doi.org/10.3851/imp1622

Hepatitis B Surface Antigen. Immunopedia.Org – Advancing global Immunology Education. https://www.immunopaedia.org.za/treatment-diagnostics/diagnostic-tests/

Jagtar Singh and Shweta Sinha (2015). Emerging of RNA viruses: a threat of epidemics around-the-clock International Journal of Applied Biology and Pharmaceutical Technology, 6(3): 80-92

Lilit Garibyan and Nidhi Avashia. Research Techniques Made Simple: Polymerase Chain Reaction (PCR). J Invest Dermatol. 2013 Mar; 133(3): e6. https://dx.doi.org/10.1038%2Fjid.2013.1

Livia Melo Villar, Helena Medina Cruz, Jakeline Ribeiro Barbosa, Cristianne Sousa Bezerra, Moyra Machado Portilho, Letícia de Paula Scalioni. Update on hepatitis B and C virus diagnosis. Review World J Virol. 2015 Nov 12;4(4):323-42. https://doi.org/10.5501/wjv.v4.i4.323

Mallika Ghosh, Srijita Nandi, Shrinwanti Dutta, and Malay Kumar Saha. Detection of hepatitis B virus infection: A systematic review. World J Hepatol. 2015 Oct 18; 7(23): 2482–2491. https://dx.doi.org/10.4254%2Fwjh.v7.i23.2482

Mubashir Nazir, Roomi Yousuf, Muzafar Amin*, Syed Khurshid, Arshi Syed and Talat Masoodi. A Comparative Study of Screening of Hepatitis B by Two Different Immunochromatographic Methods among Patients Attending a Tertiary Care Hospital. Int.J.Curr.Microbiol.App.Sci (2019); 8(4): 1506-1513. https://doi.org/10.20546/ijcmas.2019.804.176

https://www.mybiosource.com/learn/westernblotting, 2006- 2020

P. T. Jokelainen, Kai Krohn, A. M. Prince and N. D. C. Finlayson (1970). "Electron Microscopic Observations on Virus-Like Particles Associated with SH Antigen". J Virol. 6 (5): 685-689. ISSN 1098-5514.

Pradeep Bhaumik. Epidemiology of Viral Hepatitis and Liver Diseases in India. Euroasian J Hepatogastroenterol. 2015 Jan-Jun; 5(1): 34–36.

Shevanthi Nayagam & Mark Thursz. Strategies for Global Elimination of Chronic HBV Infection: 2019 Update.Current Hepatology Reports 2019; 18: 300–309. https://doi.org/10.1007/s11901-019-00478-w

Shipeng Sun, Shuang Meng, Rui Zhang, Kuo Zhang, Lunan Wang & Jinming Li et al. Development of a new duplex real-time

polymerase chain reaction assay for hepatitis B viral DNA detection. Virology Journal 2011; 8(227). http://www.virologyj.com/content/8/1/227

Tam, Y.J., Zeenathul, N.A., Rezaei, M.A., Mustafa, N.H., Azmi, M.L.M., Bahaman, A.R., Lo, S.C., Tan, J.S., Hani, H., Rasedee, A., 2017. Wide dynamic range of surface-plasmon-resonance-based assay for hepatitis b surface antigen antibody optimal detection in comparison with elisa. Biotechnology and applied biochemistry 64, 735–744.

Wang, X., Li, Y., Wang, H., Fu, Q., Peng, J., Wang, Y., Du, J., Zhou, Y., & Zhan, L. (2010). Gold nanorod-based localized surface plasmon resonance biosensor for sensitive detection of hepatitis B virus in buffer, blood serum and plasma. *Biosensors & bioelectronics*, *26*(2), 404–410.

Western Blot for Diagnosis of Hepatitis Infection https://www.mybiosource.com/learn/westernblotting

WHO: Global hepatitis report 2017. ISBN 978-92-4-156545-5. https://apps.who.int/iris/bitstream/handle/10665/255016/9789241565455-eng.pdf;jsessionid=BD2EF9BD0733EDD287CA135447A41366?sequence=1

Yao CY, Fu WL. Biosensors for hepatitis B virus detection. World J Gastroenterol 2014; 20(35): 12485-12492. DOI: https://dx.doi.org/10.3748/wjg.v20.i35.12485

MICROBIAL BIOSENSORS FOR EFFICACIOUS DISEASE DIAGNOSIS AND MONITORING

Rasanpreet Kaur[1], Parul Yadav[2] and Jagdip Singh Sohal[3*]

[1]Student, Amity Center for Mycobacterial Disease Research, Amity Institute of Microbial Technology, Amity University Rajasthan
[2]Assistant Professor, AUSIC, Amity University Rajasthan
[3]Assistant Professor, Amity Center for Mycobacterial Disease Research, Amity Institute of Microbial Technology, Amity University Rajasthan

[*]Corresponding Author: jssohal@jpr,amity.edu

INTRODUCTION

A microbial biosensor is a biosensor that uses microorganisms which consists of numerous enzymes as the bioelements. The enzymes in the living cells can produce a response to the analytes specifically and selectively, without neither the necessity of time-consuming and costly purification nor the negative effects of the operating environment (Su et al., 2011). In order to transfer the responses from the recognition elements to the transducers, the immobilization between the bioelements and the transducers must be intimate and stable. Integrating the microorganisms onto the transducer is the basic requirement of achieving a reliable microbial biosensor (Lei et al., 2006). Immobilization determines not only the quality of the signal transferred from the microorganisms to the transducer but also the reusability of the microbial biosensor. Therefore, immobilization plays an important role in developing a microbial biosensor (D'Souza, 2001). The conventional immobilization methods include adsorption, entrapment, encapsulation, covalent binding, and cross linking. However, all of these methods suffer from either poor long-term stability or negative effects from being exposed to harsh reaction conditions. Advances in nanotechnology offer an alternative for better immobilization by using nanomaterial such as nanoparticles, nanotubes, and fiber optics, which promote higher reliability and

stability of the bioelements (Tuncagil et al., 2011). The transducer is another critical part of the microbial biosensor for converting the biological response to a measurable signal.

Synthetic biology aims to engineer living organisms, such as microbial biosensors, to execute new functions to solve real-world problems (Konig et al., 2013). The growing burden of complex, multifactorial human diseases, such as inflammation, cancer, neurodegeneration, and cardiovascular disease, will likely require more sophisticated drugs that can adapt to each patient's specific conditions, thus enabling precision medicine. By designing genetic circuits, a sense-compute-respond paradigm can be introduced into living cells, similar to frameworks used in other engineering fields (Tabor et al., 2009). Through iterative design-build-test-learn cycles, these genetic circuits can be continuously improved to meet specific performance criteria (Appleton, 2006). Although biological systems are not naturally modular, synthetic biologists aim to design parts and circuits that can function reliably across many contexts.

Type of Microbial Biosensors

Fluorescent Microbial Biosensor

Fluorescent microbial biosensors are widely used in analysis processes, which can emit fluorescent light that is directly proportional to the analyte's concentration at a low level. The basis of the fluorescent microbial biosensor is to fuse an inducible promoter to a reporter gene to encode a fluorescent protein which can emit detectable fluorescence in a genetically engineered microorganism (Su et al., 2011). Due to the advantages of stability and sensitivity, green fluorescent protein is most commonly used in fabrication of fluorescent microbial biosensors (Pickup et al., 2005).

Bioluminescent Microbial Biosensor

Bioluminescence based microbial biosensors have been extensively used in environmental monitoring for detection of toxicity due to its ability to closely reflect to toxicity (Steinberg et al., 1995). As a proportional response to the concentration of the analytes, the changes

in the density of the bioluminescence emitted by the living cells can be measured by the bioluminescent microbial biosensor. According to the mechanism of production of bioluminescence, the method to control the expression of the lux gene can be divided into two manners: the constitutive manner and the inducible manner. In the constitutive manner, the bioluminescence caused by lux gene-coded luciferase exists constitutively as long as the organism is active. As the density of the bioluminescence can be affected by the additional compounds such as the toxicity, it can be used as a parameter to determine the additional compounds. In the inducible manner, the lux gene is fused with a promoter regulated by the concentration of the analytes. Based on this mechanism, the bioluminescence cannot be detected until the concentration of the analytes approaches a critical value (Lei et al., 2006). Several bioluminescent microbial biosensors have been developed in recent years. A whole-cell bioluminescent biosensor, based on genetically engineered *Escherichia coli* bacteria, carrying a recA::lucCDBAE promoter-reporter fusion, was developed for the detection of water toxicity (Daniel et al., 2008).

Colorimetric Microbial Biosensor

Colorimetric microbial biosensors make use of the changes in the color of the special compound to determine the concentration of the target analytes. Methyl parathion can be hydrolyzed by bacterium into chromophoric product, p-nitrophenol (PNP), which can be measured by a colorimetric method. Based on this mechanism, colorimetric transducers have been widely used in developing microbial biosensors for the detection of methyl parathion. A colorimetric microbial biosensor based on the immobilization of *Flavobacterium* sp. in glass fiber filter was constructed for the detection of methyl parathion with a detection limit of 0.3 μM and a linear range from 4 - 80 μM (Kumar et al., 2006). Further, Kumar et al., 2006 immobilized *Sphingomonas* bacteria onto the surface of the wells of polystyrene microplates (96 wells) to construct a colorimetric microbial biosensor, which had the same linear range to methyl parathion but achieved an advantage of multiple detections (Kumar & D'Souza, 2010). By immobilizing the *Sphingomonas* bacteria on inner epidermis of onion bulb scale, a colorimetric microbial biosensor for detection of methyl parathion was

developed and achieved a stable characteristic (Kumar & D'Souza, 2011).

APPLICATIONS OF MICROBIAL BIOSENSORS IN BIOMEDICINE

Microbial biosensors take advantage of the ability of microbes to adapt and respond to their environment (Inda et al., 2019). Key performance characteristics that can be achieved by microbial biosensors can make them useful for real-world applications, including robustness, evolvability, high sensitivity and specificity, continuous sensing, non-invasiveness, and scalability. However, these features are not inherent to microbial systems and may require engineering or evolutionary strategies to be achieved.

Robustness

Microbial biosensors must function reliably in real-world environments, which are often harsh. For example, natural systems that sense biomarkers of inflammation, such as nitric oxide (Archer et al., 2012), thiosulfate (Daeffler et al., 2017), tetrathionate (Riglar et al., 2017), and blood (Mimee et al., 2018), have been engineered into microbial biosensors. In particular, one of those tetrathionate biosensors was introduced in a commensal murine *Escherichia coli* strain, which was used to follow the progression of inflammation in mice for over 6 months (Riglar & Silver, 2018). However, environmental conditions can be detrimental to these living biosensors. For microbial biosensors to work in the gut, they need to survive passage through the gastrointestinal (GI) tract, which includes challenging conditions such as rapid changes to extreme pHs (1.5 to 8.5), proteases, and bile salts. With inflammation in the gut, reactive oxygen species (ROS) and other antimicrobial molecules (Persson et al., 2010) can create an even more hostile environment. Cells can be engineered or evolved to enable enhanced robustness in challenging environments. For example, the gene clusters that encode acid-resistance systems (e.g., AR1, AR2, and AR3 from *E. coli*) can be introduced to enhance the viability of engineered microbes in the host's stomach, protecting them from the stress of acidic conditions

(Richard & Foster, 2003). Similarly, evolved osmotolerant *E. coli* mutants, selected under high NaCl stress, can provide protection from high concentration of salts (0.80 M NaCl) during transit through the GI tract (Crook et al., 2019).

Evolvability

Directed evolution is an iterative process that generates genetic diversity, followed by screening for functional gene variants with desired functions (Packer & Liu, 2015). Genetic circuits that comprise bacterial sensors can be optimized via the directed evolution of each component, such as promoters, protein sensors and gene circuits, or at the whole cell level to improve features such as bacterial colonization or stress tolerance.

However, this capacity to evolve can also be a disadvantage for the long-term stability of living biosensors, since the taxing of cellular resources to support synthetic genetic circuits can result in compensatory genetic mutations, loss of engineered functions, and impaired growth of the recombinant strain in a host- and environment-dependent manner. To limit the burden imposed on the cell, gene expression designs with reduced burden are needed. Measuring the expression of a green fluorescent protein (GFP)-based capacity monitor in *E. coli* enables the assessment of the burden that heterologous gene expression exerts on the cell, and this information can be used to design constructs with less impact on the host (Ceroni et al., 2015).

For example, through directed evolution, Meyer et al., 2019 developed Marionette, a platform for simultaneously optimizing sensors for complex profiling of multiple small-molecule signals in one cell. Marionette was applied to generate a single array of 12 high-performance sensors that exhibit >100-fold induction with low cross-reactivity. Cellular resources were optimized by simultaneous selection for lower basal expression levels (i.e., low gene expression in the absence of the desired stimulus), high dynamic range, increased sensitivity, and low cross talk. For this optimization, a genetically diversified library of sensors was cloned upstream of an operon

containing a mutant of the phenylalanine aminoacyl tRNA-synthetase (PheS) and a thermostable DNA polymerase (DNAP) on a dual-selection plasmid. The PheS cassette enables selection against leakiness in the absence of inducer, while the DNAP cassette enables selection for enhanced sensitivity. After induction, the cells were encapsulated, lysed, and amplified by polymerase chain reaction (PCR) with primers that immediately flank the sensor library using the DNAP expressed by the sensor, such that more amplification was achieved, the more DNAP was expressed. In the absence of inducer, leaky transcription of PheS leads to the charging of phenylalanyl tRNA with the non-canonical amino acid 4-chloro-d-phenylalanine. Adding more of this amino acid increases the stringency of the negative selection against leakiness by making PheS transcription more toxic. For positive selection to enhance the sensitivity to inducer, the sensors responsible for the greatest expression of DNAP are mostly amplified by emulsion PCR. Thus, multiple properties of the sensor response function can be improved through one cycle of negative and positive selection. Chemical specificity can be achieved by adding a potentially cross-reactive inducer during the negative selection, thus selecting against cross-reactive mutants. Chemical antagonism can be selected against by adding a potentially antagonistic inducer to the positive selection, thus selecting for mutants that are less strongly antagonized (Meyer et al., 2019).

Sensitivity and Specificity

Bacteria are naturally equipped with sensors that detect a large number of molecules in their environment (e.g., gut, skin, or mouth) within biologically relevant concentrations [e.g., bacterial sensors can detect tetrathionate (Daeffler et al., 2017), nitric oxide (Archer et al., 2012), thiosulfate (Daeffler et al., 2017), and ROS (Müller et al., 2019) in the micromolar range]. For protein biosensors, specific recognition sites within the protein can bind to different small-molecule signals and potentially discriminate between related ones. This sensor allows bacteria to control the expression of cognate nitric oxide reductases that metabolize and detoxify nitric oxide inside the gut.

However, not all-natural sensors are ready for real-world applications, since their original functions may not match the desired use cases. Strategies for improving their sensitivity and specificity include directed evolution and rational engineering of the protein components themselves or the introduction of circuit-level designs, such as digitalizing, amplifying, and multiplexing signal detection (Courbet et al., 2015).

For example, unwanted cross talk between different signals can confound the interpretation of the responses of microbial biosensors (Daeffler et al., 2017). The TtrSR two-component system (TCS) from Salmonella enterica Typhimurium (Price-Carter et al., 2001), which responds to tetrathionate, is composed of the TtrS kinase sensor and the TtrR response regulator, which controls downstream transcription via the ttrB promoter (PttrB). The global regulator FNR (fumarate and nitrate reductase regulator) is required for transcription from PttrB and is repressed by O_2. Thus, using PttrB as a readout for tetrathionate sensing is potentially confounded by O_2 levels due to FNR regulation. This cross-regulation could compromise the performance of TtrSR-based tetrathionate sensing in the gut, where O_2 levels may vary depending on their proximity to the epithelial mucosal boundary. To avoid this cross-repression, Daeffler et al., 2017 computationally identified a novel tetrathionate sensor from the marine bacterium *Shewanella baltica*, which bypasses the FNR system. By expressing this sensor in *E. coli*, they provided an alternative tetrathionate sensor with improved performance sensing in the gut. The studies demonstrated the specificity of the sensor by showing that it has selectivity for tetrathionate over other terminal electron acceptors (Inda & Lu, 2020).

Orthogonality

In this aspect, orthogonality is defined as the lack of interference between one gene circuit component and another when they are put together in a cell, or between one gene circuit component and a component of the endogenous cellular biology. Orthogonality is not a given in biological systems, especially when multiple signaling networks interoperate within environments that are not physically

segregated. Thus, orthogonality is a crucial element of design when using multiple sensors in the same cell (Meyer et al., 2019). For example, for thermal biosensors, temperature-dependent transcriptional repressors have been shown to be less susceptible to cross talk compared to microbial heat shock promoters, which can be activated not only by temperature changes but also by other chemical stressors (Piraner et al., 2017).

Unanticipated cross talk between different pathways may interfere with the proposed functioning of engineered gene networks. To make signal engineering more predictable, Schmidl et al., 2019 rewired bacterial TCSs by modular DNA-binding domain swapping, achieving robust signaling even in heterologous hosts. These authors showed that the two largest families of response regulator DNA-binding domains (OmpR/PhoB and NarL/FixJ families) can be interchanged, enabling the corresponding TCSs to be rewired to synthetic output promoters. This remarkable flexibility has been exploited to eliminate cross-regulation, un-silence a gram-negative TCS in a gram-positive host, and engineer a system that can achieve an increase in activation of over 1,300-fold (Schmidl et al., 2019).

In addition to choosing or designing better sensor parts, synthetic gene networks can be engineered to compensate for cross talk. For example, a common approach is to insulate signal transduction pathways by identifying and minimizing responsible interactions between the genetic components. Alternatively, one can introduce new gene network connections to cancel out cross talk at the network level. After quantitatively mapping the degree of cross talk, we designed gene circuits that introduced compensatory cross talk at the gene network level, essentially cancelling out the non-cognate ROS contribution to the output and improving the specificity of the overall circuit to the cognate ROS molecule (Müller et al., 2019)

Continuous Sensing

Microbial biosensors can continuously monitor their environments without the need for interrupted or periodic sampling. Sensing biomarkers *in situ* as they are produced could aid in elucidating

underlying mechanisms of disease, studying disease dynamics (Lubkowicz et al., 2018), and improving disease management. For example, an ingestible microbio-electronic device was developed to detect blood in the GI tract (Mimee et al., 2018). Specifically, probiotic *E. coli* Nissle 1917 (EcN) was engineered as a microbial biosensor to produce bioluminescence in the presence of heme, once imported into the cell. The biosensor was packaged in a capsule with miniaturized electronics that transmitted the detected luminescence signal as information wirelessly to an external device.

One important caveat is that microbial biosensors that rely on gene expression may have slower signal transduction than enzyme-based detection methods, although the former have the advantage that they can be coupled to biological responses. Another challenge with gene-expression based sensors is how to build them to detect extracellular analytes that do not penetrate into cells. To address this problem, Chang et al., 2018 developed a bacterial transmembrane receptor using single-domain antibodies for extracellular ligand detection. These receptors consist of a ligand-induced dimerization domain fused to a single-domain antibody in the periplasm and a monomeric DNA-binding domain inside the cell. In the presence of the stimulus, the chimeric receptor undergoes ligand-induced dimerization and activates downstream reporter gene expression. The authors successfully expressed a synthetic alpha-rep (helicoidal repeat protein) binder recognizing GFP and found that it could bind recombinant enhanced GFP in *E. coli* cells with a deficient outer membrane. The transmembrane receptor principle may be general enough to be used in other bacterial hosts, such as gram positives, that do not have an outer membrane. In that case, the receptor sensing domain would be directly exposed on the cell surface and accessible to extracellular ligands (Chang et al., 2018).

Non-invasiveness

Given their micron-scale size, microbial biosensors can be readily deployed in difficult-to-access sites of the body (Isabella et al., 2018). Even in remote areas [e.g., the small intestine or the center of a tumor, as has been reported for *Salmonella* bacteria naturally infecting solid

tumors (Saltzman et al., 1996) and EcN as a living biotherapeutic for the treatment of cancer (Leventhal et al., 2020)], microbial biosensors can detect biologically relevant labile compounds that would be otherwise metabolized, absorbed by the host, or inactivated in biological samples (serum, urine, or feces) (Sands, 2015). However, choosing bacterial chassis that are well adapted to the environment of interest is important for ensuring the accessibility and function of bacterial sensors. For example, microbiome studies often use antibiotics to clear out the native host microbiome to enable exogenously delivered bacteria to engraft, since exogenous bacteria are often unable to compete with the endogenous flora. However, this is not ideal if one wishes to use microbial biosensors to study the native host microbiome. An alternative is to directly engineer bacteria that are already well adapted to the microbiome of interest and then introduce these bacteria into the host, or to use bacteriophages that deliver gene circuits directly into bacteria already residing in the specific environment of interest. These last approaches may be of special interest in the search of new cancer therapies. Recent research that characterized the tumor microbiome has shown that each tumor type (breast, lung, ovary, pancreas, melanoma, bone, and brain tumors and the normal surrounding tissues were studied) has a distinct composition of bacteria, residing intracellularly either in cancer or immune cells, with potential consequences in the patient's response to immunotherapy (Nejman et al., 2020).

Scalability

The manufacturing of microbial biosensors is potentially cost-effective because microbial cells can self-renew and can be scaled up in industrial processes. These features can drive down the cost of deployment into real-world environments, which may be especially important for applications in the developing world. For example, Mao et al., 2018 used a probiotic-based strategy to detect cholera and trigger therapeutic functionality in an approach that could be deployed in the developing world. Specifically, these studies engineered *Lactococcus lactis* to detect quorum-sensing signals produced by *Vibrio cholerae* in the gut and then trigger the expression of an enzymatic reporter that is readily detected in fecal samples. The

investigators used a nitrocefin-based β-lactamase assay to generate a visible color change from yellow to red as the output. However, the potential for horizontal gene transfer of the β-lactamase gene to pathogenic bacteria restricts the use of this specific construct in the field. Therefore, a food-grade enzymatic reporter is needed that is not degraded in the human gut and can be detected with a cost-effective substrate to enable its use in low-resource settings.

By further engineering these probiotics to produce lactic acid, Mao et al., 2018 showed that the probiotic bacteria could reduce the intestinal *V. cholerae* burden and improve the survival of infected infant mice. Preventive dietary interventions with this technology could help block cholera progression and provide community-level surveillance of cholera cases in populations at risk of outbreaks. In addition to horizontal gene transfer, challenges posed by the self-renewing ability of microbial biosensors include the potential need for biocontainment due to regulatory demands as well as the possibility of genetic drift over time (Jimenez et al., 2019).

FUTURE PROSPECTS

The emerging field of synthetic biology offers a potential means to address major challenges in human health, from prevention to diagnosis to treatment. Although microbial biosensors offer several benefits compared to other sensing technologies, microbial biosensor engineering is still in its beginning, and advancements of biosensors into clinical settings will be imperative to identify areas needing improvement in terms of their performance and safety. Important bottlenecks hampering the deployment of these technologies into the real world are in the lack of well-validated disease biomarkers and obstacles for clinical translation. As the underlying design-build-test-learn synthetic biology cycle is expanding at a rapid pace, it will be important for regulators, industrialists, and clinicians to help speed up the testing of these concepts in clinical and industrial locales.

REFERENCES

Appleton, E. (2016). *A design-build-test-learn tool for synthetic biology* (Doctoral dissertation, Boston University).

Archer, E. J., Robinson, A. B., & Süel, G. M. (2012). Engineered E. coli that detect and respond to gut inflammation through nitric oxide sensing. *ACS synthetic biology*, *1*(10), 451-457.

Ceroni, F., Algar, R., Stan, G. B., & Ellis, T. (2015). Quantifying cellular capacity identifies gene expression designs with reduced burden. *Nature methods*, *12*(5), 415-418.

Chang, H. J., Mayonove, P., Zavala, A., De Visch, A., Minard, P., Cohen-Gonsaud, M., & Bonnet, J. (2018). A modular receptor platform to expand the sensing repertoire of bacteria. *ACS synthetic biology*, *7*(1), 166-175.

Courbet, A., Endy, D., Renard, E., Molina, F., & Bonnet, J. (2015). Detection of pathological biomarkers in human clinical samples via amplifying genetic switches and logic gates. *Science translational medicine*, *7*(289), 289ra83-289ra83.

Crook, N., Ferreiro, A., Gasparrini, A. J., Pesesky, M. W., Gibson, M. K., Wang, B., ... & Dantas, G. (2019). Adaptive strategies of the candidate probiotic E. coli Nissle in the mammalian gut. *Cell host & microbe*, *25*(4), 499-512.

Daeffler, K. N. M., Galley, J. D., Sheth, R. U., Ortiz-Velez, L. C., Bibb, C. O., Shroyer, N. F., ... & Tabor, J. J. (2017). Engineering bacterial thiosulfate and tetrathionate sensors for detecting gut inflammation. *Molecular systems biology*, *13*(4), 923.

Daniel, R., Almog, R., Ron, A., Belkin, S., & Diamand, Y. S. (2008). Modeling and measurement of a whole-cell bioluminescent biosensor based on a single photon avalanche diode. *Biosensors and Bioelectronics*, *24*(4), 882-887.

D'souza, S. F. (2001). Immobilization and stabilization of biomaterials for biosensor applications. *Applied biochemistry and biotechnology*, *96*(1-3), 225-238.

Inda, M. E., & Lu, T. K. (2020). Microbes as biosensors. *Annual Review of Microbiology*, *74*, 337-359.

Inda, M. E., Mimee, M., & Lu, T. K. (2019). Cell-based biosensors for immunology, inflammation, and allergy. *Journal of Allergy and Clinical Immunology*, *144*(3), 645-647.

Isabella, V. M., Ha, B. N., Castillo, M. J., Lubkowicz, D. J., Rowe, S. E., Millet, Y. A., ... & Reeder, P. J. (2018). Development of a synthetic live bacterial therapeutic for the human metabolic disease phenylketonuria. *Nature Biotechnology*, *36*(9), 857-864.

Jimenez, M., Langer, R., & Traverso, G. (2019). Microbial therapeutics: New opportunities for drug delivery. *The Journal of experimental medicine*, *216*(5), 1005-1009.

Konig, H., Frank, D., Heil, R., & Coenen, C. (2013). Synthetic genomics and synthetic biology applications between hopes and concerns. *Current genomics*, *14*(1), 11-24.

Kumar, J., & D'Souza, S. F. (2011). Immobilization of microbial cells on inner epidermis of onion bulb scale for biosensor application. *Biosensors and Bioelectronics*, *26*(11), 4399-4404.

Kumar, J., & D'Souza, S. F. (2010). An optical microbial biosensor for detection of methyl parathion using Sphingomonas sp. immobilized on microplate as a reusable biocomponent. *Biosensors and Bioelectronics*, *26*(4), 1292-1296.

Kumar, J., Jha, S. K., & D'souza, S. F. (2006). Optical microbial biosensor for detection of methyl parathion pesticide using Flavobacterium sp. whole cells adsorbed on glass fiber filters as disposable biocomponent. *Biosensors and Bioelectronics*, *21*(11), 2100-2105.

Lei, Y., Chen, W., & Mulchandani, A. (2006). Microbial biosensors. *Analytica chimica acta*, *568*(1-2), 200-210.

Leventhal, D. S., Sokolovska, A., Li, N., Plescia, C., Kolodziej, S. A., Gallant, C. W., ... & Momin, M. (2020). Immunotherapy with engineered bacteria by targeting the STING pathway for anti-tumor immunity. *Nature Communications*, *11*(1), 1-15.

Lubkowicz, D., Ho, C. L., Hwang, I. Y., Yew, W. S., Lee, Y. S., & Chang, M. W. (2018). Reprogramming probiotic Lactobacillus reuteri as a biosensor for Staphylococcus aureus derived AIP-I detection. *ACS synthetic biology*, *7*(5), 1229-1237.

Mao, N., Cubillos-Ruiz, A., Cameron, D. E., & Collins, J. J. (2018). Probiotic strains detect and suppress cholera in mice. *Science Translational Medicine*, *10*(445) eaao2586.

Meyer, A. J., Segall-Shapiro, T. H., Glassey, E., Zhang, J., & Voigt, C. A. (2019). Escherichia coli "Marionette" strains with 12 highly optimized small-molecule sensors. *Nature chemical biology*, *15*(2), 196-204.

Mimee, M., Nadeau, P., Hayward, A., Carim, S., Flanagan, S., Jerger, L., ... & Bulović, V. (2018). An ingestible bacterial-electronic system to monitor gastrointestinal health. *Science*, *360*(6391), 915-918.

Müller, I. E., Rubens, J. R., Jun, T., Graham, D., Xavier, R., & Lu, T. K. (2019). Gene networks that compensate for crosstalk with crosstalk. *Nature communications*, *10*(1).

Nejman, D., Livyatan, I., Fuks, G., Gavert, N., Zwang, Y., Geller, L. T., ... & Meltser, A. (2020). The human tumor microbiome is composed of tumor type–specific intracellular bacteria. *Science*, *368*(6494), 973-980.

Packer, M. S., & Liu, D. R. (2015). Methods for the directed evolution of proteins. *Nature Reviews Genetics*, *16*(7), 379-394.

Persson, B. A., Lund, M., Forsman, J., Chatterton, D. E., & Åkesson, T. (2010). Molecular evidence of stereo-specific lactoferrin dimers in solution. *Biophysical chemistry*, *151*(3), 187-189.

Pickup, J. C., Hussain, F., Evans, N. D., & Sachedina, N. (2005). In vivo glucose monitoring: the clinical reality and the promise. *Biosensors and Bioelectronics*, *20*(10), 1897-1902.

Piraner, D. I., Abedi, M. H., Moser, B. A., Lee-Gosselin, A., & Shapiro, M. G. (2017). Tunable thermal bioswitches for in vivo control of microbial therapeutics. *Nature chemical biology*, *13*(1), 75-80.

Price-Carter, M., Tingey, J., Bobik, T. A., & Roth, J. R. (2001). The Alternative Electron Acceptor Tetrathionate Supports B12-Dependent Anaerobic Growth ofSalmonella enterica Serovar Typhimurium on Ethanolamine or 1, 2-Propanediol. *Journal of bacteriology*, *183*(8), 2463-2475.

Richard, H. T., & Foster, J. W. (2003). Acid resistance in Escherichia coli. *Advances in applied microbiology*, *52*, 167-186.

Riglar, D. T., & Silver, P. A. (2018). Engineering bacteria for diagnostic and therapeutic applications. *Nature Reviews Microbiology*, *16*(4), 214-225.

Riglar, D. T., Giessen, T. W., Baym, M., Kerns, S. J., Niederhuber, M. J., Bronson, R. T., ... & Silver, P. A. (2017). Engineered bacteria can function in the mammalian gut long-term as live diagnostics of inflammation. *Nature biotechnology*, *35*(7), 653-658.

Saltzman, D. A., Heise, C. P., Hasz, D. E., Zebede, M., Kelly, S. M., Curtiss III, R., ... & Anderson, P. M. (1996). Attenuated Salmonella typhimurium containing interleukin-2 decreases MC-38 hepatic metastases: a novel anti-tumor agent. *Cancer biotherapy & radiopharmaceuticals*, *11*(2), 145-153.

Sands, B. E. (2015). Biomarkers of inflammation in inflammatory bowel disease. *Gastroenterology*, *149*(5), 1275-1285.

Schmidl, S. R., Ekness, F., Sofjan, K., Daeffler, K. N. M., Brink, K. R., Landry, B. P., ... & Tabor, J. J. (2019). Rewiring bacterial two-component systems by modular DNA-binding domain swapping. *Nature chemical biology*, *15*(7), 690-698.

Steinberg, S. M., Poziomek, E. J., Engelmann, W. H., & Rogers, K. R. (1995). A review of environmental applications of

bioluminescence measurements. *Chemosphere*, *30*(11), 2155-2197.

Su, L., Jia, W., Hou, C., & Lei, Y. (2011). Microbial biosensors: a review. *Biosensors and bioelectronics*, *26*(5), 1788-1799.

Tabor, J. J., Groban, E. S., & Voigt, C. A. (2009). Performance characteristics for sensors and circuits used to program E. coli. In *Systems Biology and Biotechnology of Escherichia coli* (pp. 401-439). Springer, Dordrecht.

Tuncagil, S., Ozdemir, C., Demirkol, D. O., Timur, S., & Toppare, L. (2011). Gold nanoparticle modified conducting polymer of 4-(2, 5-di (thiophen-2-yl)-1H-pyrrole-1-l) benzenamine for potential use as a biosensing material. *Food chemistry*, *127*(3), 1317-1322.

DEVELOPMENT OF ANTIBIOTIC RESISTANCE IN ESKAPE PATHOGEN

Jyoti Yadav[1], Anupam Jyoti[2], Vijay Kumar Srivastava[2], Vinay Sharma[3], Sanket Kaushik*[2]

[1]Student, Amity Institute of Biotechnology, Amity University, Jaipur, Rajasthan, India

[2] Assistant Professor, Amity Institute of Biotechnology, Amity University, Jaipur, Rajasthan, India

[3] Professor, Amity Institute of Biotechnology, Amity University, Jaipur, Rajasthan, India

*Corresponding Author: skaushik@jpr.amity.edu

INTRODUCTION

Nosocomial infections also called as health care associate infections are caused by microorganisms that are resistant to antibiotics (Khan et al., 2015). Group of nosocomial pathogens are called ESKAPE and it includes *Enterococcus faecium, Staphylococcus aureus, Klebisilla pneumonia, Acinetobacter baumanii, Pseudomonas aeruginosa and Enterobacter species*(Santajit et al., 2016). In these bacteria antibiotic resistant genes are found on the bacterial chromosome, plasmid, or transposons (Bennett, 2008). In hospital settings bacteraemiahas emerged as one of a major problem is defined as presence of bacteria in blood (Chiang et al., 2017). Major bacteremia in cancer patients was found to be because of the ESKAPE pathogens. Various studies indicated that about 54% was found to be only because of ESKAPE pathogens (Santajit et al., 2016). Moreover about 4.7% comprised of drug-resistant ESKAPE pathogens (rESKAPE) the resistant strains including vancomycin-resistant *Enterococcus faecium*, extended-spectrum beta-lactamase (ESLB)-producing *Klebsiella pneumoniae* methicillin-resistant *Staphylococcus aureus* (MRSA) , carbapenem and quinolone-resistant

ISBN: 978-81-950423-8-8

Pseudomonas aeruginosa, carbapenem-resistant *Acinetobacter baumannii* and β-lactam and ESBL-producing *Enterobacter* species. The *mecA* gene is a gene responsible for methicillinresistance in *Staphylococcus aureus*. This gene is responsible for production of penicillin-binding protein 2a (PBP2a; also called PBP2′) (Łęski and Tomasz, 2005).

Generally, the bacteremia due to these resistant strains have been found in people who suffered from more than one infections, were given inappropriate antibiotic treatment or had urinary tract infections (Wong et al., 2017). Several *E. faecium* species were detected VAN resistant, as they contained *vanA* gene (Szakacs et al., 2014). About 80% of the total ventilator-associated pneumonia (VAP) infections were reported to occur because of ESKAPE pathogens. Although ESKAPE pathogens are resistant to several antibiotics however some of them including Colistin and fosfomycin (the revived antibacterials) and early-phase neoglycoside (ACHN-490) were found to be the therapeutic options for treating the MDR in 2010. Also, polymyxins represented as polymyxin E (colistin) were found to be most potent in fighting the gram negative MDR strains. Fosfomycin is found to be very active against the Enterobacteriaceae MDR strains. Tigecycline also is found to be active against the Enterobacteriaceae and *Acinetobacter baumannii* MDR strains. ACHN-490 is still in early phase II trials and has an activity against the *K. pneumoniae* MDR strain (Cerceo et al., 2016). Two antibiotic peptides namely WLBU2 and WR12 are synthesized *de novo* and are found to work against the 142 MDR pathogen strains. Their working is found to be more efficient when compared to colistin and LL37 (Ma et al., 2020). In this regard with the development of molecular biology techniques it is now becoming possible to get detailed information about the important proteins and the unique pathways present in several organisms. With the emergence of MDR strains we can now target important proteins in the bacteria for drug development.

ESKAPE Pathogens

The ESKAPE group consists of six hospital borne pathogens that are multidrug resistant: *Enterococcus faecalis*, *S aureus*, *K pneumoniae*, *K pneumoniae*, *A baumannii*, *P aeruginosa* and *Enterobacter* species (Fig. 1). ESKAPE pathogens which are a reason for utmost nosocomial infections are associated with high motility rate that also increase the actual cost of intensive care (Founou et al., 2017).The development of new antimicrobial options by innovative strategies provide the in-depth knowledge of virulence, resistance transmission and pathogenesis of these microbes.Detailed structural information of the proteins drug targets will be beneficial to understand the detailed functional mechanism of these proteins. Therefore, directing attention towards the ESKAPE pathogens will help to permit critical assessment of novel antibacterial agents (Pendleton et al., 2013).

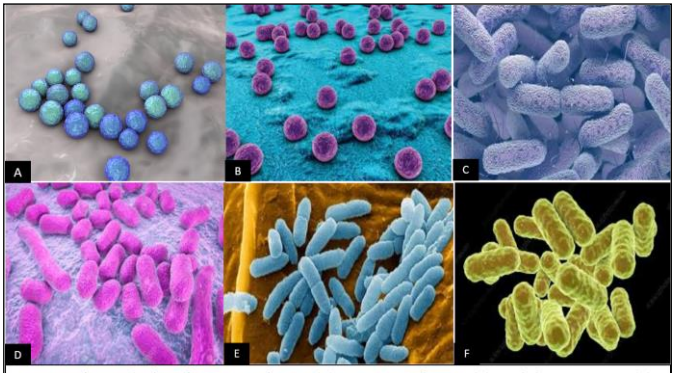


Figure 1. Microscopic view of ESKAPE pathogens: (A) *Enterococcus faecium*; (B) *Staphylococcus aureus*; (C) *Klebsiella pneumonia*; (D) *Acinetobacter baumannii*; (E) *Pseudomonas aeruginosa* and (F) *Enterobacter* spp. (Bacterial images adopted from internet)

***Enterococcus faecium* (E)**

E faecium a gram positive, opportunistic multidrug resistant bacterium is mostly present in human gut and animals. There are 20 enterococcal species of which *E faecalis* and *E faecium* are prominent cause of health care associated infections (Elsner et al., 2000). Over the past years, there was increase in vancomycin-resistant enterococcal infections and ampicillin resistance around the globe. Vancomycin- enterococci (VRE) emerged during late 1980's and was associated with *E. faecium* isolates which increased upto 61% in north America by 2002 (Uttley et al., 1988). Van- A-E and Van G are the six types of VRE upon which Van-A exhibit highest resistance level among all glycopeptide antibiotics. VRE is responsible for blood stream infection due to which high mortality rate, high cost of treatment and hospital duration occur (Chiang et al., 2017). Galloway-Pena and her colleagues exhibited two clades: Clade A and Clade B which are genetically different. Clinical isolates correlated with hospitals represented as Clade A and isolates correlated with community represented as Clade B. In their study they found both the clades reveal low- affinity penicillin- binding proteins which shows weak binding towards β-lactam (Arias and Murray, 2012).

***Staphylococcus aureus* (S)**

S aureus a gram positive bacterium is a representative of skin microbiota which is commonly isolated from moist areas. *S. aureus* with high carriage rates is an important wound pathogen which is responsible for the chronic and acute infections up to formation of biofilms (Pendleton et al., 2013). *S. aureus* biofilms are the main reason for chronic infection such as insertion of implanted medical devices like medical heart valves and orthopedic devices. Over the past two decades, due to β- lactamase produced by staphylococcus species, approximately 80% incidents in hospital associated and community infections were enhanced (Wu et al., 2010). All the strains which secreted collagenase, hemolysin and proteases hyaluronidase accommodate establishment on the host by destroying the tissue for nutrition (Boubaker et al., 2004). Medical strains of

Methicillin-resistant *Staphylococcus aureus* (MRSA) were tested for *S. aureus* isolates (25%) or more than 50% in some areas. Therefore, directing attention towards MRSA is a major concern and priority for healthcare system which help us to combat wide challenge of multidrug resistant bacteria. (Indrawattana et al., 2013).

Klebsiella pneumoniae (K)

K pneumoniae an opportunistic pathogen belonging to the Enterobacteriacae family along with *E. coli*. The infections caused by *Klebsiella* species may be acquired or endogenous, it spread through direct contact with an infected person and mostly these infections are associated with healthcare settings. (Santajit & Indrawattana, 2016). Strains of *K. pneumoniae* have aquired β-lactamase enzymes which have an ability to destroy the β-lactam antibiotics like cephalosporins carbapenems and penicillins. To treat determined infections produce by gram negative bacteria, carbapenems conventionally used which lead to increased prevalence of carbapenem-resistant strains (Queenan and Bush, 2007). Occurrence of carbapenemase-mediated MDR strains is a major concern therefore an effective treatment option is required to overcome these pathogens.

Acinetobacter baumannii (A)

A. baumannii a gram- negative bacterium are most often present in healthcare facilities such as incentive care units and surgical wards. *A. baumannii* have an ability to survive for a very long time on fingertips which may cause high rate of nosocomial cross-contamination (Pendleton et al., 2013). Due to the emergence of carbapenemase, imipenemmetallo- β-lactamases carried by *A. baumannii* strains were reported (Vila et al., 2007). The combined action of resistant genes makes them competent to avoid the action of most traditionally occurring antibiotic compounds (Boucher et al., 2009). This combination of factors of multidrug resistance and intrinsic virulence, *A. baumannii* become a global epidemic (Boucher et al., 2009).

Pseudomonas aeruginosa (P)

P. aeruginosa a Gram negative, rod-shaped, human pathogen are responsible for the severe respiratory infections (Diggle and Whiteley, 2020). In general population carriage rates are moderate but higher in immunocompromised individuals. Over a decade, *P. aeruginosa* remains feasible in the lungs oftenest diagnosed with Cystic fibrosis (CF). Polymyxin and carbapenem are the two widespread class of antibiotics shows resistance by *P. aeruginosa* isolates (Xipell et al., 2017). Due to increase in the multi drug resistant isolates of *P. aeruginosa* an effective antimicrobial therapy is urgently required (Boucher et al., 2009).

Enterobacter species (E)

Enterobacter species are gram-negative opportunistic pathogens which are commonly found in the respiratory tracts and bloodstream infections. They are responsible for the opportunistic infections in hospitalized patients and immunocompromised individuals with a broad range of antibiotic resistance mechanisms (Mehrad et al., 2015). Enterobacter species are most common in urinary and respiratory tracts along with bloodstream infections leading to serious nosocomial infections (Polin andSaiman, 2003).

Infections caused by ESKAPE Pathogen

ESKAPE pathogens are cause nosocomial infections which are capable of 'escaping' the action of antimicrobial agents. Infections caused by ESKAPE pathogens are recently been identified as a serious emerging problem to healthcare settings (Bodro et al., 2013). Due to ESKAPE pathogens ventilator- associated pneumoniae (VAP)continues to increases in frequency with increase in resistance profile of the pathogen in ICUs worldwide. VAP being the second most common nosocomial infection in critically ill patients therefore they are at higher risk of suffering from VAP, (Sandiumenge andRello, 2012). In Enterobacter species, *E. cloacae* causes' bloodstream infection and these infections are widespread in neonates and elderly individuals. *E. hormaechei* is emerging as most important pathogens and clinically relevant within the *E. cloacae*

unit (Annavajhala et al., 2019). In Asia, MRSA caused 50% of *S. aureus* bloodstream infections and approximately 40% of the population has *S. aureus* as a commensal organism (Turner et al., 2019). Patients with lung infections like bronchiectasis and also cystic fibrosis are highly vulnerable to persistent pulmonary infection. *P. aeruginosa* remains viable in the lungs of patients diagnosed with Cystic fibrosis almost over a decade (LaFayette et al., 2015).

Development of antibiotic resistance in ESKAPE Pathogen

According to World Health Organization (WHO) new antibiotics are needed against bacteria listed as ESKAPE pathogens. As per the urgency of need for antibiotic the pathogens are categorized as Critical, High and Medium (Tacconelli et al., 2018). The critical priority list of pathogen consists of carbapenem resistant *A. baumannii* and *P.aeruginosa*, *K.pneumoniae* and *Enterobacter* spp. whereas, high priority group methicillin and vancomycin resistant *S. aureus* (MRSA and VRSA) are listed. There are several examples of antibiotics for ESKAPE pathogens that developed resistance such as oxazolidines, macrolides, tetracycline, β- lactamase inhibitor combinations, fluoroquinolones and carbapenems and glycopeptides are the last line of defense (Naylor et al., 2018). The mechanisms used by the nosocomial ESKAPE pathogens consists of Drug inactivation and alteration, modification drug binding site, Reduced intracellular Drug Accumulation and formation of biofilm. (Santajit and Indrawattana, 2016).

Drug inactivation and alteration: Aminoglycoside-modifying enzymes, β-lactamases and chloramphenicol acetyltransferases are some bacterial enzymes which inactivate the antibiotics and modify the enzyme irreversibly. β-lactamases is well characterized enzyme which is classified according to two main systems: The Ambler scheme and the Bush-Jacoby-Medeiros system. The Ambler scheme consists of four group classes: Cephalosporinase, Penicillinase, extended-spectrum β- lactamases (ESBLs), broad-spectrum β-lactamases, and carbapenemases are the bacterial enzymes produce

by Ambler class A. β-lactamase are the inhibitors that inhibits the Class A enzymes., example sulbactam and tazobactam (Giedraitiene et al., 2011). Bacteria that produce Ambler class B enzymes exhibit resistance to β-lactams such as cephalosporins, carbapenems, penicillins and β-lactamase inhibitors, except aztreonam. The Ambler class C group includes cephalosporinase and penicillinase, such as AmpC β-lactamase, which results in low level resistance to narrow-spectrum cephalosporin drugs (Jacoby, 2009). The most common members of Ambler class D enzymes consist of oxacillin hydrolyzing enzymes (OXA) such as s OXA-11, OXA-14, and OXA16 (Dzidi et al., 2008).

Modification of Drug binding site

Another common mechanism of multi-drug resistant by ESKAPE microbes is to modify the target sites of antibiotic which include mechanism such as modification of target enzyme, Alterations of ribosomal target site and Alterations of cell wall precursor (Sanchit et al., 2016). Mutation in the gene coding for penicillin- binding proteins results in the production of mutated protein with unique properties. Penicillin- binding proteins are enzymes, in bacteria generally they are present on the membrane of cytoplasm of the cell wall. It functions in assembly and control of the latter stages of the cell wall building (Pucci and Dougherty, 2002). ESKAPE pathogens also acquire multi-drug resistant through enzyme targets modification of fluoroquinolone group of antibiotics. Ciprofloxacin and norfloxacin are the fluoroquinolones which shows some of the most widely advised antimicrobial agents (Hooper, 2001).

Decreased intracellular Drug Accumulation

The stability between uptake of antibiotic and their removal regulate vulnerability of a particular drug. Therefore, mechanism by which the incidence of moderated protein channels on the bacterial outer membrane decrease the entry of drug and to decrease the quantity of drug accumulation in the cell efflux pumps present (Sanchit et al., 2016). Porins are the proteins which are present on the outer membranes of gram negative bacteria that form channels through

which hydrophilic and small molecules are allowed to pass. OprD porin associated to reduced carbapenem susceptibility by loss or modification of *P. aeruginosa* (Cui et al., 2003). The proteins of membrane that play a role as exporters are known as efflux pumps. Resistance-nodulation-division (RND), multidrug and toxic compound extrusion (MATE), major facilitator superfamily (MFS), ATP-binding cassette (ABC), small multidrug resistance (SMR and proteobacterial antimicrobial compound efflux (PACE) are the six main efflux pump families which are categories (Li et al., 2015).

Biofilm formation

Biofilms are the surface associated microorganisms attached to a surface coated with extracellular matrix which secretes extracellular polymeric substances (EPS). These EPS comprise of polysaccharides, proteins and lipids (Sharma et al., 2014). The major processes by which biofilm mediated antibiotic resistance increases are; limited antibiotic penetration through ECM, antibiotic- modifying enzymes excretion, increased filamentous bacteriophage growth, communication among different microbial species in biofilms, exposure of persister cells, biofilm associated efflux, increased in mutations and horizontal gene transfer and altered metabolic activity (Secor et al., 2018). Biofilms of *S. aureus, P. aeruginosa, A. baumannii,* and *K. pneumonia* are the pathogens which are most commonly present in healthcare settings (Høiby et al., 2010).

Treatment and control

Antimicrobial drug discovery is extremely challenging and current escalation in antimicrobial resistant has prompted us to find alternative methods for treatment (Payne et al., 2007). The occurrence of multi drug resistance (MDR) and extensive drug resistance (XDR) are now posing a crucial challenge to healthcare community. The general antibiotic therapy used to efficiently treat infections caused by bacteria involves the use of antibiotics either singly or in combination (Mulani et al., 2019). There are several alternative therapies such as use of Bacteriophage therapy,

Antimicrobial peptides in therapy, Photodynamic light therapy and Silver Nanoparticles in therapies (Fig. 2) **(**Mandal et al., 2014).

The Bacteriophage therapy

Bacteriophages the viruses which infect bacteria are also utilized as therapeutic agents for the treatment of bacterial infections. Bacteriophages used for therapy shows many advantages which includes high specificity on host, treatment by using low dosages, rapid proliferation inside the host bacteria, etc. (Domingo-Calap and Delgado-Martínez, 2018). *In vitro* phages have shown to be efficient as antibacterial agents against biofilm and planktonic cells of ESKAPE. *In vitro* studies have also demonstrated efficacy and safety of phages which were used in treatment of bacterial infections (Jamal et al., 2019). Endolysin produced by bacteriophages, has been established as beneficial for destruction of bacterial cell wall and facilitating entry of the antibiotic. Reduction of biofilm formation in bacteria occurs when antibiotics are used in combination with phage therapy (Chaudhry et al., 2017).

Use of Antimicrobial peptides (AMPs) in therapy

Antimicrobial peptides (AMPs) commonly called as host defense peptides are short, charged, varied host defense oligopeptides formed by all living organisms. They offer a wide spectrum of activity against a broad range of pathogen. AMPs are also found to have promising in vivo activity against ESKAPE pathogens (Mulani et al., 2019). It has been reported that HLR1 shows anti-inflammatory and noncytotoxic effects to treat skin infections against MRSA infected wound excision model in rat which is a new therapeutic alternative (Björn et al., 2016). Telavancin, vancomycin, teicoplanin, telaprevir, enfuvirtide, dalbavancin, daptomycin, bacitracin etc are examples of some useful peptides such which can be used for treatment either alone or in combination with other antibiotics (Gomes et al., 2018).

Use of Photodynamic Light in therapy

Photodynamic light therapy can be used either alone or combined with a photosensitizer (PS), which leads to microbial death, it is widely utilized in treatment of dental, skin and soft tissue infections (Cieplik et al., 2018). Time dependant effect of the therapy on *P. aeruginosa* demonstrated that regular exposure of light emitting diode (LED) increased the inhibitory effect on bacterial growth (Sueoka et al., 2018). Extensive research has been carried out to design the PSs with increased inhibitory effect. For antimicrobial therapy, an ideal PS should have better permeability to cross the bacterial cell membrane, selective toxicity with minimal harm to the human tissue and for effective penetration at the site of action absorption coefficient is appropriate (Cieplik et al., 2018). Combined effect of using aPDT-antibiotic has proved more effective to inactivate various virulence factors in several bacterial isolates (Fila et al., 2017).

Use of Nanoparticles in Therapy

It is well known that metal nanoparticles have diverse biomedical applications for treating drug resistant pathogens. Due to their multi-targeted approach, silver nanoparticles (AgNPs) synthesized using physical, chemical or biological methods have shown promising antibacterial activity (Siddiqi et al., 2018). It has been showed that AgNPs utilized in combination with different antibiotics provide synergistic antibacterial activities against different bacteria including ESKAPE pathogens. Despite the use of AgNPs as a potential therapeutic agent, they are not used commonly due to the lack of data suggesting the toxicity of these nanoparticles on host cells. It is believed AgNPs can have a toxic effect on the human cell as well (Mulani et al., 2019). Although numerous studies are being carried out to understand the toxic effect of AgNPs, more such investigation are required to understand the mechanism of toxicity of nanoparticles.

Conclusion and Future prospective

Multidrug resistance is becoming a major threats to medical community, which usually caused by too much drug usage,

improper use of antibiotics etc. ESKAPE has become important human pathogens because of the increase in number of infections caused by these organisms and the appearance of multidrug resistant strains. These pathogens are mostly found in hospital acquired infections and are developing resistance against many commonly occurring antibiotics.

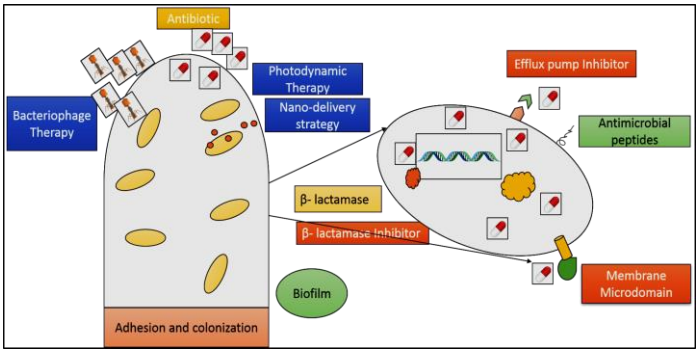


Figure 2. Treatment and control of ESKAPE pathogen

The mechanisms used by the nosocomial ESKAPE pathogens to acquire antibiotic resistance includes modification of drug targets, enzymatic inactivation, altered cell permeability through porin loss or increase in expression of efflux pumps, and mechanical protection provided by biofilm formation. In this regard there is an increased use novel therapeutic agent such as phage therapy, antimicrobial peptides, metal nanoparticles, and photodynamic light to fight the ESKAPE infections. In addition to this with increase in the structural information of important protein drug targets from different bacteria it is now possible to target an important protein of the bacteria for drug development. Protein drug targets are the molecules which are crucial for the survival of the bacterium and their inhibition will hinder bacterial growth leading to its destruction.

References

Arias, C.A. and Murray, B.E., 2012. The rise of the Enterococcus: beyond vancomycin resistance. *Nature Reviews Microbiology*, *10*(4), pp.266-278.

Bennett, P.M., 2008. Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. *British journal of pharmacology*, *153*(S1), pp.S347-S357.

Beyth, N., Houri-Haddad, Y., Domb, A., Khan, W. and Hazan, R., 2015. Alternative antimicrobial approach: nano-antimicrobial materials. *Evidence-based complementary and alternative medicine*, *2015*.

Björn, C., Mahlapuu, M.,Mattsby-Baltzer, I. and Håkansson, J., 2016. Anti-infective efficacy of the lactoferrin-derived antimicrobial peptide HLR1r. *Peptides*, *81*, pp.21-28.

Boubaker, K., Diebold, P., Blanc, D.S., Vandenesch, F., Praz, G., Dupuis, G. and Troillet, N., 2004. Panton-valentine leukocidin and staphyloccoccal skin infections in schoolchildren. *Emerging infectious diseases*, *10*(1), p.121.

Boucher, H.W., Talbot, G.H., Bradley, J.S., Edwards, J.E., Gilbert, D., Rice, L.B., Scheld, M., Spellberg, B. and Bartlett, J., 2009. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clinical infectious diseases*, *48*(1), pp.1-12.

Cerceo, E., Deitelzweig, S.B., Sherman, B.M. and Amin, A.N., 2016. Multidrug-resistant gram-negative bacterial infections in the hospital setting: overview, implications for clinical practice, and emerging treatment options. *Microbial Drug Resistance*, *22*(5), pp.412-431.

Chaudhry, W.N., Concepcion-Acevedo, J., Park, T., Andleeb, S., Bull, J.J. and Levin, B.R., 2017. Synergy and order effects of antibiotics and phages in killing Pseudomonas aeruginosa biofilms. *PloS one*, *12*(1), p.e0168615.

Chiang, H.Y., Perencevich, E.N., Nair, R., Nelson, R.E., Samore, M., Khader, K., Chorazy, M.L., Herwaldt, L.A., Blevins, A.E., Ward, M.A. and Schweizer, M.L., 2017. Incidence and outcomes associated with infections caused by vancomycin-

resistant enterococci in the United States: systematic literature review and meta-analysis.

Cieplik, F., Deng, D., Crielaard, W., Buchalla, W., Hellwig, E., Al-Ahmad, A. and Maisch, T., 2018. Antimicrobial photodynamic therapy–what we know and what we don't. *Critical Reviews in Microbiology*, *44*(5), pp.571-589.

Cui, L., Ma, X., Sato, K., Okuma, K., Tenover, F.C., Mamizuka, E.M., Gemmell, C.G., Kim, M.N., Ploy, M.C., El Solh, N. and Ferraz, V., 2003. Cell wall thickening is a common feature of vancomycin resistance in Staphylococcus aureus. *Journal of clinical microbiology*, *41*(1), pp.5-14.

DiazGranados, C.A. and Jernigan, J.A., 2005. Impact of vancomycin resistance on mortality among patients with neutropenia and enterococcal bloodstream infection. *The Journal of infectious diseases*, *191*(4), pp.588-595.

Diggle, S.P. and Whiteley, M., 2020. Microbe Profile: Pseudomonas aeruginosa: opportunistic pathogen and lab rat. *Microbiology*, *166*(1), pp.30-33.

Domingo-Calap, P. and Delgado-Martínez, J., 2018. Bacteriophages: protagonists of a post-antibiotic era. *Antibiotics*, *7*(3), p.66.

Džidić, S., Šušković, J. and Kos, B., 2008. Antibiotic resistance mechanisms in bacteria: biochemical and genetic aspects. *Food Technology & Biotechnology*, *46*(1).

Elsner, H.A., Sobottka, I., Mack, D., Laufs, R., Claussen, M. and Wirth, R., 2000. Virulence factors of Enterococcus faecalis and Enterococcus faecium blood culture isolates. *European Journal of Clinical Microbiology and Infectious Diseases*, *19*(1), pp.39-42.

Founou, R.C., Founou, L.L. and Essack, S.Y., 2017. Clinical and economic impact of antibiotic resistance in developing countries: A systematic review and meta-analysis. *PloS one*, *12*(12), p. e0189621.

Giedraitienė, A., Vitkauskienė, A., Naginienė, R. and Pavilonis, A., 2011. Antibiotic resistance mechanisms of clinically important bacteria. *Medicina*, *47*(3), p.19.

Gomes, B., Augusto, M.T., Felício, M.R., Hollmann, A., Franco, O.L., Gonçalves, S. and Santos, N.C., 2018. Designing improved active peptides for therapeutic approaches against infectious diseases. *Biotechnology advances*, *36*(2), pp.415-429.

Høiby, N., Bjarnsholt, T., Givskov, M., Molin, S. and Ciofu, O., 2010. Antibiotic resistance of bacterial biofilms. *International journal of antimicrobial agents*, *35*(4), pp.322-332.

Indrawattana, N., Sungkhachat, O., Sookrung, N., Chongsa-Nguan, M., Tungtrongchitr, A., Voravuthikunchai, S.P., Kong-Ngoen, T., Kurazono, H. and Chaicumpa, W., 2013. Staphylococcus aureus clinical isolates: antibiotic susceptibility, molecular characteristics, and ability to form biofilm. *BioMed Research International*, *2013*.

Jamal, M., Andleeb, S., Jalil, F., Imran, M., Nawaz, M.A., Hussain, T., Ali, M., ur Rahman, S. and Das, C.R., 2019. Isolation, characterization and efficacy of phage MJ2 against biofilm forming multi-drug resistant Enterobacter cloacae. *Folia microbiologica*, *64*(1), pp.101-111.

Ma, Y.X., Wang, C.Y., Li, Y.Y., Li, J., Wan, Q.Q., Chen, J.H., Tay, F.R. and Niu, L.N., 2020. Considerations and caveats in combating ESKAPE pathogens against nosocomial infections. *Advanced Science*, *7*(1), p.1901872.

Mandal, S.M., Roy, A., Ghosh, A.K., Hazra, T.K., Basak, A. and Franco, O.L., 2014. Challenges and future prospects of antibiotic therapy: from peptides to phages utilization. *Frontiers in pharmacology*, *5*, p.105.

Mulani, M.S., Kamble, E.E., Kumkar, S.N., Tawre, M.S. and Pardesi, K.R., 2019. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. *Frontiers in microbiology*, *10*, p.539.

Naylor, N.R., Atun, R., Zhu, N., Kulasabanathan, K., Silva, S., Chatterjee, A., Knight, G.M. and Robotham, J.V., 2018. Estimating the burden of antimicrobial resistance: a systematic literature review. *Antimicrobial Resistance & Infection Control*, *7*(1), p.58.

Khan, H.A., Ahmad, A. and Mehboob, R., 2015. Nosocomial infections and their control strategies. *Asian pacific journal of tropical biomedicine*, *5*(7), pp.509-514.

Łęski, T.A. and Tomasz, A., 2005. Role of penicillin-binding protein 2 (PBP2) in the antibiotic susceptibility and cell wall cross-linking of Staphylococcus aureus: evidence for the cooperative functioning of PBP2, PBP4, and PBP2A. *Journal of bacteriology*, *187*(5), pp.1815-1824.

Li, X.Z., Plésiat, P. and Nikaido, H., 2015. The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. *Clinical microbiology reviews*, *28*(2), pp.337-418.

Lima, R., Del Fiol, F.S. and Balcão, V.M., 2019. Prospects for the use of new technologies in combating multidrug-resistant bacteria. *Frontiers in pharmacology*, *10*, p.692.

Payne DJ, Gwynn MN, Holmes DJ, Pompliano DL. 2007. Drugs for bad bugs: confronting the challenges of antibacterial discovery. Nat Rev Drug Discov 6:29 – 40.

Pendleton, J.N., Gorman, S.P. and Gilmore, B.F., 2013. Clinical relevance of the ESKAPE pathogens. *Expert review of anti-infective therapy*, *11*(3), pp.297-308.

Polin, R.A. and Saiman, L., 2003. Nosocomial infections in the neonatal intensive care unit. *NeoReviews*, *4*(3), pp.e81-e89.

Pucci, M.J. and Dougherty, T.J., 2002. Direct quantitation of the numbers of individual penicillin-binding proteins per cell in Staphylococcus aureus. *Journal of Bacteriology*, *184*(2), pp.588-591.

Queenan, A.M. and Bush, K., 2007. Carbapenemases: the versatile β-lactamases. *Clinical microbiology reviews*, *20*(3), pp.440-458.

Sandiumenge, A. and Rello, J., 2012. Ventilator-associated pneumonia caused by ESKAPE organisms: cause, clinical features, and management. *Current opinion in pulmonary medicine*, *18*(3), pp.187-193.

Santajit, S. and Indrawattana, N., 2016. Mechanisms of antimicrobial resistance in ESKAPE pathogens. *BioMed research international*, *2016*.

Secor, P.R., Michaels, L.A., Ratjen, A., Jennings, L.K. and Singh, P.K., 2018. Entropically driven aggregation of bacteria by host polymers promotes antibiotic tolerance in Pseudomonas aeruginosa. *Proceedings of the National Academy of Sciences*, *115*(42), pp.10780-10785.

Sharma, G., Rao, S., Bansal, A., Dang, S., Gupta, S. and Gabrani, R., 2014. Pseudomonas aeruginosa biofilm: potential therapeutic targets. *Biologicals*, *42*(1), pp.1-7.

Siddiqi, K.S., Husen, A. and Rao, R.A., 2018. A review on biosynthesis of silver nanoparticles and their biocidal properties. *Journal of nanobiotechnology*, *16*(1), p.14.

Szakacs, T.A., Kalan, L., McConnell, M.J., Eshaghi, A., Shahinas, D., McGeer, A., Wright, G.D., Low, D.E. and Patel, S.N., 2014. Outbreak of vancomycin-susceptible Enterococcus faecium containing the wild-type vanA gene. *Journal of clinical microbiology*, *52*(5), pp.1682-1686.

Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D.L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y. and Ouellette, M., 2018. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *The Lancet Infectious Diseases*, *18*(3), pp.318-327.

Turner, N.A., Sharma-Kuinkel, B.K., Maskarinec, S.A., Eichenberger, E.M., Shah, P.P., Carugati, M., Holland, T.L. and Fowler, V.G., 2019. Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research. *Nature Reviews Microbiology*, *17*(4), pp.203-218.

Xipel, M., Bodro, M., Marco, F., Losno, R.A., Cardozo, C. and Soriano, A., 2017. Clinical experience with ceftazidime/ avibactam in patients with severe infections, including meningitis and lung abscesses, caused by extensively drug-resistant Pseudomonas aeruginosa. *International Journal of Antimicrobial Agents*, *49*(2), pp.266-268.

Vila, J., Martí, S. and Sanchez-Céspedes, J., 2007. Porins, efflux pumps and multidrug resistance in Acinetobacterbaumannii. *Journal of antimicrobial chemotherapy*, *59*(6), pp.1210-1215.

Wong, D., Nielsen, T.B., Bonomo, R.A., Pantapalangkoor, P., Luna, B. and Spellberg, B., 2017. Clinical and pathophysiological overview of Acinetobacter infections: a century of challenges. *Clinical microbiology reviews*, *30*(1), pp.409-447.

Wu, D., Wang, Q., Yang, Y., Geng, W., Wang, Q., Yu, S., Yao, K., Yuan, L. and Shen, X., 2010. Epidemiology and molecular characteristics of community-associated methicillin-resistant and methicillin-susceptible Staphylococcus aureus from skin/soft tissue infections in a children's hospital in Beijing, China. *Diagnostic microbiology and infectious disease*, *67*(1), pp.1-8.

Zaman, S.B., Hussain, M.A., Nye, R., Mehta, V., Mamun, K.T. and Hossain, N., 2017. A review on antibiotic resistance: alarm bells are ringing. *Cureus*, *9*(6).

DESERT PLANTS: A POTENTIAL SOURCE TO COMBAT URINARY TRACT INFECTIONS

Neha Singh[1], Neelam Jain[2], and G K Aseri*[3]

[1]Ph.D. Scholar, Amity Institute of Microbial Technology, Amity University Rajasthan, Jaipur-303002

[2]Professor, Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur-303002

[3]Professor, Amity Institute of Microbial Technology, Amity University Rajasthan, Jaipur-303002

*Corresponding Author: gkaseri@jpr.amity.edu

Introduction

Urinary tract infections (UTI) are the second most common disease in long-term care facilities and community medical practice (Chau *et al*., 2016). It is defined as an inflammatory syndrome caused by several microorganism invading the urinary tract. More than eight million urinary tract infection (UTI) cases are reported yearly, and, likely, this figure is substantially under-estimated. Approximately two in six people globally are estimated to be infected with UTI at least once annually. Antibiotic-resistant infections are associated with more significant morbidity and mortality. They affect various patients ranging from young children to the elderly and from healthy men and women to the compromised ones. Urinary tract infections are the most prevalent bacterial disease and have a vital massive economic and societal burden on health care sectors across the globe (Mobley *et al.*, 1996). In community practice, high morbidity and financial infectious disease cost more than 5 billion US $ (Prakash *et al*., 2013). It is classified into uncomplicated and complicated infections in the kidney, urethra, and bladder (Raka *et al*., 2016). About 95% of the

ISBN: 978-81-950423-8-8

microorganisms are responsible for such conditions as *Klebsiella*, *Enterococcus*, *Staphylococcus* and *Proteus*, *chlamydia trachomatis* and *Neisseria gonorrhoeae*, and some of the fungi are responsible. Among these bacteria, *E-coli* is the predominant pathogen (Kavitha *et al.*, 2014), which causes 80% of all UTI to use antibiotics and increases urinary tract infection chances in Immuno compromised patients. The suppressed immune system, poor nutrition, unhygienic conditions like improper washing of hands, indiscriminated patients (HIV/Diabetes) are more prone to these infections (Mithraja *et al.*, 2012). Antibiotic resistance is a critical threat that is no prolonged expected by the world health organization. It's commonly found in many countries and can harm anyone of any age in the world (WHO, 2014).

Antibiotic drugs are responsible for most of severe bacterial resistance as far as global public health is concerned. In developing countries where drug availability is insufficient, and resistance is high, it is more critical (Ventola *et al.*, 2015). There are several approaches to the treatment and management of UTIs. Therefore, we need to look elsewhere for new antibiotics sources, and plant material is an obvious alternative. For thousands of years, herbal products have performed an essential role in treating and preventing human infections and earlier served humankind as the source of all drugs (Thakur *et al.*, 2016).

Healing Plants have been accepted as a universal repository of bioactive elements with various operative combinations in their formation. Their antimicrobial activity is attributed to multiple tools Willing to human well-being (Kavitha *et al.*,2014). In the international market, approximately 75% of medicinal plant drugs are open in traditional medicinal knowledge. About 70% of novel drugs are synthetically modified and begun from natural sources occurring in India (Pan *et al.*, 2014).Throughout the world, there are several reports for herbal treatment of UTIs. There are many herbs present in Ayurveda that help in the healing of these urinary tract infections.

Interestingly, it is essential to note that about 60% of cancer medicines and 80% of cardiovascular disease drugs, antimicrobial, and immunosuppressive available in the market are natural products. About 11% of the 433 vital drugs selected by the World Health Organization are obtained from plants, whereas Globally, nearly one-quarter of guided medicines are herbal based (WHO 2017). This has triggered an interest in finding new antimicrobial agents possessed antibacterial, antifungal, antidiabetic, antioxidant, anti-nociceptive, and shown anti-inflammatory activity. Desert medicinal plants are having a commendable knowledge of the medicinal values. The vast majority is still untouched and waiting to be explored for their bioactive constituents. This chapter seeks to combine antimicrobial studies of desert medicinal plants indigenous to Thar desert against urinary pathogens, relevant to public health and previously reported good to excellent antimicrobial activities

Pathogenesis of UTI

It is removing water and waste from the body by the urinary tract. It is constituted of the urethra, kidneys, bladder, ureters, and prostate in males. The function of kidneys is to remove waste and purify the blood. (Raka *et al*., 2016). The UTI pathogenesis, defined by the body of the urinary tract and is a result of the interplay between virulence parts of microorganisms and the host support system (Figure:1).

Microorganisms Infecting Urinary Tract Infection

UTI founds in many patients of all ages but more common with ladies; about 60 % of ladies have undergone Urinary tract infections throughout their life. Urinary tract infection shortens a huge gathering of requirements that cover pyelonephritis, cystitis, urethritis and prostatitis (Table 1).

Treatment of UTIs

As bacteria generally cause UTI, all are usually managed with antimicrobial antibiotics. But, there are various limitations associated with antibiotics, such as a variety of remedy and period

of therapy depends on the nature of bacteria, its level of susceptibility, symptoms, history, and immune status of the patient and also emerging cases of antimicrobial drug resistance has led to use of alternative therapies.

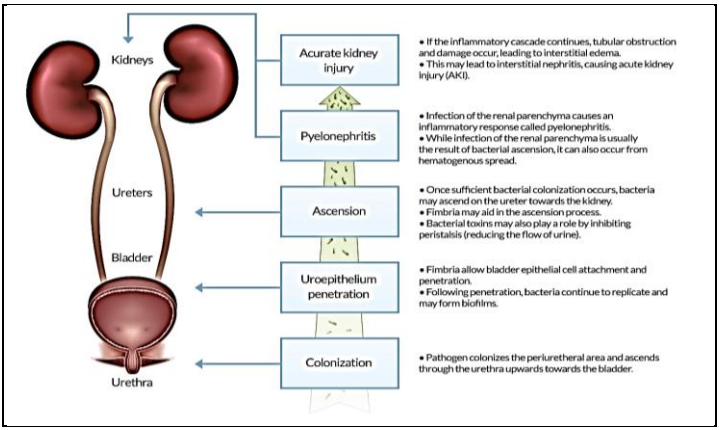


Figure 1: Pathogenesis of UTI

Allopathic medicines have serious side effects on health. Some of the existing antibiotic drugs like *Bactrim, Amoxicillin, Ampicillin, sulphamethoxazole, and Ciprofloxacin* may cause several future problems; they kill bacteria indiscriminately throughout the body when taken repeatedly, they make the immune system weaker instead to give strength (Wagenlehner and Naber, 2005). Microbial resistance is increasing, and the outlook for antimicrobial medicines in the future is still uncertain. Therefore, appropriate footsteps need to be taken to overcome this problem, such as controlling the treatment of antibiotics, developing research to understand the genetic mechanisms of resistance better, and continuing investigations to develop new drugs (Jaradat *et al.*,2020).

Traditional uses and biological effects of the desert plants

Desert Medicinal plants are known to produce individual bioactive molecules that react with other organisms in the environment, inhibiting bacterial or fungal growth (Jaradat *et al*., 2020).

Table 1: Microbial agents of Urinary tract infections (Raka *et al*., 2016).

Types of UTI	Etiological Agents
Cystitis & Pyelonephritis	*Gram negative bacilli*: *Acinetobacter* species, *Escherichia coli, Enterobacter spp, Klebsiella spp., Pseudomonas aeruginosa, Proteus mirabilis*
	Gram positive cocci: *Enterococcus spp., Staphylococcus aureus,* Coagulase negative Staphylococci, *Staphylococcus saprophyticus, Leptospira interrogans, Aerococcus urinae*
	Corynebacterium urealyticum, Salmonella typhi
	Fungi: *Candida glabrata, Candida albicans,*
	Parasites: *Schistosoma haematobium*
	Viruses: *Herpes simplex virus, Adenoviruses*
Urethritis	*Trichomonas vaginalis, Candida spp., Chlamydia trachomatis, Neisseria gonorrhoeae, Ureaplasma urealyticum*
	Mycoplasma hominis, Mobiluncus spp., Gardnerella vaginalis
Prostatitis	*Cryptococcus neoformans, Escherichia coli, Neisseria gonorrhoeae, Klebsiella spp., Candida spp., Proteus mirabilis, Enterococcus spp., Enterobacter spp.*

The use of plant phytochemicals, including extracts, both with known antimicrobial assets, can be a great significance in treatments.The herbal drug has been shown to have actual utility, and around 80% of the rural community depends on it as primary

health care (WHO 2017). The medicinal plants of this region have great potential to be used in the drug and pharmaceutical industries. The Discovery of new plant-based antimicrobial compounds that will help develop new preparations for an infectious disease like urinary tract infection cases is quite prevalent, particularly in rural areas.

Desert medicinal plant used to treat urinary tract infections

Thar Desert is very rich in medicinal plant capital which is documented in this table (Table-2). Medicinal plant from both arid and semi-arid regions have been used from ancient times for the management and treatment of UTIs (Fig 2) because of their less side effect, cost effectiveness, easy availability, and absence of bacterial resistance.

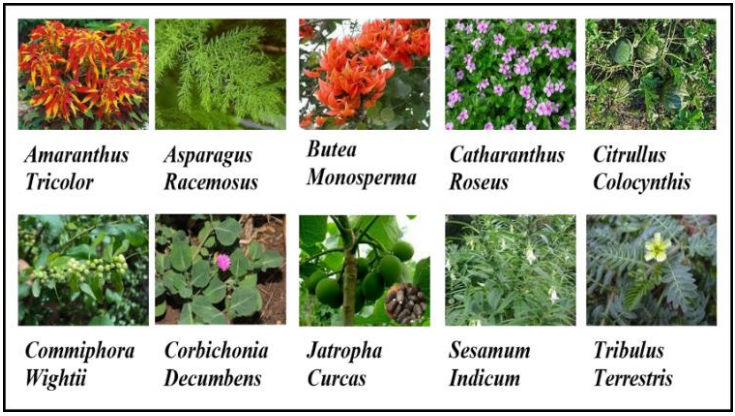


Figure 2. Few Desert Medicinal plants used to treat urinary tract infection

The leaves of *Amaranthus tricolor* (L) contains betalain pigments rich in amaranthin, betaxanthin, methyl derivative of arginine betaxanthin, and betalamic acid (Mousumi *et al.*, 2013) with potential pharmacological activity against bacterial pathogens related to UTIs. The roots of the *Asparagus racemosus* plants have been widely used in traditional medicine so as to cure large number

of diseases which include nervous disorders, cancers, and urinary tract infections etc. Large number of people treat dysuria and UTI by consuming boiled water or milk along with roots of *Asparagus racemosus* (Goyal *et al.,* 2003). Gum of *Butea monosperma* has been used in folk medicines in order to treat both microbial as well as fungal infection. Root bark has been largely used as an aphrodisiac, analgesic and antihelmintic while the leaves hold antimicrobial property (Kasture *et al.,* 2000). Conventionally, *Catharanthus roseus* has been used in folk medication for the management of diabetes, high blood pressure, anticancer activities and they possess unique antibacterial bio-active compounds against human pathogenic bacteria. (Abdulkarim *et al.,* 2005) *Citrullus colocynthis* roots are used as purgative against various diseases like jaundice, urinary diseases, rheumatism (Uma & Sekar *et al.,* 2014). Herbal extracts of *Commiphora wightii* have shown inhibitory action against struvite crystals which is one of the major components of urinary stones (Chauhan *et al.,*2009). *Corbichonia decumbens* is considered as a plant with high degree of antimicrobial activity which seems to be confirmed by the folk therapy of infections and traditional therapeutic claims of this herb. This plant has been used for the management of kidney stone problems. In tri bels the leaves of a plant are being crushed and are consumed orally in case of kidney stone problem (Uma *et al.,* 2013). *Jatropha's* oil seeds have been used for their purgative properties by expelling intestinal parasites (Suhaili *et al*., 2011). The antimicrobial effectiveness of Sesame oil and its products against bacterial infection have been reported (Shasmita *et al*.,2015). *Tribulus terrestris* plant has been conventionally used as a remedy for stomach-ache and urinary tract infections as practised ethno medically over the globe (Usman *et al*., 2007).

Discussion

Regular applications of Desert healing therapeutic plants, well-organized experimental research, and identification of bioactive natural plant compounds and their assets are expected to produce new therapies and nature-based herbal goods, these findings suggest that these Desert medicinal plants may be considered a promising

source of new antibacterial agents and further studies should be performed to characterize their mode of action. Many great new drugs have been obtained from natural origins, many based on their traditional medicine application (Murugan & Mohan, 2011). Analysis of the chemical and biological features of herbal remedies outcomes over the past two centuries has not solely yielded drugs for the therapy of human diseases but also triggered the development of new synthetic natural chemistry to discover innovative and more efficient therapeutic agents. A unique plant may contain many bio-active compounds and other medicinal properties. Various strategies have been taken towards the therapy and management of UTIs. Among these, the use of herbal agents has proposed a new way of blocking and managing infections. Improvements in phytochemistry and the testimony of bioactive compounds from desert plants effective against disease have revived interest in treating UTIs with herbal remedies.

Table 2: Desert medicinal plant used to treat urinary tract infection

Botanical name	Family	Plant part	Phytochemical	Traditional use	Reference
Amaranthus Tricolor	Amaranthaceae	Leaves	Alkaloids, Cardiac Glycoside, Saponins, Tannins, Aminoacids, Flavonoids, Saponins,	Diuretic Astringent	Pulipati *et al*., 2015
Asparagus Racemosus	Liliaceae	Leaves	Saponins, Flavonoids, Saponins, Phenols, Alkaloids, Carbohydrates	Rejuvenative, Aphrodisiac Galactagogue Antispasmodic, Duretic, Antitumor.	Jose & Devassykutty *et al*., 2016
Butea Monosperma	Fabaceae	Leaves	Alkaloids, Carbohydrates, Flavonoids, Glycoside, Proteins, Saponins, Sterols, Starch, Tannins	Anthelmintic, anticonvulsive, anti-inflammatory, antimicrobial, antiperoxidative	Kaur *et al*., 2017
Catharanthus roseus	Apocynaceae	Stem	Alkaloids, Terpenoids, Phenols, Tannins,	Antidiabetic, antifungal, antiasthmatic,	Gupta *et al*., 2015

Botanical name	Family	Plant part	Phytochemical	Traditional use	Reference
			Saponins, Flavonoids, Quinines, Proteins,	antibacterial, Anti-inflammatory.	
Citrullus colocynthis	Cucurbitaceae	Fruit	Alkaloids, Carbohydrates, Flavonoids, Saponins, Proteins, Terpenoids, Anthranol, Glycoside	Anticandidal and antibacterial, antioxidant, anti-inflammatory	Khatibi & Teymorri 2011
Commiphora wightii	Burseraceae	Resin	Flavonoids, Saponins, Terpenoids, Tannins, Phenols, Carbohydrates	Anti-inflammatory, Antibacterial, Antidiabetic Antiproliferative	Rajeev *et al*.,2016
Corbichonia decumbens	Molluginaceae	Root, stem	Alkaloids, Tannins, Starch, Flavonoids, Carbohydrates	Antimicrobial, antibacterial, antifungal	Uma *et al.*, 2013
Jatropha curcas	Euphorbiaceae	Seed	Alkaloids, Saponins, Tannins, Flavonoids, Phenols, Coumarins.	Antibiotic, abortifacient, antidermatitic, anti-inflammatory, antimicrobial	Suhaili *et al*., 2011
Sesamum indicum	Pedaliaceae	Seed	Proteins, Alkaloids, Flavonoids, Terpenoids, Glycoside	Anticancer, lactogogue, and diuretic, hepatoprotective and laxative	Shasmita *et al.*, 2015
Tribulus terrestris	Zygophyllaceae	Leaves	Alkaloids, Starch, Saponins, Flavonoids, Tannins, Proteins, Cardiac Glycoside.	Urinary tract infections, urolithiasis, dysmenorrhea	Priyadarshini *et al*.,2015

Conclusion

The use of antibiotics for the treatment and prevention of Urinary Tract Infections has given to antibiotic-resistant gain. The results described here might be recognized sufficiently to identify and isolate the bioactive compound accountable for the inhibition of bacterial activity against uropathogenic bacteria and estimate its

pharmaceutical significance. By documenting a variety of excellent technologies applied for the separation, removal, and characterization of bioactive mixtures from chosen market Desert therapeutic plants, Practice of phytochemical treatment researchers and trade professionals interested in the improvement of proper methods and plans for mixture removal and for increasing their yield with future applications in mind and purification. Using these alternative approaches in regulating Urinary Tract Infections could avoid the risk of drug resistance.

References

Abdulkarim A, Sadiq Y, Gabriel OA, Abdulkadir UZ, Ezzeldin MA. Evaluation of five medicinal plants used in diarrhea treatment in Nigeria. J Ethno Pharmacol 2005; 101: 27-30.

Chauhan, C. K., Joshi, M. J., & Vaidya, A. D. B. (2009). Growth inhibition of struvite crystals in the presence of herbal extract Commiphora wightii. *Journal of Materials Science: Materials in Medicine*, *20*(1), 85.

Chou, S. T., Lo, H. Y., Li, C. C., Cheng, L. C., Chou, P. C., Lee, Y. C., ... & Hsiang, C. Y. (2016). Exploring the effect and mechanism of Hibiscus sabdariffa on urinary tract infection and experimental renal inflammation. *Journal of ethnopharmacology*, *194*, 617-625.

Goyal RK, Singh J, Lal H. *Asparagus racemosus* - An update. Indian J Med Sci. 2003;57(9):408-14.

Gupta, S., Chourey, A., Gupta, D., Agrawal, A., & Gupta, S. (2015). Biocontrol of clinical bacterial isolates associated with urinary tract infection using wild medicinal plant extract. *Nat. Prod. Plant Resour*, 5 (3), 23-30.

Jaradat, N. (2020). Phytochemistry, traditional uses and biological effects of the desert plant Styrax officinalis L. Journal of Arid Environments, 182, 104253.

Jose, J., & Devassykutty, D. (2016). Evaluation of antibacterial activity of Asparagus racemosus in urinary tract infection.

National Journal of Physiology, Pharmacy and Pharmacology, *6*(6), 596.

Kasture VS, Chopde CT, Deshmukh VK. Anticonvulsive activity of Albizzia lebbeck, Hibiscus rosa sinesis and Butea monosperma in experimental animals. J Ethnopharmacol 2000; 71:65–75.

Kaur, V., Kumar, M., Kaur, P., Kaur, S., Singh, A. P., & Kaur, S. (2017). Hepatoprotective activity of Butea monosperma bark against thioacetamide-induced liver injury in rats. *Biomedicine & Pharmacotherapy*, *89*, 332-341.

Kavitha, K. S., & Satish, S. (2014). Antibacterial activity of seed extracts of Callistemon lanceolatus DC on uropathogenic bacteria. *Journal of Acute Medicine*, *4*(1), 6-12.

Khatibi, R., & Teymorri, J. (2011). Anticandidal screening and antibacterial of Citrullus colocynthis in South East of Iran. *Journal of Horticulture and Forestry*, *3*(13), 392-398.

Mendonça-Filho, R. (2006). Bioactive phytocompound: New approaches in the phytosciences. Medicinal Plants into Drugs. Wiley-VCH, 1–24.

Mithraja, M. J., Irudayaraj, V., Kiruba, S., & Jeeva, S. (2012). Antibacterial efficacy of Drynaria quercifolia (L.) J. Smith (Polypodiaceae) against clinically isolated urinary tract pathogens. *Asian Pacific Journal of Tropical Biomedicine*, *2*(1), S131-S135.

Mobley, H. L., & Warren, J. W. (Eds.). (1996). *Urinary tract infections: molecular pathogenesis and clinical management*. Zondervan

Mousumi B, Satyahari D, Ramkrishna S (2013). Betalains from *Amaranthus tricolor* L. J. Pharmacogn. Phytochem. 1:87-95.

Murugan, M., & Mohan, V. R. (2011). Evaluation of phytochemical analysis and antibacterial activity of Bauhinia purpurea L. and Hiptage benghalensis L. Kurz. *Journal of Applied Pharmaceutical Science*, *1*(9), 157.

Pan, Y., Cheng, T., Wang, Y., & Bryant, S. H. (2014). Pathway analysis for drug repositioning based on public database mining. *Journal of chemical information and modeling*, *54*(2), 407-418.

Prakash, D., & Saxena, R. S. (2013). Distribution and antimicrobial susceptibility pattern of bacterial pathogens causing urinary tract infection in urban community of meerut city, India. *International Scholarly Research Notices*, *2013*.

Priyadarshini, Prajna, Pritilata, P., Banojini, P., & Susmita Kumari, S. (2015). Antimicrobial effect of Hydro-Alcohol extracts of Tribulus Terrestris, Phyllanthus Amarus and Hemidesmus Indicus against common Bacterial Urinary Pathogens-AN In Vitro Study. *International Journal of Ayurveda and Pharma Research*, *3*(11).

Pulipati, S. (2015). Phytochemical analysis and antibacterial efficacy of Amaranthus tricolor (L) methanolic leaf extract against clinical isolates of urinary tract pathogens. African Journal of Microbiology Research, 9(20), 1381-1385.

Rajeev, K., Rakesh, R., Sharma, p. K., & Gupta, A. K. (2016). A clinical study to evaluate the efficacy of Trikantakadi guggulu in the management of vatashthila wsr to benign prostatic hyperplasia (bph). International Journal of Technical Research and Applications 4, (3), 43-47.

Raka, Lul & Mulliqi-Osmani, Gjyle & Kurti, Arsim & Bajrami, Rrezarta & Lila, Greta. (2016). Urinary Tract Infections.

Shasmitha, R. (2015). Health benefits of Sesamum indicum: A short review. *Asian J Pharm Clin Res*, *8*(6),1-3.

Suhaili, Z., Yeo, C. C., Yasin, H. N., Badaludin, N. A., & Zakaria, Z. A. (2011). Antibacterial profile of Jatropha curcas latex extracts against selected human pathogenic bacteria. *African Journal of Microbiology Research*, *5*(29), 5147-5154.

Thakur, M., Asrani, R. K., Thakur, S., Sharma, P. K., Patil, R. D., Lal, B., & Parkash, O. (2016). Observations on traditional usage of ethnomedicinal plants in humans and animals of Kangra and Chamba districts of Himachal Pradesh in north-western Himalaya, India. *Journal of ethnopharmacology*, *191*, 280-300.

Thakur, P., Chawla, R., Narula, A., Goel, R., Arora, R., & Sharma, R. K. (2016). Assessment of aquo-ethanolic extract of Camellia sinensis against Carbapenem Resistant Escherichia coli: In Vivo Trials in a Murine Model. *Biomedicine & Pharmacotherapy*, *79*, 273-283.

Uma, C., & Sekar, K. G. (2014). Phytochemical analysis of a folklore medicinal plant Citrullus colocynthis L (bitter apple). *Journal of pharmacognosy and Phytochemistry*, *2*(6).

Uma, G., Prabakaran, R., Kalimuthu, K., Chinnadurai, V., & Balasubramaniam, V. (2016). Effects of Exogenous Growth Regulators on Direct and Indirect Micropropagation of Corbichonia decumbens (Forssk.) Exell (Molluginaceae). *European Journal of Medicinal Plants*, 1-9.

Usman, H., Abdulrahman, F. I., & Ladan, A. H. (2007). Phytochemical and antimicrobial evaluation of Tribulus terrestris L. (Zygophylaceae). Growing in Nigeria. *Res. J. Bio. Sci. Medwell Journals*, *2*(3), 244-247.

Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. *P T*. 2015;40(4):277-283.

Wagenlehner, F. M. E., & Naber, K. G. (2005). Fluoroquinolone antimicrobial agents in the treatment of prostatitis and recurrent urinary tract infections in men. *Current Infectious Disease Reports*, *7*(1), 9-16.

World Health Organization. (2014). *Antimicrobial resistance: global report on surveillance*. World Health Organization.

World Health Organization. (2018). Global antimicrobial resistance surveillance system (GLASS) report: early implementation 2017-2018.

BIOMEDICAL ROLE OF MICROBIAL SURFACTANTS

Vikrant Sharma[1], Mehak Manzoor[2], Deepti Singh[3] and [*]Deepansh Sharma[3]

[1] Student, Amity Institute of Microbial Technology, Amity University Rajasthan, Jaipur-303002

[2]Ph.D. Scholar, Amity Institute of Microbial Technology, Amity University Rajasthan, Jaipur-303002

[3] Assistant Professor, Amity Institute of Microbial Technology, Amity University Rajasthan, Jaipur-303002

Corresponding Author: deepanshsharma@gmail.com

INTRODUCTION

Biosurfactant is the group of amphipathic molecules with distinct structures and properties and obtained from several microorganisms. Biosurfactant is mainly produced as the secondary metabolite and play a critical role in the growth and survival of the microorganism and it is responsible for their production by helping in the nutrition uptake, interfering with the microbe-host interaction and sometimes by acting as the biocide agent (Banat et. al., 2012). Biosurfactant comprises of a hydrophilic moiety, which includes an acid, peptide cations, or anions, mono-, di- or polysaccharides along with a hydrophobic moiety of unsaturated or saturated hydrocarbon chains of fatty acids. These units confer a wide range of properties, including the ability to lower surface and interfacial tension of liquids and to form micelles and microemulsions between two different phases (Banat et. al., 2010). Biosurfactants are more significant as compared to their synthetic counterparts because of their microbial origin, biodegradability, low toxicity, and thus they are mainly explored in the food and cosmetic industries, enhanced oil recovery, and bioremediation (Banat et. al., 2012). Biosurfactants

are broadly classified into two categories, low (including glycolipids and lipopeptides) and high (polysaccharides, proteins, lipoproteins) molecular weight biosurfactants. Low molecular weight biosurfactants are considered to have excellent surface-active properties.

With this wide range of properties, biosurfactant becomes a suitable option for their application as biomedicals. Each year, around 2 million people in the United States acquire threatening infections with bacteria that are resistant to one or more of the antibiotics designed to treat those infections. About 23,000 people die each year as an impact of these antibiotic-resistant infections and also more die from other conditions that were complicated by an antibiotic-resistant infection (Ventola 2015). Bacteria acquire resistance against antibiotics when they get in regular exposure to them. There are four main mechanisms by which bacteria acquire resistance drug efflux, modification of cell wall protein, drug inactivation enzyme activation, and modified target molecule. In the case of biosurfactants, the molecules interact with the cells non-specifically, this inhibits the cells from generating either of the mechanism for the development of resistance. Synthetic surfactants are used in the cosmetics and healthcare industry for a long time. They use surfactants for making lotions and creams which are oil-based as it reduces the surface tension between the two phases, it results in a fine-textured end product. They hold their downside as well, on regular exposure to the surfactant the molecules start entering the cell and inhibit the enzymatic functioning of the cell. Surfactants have toxicity level and thus they tend to get accumulated in the system, and thus becomes difficult to degrade (Zhu et. al., 2014). Biosurfactant is biological counterparts of the synthetic surfactants which serves the same purpose with certain advantages. Since biosurfactants are biological in nature it can be easily degraded by the cellular enzymes preventing them from getting accumulated inside the cellular system.

Among all biomedical properties of biosurfactants, antimicrobial (Gudiña et. al., 2015), antifungal (Sharma et. al., 2018); (Mnif et.

al., 2015), antitumor (Duarte et. al., 2014) properties besides their anti-adhesive nature against various pathogens make them more appropriate for application in the health care sectors. Some studies have shown that biosurfactants can be a suitable alternative to synthetic medicines and antimicrobials and can be used as a therapeutically safe alternative. Biosurfactant can be used possibly in various ways such as gene transfection, adjuvants for antigens, an inhibitor for the fibrin clot formation (Stipcevic et. al., 2001A) and also its activator, can also be used as the biomaterial for the preparation of the anti-adhesive coatings and can be incorporated in the formulation against urogenital tract infection and for pulmonary immunotherapy (Rodrigues et. al., 2006). Recently, biosurfactants have also been explored in the field of cancer therapy, lipopeptide surfactin is shown to induce apoptosis in breast cancer cells. Similarly, glycolipid mannosylerythriol lipid (MELs) and succinyl trehalose lipid (STLs) were found to arrest the growth of the carcinoma cells and induces apoptosis of the tumor cells (Cao et. al., 2010); (Zhao et. al., 2000).

Nevertheless, even if it seems that biosurfactants are valuable with wide-scale therapeutic applicability, some may constitute a risk for humans and should be carefully examined before heading for the direct application. For instance, it is recognized that *Pseudomonas aeroginosa* is responsible for severe nosocomial infection, yet the strain produces a strong glycolipid (Rhamnolipid) for several medical-related applications (Hoskova et. al., 2013), (Abbasi et. al., 2013). This book chapter review discusses the current state of biosurfactant research and emphasizes the potential therapeutic applications in biomedicals. The aim is to provide novel insight into the latest research in the biomedical sector and to give a new insight into the mechanism and the functioning related to the specific biomedical applications of biosurfactants.

ANTIMICROBIAL POTENTIAL

The circumspect raise of resistance in pathogenic microorganisms especially the incidence of bacteria globally, compromising the cogency of conventional antibiotics. The antibiotic resistance

problem has been indorsed to the overdosing and abuse of antibiotics, as well as a shortage of novel drug advances due to reduced cost-effective reasons and puzzling regulatory necessities. Each year, at least 2 million people acquire serious infections with bacteria that are resistant to one or more of the antibiotics designed to treat those infections in the United States. Many more die from other conditions that were complicated by an antibiotic-resistant infection (Ventola 2015). Biosurfactant has been used as an antimicrobial agent in different prospects such as used as an antimicrobial agent in surgical implants (Satpute et. al., 2019) and in the prevention of biofouling (Kim et. al., 2015). Biosurfactants obtained from probiotic LAB primarily considered as GRAS without having any significant toxicity to human and animal health (Sharma et .al., 2015). Biosurfactant obtained from many microorganisms have been found potent antimicrobials to control various human pathogens (Sharma et. al., 2015); (Díaz De Rienzo et. al., 2015); (Cameotra and Makkar 2004); (Reid et. al., 2001); (Rodrigues et. al., 2004). The mechanism of biosurfactant action still rests imprecise, but the various modes of action and assumptions were hypothesized such as;

- Biosurfactants initiating loss of cell membrane integrity leads to consequent failure of the nutrition management within cell and biosurfactants are also capable to form cellular pore which interrupts the cell membrane functionality. The pores cause trans-membrane ion influx of Na+ and K+, which ultimately results in membrane disruption and cell death (Ines and Dhouha, 2015).
- The fatty acid composition of the biosurfactants introducing into the plasma membrane initiating rise of membrane size and change in ultra-structural were also observed (Gomaa, 2013).
- Acyl tails of the microbial biosurfactant triggering disruptions of the cell membrane, allowing the cell membrane to separate away from the cytoplasmic content (Desai and Banat, 1997).

Biosurfactants obtained from the *L. plantarum* CFR 2194 has shown antimicrobial activity against the various foodborne pathogens (Madhu and Prapulla, 2013). Aqueous extract biosurfactant showed

an effective antimicrobial property against food-borne pathogen such as *E. coli*, *Salmonella typhi*, *Yersinia enterocolitica*, and *S. aureus*. More prominently, the biosurfactant displayed antiadhesive significance against food-borne diseases. The findings indicate the significance of developing approaches to inhibit bacterial establishment on food contact surfaces and biomedical equipment and tools.

Inhibition of rising multi-drug resistant pathogens is one of the chief apprehensions in combating opportunistic pathogens. Xylolipid biosurfactant produced by *L. lactis* displayed antibacterial property against clinical isolates of *E. coli* and *S. aureus* (Saravanakumari and Mani, 2010). Biosurfactant obtained from LAB has been commended as a broad-spectrum antibacterial. Biosurfactant derived from *L. lactis* is nontoxic for usage in therapeutic and food formulations and regarded as GRAS due to its probiotic origin. Biosurfactants displayed significant antibacterial potency and are safe to consume orally and topically. So, it could be exploring as a therapeutic molecule in industrial applications and additives. Antimicrobial properties of biosurfactants or LAB origin may open up possibilities for unconventional therapeutic for the control of hospital and clinical associated infections. Biosurfactants can prevent the adhesion of the microbes on the surface as they alter the interfaces of the fluid phases with different polarizations and hydrogen bonding (Rodrigues, 2011), (Sodagari, et. al., 2013). Also, these compounds can rupture the cell membranes that lead to cell lysis due to increased membrane permeability which further leads to the leakage of the intracellular metabolites (Bharali, et. al., 2013). Alterations can also be made in the physical structure of the membrane which leads to the disturbance in the various primary functions of the cell membrane such as nutrient transport and energy generation.

ANTI-ADHESION ACTIVITY

Biofilm is a consortium of microorganisms in which cells are adherent to each other or with a surface, employing exopolysaccharides (EPS). Biofilms can be formed on both living or

non-living surfaces thus, it can be prevalent in natural, industrial, or hospital settings (Hall-Stoodly et. al., 2004); (Lear et. al., 2012). Microbial adhesion to solid surfaces and the subsequent biofilm development has been known to diverse environments. Biofilm formation by the pathogenic microorganisms to solid surfaces such as biomedical implants, surgical & biomedical tools, and food processing surfaces is a commonly occurring problem (Sharma et. al., 2015); (Rodrigues, 2011); (Naughton et. al., 2019). Bacteria generally settle down to solid surfaces to form biofilms as a tactic to safeguard planktonic cells from various environmental stress like high temperature, salt concentration, and presence of inhibitory compounds such as antibiotics and toxins (Liu et. al., 2012). In the food industry, biofilms development may be a foundation of intractable contaminations, leads to food decay, and are a potential threat to public health like epidemics of foodborne diseases (Fouladkhah and Henry, 2019). Biofilms formation in the food formulation area is challenging to exterminate because of their resistant structural communities which can be a sheath, a mat, a slimy film, or a glue-like film. (Stoodley et. al., 2002); (Bucker et. al., 2014); (Sharma et. al., 2016). According to the National Institute of Health (NIH), out of the total number of microbial infections, 65% are caused because of biofilms (Lewis, 2001). Some common biofilm infection includes urinary tract infection caused by *Escherichia coli*, catheters infection caused by *Staphylococcus aureus*, child middle-ear infections caused by *Haemophilus influenza*. Few cases have been reported where the stones formed in the UTI were microcolonies of microbes surrounded by a matrix composed of crystallized minerals. Infection of *Staphylococcus epidermidis* causes inflammation (endocarditis), as they grow on the cuff at a mechanical heart valve. Leptospirosis is another biofilm-associated disease that is caused by *Leptospira interrogans* which is a rare bacterial infection that eventually results in meningitis. (Marrison et. al., 1999); (Stewart and Hirani, 2007); (Sestrich et. al., 2008); (Marshall, 2006). Current strategies to combat biofilm formation are inadequate in terms of their effectiveness and residual nature on food surfaces specifically. Biofilm formation by pathogenic bacteria is a serious problem in the emerging clinically

resistance amongst hospital-acquired pathogens. Though, conventional biofilm disrupting agents such as Bismuth (Domenico et. al., 1991, 1992), Carvacrol (Burt et. al., 2014), derivatives of 2-aminoimidazoles (Richards and Melander, 2009), Ultrasound (Baumann et. al., 2009), Ionizing radiation (Niemira and Solomon, 2005), atmospheric plasma inactivation (Vleugels et. al., 2005), may also contribute to ineffective biofilm removal. Sterilization and disinfection of the industrial processing unit is a pivotal aspect of an industrial setup, but the conventional methods and material used are not proficient to abolish the preformed biofilms and do not avert microorganisms to colonize on the surface (Simões et. al., 2010; Sharma and Saharan, 2016). These above-mentioned approaches can exterminate around 90% of the microbial population, but comprehensive removal practically not achievable (Taomina and Beuchat, 2002; Sharma et. al., 2015). Along with this, the application of the disinfectant over the permissible limit will result in the increasing problem of residual disinfectant concentration in the production and processing area which eventually hampers the quality of the product (Torlak and Sert, 2013; Ortega-Morales et. al., 2013). Novel biofilm control approaches are continuously evolving such as various enzymes (Thallinger et. al., 2013), bacteriophages (Nannapaneni and Soni, 2015), bacteriocins (Bolocan et. al., 2016), and biosurfactants (Sharma, 2016; Johny, 2013; Sabaté and Audisio, 2013) microbial source. Biosurfactants derived from LAB has gained huge attention in biomedical and food formulations sectors because of their antibiofilm and antiadhesive potential to prevent biofilm formation. The probiotic origin, GRAS status with significant dispersal potential of biosurfactants obtained from LAB makes them suitable candidates for usage as effective green biofilm removal agents (Sharma et. al., 2016; Sharma, 2016).

Biosurfactants exhibit antiadhesive property which are extensively explored and reported and so is the antimicrobial property of these molecules. These are the two basic properties that make biosurfactants a strong alternative to synthetic or chemical counterparts. Since these molecules are biological from origin thus, they have low toxicity and are biodegradable. Considering all these

properties applications of these molecules in surgical implants become one more area where its application can be explored. With the antiadhesive nature of these molecules, the life of the implants can be increased as it won't allow the colonization of bacterial on or in the implants, and with the antimicrobial nature of the microbial growth can be easily prevented (Banat M, et al 2010). Silicon (medical grade) based implants are tested against the *Candida albicans* using the *Lactobacilli* sp. derived biosurfactant. The experiment was designed in a manner where the pathogen was tested against its biofilm-forming ability and adhesive nature, the cells were tested in a co-incubation mode where the cells (pathogen) and the biosurfactant solution are involved in the culturing system. The second method was pre-coated plated where the biosurfactant solution was pre-coated on the silicon discs and those discs were transferred in the media containing the proliferating pathogens, co-incubation assays, BS induced a significant reduction of biofilm formation of about 90% wherein, in pre-coating experiments, the highest performance was observed during *C. albicans* adhesion phase, whereas during the biofilm formation phase, the inhibition was lower (about 50 %) but still significant (Ceresa, C. et al 2015).

Food processing industries are the ones where microorganisms are indigenous to certain foods where they do not induce any harmful molecules which may harm the consumer but instead add in certain beneficiary molecules for example the fermented food where certain microbes are intentionally introduced as consumables because for their nutritive value. Efforts are made for their control when they show overgrowth and results in visible spoilage. Biofilm formation by pathogenic and spoilage causing microorganisms serves as a reservoir of pathogenic microorganism which may contaminate raw materials and food products during processing, resulting in food spoilage which eventually results in economic losses for the producers (Winkelströter et al., 2014). The regular detection of such microbes in the industrial setup signals towards antimicrobial and disinfectant resistance. The contaminated food not only causes economic loss but also results in imparting harmful effect on the health of the consumer. In the industrial setup these bacteria are not observed in the colony form instead they tend to form biofilms.

Biofilm is the aggregation of similar or closely related microbes which is formed of exo-polysaccharides. This is the response of the microbes towards the environment as a protective reservoir. Since the nature of these biofilms is a polysaccharide, it is hydrophobic in nature and thus doesn't get washed off with regular wash also it results in the blocking of the pipelines and other important channels of the industrial instruments. These are certain methods for the cleaning of these blockings but some are not effective ubiquitously and some are not very cost-effective. One of the basic methods used for the removal of the biofilm is using physical methods which include scrubbing, water jets, and turbulent flow in pipelines are used to administer force to susceptible surfaces during cleaning protocols (Seafood, 2012).

In line with the treatment of medical devices, biosurfactants have been used in the pre-treatment of food processing planes (Kim *et al.,* 2006). Not even silicone tubing and solid surfaces, biosurfactant treated stainless steel surfaces can also be sanitized. Biosurfactants produced by *L. helveticus* declined the population of *L. monocytogenes* on metal and polypropylene surfaces (Meylheuc *et al.,* 2006). LAB-derived biosurfactants are also documented for removal of clinically hazardous microorganisms like *Staphylococcus epidermidis* and *S. aureus* (Walencka *et al.,* 2008). Prevention of biofilm formation by oral microflora also significant merit of the LAB-derived biosurfactants (Meurman and Stamatova, 2007; Koll *et al.,* 2008). It has been also observed that LAB-derived biosurfactants are very effective in the removal of biofilms form by *Streptococcus sobrinus* and *Streptococcus mutans* (Van Hoogmoed *et al.*, 2004; 2006).

ANTITUMOR AND ANTICANCER ACTIVITY

Biosurfactants are the molecules that have been proved to be involved in several intracellular activities such as, signal transduction, cell differentiation, and cellular immune response (Rodrigues. et al 2006). For instance, glycolipids were found to be involved in arresting the growth and apoptosis of the malignant B16 cells in mice along with condensation of the choromatin and DNA

fragmentation (Zhao. et al 2000). Sophorolipids have been also reported for triggering cell differentiation instead of cell proliferation which leads to inhibition of the protein kinase C (PKC) activity in HL60 leukemia cell lines and these activities are not merely characterized as the detergent-like effect but these are the result of specific plasma membrane interactions (Isoda, H et al 1995). Different sophorolipids derivative exhibits different anticancer activities; the one with one double bond in the fatty acid part had great cytotoxic activity, whereas the acidic sophorolipids exhibited remarkable antitumor activity (Fu. et al 2008, Shao. et al 2012).

Cancer is one of the major issues and concerns majorly because of its unpredictable nature. Many approaches have been made in developing and searching for new biomarkers and therapeutic methods but still, the search for a non-toxic therapy has not stopped. Chemotherapy is one of the approaches which is widely used and it is mainly based on usage of the highly cytotoxic chemicals with therapeutic attributes that nonspecifically target the dividing cells in the body. This shows a slight improvement in the patient's survival but in the long run, it comes with certain non-repairable damage, also the treatment is non-specific, non-selective thus multidrug-resistant cancer cells remain a great challenge. This outcome of the presently available drugs in a reason the natural products and their derivatives are now under clinical practice (Lee. et al 2010), (Xu. et al2011), (Janek. et al 2013), (Takeuchi. 2011). Following the concern, the systematic exploration of natural sources has been carried out and the marine and environmental microbiota were screened for certain interesting biological activities that involved anticancer activity (Xie. et al 2011),(Yamazaki. et al 2007).

As mentioned earlier, biosurfactants are those microbial compounds that exhibit remarkable biological activities (Poulsen, 2011). In particular lipopeptides and glycolipids were proved to show the anticancer potential by interfering with the cancer progression processes (Das et al 2012). These molecules get involved in several intracellular molecular steps which result in inhibition with the steps

which are involved in the signal transduction, cell differentiation and cellular immune response (Table 1) (Ma. et al 2006), the big advantage which comes along with their application are its low toxicity, high efficacy and easy biodegradability which makes them a promising alternative to the traditional drugs available. There are different mechanisms for the anticancer efficacy of the biosurfactants, including delay of the cell cycle progression, inhibition of several signaling pathways like Akt, extracellular signal-regulated kinase/ c-Jun N-terminal kinase (ERK/JNK), and Janus kinase/ signal transducer and activator for transcription (JAK/STAT); activation of natural killer T cells (NKT) cells, and induction of apoptosis via death receptors in carcinogenic cells. Cell membrane disruption being the natural property of biosurfactants, induces cell lysis, increased membrane permeability, and metabolic leakage also aids in the mechanism for its anticancer activity (Nunnery. et al., 2010). Following is the graphical representation of the types of biosurfactants that have been explored for the anticancer property against various types of human cancer (Fig.1).

Table1. List of biosurfactants with anticancer properties against human cancer

Biosurfactant	Cell Lines	Activity	Refrence
Mannosylerythritol lipids (MELs)	K562-Myelogenous leukemia	Growth inhibition inhibits cell differentiation	(Isoda, and Nakahara, 1997)
Succinoyl trehalose lipids (STLs)	HL60-Promylocytic leukemia	Growth inhibition inhibits cell differentiation	(Sudo, et al 2000)
	KU812-Basophillic leukemia	Growth inhibition	(Isoda. et al 1995)
Sophorolipids Surfactin	HL60-Promylocytic leukemia	Plasma membrane disintegration	(Isoda. et al 1997)
	H7402-Liver cancer	Cell cycle arrest, growth inhibition, apoptosis induction	(Chen. et al 2006)

	A549-Lung cancer	Apoptosis	Chen, et al 2006
	HPAC-Pancreatic cancer	Necrosis	(Fu, et al 2008)
	KYSE109/KYSE450-Esophageal cancer	Growth inhibition	(Shao. et al 2012)
	BEL7402-Hepatocellular cancer	Apoptosis induction, growth inhibition	(Cao. et al 2009)
	K562-Mylogenous leukemia	Cell cycle arrest, growth inhibition, apoptosis induction	(Cao, et al 2009), (Wang, et al 2007)
	LoVo-Colon adenocarcinoma	Apoptosis induction, growth inhibition	(Kim, et al 2007)
	MCF7-Breast cancer	Apoptosis induction, growth inhibition	(Cao, et al 2010), (Lee, et al 2012), (Cao. et al 2011)
	Caco2-Colorectal cancer	Apoptosis induction, growth inhibition	(Wang. et al 2012)
	HCT15/HT29-Colon cancer	Growth inhibition	(Sivapathasekara et al 2010)
	Ehrlich ascites-Ehrlich ascites cancer	Cytolytic activity	(Kameda and Kanatomo 1965), (Kameda et al 1974)
	MCF7-Breast cancer	Migration inhibition	(Park et al 2013)
Sophorolipids Surfactin	T47D- Breast cancer	Growth inhibition, Cell cycle arrest	(Duarte et al 2014)
	MDA-MB-231-Breast cancer	Migration inhibition	(Park et al 2013)
	MDA-MB-231-Breast cancer	Growth inhibition, Cell cycle arrest	(Duarte et al 2014)
	Bcap-37- Breast cancer	Growth inhibition, apoptosis induction	(Liu et al 2010)
	K562- Leukemia	Growth inhibition, Cell cycle arrest,	(Wang et al

		Apoptosis induction	2007)
	K562- Leukemia	Apoptosis induction	(Wang et al 2009)
	BEL7402-Hepatocellular Cancer	Growth inhibition	(Liu, et al 2010)
	HepG2-Hepatocellular Cancer	Apoptosis induction	(Wang et al 2013)
	HeLa- Cervical cancer	Growth inhibition	(Liu , et al 2010)
	HeLa- Cervical cancer	Growth inhibition	(Nozhat et al 2012)
	KB-3-1- Oral epidermoid cancer	Growth inhibition	(Liu , et al 2010)
	SW-1990-Pancreatic cancer	Growth inhibition	(Liu, et al 2010)
	B16- Rat melanoma	Growth inhibition	(Liu, et al 2010)
Viscosin	PC3M-Metastatic prostate cancer	Migration inhibition	(Saini. et al 2008)

NEUTRACEUTICAL APPLICATION OF BIOSURFACTANTS

Healing of the damaged tissues is one of the unique features which are possessed by the human body. The uniqueness of the process is also signified by the highly coordinated and regulated yet precise cascade of the cellular responses and interaction of various cells with each other for a long duration of time. The repair mechanism of the body is divided into different phases wherein the initial phase, the superficial cutaneous wound healing is performed which is observed as the inflammatory response from the circulation to the site of injury.

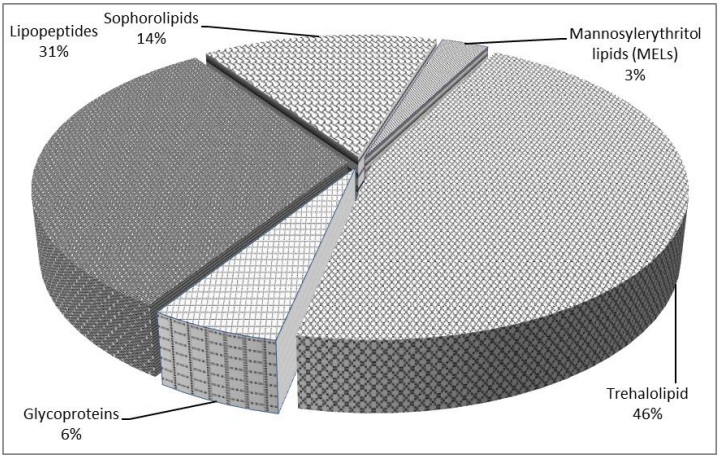


Fig.1. Distribution of biosurfactant based on applications as an anticancer agent (Source: Gudina, et al., 2013)

Activated macrophages play a variety of roles in wound healing, including proteases release for the wound debridement, phagocytosis of the debris, the release of cytokines, and growth factors which results in regulation of the cells involved in the tissue repair (Waldrof. et al 1995). The expressed growth factors activate the fibroblast and keratinocytes and the inflammatory phase is followed by the proliferative phase. At this point of the wound healing, the vascular integrity is re-sustained, old tissues are replaced by the new connective tissues produced by fibroblast and a new layer of the epithelium is formed over the damaged surface. All the processes work as a cascade thus forming granulation tissue with capillary loops, fibroblasts, inflammatory cells, and matrix proteins (Martin, 1997). At this stage, the provisional matrix is now replaced by the actual matrix which consists of, collagen, glycosaminoglycans (GAGs), proteoglycans (PGs), and elastin, with collagen being the primary protein and in the end, the proliferative stage ends with the epithelization stage, where the migration, proliferation, and differentiation of the epithelium occurs to resurface the damage. In case of severe burn wounds, a bed of

granulation tissue is first formed and this is followed by the epithelization stage which is the reason for the delay of the last phase in case of burnt wounds (Waldrof, et al 1995).

Biosurfactants have been proved to have strong killing action on the microbial cells thus establishing its antimicrobial activity but a few studies have been carried out to show the wound healing capability of the biosurfactants (Stipcevic, et al 2006, Piljac, et al 2008). The results of these studies showed that low concentrations (0.1%) of these molecules can treat ulcers and burns. This is a new and promising sector for the application of the biosurfactant and this can be used as a cheap alternative to the regular commercial burn healing ointments as an over-the-counter product. The reports which characterize biosurfactant with wound healing property mainly focused upon di-rhamnolipids BAC-3 isolated from *Pseudomonas aeroginosa* (Pijac, et al 1995). The concentration of the BAC-3 is one of the major aspects of any therapeutic formulation because the varying concentration of the molecule puts a different impact on a different mechanism of the body. In vitro studies revealed that BAC-3, when exposed under a determined concentration results in proliferation of keratinocytes, is stimulated in the presence of serum while, the proliferation of the fibroblasts is inhibited (Stipcevic, et al 2001A, Stipcevic, et al 2001B). BAC-3 in the absence of calf serum at a concentration of 0.1mg/ml, inhibits DNA synthesis in A431 human epidermal cells in vitro (Piljac, et al 1995). When the biosurfactant is exposed to the fetal calf serum at 50µl/ml di-rhamnolipid produced stimulation for the production of differentiated keratinocyte colonies 34% above the untreated control wherein the proliferation of the fibroblast was inhibited at the same concentration in 23% below control (Stipcevic, et al 2001A). Considering the reported data, it can be deduced that with a particular concentration of di-rhamnolipids the fibroblast proliferation can be inhibited whereas, the proliferation of keratinocytes can be stimulated. The effect of BAC-3 along with the presence of serum resulted in the proliferation of keratinocytes suggested the in vivo effect of the di-rhamnolipid on wound healing (Waldrof. et al 1995).

References

Abbasi H, Noghabi KA, Hamedi MM, Zahiri HS, Moosavi-Movahedi AA, Amanlou M, Teruel JA, Ortiz A. Physicochemical characterization of a monorhamnolipid secreted by Pseudomonas aeruginosa MA01 in aqueous media. An experimental and molecular dynamics study. Colloids and Surfaces B: Biointerfaces. 2013 Jan 1;101:256-65.

Banat IM, Franzetti A, Gandolfi I, Bestetti G, Martinotti MG, Fracchia L, Smyth TJ, Marchant R. Microbial biosurfactants production, applications and future potential. Applied microbiology and biotechnology. 2010 Jun 1;87 (2):427-44.

Bharali P, Saikia JP, Ray A, Konwar BK. Rhamnolipid (RL) from Pseudomonas aeruginosa OBP1: a novel chemotaxis and antibacterial agent. Colloids and Surfaces B: Biointerfaces. 2013 Mar 1;103:502-9.

Cameotra SS, Makkar RS. Recent applications of biosurfactants as biological and immunological molecules. Current opinion in microbiology. 2004 Jun 1;7(3):262-6.

Cao, X.H. et al. (2009) Surfactin induces apoptosis and G2/M arrest in human breast cancer MCF-7 cells through cell cycle factor regulation. Cell Biochem. Biophys. 55, 163–171.

Cao, X.H. et al. (2010) Surfactin induces apoptosis in human breast cancer MCF-7 cells through a ROS/JNK-mediated mitochondrial/ caspase pathway. Chem. Biol. Interact. 183, 357–362

Cao, X.H. et al. (2011) ROS–Ca2+ is associated with mitochondria permeability transition pore involved in surfactin-induced MCF-7 cells apoptosis. Chem. Biol. Interact. 190, 16–27

Ceresa C, Tessarolo F, Caola I, Nollo G, Cavallo M, Rinaldi M, Fracchia L. Inhibition of Candida albicans adhesion on medical-grade silicone by a Lactobacillus-derived biosurfactant. Journal of applied microbiology. 2015 May;118(5):1116-25.

Chen, J. et al. (2006) Sophorolipid produced from the new yeast strain Wickerhamiella domercqiae induces apoptosis in H7402 human liver cancer cells. Appl. Microbiol. Biotechnol. 72, 52–59

De Rienzo MA, Banat IM, Dolman B, Winterburn J, Martin PJ. Sophorolipid biosurfactants: possible uses as antibacterial and antibiofilm agent. New biotechnology. 2015 Dec 25;32(6):720-6.

Desai JD, Banat IM. Microbial production of surfactants and their commercial potential. Microbiology and Molecular biology reviews. 1997 Mar 1;61(1):47-64.

Duarte C, Gudiña EJ, Lima CF, Rodrigues LR. Effects of biosurfactants on the viability and proliferation of human breast cancer cells. AMB express. 2014 Dec 1;4(1):40.

Fu, S.L. et al. (2008) Sophorolipids and their derivatives are lethal against human pancreatic cancer cells. J. Surg. Res. 148, 77–82

Gomaa EZ. Antimicrobial and anti-adhesive properties of biosurfactant produced by lactobacilli isolates, biofilm formation and aggregation ability. The journal of general and applied microbiology. 2013;59(6):425-36.

Gudiña EJ, Rangarajan V, Sen R, Rodrigues LR. Potential therapeutic applications of biosurfactants. Trends in pharmacological sciences. 2013 Dec 1;34(12):667-75.

Gudiña EJ, Rodrigues AI, Alves E, Domingues MR, Teixeira JA, Rodrigues LR. Bioconversion of agro-industrial by-products in rhamnolipids toward applications in enhanced oil recovery and bioremediation. Bioresource technology. 2015 Feb 1;177:87-93.

Hall-Stoodley, L., Costerton, J.W. and Stoodley, P. (2004) Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol 2, 95–108.

Hošková M, Schreiberová O, Ježdík R, Chudoba J, Masák J, Sigler K, Řezanka T. Characterization of rhamnolipids produced by non-pathogenic Acinetobacter and Enterobacter bacteria. Bioresource technology. 2013 Feb 1;130:510-6.

Inès M, Dhouha G. Lipopeptide surfactants: production, recovery and pore forming capacity. Peptides. 2015 Sep 1;71:100-12.

Isoda, H. et al. (1995) Succinoyl trehalose lipid induced differentiationof human monocytoid leukemic cell line U937 into monocyte macrophages. Cytotechnology 19, 79–88.

Isoda, H. et al. (1997) Microbial extracellular glycolipid induction of differentiation and inhibition of protein kinase C activity of

human promyelocytic leukaemia cell line HL60. Biosci. Biotechnol. Biochem. 61, 609–614

Kameda Y, Ouhira S, Matsui K, KANATOMO S, HASE T, ATSUSAKA T. Antitumor activity of Bacillus natto. V. Isolation and characterization of surfactin in the culture medium of Bacillus natto KMD 2311. Chemical and Pharmaceutical Bulletin. 1974 Apr 25;22(4):938-44.

Kim HS, Jeon JW, Kim BH, Ahn CY, Oh HM, Yoon BD. Extracellular production of a glycolipid biosurfactant, mannosylerythritol lipid, by Candida sp. SY16 using fed-batch fermentation. Applied microbiology and biotechnology. 2006 Apr 1;70(4):391-6.

Kim LH, Jung Y, Kim SJ, Kim CM, Yu HW, Park HD, Kim IS. Use of rhamnolipid biosurfactant for membrane biofouling prevention and cleaning. Biofouling. 2015 Feb 7;31(2):211-20.

Kim, S.Y. et al. (2007) Surfactin from Bacillus subtilis displays anti-proliferative effect via apoptosis induction, cell cycle arrest and survival signaling suppression. FEBS Lett. 581, 865–871

Liu X, Ren B, Gao H, Liu M, Dai H, Song F, Yu Z, Wang S, Hu J, Kokare CR, Zhang L. Optimization for the production of surfactin with a new synergistic antifungal activity. PloS one. 2012 May 18;7(5):e34430.

Madhu AN, Prapulla SG. Evaluation and functional characterization of a biosurfactant produced by Lactobacillus plantarum CFR 2194. Applied biochemistry and biotechnology. 2014 Feb 1;172(4):1777-89.

Meylheuc T, Methivier C, Renault M, Herry JM, Pradier CM, Bellon-Fontaine MN. Adsorption on stainless steel surfaces of biosurfactants produced by gram-negative and gram-positive bacteria: consequence on the bioadhesive behavior of Listeria monocytogenes. Colloids and Surfaces B: Biointerfaces. 2006 Oct 1;52(2):128-37.

Mnif I, Grau-Campistany A, Coronel-León J, Hammami I, Triki MA, Manresa A, Ghribi D. Purification and identification of Bacillus subtilis SPB1 lipopeptide biosurfactant exhibiting antifungal activity against Rhizoctonia bataticola and

Rhizoctonia solani. Environmental Science and Pollution Research. 2016 Apr 1;23(7):6690-9.

Naughton PJ, Marchant R, Naughton V, Banat IM. Microbial biosurfactants: current trends and applications in agricultural and biomedical industries. Journal of applied microbiology. 2019 Jul;127(1):12-28.

Piljac A, Stipčević T, Piljac-Žegarac J, Piljac G. Successful treatment of chronic decubitus ulcer with 0.1% dirhamnolipid ointment. Journal of cutaneous medicine and surgery. 2008 May;12(3):142-6.

Piljac G, Piljac V, inventors; Piljac, Goran, Visnja, assignee. Pharmaceutical preparation based on rhamnolipid. United States patent US 5,455,232. 1995 Oct 3.

Reid G, Zalai C, Gardiner G. Urogenital lactobacilli probiotics, reliability, and regulatory issues. Journal of Dairy Science. 2001 Jun 1;84:E164-9.

Rodrigues L, Banat IM, Teixeira J, Oliveira R. Biosurfactants: potential applications in medicine. Journal of Antimicrobial Chemotherapy. 2006 Apr 1;57(4):609-18.

Rodrigues L, Van der Mei H, Teixeira JA, Oliveira R. Biosurfactant from Lactococcus lactis 53 inhibits microbial adhesion on silicone rubber. Applied microbiology and biotechnology. 2004 Dec 1;66(3):306-11.

Satpute SK, Mone NS, Das P, Banat IM, Banpurkar AG. Inhibition of pathogenic bacterial biofilms on PDMS based implants by L. acidophilus derived biosurfactant. BMC microbiology. 2019 Dec;19(1):1-5.

Shao, L. et al. (2012) Bioactivities of sophorolipid with different structures against human esophageal cancer cells. J. Surg. Res. 173, 286–291

Sharma D, Saharan BS, Chauhan N, Procha S, Lal S. Isolation and functional characterization of novel biosurfactant produced by Enterococcus faecium. SpringerPlus. 2015 Dec;4(1):1-4.

Sharma D, Saharan BS. Functional characterization of biomedical potential of biosurfactant produced by Lactobacillus helveticus. Biotechnology Reports. 2016 Sep 1;11:27-35.

Sharma D, Saharan BS. Functional characterization of biomedical potential of biosurfactant produced by Lactobacillus helveticus. Biotechnology Reports. 2016 Sep 1;11:27-35.

Sharma VI, Garg MU, Devismita T, Thakur PA, Henkel MA, Kumar G. Preservation of microbial spoilage of food by biosurfactant based coating. Asian J. Pharm. Clin. Res. 2018;11(2):98.

Simoes M, Simões LC, Vieira MJ. A review of current and emergent biofilm control strategies. LWT-Food Science and Technology. 2010 May 1;43(4):573-83.

Sivapathasekaran, C. et al. (2010) Marine bacterium derived lipopeptides: characterization and cytotoxic activity against cancer cell lines. Int. J. Pept. Res. Ther. 16, 215–222

Sodagari M, Wang H, Newby BM, Ju LK. Effect of rhamnolipids on initial attachment of bacteria on glass and octadecyltrichlorosilane-modified glass. Colloids and Surfaces B: Biointerfaces. 2013 Mar 1;103:121-8.

Stewart R, Hirani V. Dental health and cognitive impairment in an English national survey population. Journal of the American Geriatrics Society. 2007 Sep;55(9):1410-4.

Stipcevic T, Piljac A, Piljac G. Enhanced healing of full-thickness burn wounds using di-rhamnolipid. Burns. 2006 Feb 1;32(1):24-34.

Sudo, T. et al. (2000) Induction of the differentiation of human HL-60 promyelocytic leukemia cell line by succinoyl trehalose lipids Cytotechnology 33, 259–264

Taormina PJ, Beuchat LR. Survival of Listeria monocytogenes in commercial food-processing equipment cleaning solutions and subsequent sensitivity to sanitizers and heat. Journal of Applied Microbiology. 2002 Jan;92(1):71-80.

Van Hoogmoed CG, Dijkstra RJ, Van der Mei HC, Busscher HJ. Influence of biosurfactant on interactive forces between mutans streptococci and enamel measured by atomic force microscopy. Journal of dental research. 2006 Jan;85(1):54-8.

Van Hoogmoed CG, Van der Mei HC, Busscher HJ. The influence of biosurfactants released by S. mitis BMS on the adhesion of

pioneer strains and cariogenic bacteria. Biofouling. 2004 Sep 1;20(6):261-7..

Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. Pharmacy and therapeutics. 2015 Apr;40(4):277.

Walencka E, Różalska S, Sadowska B, Różalska B. The influence of Lactobacillus acidophilus-derived surfactants on staphylococcal adhesion and biofilm formation. Folia microbiologica. 2008 Jan 1;53(1):61.

Wang, C. et al. (2013) Surfactin-induced apoptosis through ROS–ERS– Ca2+–ERK pathways in HepG2 cells. Cell Biochem. Biophys.

Wang, C.L. et al. (2007) Induction of apoptosis in human leukemia K562 cells by cyclic lipopeptide from Bacillus subtilis natto T-2. Peptide 28, 1344–1350

Winkelströter LK, dos Reis Teixeira FB, Silva EP, Alves VF, De Martinis EC. Unraveling microbial biofilms of importance for food microbiology. Microbial ecology. 2014 Jul 1;68(1):35-46.

INTESTINAL AMOEBIASIS: TACKLING THE RAMPANT PROTOZOAN DISEASE

Mrinalini Roy[1], Anupam Jyoti[1], Sanket Kaushik[1], Vijay Kumar Srivastava[1]*

[1]Ph.D.Scholar, Amity Institute of Biotechnology, Amity University Rajasthan
[2]Assistant Professor, Amity Institute of Biotechnology, Amity University Rajasthan, Kant Kalwar, NH-11C, Jaipur-Delhi Highway, Jaipur, India

Corresponding Author: vksrivastava@jpr.amity.edu

INTRODUCTION

The single-celled amoebae were one of the earliest life forms on Earth and have lived in a constant state of emergency. Their survival is an ode to their efficient acquisition of an arsenal of genes giving them the survival edge. Amoebic defences represent the earliest iterations of the immune system. These pathogenic effectors gradually evolved into a multifaceted strategy for self-defence and host-takeover by amoeboid protists, and now hold ranks among the deadliest eukaryotic pathogens plaguing humanity. This chapter focuses on intestinal amoebic dysentery caused by the protozoa, *Entamoeba histolytica* (*Eh*) and ways to mitigate it.

ENTAMOEBA HISTOLYTICA: THE CAUSATIVE AGENT OF INTESTINAL AMOEBIASIS

It has been 160 years since the first detection of intestinal amoebiasis (IA) and we have come a long way since 1883 when Koch demonstrated that intestinal lesions are caused by amoeba in

the human ulcerated bowel (S Cummings & Angeles, 1913). The name *Entamoeba histolytica* was established by Schaudinn in 1903 based on its ability to cause tissue lysis in dysentery (Pinilla, López, & Viasus, 2008). Three non-pathogenic *Entamoeba* species, *E.dispar*, *E.moshkovskii* and *E. bangladeshi* have also been identified that are commensals of the human gastrointestinal tract (Carrero et al., 2020). The only known natural host of *E.histolytica* (*Eh*) is the human with the large intestine as a major target organ (Ximénez et al., 2011). This parasite accounted for 55,500 deaths and 2.237 million disability-adjusted life years (i.e., the sum of years of life lost and years lived with disability) in the year 2010 alone (Turkeltaub, McCarty, & Hotez, 2015), indicating the massive burden of *Eh* on the human population (Fig. 1).

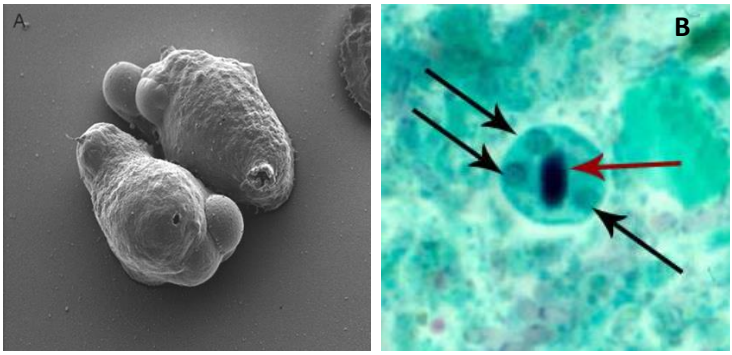


Figure 1. (A) Scanning electron micrograph of *Entamoeba histolytica* trophozoites which are the amoeba cells responsible for infection in the human host. It uses the pseudopodia to sequester and phagocytose the bacteria and the epithelium cell debris in the intestine for nutrition (García et al., 2017). (B) Cyst of *E. histolytica* stained with trichrome. Three out of the four nuclei in the tetra-nucleate cyst are visible in the focal plane (black arrows), and a chromatoid body with typically blunted ends is also visible in the cyst (red arrow)("CDC - DPDx - Amebiasis," n.d.).

The life of Eh is biphasic and does not require a vector for its transmission. One phase in the lifecycle is the trophozoite stage, and the other is the tetra-nucleate cyst stage, responsible for infection and transmission, respectively (Aguilar-Díaz, Carrero, Argüello-García, Laclette, & Morales-Montor, 2011). There is no sexual reproduction cycle for the multiplication of the trophozoites, and the overall population of Eh, created by binary fission, is believed to be clonal. Infection occurs usually when the host ingests the cysts of Eh, orally, the dormant form of the microbe. Due to their wall composition, these cysts are capable of surviving hostile conditions (e.g. pH, adverse temperatures, osmotic pressure and nutrient distress) and, can progress through antagonistic conditions in the gastrointestinal tract, specifically in the stomach and the intestine. The outer surface of the cysts is composed of a network of dense intertwined micro-fibrillar structures, majorly composed of carbohydrates and chitin-binding lectin proteins (Rawat, Singh, Jyoti, Kaushik, & Srivastava, 2020; Van Dellen et al., 2006). Once these cysts reach the terminal ileum region of the small intestine, excystation occurs and the trophozoites emerge as a mass of proliferating polyploid cells with an average diameter of about 10–60 μm. While some trophozoites continue to invade and colonize the intestinal tract, specifically colonic mucus, other trophozoites differentiate to the dormant forms, cysts, by the process of 'encystation'. Before encystation, the trophozoites suspend all cellular uptake activities and transform into non-motile cells prior to agglutination. Agglutination of the cells is a prerequisite for encystations. Trophozoites in the G2 phase of the cell cycle receive the stimulus for encystation

and transform into a tetra-nucleate cyst. These cysts are about 10-15µm in diameter.

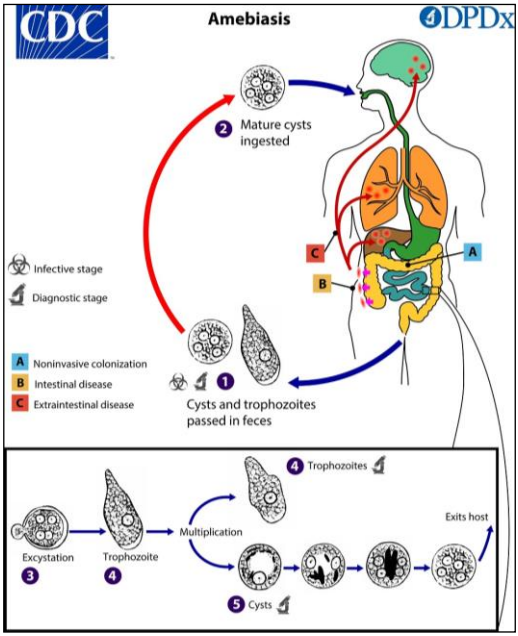


Figure 2. Lifecycle of *E. histolytica.* The cysts and trophozoites are passed in formed and diarrheal faeces respectively (1). The ingestion of the of mature cysts (2) starts the infection process and can take any of the three manifestations- A) Non-invasive or luminal colonization, B) Intestinal amoebiasis by invasion of intestinal mucosa, and C) Extraintestinal manifestations of ALA, pulmonary abscess and brain abscess. Excystation of the cysts (3) occurs in the intestine where each quadrinucleate cyst divides into eight actively growing trophozoites (4). Some of the trophozoites undergo encystation (5) to form dormant cysts that are the main diagnostic and transmission stage of *E. histolytica* pathogen.("CDC - DPDx - Amebiasis," n.d.)

Under favourable conditions, each tetra-nucleate cyst gives rise to eight trophozoites (Espinosa-Cantellano, Marti´nez, & Marti´nez-Palomo, 2000). The nutrition of the trophozoites is primarily dependent upon the natural microbiota, specifically the bacteria which act as food for *Eh*. This is proved by the fact that germ-free animals, when infected with Eh, fail to develop amoebiasis (Phillips & Wolfe, 1959). Thus, we can speculate that the composition of the human gut microbiome can affect the prognosis of this protozoan disease (Fig. 2).

SYMPTOMS AND SPREAD OF INTESTINAL AMOEBIASIS

The symptoms of Intestinal Amoebiasis (IA) range from abdominal pain and ulcerative colitis with mucous and blood (amoebic dysentery) to appendicitis and distinct flask-shaped ulcers along the colon epithelium (amoebic colitis/amoeboma). Occasionally, the parasites travel through the portal vein into the liver and produce the main extraintestinal infection, amoebic liver abscess (ALA). It can also travel to the lungs and brain, mainly in immunocompromised patients, and cause fatal infections(Baxt & Singh, 2008)(Carrero et al., 2020)(Tharmaratnam et al., 2020).

Transmission and spread of *E. histolytica* occur when contamination of food and water with cyst-containing faeces occurs, which is subsequently ingested by a susceptible human host. Cysts are the main transmission stage because they can survive in harsh external environment for long durations due to their protective cyst wall. The common reasons for spread are listed below.

- Faecally-contaminated drinking water.
- Raw food like salads and fruit juices; cysts are destroyed when food is cooked at boiling temperature.
- Swimming in dirty contaminated waterbodies.
- Flies can spread cysts stuck to their legs and body.
- Food handlers can spread infection if proper hygiene is not maintained.

- Auto infection can also occur by faeco-oral route by not maintaining proper personal hygiene.

Infection can be prevented by sincere efforts in improving sanitation and the provision of safe drinking water. Launching public health campaigns to promote hygienic practices like proper hand-washing and personal hygiene can be a big step towards prevention. By improving latrine facilities, provision of cleaning supplies, promoting safe consumption of alcoholic beverages as harm reduction for ALA and food safety are other ways to mitigate the amoebiasis burden. WHO provides a complete guideline on keys to food safety that involves the utilization of clean water and utensils when cooking, separation of raw and cooked foods, ensuring foods are kept at the appropriate temperature and are cooked thoroughly(World Health Organization, 2007).

IA ravages through developing countries like India, mainly because of the poor quality of drinking water and thus, there is a dire need to increase hygiene standards of drinking water in areas endemic to *E. histolytica*. The WHO 'Guidelines for Drinking Water Quality' shows that *E. histolytica* has a high resistance to low-dose chlorination practices traditionally used in potable water treatment. WHO highly recommends the boiling of drinking water for 1 min or the addition of iodine to drinking water supplies to sanitize water sources of *Eh* cysts before human consumption (World Health Organization, 2017).

With the SARS-Cov-2 pandemic plaguing the entire world, it is natural to draw the parallels between Covid19 and Intestinal Amoebiasis. Both these conditions have severe prognosis but can be prevented by the simple practice of proper handwashing, and sanitization. This underpins the importance of public hygiene, availability of sanitary facilities and awareness about public health to curb the morbidity and economic burden of a deadly disease.

MECHANISMS OF PATHOGENICITY

E. histolytica protozoan effectors

Infection cycle begins with ingestion of Eh cysts by the human host. On reaching the ileum region of the small intestine, Eh trophozoites (parasitic amoeba cells) are released from the cysts and adhere to the mucus layer of the colon through the surface lectin molecules called galactose/N-acetyl-galactosamine (Gal/GalNAc) lectin(Hou, Mortimer, & Chadee, 2010). The mucus gel constitutes a matrix for antimicrobial peptides and other molecules, such as secretory IgA antibodies (sIgA Abs). However, E. histolytica subverts this first line of defence by secreting glycosidases and cysteine proteases (EhCPs) that degrades the mucin polymer network, sIgA Abs, and other antimicrobial molecules (Carrero et al., 2020)(Hou et al., 2010).

The amoebae then colonize the large intestine through an active process of adhesion to the surface of the intestinal epithelium. The parasitic cells induce human host cell lysis via a cell-contact mechanism, releasing lytic molecules at the membrane of the target cell, leading to the dissolution of the extracellular matrix (ECM), lysis of the colon epithelial cells, and penetration of the mucosa. The trophozoites phagocytose the cell debris as a source of macromolecules. The adhesion process has four essential effectors, namely the 220 kDa lectin, a 112 kDa adhesion/cysteine protease (Rodríguez, Hernández, Santos, Valdez, & Orozco, 1989), the Gal/GalNAc lectin (Ravdin & Guerrant, 1981), and a family of serine-rich proteins (SR*EH*Ps) with tandem repeats (Carrero et al., 2020). Lectins act as a pathogen-associated molecular pattern (PAMP) and trigger the innate immune response against *Eh* cells. A new mechanism of pathogenicity was reported by (Ralston et al., 2014) for *E. histolytica* trophozoites, which consists of the ingestion of pieces of living cells, a process known as trogocytosis. This causes an elevation of intracellular calcium levels and ultimately leads to cell death. In this manner, trogocytosis contributes to intestinal tissue invasion and further colon epithelium damage.

Human host immunopathology

E. histolytica pathogenesis is multifactorial requiring both parasite virulent molecules and host-induced innate immune responses. *Eh*-induced host pro-inflammatory responses play a critical role in disease pathogenesis by causing damage to tissues and allowing the parasites access to systemic sites (Shahi, Moreau, & Chadee, 2019). The initial pro-inflammatory response involves the cellular activity of neutrophils, which further is succeeded by the activation of mast cells, macrophages and the natural killer T cells (NKTs). This innate immune response is triggered after the lectin of the parasite has engaged with the N-acetyl-D-galactosamine on the cell-surface O-linked oligosaccharides of the host cells. Inflammation is the prime driver of tissue damage, marked by the activation of transcription factor NFκB and tumour necrosis factor-alpha (TNFα) that downstream activate a cascade of sustained inflammation in the host GIT. The continuous degradation of GIT mucosal sublayers by *Eh* cysteine proteases induces an immune response. This triggers the release of more pro-inflammatory mediators by the epithelial cells generating a cytokine storm. The cytokines help severely disrupt the intestinal tissue and characteristic flask-shaped ulcers form in the bowel lining (ulcerative colitis)(Rawat et al., 2020).

Recently, another immunopathology mechanism has been discovered for invasive amoebiasis in which *E. histolytica* trophozoites trigger the neutrophil extracellular traps (NETs) but not *E. dispar* trophozoites. NETs are a meshwork of chromatin fibres that are decorated with neutrophil-derived antimicrobial peptides and enzymes such as neutrophil elastase, cathepsin G, and myeloperoxidase (MPO). These structures represent an important strategy to immobilize and kill invading microorganisms but are highly pro-inflammatory, with proposed involvement in exacerbation of autoimmune and inflammatory disorders, such as glomerulonephritis, chronic lung disease, and sepsis. In the context of intestinal amoebiasis and amoebic liver abscess, studies have

revealed the molecular pathways involved in NET activation and describe the possibility of the participation of NETs in amoebic pathogenesis (Díaz-Godínez et al., 2018; Fonseca et al., 2018).

DIAGNOSIS OF *ENTAMOEBA HISTOLYTICA* INFECTIONS

Microscopy

The dormant cysts of *E. histolytica* are solely responsible for the transmission of the disease, intestinal amoebiasis. These cysts are not only crucial to the spread of the amoeba but in diagnosis as well. In developing countries like India, the gold standard of IA diagnosis is based on the identification of the mature quadrinucleate cysts with the wet-mount examination of stool samples under a light microscope. Microscopic diagnostics have critical drawbacks, including poor sensitivity (approximately 60%) and the inability to distinguish pathogenic *E. histolytica* from the morphologically identical but nonpathogenic *E. dispar, E. moshkovskii* and *E. Bangladeshi* (Carrero et al., 2020) (Tanyuksel & Petri, 2003).

Isozyme Analysis

Another diagnostic technique is isozyme analysis which exploits the fact that isolates from *E. histolytica* have a genetically different hexokinase enzyme from isolates of *E. dispar*. Although this is a more sensitive method than wet-mount microscopy, it is a laborious, expensive and time-consuming technique, taking between one and two weeks with a success rate of 50–70%. These reasons have curtailed the use of isozyme analysis diagnosis as a routine practice in clinical laboratories (Tanyuksel & Petri, 2003).

ELISA and PCR diagnostics

ELISA tests have also been used for the detection of anti-amoeba antibodies in serum and amoebic antigens in faeces obtained from the patient. The difficulty of differentiating a past infection from an active one has considerably limited its use in clinical diagnosis, but instead, it has been used for seroepidemiological studies(Carrero et

al., 2020). PCR amplification and its variants have been widely used for the molecular diagnosis of amoebiasis. These are highly sensitive and can efficiently differentiate *E. histolytica* from other *Entamoeba* species. However, the main disadvantages of the PCR methods are the cost of specialized equipment and requirement of skilled personnel (Roy et al., 2005).

Molecular diagnostics has led to the discovery of other *Entamoeba* species, such as *E. moshkowskii*, which can also infect the human intestine with a significant frequency(Ximénez et al., 2011). Another interesting finding was the detection of *E. dispar* DNA sequences in samples from patients with amoebic liver abscess. This presents a new facet to this commensal protozoan in the context of invasive amoebiasis and further studies might reveal more about the complex relationship of this protest with the human GIT (Caliari, Gomes, Neumann, & Oliveira, 2015). However, much of the epidemiology and its contribution to the morbidity of *Entamoeba* infections remains unknown. This presents a dire need for new diagnostic tools.

CRISPR based diagnostics

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/ Cas (CRISPR Associated proteins) technology offers the solution for precise diagnostics. The technology has been able to differentiate between two strains of the Zika virus and recently has also been used to create a rapid diagnostic kit for SARS-Cov-2 using CRISPR/Cas12 system (Broughton et al., 2020). Developing a specific and sensitive diagnostic method for *E. histolytica* will help detect *Eh* in asymptomatic and mildly symptomatic hosts. This will easily enable early treatment and clearance of infection before the disease prognosis worsens. Decreased prevalence of *Eh* will ultimately reduce disease burden because the number of asymptomatic carriers will reduce and spread of the pathogen to susceptible weaker hosts will decline. It is always essential to break the chain of transmission whether it is amoebiasis or covid19. We are still not clear about the factors triggering extra-intestinal invasive behaviour of some *Eh* strains. Mortality due to intestinal

amoebiasis is low but mortality due to hepatic invasions and amoebic liver abscess (ALA) is as high as 75%. Thus it is imperative to have precise and differential diagnosis of ALA with other liver pathologies, especially pyogenic liver abscess (PLA), caused by bacteria, which is a common occurrence in clinical practice (Ximénez et al., 2011).

CONVENTIONAL TREATMENT STRATEGIES

Treatment strategies in clinical practice use a wide range of anti-amoebic compounds that can be categorized based on their mode of action that primarily occurs on two distinct levels: (a) luminal level: compounds acting only at the level of the intestinal lumen, useful in managing amoebic non-dysenteric colitis (e.g. paromomycin and diloxanide), and (b) systemic level: these compounds are absorbed and released into the bloodstream, from where they are transported to tissues, which are the actual site of action (e.g. nitroimidazoles). Some compounds act at both levels such as 5-Nitroimidazoles, a large group of anti-amoebic compounds. Another group of drugs belonging to the diiodohydroxyquinoline group, including iodoquinol, and clioquinol were once used for IA, but the neurotoxicity caused by these compounds has led to their withdrawal as therapeutics (Rawat et al., 2020).

Metronidazole is the drug of choice for treatment of amoebic dysentery and ALA due to its effectiveness, availability and low cost. Tinidazole is another popular drug for managing IA and belongs to the 5- nitroimidazole group. However, one cannot negate the side effects of these drugs which include nausea, leucopenia, metallic-taste in the mouth and other allergic reactions (Carrero et al., 2020). The substantial length of the treatment and use of other drugs like paromomycin, a luminal amoebicide in addition to metronidazole, provokes patients to desert the treatment half-way and the entire infection is not

cleared. This perpetuates the vicious cycle of *Eh* transmission and infection and leads to the emergence of drug resistance.

EXPLORING ALTERNATIVES TO CONVENTIONAL TREATMENTS

Eh L-Cysteine and *Eh* Cysteine Proteases as Drug Targets

L-Cysteine is an essential amino acid for *E. histolytica* and is implicated in several important pathogenic processes including attachment, motility, proliferation, and anti-oxidative defence. We cannot directly target the destruction of L-cysteine because it is an essential amino acid for humans, thus we target the cysteine biosynthetic pathway specific to *E. histolytica*. Researchers have found a fungal metabolite pencolide as the first compound that inhibits cysteine synthase and arrests amoebic cell growth in a cysteine-dependent manner with relatively low mammalian cytotoxicity (Mori et al., 2018).

Cysteine proteases are the most abundant proteases in *E. histolytica*. With more than 50 genes coding for this crucial virulence factor, they are an attractive drug target. An assortment of functions can be attributed to cysteine proteases, including the accession of nutrients, degradation of the mucosal layer and ECM of the enteric lining of the host, destruction of liver tissue, induction of an inflammatory response, and involvement in the stage transitional processes in the parasite's lifecycle. Five cysteine proteases are highly expressed in *Eh*, namely *Eh*CP1, *Eh*CP2, *Eh*CP5, *Eh*CP4 and *Eh*CP7. The amoeba regulates its cysteine proteases in a very controlled manner to illicit the exact amount of toxic and inflammatory effects at the desired location. A newly discovered mechanism used by *Eh* to prevent inflammation during the invasion of the gut is via the production of a cyclooxygenase-like protein (*Eh*Cox). *Eh*Cox alters the activity of *E. histolytica* cysteine proteases that generate inflammation in the body (Shahi et al., 2019). Further inspection of *Eh*cox can be done, to establish its potential as a drug to control immunopathology of *Eh.*

Another protein of interest is the *Eh*Vps29, a zinc-binding metalloprotein, that helps maintain the structural integrity of major proteins in *E. histolytica*. Experimental analysis of *Eh*Vps29 revealed that it perhaps titrates out some of the key regulatory factors required for retromer functioning in *Entamoeba* and hence leads to decreased cysteine protease activity. The decline in activity of crucial *Eh*CPs downregulates the pathogenesis of *Entamoeba* (Srivastava et al., 2017).

Cysteine proteases are implicated in several human pathologies and CP inhibitors could be profitable targets for drugs. The drugs based on CP mode of action can also be useful for treating other protozoal diseases which are a great threat to humans, like malaria and leishmaniasis. We have observed before that treatment for one protozoan can be applied to other protozoans as well. Studies have reported that the anti-malarial drug mefloquine (4-methanolquinoline compound), has a better and more rapid amoebicidal in-vitro action against the trophozoites as well as the cysts, compared to metronidazole, which fails to cleave the cysts. Mefloquine also has a much longer half-life in human patients in contrast to metronidazole (Rawat et al., 2020). Further research is being carried out for exploring the potential of CP-derived drugs for ameobicidal activity. Since *Eh*CPs are broad-spectrum, a therapeutic based on CPs will help tackle even the unidentified strains of pathogenic entamoebae.

Halting Encystation and Excystation Processes for IA Control

The interaction between the protozoa and human host is multifaceted. However, the critical stages for the pathogenesis of *Eh* are the processes of encystation and excystation, and these processes can be targeted to stop transmission of the disease. During encystations, several factors come into play, one such factor is the heat shock protein Hsp90. The mRNA repertoire of Hsp90 and its co-chaperones is drastically downregulated. This establishes the role of Hsp90 as a negative regulator of encystations and a potential drug target. The transcription factor ERM-BP (encystation regulatory motif-binding protein) is a novel and prime regulator

of stage transition in *Entamoeba*. It regulates the development of mature cysts by directly regulating the occupancy of the promoter. Another effector protein, the *Ei*CSpk (*Entamoeba invadens* cyst specific protein kinase) is an encystation-specific kinase that induces the production of a cell division protein kinase (accession no: *EI*N_530930), expressed only during encystations stage.

The novel proteins, ERM-BP and *Ei*CSpk should be investigated as therapeutics for IA, since these are specific to *Entameoba* and might elicit only minimal side effects in humans, in contrast to the existing nitroimidazole therapies. Thus (Rawat et al., 2020).

Understanding Vesicular Trafficking in *E.histolytica* for development of therapeutics

Vesicular trafficking is crucial for the survival of any eukaryotic cell. The *E. histolytica* genome encodes 91 Rab guanosine triphosphatases (GTPases) that belong to the Ras superfamily of GTPases, which is linked with the regulation of vesicular trafficking pathways and various signalling pathways associated with metabolism and virulence. Researchers have discovered Rab GTPases unique to *E. histolytica*, namely the *Eh*RabX37, *Eh*RabX38 and *Eh*RabX42 which have a non-conservative amino acid substitution in the G2, G3 or G5 motifs, and can be thus labelled as pseudoGTPases. These play an important role in nucleotide binding and hydrolysis(Srivastava, Chandra, Saito-Nakano, Nozaki, & Datta, 2016). GTPases like the *Eh*RabX3 are associated with the regulation of molecular domains of other GTPases, which are the prime regulators of vesicular trafficking in *Eh*, and hence crucial for metabolism in the parasite (Mitra, Saito-Nakano, Nakada-Tsukui, Sato, & Nozaki, 2007). Rab11B is another GTPase that regulates the production of cysteine proteases (CPs) as overexpression of Rab11b resulted in a dramatic increase in both intracellular and secreted CP activity and an increase in cell monolayer destruction (Carrero et al., 2020)(Mitra et al., 2007).

The biochemical and structural data on these unique Rab GTPases will provide a basis for understanding novel features about the

GTPases, which are unique to the parasite and determine ways to exploit these enzymes efficiently, as drug targets.

Vaccine development

Unabated efforts for vaccine development for *E.histolytica* are underway, yet we have not been able to produce it for clinical use. One potential vaccine candidate is the formulation of Gal/GalNAc lectin and CpG oligodeoxynucleotides administered through injection. This guarded against *E. histolytica* challenge in both gerbils and mice. CpG was used as an adjuvant because it can activate the ideal branches of the immune system required for a vaccine. Protective immunity to ALA was observed in gerbils when trophozoite DNA was used in the vaccine. Parasitic DNA activated the macrophages in a toll-like receptor (TLR)-9-dependent manner. Immunization with a combination of trophozoite DNA and Gal/GalNAc lectin was able to provide complete protection against *E. histolytica* challenge (Ivory, Prystajecky, Jobin, & Chadee, 2008).

Advancements in vaccine research are required, and the impetus for that will be provided by unravelling the complex pathology of *E. histolytica* and elucidation of the genes involved in the virulence processes. Understanding the immunopathological cascade of the human host and its regulatory checkpoints will help decode the innate immune response mounted to clear the parasite and thereby, present us with vaccine candidates.

CURRENT PERSPECTIVE: PARALLELS BETWEEN AMOEBIC DYSENTERY AND COVID19

The SARS-Cov-2 pandemic has proved to be an unprecedented phenomenon, crippling economies and healthcare systems with an alarming death toll. Amoebic dysentery albeit is not as fatal but it is extremely prevalent in developing countries but not limited to them. The ease and frequency of air travel have made emigration a global phenomenon that is modifying the epidemiology of infectious diseases worldwide. Amoebic dysentery, quintessentially known as “travellers’ diarrhoea” has now spread to the developed

world owing to the global travel network. Similarly, the massive scale of covid19 spread is solely due to this reason. Another interesting parallel between covid19 and IA is the role of uncontrolled inflammation in the human body that leads to the severe pathologies associated with these diseases. Most cases of covid19 and amoebic dysentery are mild or asymptomatic and thereby increasing manifold the challenges in managing these conditions. Perhaps the most intuitive parallel between the diseases is the simple prevention by taking care of hygiene and sanitation and breaking the transmission chain (Hiscott et al., 2020).

SUMMARY AND FUTURE PROSPECTS

Entamoeba histolytica is the parasitic protozoan responsible for the human diseases intestinal amoebiasis, amoebic colitis and life-threatening conditions of hepatic and pulmonary amoebic abscesses. The research in the pathologies and prevention of these morbidities has been a challenge because of the complex multifarious relationship between *E. histolytica* and the human host during the distinct stages of the disease. To further complicate matters, the composition of gut microbiota and the host acute inflammatory response play a role in the prognosis of the disease. This is the reason that to date, no suitable animal models have been established that can mimic the whole cycle of the human amoebiasis.

To overcome this lack of model organisms, sophisticated technologies like the CRISPR-Cas systems can be exploited to discover novel and crucial molecules that regulate the essential pathogenic pathways associated with the transmission of the disease. Alternative treatment methodologies are constantly being explored and developed to create feasible, inexpensive, non-toxic and safe therapeutics for *E. histolytica* infections. Precise diagnostics are also being developed to allow specific diagnosis of *Eh* and removing the chance for the erroneous treatment of another similar disease and to enable early-stage clearance of amoebic pathogens. Further research in *Entamoeba*

pathologies can help in devising short effective treatment strategies for amoebiasis and liver abscesses and reducing the global burden of this 160-year-old disease in the future.

REFERENCES

Aguilar-Díaz, H., Carrero, J. C., Argüello-García, R., Laclette, J. P., & Morales-Montor, J. (2011). Cyst and encystment in protozoan parasites: Optimal targets for new life-cycle interrupting strategies? Trends in Parasitology. https://doi.org/10.1016/j.pt.2011.06.003

Baxt, L. A., & Singh, U. (2008, October). New insights into Entamoeba histolytica pathogenesis. Current Opinion in Infectious Diseases, Vol. 21, pp. 489–494. Curr Opin Infect Dis. https://doi.org/10.1097/QCO.0b013e32830ce75f

Broughton, J. P., Deng, X., Yu, G., Fasching, C. L., Servellita, V., Singh, J., … Chiu, C. Y. (2020). CRISPR–Cas12-based detection of SARS-CoV-2. Nature Biotechnology. https://doi.org/10.1038/s41587-020-0513-4

Caliari, M., Gomes, M., Neumann, E., & Oliveira, F. S. (2015). Entamoeba dispar: Could it be pathogenic. Tropical Parasitology, 5(1), 9. https://doi.org/10.4103/2229-5070.149887

Carrero, J. C., Reyes-López, M., Serrano-Luna, J., Shibayama, M., Unzueta, J., León-Sicairos, N., & de la Garza, M. (2020, January 1). Intestinal amoebiasis: 160 years of its first detection and still remains as a health problem in developing countries. International Journal of Medical Microbiology, Vol. 310. Elsevier GmbH. https://doi.org/10.1016/j.ijmm.2019.151358

CDC - DPDx - Amebiasis. (n.d.). Retrieved December 14, 2020, from https://www.cdc.gov/dpdx/amebiasis/index.html

Díaz-Godínez, C., Fonseca, Z., Néquiz, M., Laclette, J. P., Rosales, C., & Carrero, J. C. (2018). Entamoeba histolytica trophozoites induce a rapid non-classical NETosis mechanism independent of NOX2-derived reactive oxygen species and PAD4 activity. Frontiers in Cellular and Infection Microbiology, 8(JUN). https://doi.org/10.3389/fcimb.2018.00184

Espinosa-Cantellano, M., Martı´nez, A., & Martı´nez-Palomo, M. (2000). Pathogenesis of Intestinal Amebiasis: From Molecules to Disease (Vol. 13). Retrieved from http://cmr.asm.org/

Fonseca, Z., Díaz-Godínez, C., Mora, N., Alemán, O. R., Uribe-Querol, E., Carrero, J. C., & Rosales, C. (2018). Entamoeba histolytica induce signaling via Raf/MEK/ERK for neutrophil extracellular trap (NET) formation. Frontiers in Cellular and Infection Microbiology, 8(JUL), 226. https://doi.org/10.3389/fcimb.2018.00226

García, C. G., Marchat, L. A., López-Cánovas, L., Ishiwara, D. G. P., Rodríguez, M. A., & Orozco, E. (2017). Drug Resistance Mechanisms in Entamoeba histolytica, Giardia lamblia, Trichomonas vaginalis, and Opportunistic Anaerobic Protozoa. In Antimicrobial Drug Resistance (pp. 613–628). Springer International Publishing. https://doi.org/10.1007/978-3-319-46718-4_40

Hou, Y., Mortimer, L., & Chadee, K. (2010). Entamoeba histolytica cysteine proteinase 5 binds integrin on colonic cells and stimulates NFκB-mediated pro-inflammatory responses. Journal of Biological Chemistry, 285(46), 35497–35504. https://doi.org/10.1074/jbc.M109.066035

Hiscott, J., Alexandridi, M., Muscolini, M., Tassone, E., Palermo, E., Soultsioti, M., & Zevini, A. (2020). The Global Impact of

the Coronavirus Pandemic. Cytokine & Growth Factor Reviews.https://10.1016/j.cytogfr.2020.05.010

Ivory, C. P. A., Prystajecky, M., Jobin, C., & Chadee, K. (2008). Toll-like receptor 9-dependent macrophage activation by Entamoeba histolytica DNA. Infection and Immunity, 76(1), 289–297. https://doi.org/10.1128/IAI.01217-07

Mitra, B. N., Saito-Nakano, Y., Nakada-Tsukui, K., Sato, D., & Nozaki, T. (2007). Rab11B small GTPase regulates secretion of cysteine proteases in the enteric protozoan parasite Entamoeba histolytica. Cellular Microbiology, 9(9), 2112–2125. https://doi.org/10.1111/j.1462-5822.2007.00941.x

Mori, M., Tsuge, S., Fukasawa, W., Jeelani, G., Nakada-Tsukui, K., Nonaka, K., … Shiomi, K. (2018). Discovery of Antiamebic Compounds That Inhibit Cysteine Synthase From the Enteric Parasitic Protist Entamoeba histolytica by Screening of Microbial Secondary Metabolites. Frontiers in Cellular and Infection Microbiology, 8, 409. https://doi.org/10.3389/fcimb.2018.00409

Phillips, B. P., & Wolfe, P. A. (1959). THE USE OF GERMFREE GUINEA PIGS IN STUDIES ON THE MICROBIAL INTERRELATIONSHIPS IN AMOEBIASIS. Annals of the New York Academy of Sciences, 78(1), 308–314. https://doi.org/10.1111/j.1749-6632.1959.tb53115.x

Pinilla, A. E., López, M. C., & Viasus, D. F. (2008, January). Historia del protozoo Entamoeba histolytica. Revista Medica de Chile, Vol. 136, pp. 118–124. https://doi.org/10.4067/s0034-98872008000100015

Ralston, K. S., Solga, M. D., MacKey-Lawrence, N. M., Somlata, Bhattacharya, A., & Petri, W. A. (2014). Trogocytosis by

Entamoeba histolytica contributes to cell killing and tissue invasion. Nature. https://doi.org/10.1038/nature13242

Ravdin, J. I., & Guerrant, R. L. (1981). Role of adherence in cytopathogenic mechanisms of Entamoeba histolytica. Study with mammalian tissue culture cells and human erythrocytes. Journal of Clinical Investigation, 68(5), 1305–1313. https://doi.org/10.1172/JCI110377

Rawat, A., Singh, P., Jyoti, A., Kaushik, S., & Srivastava, V. K. (2020). Averting transmission: A pivotal target to manage amoebiasis. Chemical Biology and Drug Design, 96(2), 731–744. https://doi.org/10.1111/cbdd.13699

Rodríguez, M. A., Hernández, F., Santos, L., Valdez, A., & Orozco, E. (1989). Entamoeba histolytica: molecules involved in the target cell-parasite relationship. Molecular and Biochemical Parasitology, 37(1), 87–99. https://doi.org/10.1016/0166-6851(89)90105-9

Roy, S., Kabir, M., Mondal, D., Ali, I. K. M., Petri, W. A., & Haque, R. (2005). Real-time-PCR assay for diagnosis of Entamoeba histolytica infection. Journal of Clinical Microbiology, 43(5), 2168–2172. https://doi.org/10.1128/JCM.43.5.2168-2172.2005

S Cummings, B. R., & Angeles, L. (1913). CALIFORNIA STATE JOURNAL OF MEDICINE CHRONIC INTESTINAL AMEBIASIS, WITHOUT DYSENTERY.* REPORT OF TWO CASES. In California State Journal of Medicine (Vol. 11). BMJ Publishing Group. Retrieved from BMJ Publishing Group website: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1640701/

Shahi, P., Moreau, F., & Chadee, K. (2019). Entamoeba histolytica Cyclooxygenase-Like Protein Regulates Cysteine Protease

Expression and Virulence. Frontiers in Cellular and Infection Microbiology, 8(JAN), 447. https://doi.org/10.3389/fcimb.2018.00447

Srivastava, V. K., Chandra, M., Saito-Nakano, Y., Nozaki, T., & Datta, S. (2016). Crystal Structure Analysis of Wild Type and Fast Hydrolyzing Mutant of EhRabX3, a Tandem Ras Superfamily GTPase from Entamoeba histolytica. Journal of Molecular Biology, 428(1), 41–51. https://doi.org/10.1016/j.jmb.2015.11.003

Srivastava, V. K., Yadav, R., Watanabe, N., Tomar, P., Mukherjee, M., Gourinath, S., … Datta, S. (2017). Structural and thermodynamic characterization of metal binding in Vps29 from Entamoeba histolytica: implication in retromer function. Molecular Microbiology. https://doi.org/10.1111/mmi.13836

Tanyuksel, M., & Petri, W. A. (2003, October 1). Laboratory Diagnosis of Amebiasis. Clinical Microbiology Reviews, Vol. 16, pp. 713–729. American Society for Microbiology Journals. https://doi.org/10.1128/CMR.16.4.713-729.2003

Tharmaratnam, T., Kumanan, T., Iskandar, M. A., D'Urzo, K., Gopee-Ramanan, P., Loganathan, M., … Tobbia, I. (2020, January 22). Entamoeba histolytica and amoebic liver abscess in northern Sri Lanka: A public health problem. *Tropical Medicine and Health*, Vol. 48, pp. 1–13. BioMed Central Ltd. https://doi.org/10.1186/s41182-020-0193-2

Turkeltaub, J. A., McCarty, T. R., & Hotez, P. J. (2015, January 12). The intestinal protozoa: Emerging impact on global health and development. *Current Opinion in Gastroenterology*, Vol. 31, pp. 38–44. Lippincott Williams and Wilkins. https://doi.org/10.1097/MOG.0000000000000135

Van Dellen, K. L., Chatterjee, A., Ratner, D. M., Magnelli, P. E., Cipollo, J. F., Steffen, M., … Samuelson, J. (2006). Unique posttranslational modifications of chitin-binding lectins of

Entamoeba invadens cyst walls. *Eukaryotic Cell*, *5*(5), 836–848. https://doi.org/10.1128/EC.5.5.836-848.2006

World Health Organization. (2007). (n.d.). Five keys to safer food manual department of food safety, zoonoses and foodborne diseases.

World Health Organization. (2017). (n.d.). Guidelines for Drinking-water Quality fourth edition incorporating the first addendum.

Ximénez, C., Morán, P., Rojas, L., Valadez, A., Gómez, A., Ramiro, M., … Oswaldo, P. (2011). Novelties on amoebiasis: A neglected tropical disease. *Journal of Global Infectious Diseases*, *3*(2), 166. https://doi.org/10.4103/0974-777X.81695

DENDRIMER: AN EMERGING NANO VEHICLE FOR THE TREATMENT OF LEISHMANIASIS

Pradeep Kumar[1]*, Gajendra Kumar Aseri[2], Vishal Saxena[3]

[1]Ph.D.Scholar, Amity Institute of Microbial Technology (AIMT), Amity University Rajasthan, Jaipur 333002
[2]Professor, Amity Institute of Microbial Technology (AIMT), Amity University Rajasthan, Jaipur 333002
[3]Associate Professor, Biological Science Department, Birla Institute of Technology and Science (BITS), Pilani 333031

*Corresponding Author: Pradeepkalgar@gmail.com

Introduction

Leishmaniasis is a protozoan derived parasitic disease which is caused by the genus leishmania from the trypanosomatidae family and transmitted by the bite of sandflies. There are two groups of sand flies which belong to the genus *Phlebotomus* in underdeveloped countries, and the genus *Lutzomyia* in more developed countries (Desjeux, P *et al.* 2004). An estimated 20 distinct species of leishmania caused this deadly disease in humans with four different clinical manifestations entities: visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and diffuse cutaneous leishmaniasis (DCL) (Murray, Henry W., et al 2005, Bhargava, P., & Singh, R. et al 2012). Visceral leishmaniasis is a distractive form of leishmaniasis which is more fetal if not cure at the appropriate time and which is caused by the Leishmania donovani, leishmania Chagasi, and Leishmania infantum species (Maltezou, H. C *et al*. 2009).

The life cycle of the parasite is started, when sandflies bite for taking blood as a meal from the host. During this sandflies ingest the macrophages which are infected with amastigotes form of infection and this amastigote is released in their digestive tract. Here the

amastigotes form is converted into the promastigotes form of infection and this promastigotes form of infection is injected into the next host at the time of the next feed. The injected promastigotes are adopted by macrophages and converted those again into the amastigotes form of infection (Gossage, Sharon M *et al*. 2003).

An estimated 350 million peoples are at risk with 1.5 million to 2.0 million new cases arising every year with 70,000 deaths from various countries due to leishmaniasis in which 75 percent of cases are only due to cutaneous leishmaniasis (Murray, Henry W *et al.* 2005). This also gives a big obstacle with (2.4 million) disability-adjusted life year (DALY) give its third ranking in disease burden by a neglected tropical disease and number second Ranking in parasitic related death after malaria (Ready, P. D *et al.* 2014). 75 percent cases of cutaneous leishmaniasis are reported in Afghanistan, Colombia, Brazil, Algeria, Peru, Costa, Rica, Iran, Syria, Ethiopia, Sudan, and 90 percent cases of visceral leishmaniasis are from India, Bangladesh, Nepal, Sudan, South Sudan, Pakistan and Brazil (Alvar, Jorge *et al.* 2012).

In India, visceral leishmaniasis is the more disseminated type of infection which is caused high morbidity with mortality and serious clinical manifestations like hepatomegaly, spleenomegaly, weakness, weight loss, anemia, darkening of the skin (that's why in India visceral leishmaniasis is known by the name of Kala-Azar means Black Fever), Wasting and Pallor. These all clinical varied, if untreated at a specific time, can cause death in humans (Collin, Simon *et al.* 2004, Davidson, R. N *et al.* 1998). In India, several cases of VL mostly come from Bihar and adjoining areas of Jharkhand, Uttar Pradesh which give half of the world burden of VL (Sundar, S., & Chatterjee, M *et al.* 2006). PKDL (Post Kala-Azar Dermal Leishmaniasis) is also a highly fatal form of leishmaniasis in India which is gesticulated in our dangerous sign after successful treatment of VL this can lead to a lengthy treatment for successful midget the effect of this malady (Desjeux, P., & Ramesh, V *et al.* 2011). Some studies on leishmanial cases show that Rajasthan is also a region where some cases of cutaneous leishmaniasis are

observed. It indicates that the range of endemic regions of leishmaniasis in India is diverse and especially in those areas where poverty is a major problem (Tyagi, B *et al.* 2004). World health organization adds leishmaniasis disease in a category, which is represented by the emerging and uncontrolled series of disease (Murray, Henry W *et al.* 2005).

The endemic region of leishmaniasis is grown so fast due to numerous changes in our lifestyle and some environmental risk factors like urbanization, lack of sanitation, deforestation, new agricultural irrigation plan and high deracination of populations from one to another place and some particular risk factor like malnutrition, traveling in those areas where leishmaniasis is endemic, feeble immunity person makes leishmaniasis an important public health problem which gives-destructive impact on social and economic values of a nation (Pink, Richard *et al.* 2005, Bhargava, P., & Singh, R *et al.* 2012). Much therapeutics are used for the treatment of leishmaniasis but serious side effects, dose-dependent toxicity, Route of administration, and effective cost are the barricade for these therapeutics (Sundar, S. 2001., & Matlashewski, Greg *et al.* 2011). Traditionally the pentavalent antimonial drug sodium stibogluconate is used for the treatment of leishmaniasis, but due to resistance issues, some alternative drugs are also used after the failure of pentavalent antimonial. Amphotericin B, miltefosine, and paromomycin have replaced the pentavalent antimonial in those areas where the resistance issue is observed (Sundar, Shyam, *et al.* 2001 & Guerin, Philippe J *et al.* 2002). Amphotericin B is a polyene antifungal antibiotic, procured from *streptomyces nodosum* have a hundred percent cure rate against Leishmaniasis but it is insoluble in water at endosomal pH (Saravolatz, Louis D *et al*. 2006). Lack of solubility in water blocks its oral delivery so it can be given either I.V. or I.M. Route of drug the administration which is because several side effects, long hospitalization, and high cost of treatment can be lead to developing a new alternative system for successful midget these obstacles.

Table 1. Anti-Leishmanial Drugs

Drugs	Content	Trade name	Route of Administration
Pentavalent Antimonial	Meglumine antimonite (MA)	Glucantim	I.V.
	Sodium Stibogluconate (SbG)	Pentostam among other	I.V.
Micelle formulation	Amphotericin B deoxycholate	Fungizone	I.V.
Miltefosine	Alkylphosphocholine	Impavido among Other	Oral
Paromomycin	Aminoglycoside antibiotics	Aminosidine	Oral
Pentamidine	Aromatic Diamine	Nebupent and more	I.V. or I.M.
Sitamaquine	8-aminoquinoline	In Clinical trails	Oral drug
Buparvaquone	Hydroxynaphtoquinone	In vivo trails	Topical
Polymer Formulation	N-(2-hydroxypropyl)-methacrylamide GFLG-Amphoterin B	In vivo trails	I.V.
Dendrimer Formulation	Mannosylated PPI-Amphotericin B	In vivo trails	I.V.
	PAMAM Dendrimer-Amphotericin B	In vitro trails	Oral
Lipid Formulations	Liposomal Amphotericin B	Ambisome	I.V.
	Amphotericin B lipid complex (ABLC)	Abelcet	I.V.
	Amphotericin B colloidal complex (ABCD)	Amphocil	I.V.

Treatment modalities for Leishmaniasis:-

For the last 3 decades, nanotechnology is an emerging and interesting field in the era of targeted delivery of drugs. Several drug delivery vehicles like nanoparticles, Dendrimers, micelles, liposomes, nanospheres, nanocapsules, hydrogels, and polymers are developed for the removal of the hurdle of bioavailability, target specific delivery, and solubility enhancement of hydrophobic drugs (Svenson, S., & Tomalia, D. A *et al.* 2012). A dendrimer is a class of hyperbranched nano polymer that has a unique architecture design with controlled synthesis, monodispersity, and low molecular weight (Tomalia, Donald A *et al.* 1985). The term dendrimer comes from the Greek word Dendron meaning tree. Dendrimer has a tree-like structure that has a core molecule as a stem with a versatile peripheral structure like branches. A dendrimer is a polymer of the 20^{th} century with great features for pharmaceuticals applications. The advanced design of dendrimer poses an internal cavity and surface functional group that makes them the first choice for biological applications like solubility enhancement of insoluble drugs and target-specific delivery of drugs (Newcome, G. R *et al.* 2001 & Kannan, R. M *et al.* 2014). PAMAM dendrimer is most starburst and a most studied class of the dendrimer in areas of drug delivery, which is mostly used for the solubility enhancement of drugs due to its unique architecture index (Kesharwani, P., Jain, K., & Jain, N. K *et al.* 2014, Gupta, Umesh *et al.* 2006).

Threats of Leishmaniasis is rapidly increasing from the last few decades due to improper lifestyle and resistance issues with parental treatment modalities so an alternative source of treatment is must needed for the successful eradication of Leishmania infection. Amphotericin B is having the great potentiality to banish the hurdle of Leishmania infection but its amphiphilic and zwitterionic nature and High $_{p}$ka properties of chemical structure block its oral delivery (Lemke, A., Kiderlen, A. F., & Kayser, O *et al.* 2005, Volmer, A. A., Szpilman, A. M., & Carreira, E. M *et al.* 2010). Several researchers are work on how to reduce the side effect of amphotericin b with the parental route and how to change the route of administration of its enhancing bioavailability but ideal treatment

systems are still so far from a common man. Versatile properties of the dendrimer especially hydrophobic interior with the hydrophilic exterior are the keen beneficial feature for solubility enhancement of AmB through dendrimer. In this chapter, we discussed and analyzed some research articles which have described the potentiality of dendrimer for the development of an ideal treatment system for the eradication of Leishmania infection.

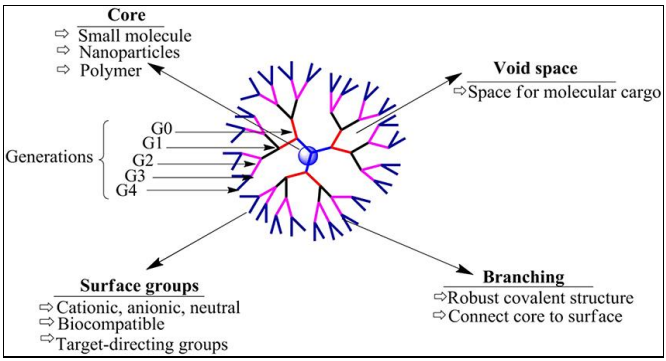


Fig 1. General structure of a Dendrimer (Source: Choudhary, Sonam *et al.* 2017)

Jobin Jose and Narayan Charyulu demonstrate that the Incorporation of AmB in PAMAM dendrimer enhances the solubility significantly. He describes that the number of an amine group on the surface of the dendrimer is responsible for the solubility of the drug. The solubility of AmB is also enhancing by concentration as well as the generation of the dendrimer. The higher generation dendrimer has a higher no. of amine group so it is more solubilize the drug as compared with the lower generation. As an earlier study illustrate that the solubility enhancement of a drug by conjugate with dendrimer is probably dependent upon the hydrophobic interactions, hydrogen bonding, internal cavities, and electrostatic interaction of the peripheral surface of the drug with a terminal group of a dendrimer. (Choudhary, Sonam *et al.* 2017). In this study they also emphasize the effect of pH on solubility enhancement, he was

conducting his study on three different pH 4.0, 7.4, 10.0 and conclude that the solubility of the drug is higher on pH 7.4>10.0>4.0 due to ionization status and electrostatic interaction and hydrogen bonding between the dendrimer amine group and OH and COOH functional group of AmB. So they conclude that the Amine terminated dendrimer is highly able to enhance the solubility of poorly water-soluble drug AmB (Jose, J., & Charyulu, R. N *et al.* 2016). Jobin Jose *et al.* did the second study on PAMAM dendrimer with AmB, his study emphasizes the solubility profile of drugs as well as release behavior of drug from dendrimer. Based on the result obtained from this study he concluded that the solubility of AmB is extensively enhanced by incorporation with dendrimer due to its surface amine group and the release behavior which is analyzed in PBS buffer of pH 7.4 at room temperature of the drug from dendrimer is highly emblem that the release kinetics depends on internal cavities of the dendrimer. The hydrophobicity of the internal cavities of the dendrimer is responsible for the delay in the release as compared to the free drug (Jose, J., & Charyulu, R. N *et al.* 2015).

Jain *et al.* have developed an optimized dendrimer-based drug delivery system for macrophage-targeted delivery of AmB with the fifth generation of PPI dendrimer. They develop a conjugate of AmB with Mannosylated PPI dendrimer because mannosylation is an advanced strategy for macrophage-targeted delivery of drugs due to the availability of mannose accepting receptors on the outer surface of macrophages (Jain, N. K., Mishra, V., & Mehra, N. K *et al.* 2013). The results of this study showed that the entrapment efficiency of the dendrimer is extensively good and the release behavior of conjugated dendrimer is higher in acetate buffer of pH 5.5 compared with PBS Buffer of pH 7.4. The cell uptake and MTT based cytotoxicity studies of dendrimer-based formulation get higher cell uptake by macrophages and negligible cytotoxicity compare with marketed liposomal formulations as well as plain drug. The *in vitro* antileishmanial activity of this conjugated dendrimer drug formulation is compared with liposomal AmB and Plain drug. The mannosylated dendrimeric formulation shows IC_{50}

value at 0.0385 µM concentration ($P<0.001$) which is lower than the IC_{50} value of the plain drug as well as liposomal formulations. The *in vivo* studies of the formulation is also show significantly good results at 1.0 mg/kg dose and the antileishmanial activity of dendrimeric formulation, liposomal Formulation, and the plain drug is in a higher to lower manner respectively. Based on this study, her group concludes that the Mannosylated Dendrimer drug formulation is a great carrier for macrophages targeted delivery of AmB drug with improved Pharmacokinetics and Biodistribution adumbration and it is also a safe delivery system for AmB with reducing cytotoxicity and improved bioavailability for successfully treatment of Leishmania infection (Jain *et al.,* 2015).

Jain *et al.* were done another study on the potentiality of dendrimer for target-specific delivery and solubility enhancement of AmB. They formulated a Conjugate of AmB with muramyl dipeptide (MDP) modified PPI (G 5.0) dendrimer. MDP is a dipeptide that is responsible for the stimulation of non-specific resistance against parasitic burden as well as an increment in metabolic activity of macrophages. So her group conjugates the PPI dendrimer with muramyl dipeptide and PPI (Muramyl dipeptide conjugated PPI dendrimer) with AmB results shown the great properties of MDP modified PPI dendrimer like drug loading as well as drug entrapment efficiency and the release behavior of this formulation is similar to past studies which are suggested that the release of drug from dendrimer is significantly faster ($P<0.01$) on acidic pH (5.5) than physiological pH (7.4). Hemolytic study and cytotoxicity studies of MDPPI dendrimer are shown less hemolysis on 20 µg/ml concentration as well as less cytotoxicity rate compared with the marketed formulation of AmB ($p<0.001$). The antileishmanial activity of this formulation is determined in vitro and in vivo conditions and the results of these studies show that the developed formulation of MDPPI with AmB enhances the parasite killing activity of AmB in comparison with the marketed drug formulation of AmB. The in vivo study was performed on Leishmania donavani infected mice model with a dose of Ambisome, fungizone, and MDPPI with AmB in 1.0 mg/kg body weight manner, and the result

of parasite killing activity of above formulation is 61.23% ±3.16%, 46.34 ± 1.71% and 89.32% ± 3.52% respectively which is shown that the MDPPI with AmB can enhance the parasite killing activity of AmB with reducing cytotoxicity. Finally, they conclude that the AmB loaded Ligand anchored Dendrimer MDPPI can reduce the toxicity of the drug by enhancing the antileishmanial potentiality of amphotericin B and an ideal vehicle for delivering a drug at target specific site this quality of the formulation of MDPPI Dendrimer with AmB is a sign to the alternative option of marketed formulation of AmB for the effective treatment of infectious disease especially Leishmaniasis (Jain, Keerti *et al.* 2015).
Mehzari *et al.* were conducted an *In vivo* study with two different antileishmanial drugs (*i.e.* Amphotericin B and Bitulinic Acid) with ALGD dendrimer and nanochiston. They conducted his studies on a mice model that is infected with Leishmania major infection. After histopathological studies of infected mice, the results indicate that amphotericin B-nanochiston (AK) at 10 mg/kg and bitulinic acid-nanochiston (BK) at 20 mg/kg dose significantly reduce the size of the organ as well as disease effects as compare to amphotericin B-dendrimer (AD) at 50 mg/kg and bitulinic acid-dendrimer (BD) at 40 mg/kg body weight doses (Zadeh Mehrizi, Tahereh *et al.* 2018).

Daftarian *et al.* also conducted a research study on successful eradication of leishmania threatens with uses AmB with Dendrimer. They use pan-DR-Binding epitope (PADRE) derivative Dendrimer (PDD) as a polymeric vehicle and conduct his studies on leishmania infected mice model, his group demonstrate that PDD dendrimer is work as an escort to liposomal AmB to antigen-presenting cells (APCs) in vivo which helps reduce the dose of the drug due to target-specific delivery as well as enhance the drug efficacy by 83 %. The developed formulation also enhances the target-specific delivery by 10 folding with reducing toxicity with the parasitic burden (Daftarian, Pirouz M *et al.* 2013).

Pirouz M. Daftarian and his team have conducted a study on successfully target-specific delivery of liposomal AmB. Amphotericin B is a widely used antibiotic for the treatment of Leishmania infection but due to some adverse side effects like

nephrotoxicity and neurotoxicity, several modified modalities of AmB are used for the treatment of this lethal infection (Sundar, Shyam *et al.* 2010). Liposomal AmB is one of the best-modified treatment modalities for reducing its nephrotoxicity problem but the high dose requirement of liposomal AmB is a big hurdle for eradication or overcome of this problem for this a target-specific delivery of drug with reduced degradation is needed so his group developed a Pan-DR-binding epitope derived dendrimer (PDD) and make its conjugate with liposomal AmB because we know Leishmania species are obligate parasite in phagocytosis so for the targeting of leishmania infected phagocytosis peptide dendrimer is highly helpful for the targeting of MHC-2 receptors so after getting the results of this study his group concludes that PDD dendrimer is an ideal vehicle for targeted delivery of L-AmB and high efficacy by 80% with great pharmacokinetics properties and stimulating t-cells response (Daftarian, P. M., Ager, A. L., & Stone, G *et al.* 2014).

A study on the potentiality of PAMAM dendrimer for targeted drug delivery of Amphotericin B for the treatment of leishmaniasis is conducted in our laboratory and based on results obtained from our study we demonstrate that the PAMAM dendrimer and its modified derivatives are an ideal vehicle for delivery of amphotericin B. (Data unpublished)

Some other study also conducted in the era of DNA vaccine development for the treatment of Leishmania infection with the help of dendrimer. DNA vaccine development is a recent immunization tool with great potentiality over any other traditional vaccine because DNA vaccine is complement with adjuvant (Tabatabaie, Fatemeh *et al.* 2018). No vaccine is approved by FDA for the treatment of Leishmania infection to date due to the non-availability of suitable adjuvant and recent studies have been proven that a nanoparticle is a group of nanomaterial's that have ideal therapeutic properties for use as an adjuvant for the delivery of any antigen. These unique properties of nanomaterial's specially dendrimer class indicating the vaccine formulations with Dendrimers are emerged area for the research community to develop an ideal vaccine for

successful elimination of Leishmania infection. Some dendrimer prodrug is also developed by scientists for the eradication of leishmaniasis (Giarolla, J., Pasqualoto, K. F. M., & Ferreira, E. I *et al.* 2013).

Conclusion

Dendrimer is an ideal polymeric vehicle for mitigating the hurdles of target-specific delivery of antileishmanial drugs. In the future, it plays a vital role in the successful development of effective treatment modalities for the treatment of Leishmania infection.

REFERENCES

Alvar, J., Vélez, I. D., Bern, C., Herrero, M., Desjeux, P., Cano, J. ...& WHO Leishmaniasis Control Team. (2012). Leishmaniasis worldwide and global estimates of its incidence. *PloS one*, *7*(5).

Bhargava, P., & Singh, R. (2012). Developments in diagnosis and antileishmanial drugs. *Interdisciplinary perspectives on infectious diseases*, *2012*.

Bhargava, P., & Singh, R. (2012). Developments in diagnosis and antileishmanial drugs. *Interdisciplinary perspectives on infectious diseases*, *2012*.

Choudhary, S., Gupta, L., Rani, S., Dave, K., & Gupta, U. (2017). Impact of dendrimers on solubility of hydrophobic drug molecules. *Frontiers in pharmacology*, *8*, 261.

Collin, S., Davidson, R., Ritmeijer, K., Keus, K., Melaku, Y., Kipngetich, S., & Davies, C. (2004). Conflict and kala-azar: determinants of adverse outcomes of kala-azar among patients in southern Sudan. *Clinical Infectious Diseases*, *38*(5), 612-619.

Daftarian, P. M., Ager, A. L., & Stone, G. (2014). A Targeted and Adjuvanted Nanoparticle for Immunochemotherapy of Leishmania Infections. *Current Tropical Medicine Reports*, *1*(3), 148-153.

Daftarian, P. M., Stone, G. W., Kovalski, L., Kumar, M., Vosoughi, A., Urbieta, M., &Boodoo, R. (2013). A targeted and adjuvanted nanocarrier lowers the effective dose of liposomal amphotericin B and enhances adaptive immunity in murine cutaneous leishmaniasis. *The Journal of infectious diseases*, *208*(11), 1914-1922.

Davidson, R. N. (1998). Practical guide for the treatment of leishmaniasis. *Drugs*, *56*(6), 1009-1018.

Desjeux, P. (2004). Leishmaniasis: current situation and new perspectives. *Comparative immunology, microbiology and infectious diseases*, *27*(5), 305-318.

Desjeux, P., & Ramesh, V. (2011). Post-kala-azar dermal leishmaniasis: facing the challenge of eliminating kala-azar from South Asia. In *Kala Azar in South Asia* (pp. 111-124). Springer, Dordrecht.

Giarolla, J., Pasqualoto, K. F. M., & Ferreira, E. I. (2013). Design and exploratory data analysis of the second generation of dendrimer prodrugs potentially antichagasic and leishmanicide. *Molecular diversity*, *17*(4), 711-720.

Gossage, S. M., Rogers, M. E., & Bates, P. A. (2003). Two separate growth phases during the development of Leishmania in sand flies: implications for understanding the life cycle. *International journal for parasitology*, *33*(10), 1027-1034.

Guerin, P. J., Olliaro, P., Sundar, S., Boelaert, M., Croft, S. L., Desjeux, P., ...&Bryceson, A. D. (2002). Visceral leishmaniasis: current status of control, diagnosis, and treatment, and a proposed research and development agenda.*The Lancet infectious diseases*, *2*(8), 494-501.

Gupta, U., Agashe, H. B., Asthana, A., & Jain, N. K. (2006). Dendrimers: novel polymeric nanoarchitectures for solubility enhancement. *Biomacromolecules*, *7*(3), 649-658.

Jain, K., Verma, A. K., Mishra, P. R., & Jain, N. K. (2015). Characterization and evaluation of amphotericin B loaded MDP conjugated poly (propylene imine) dendrimers. *Nanomedicine: Nanotechnology, Biology and Medicine*, *11*(3), 705-713.

Jain, K., Verma, A. K., Mishra, P. R., & Jain, N. K. (2015). Surface-engineered dendrimeric nanoconjugates for macrophage-targeted delivery of amphotericin B: formulation development and in vitro and in vivo evaluation. *Antimicrobial agents and chemotherapy*, *59*(5), 2479-2487.

Jain, N. K., Mishra, V., &Mehra, N. K. (2013). Targeted drug delivery to macrophages. *Expert opinion on drug delivery*, *10*(3), 353-367.

Jose, J., & Charyulu, R. N. (2015). Solubility enhancement of an antifungal agent by association with dendrimers. *Indian Journal of Research in Pharmacy and Biotechnology*, *3*(2), 171.

Jose, J., & Charyulu, R. N. (2016). Prolonged drug delivery system of an antifungal drug by association with polyamidoaminedendrimers. *International journal of pharmaceutical investigation*, *6*(2), 123.

Kannan, R. M., Nance, E., Kannan, S., & Tomalia, D. A. (2014). Emerging concepts in dendrimer-based nanomedicine: from design principles to clinical applications. *Journal of internal medicine*, *276*(6), 579-617.

Kesharwani, P., Jain, K., & Jain, N. K. (2014). Dendrimer as nanocarrier for drug delivery. *Progress in Polymer Science*, *39*(2), 268-307.

Lemke, A., Kiderlen, A. F., &Kayser, O. (2005). Amphotericin b. *Applied microbiology and biotechnology*, *68*(2), 151-162.

Maltezou, H. C. (2009). Drug resistance in visceral leishmaniasis. *BioMed Research International*, *2010*.

Matlashewski, G., Arana, B., Kroeger, A., Battacharya, S., Sundar, S., Das, P., ...&Alvar, J. (2011). Visceral leishmaniasis: elimination with existing interventions. *The Lancet infectious diseases*, *11*(4), 322-325

Mehrizi, T. Z., Mosaffa, N., Hoseini, M. H. M., Ardestani, M. S., Khamesipour, A., Shahmabadi, H. E., ...&Ramezani, A. (2018). In vivo therapeutic effects of four synthesized antileishmanial nanodrugs in the treatment of Leishmaniasis. *Archives of Clinical Infectious Diseases*, *13*(5).

Murray, H. W., Berman, J. D., Davies, C. R., &Saravia, N. G. (2005). Advances in leishmaniasis. *The Lancet*, *366*(9496), 1561-1577.

Newkome, G. R., Moorefield, C. N., &Vögtle, F. (2001). *Dendrimers and dendrons: concepts, syntheses, applications*. Wiley-VCH Verlag GmbH.

Pink, R., Hudson, A., Mouriès, M. A., &Bendig, M. (2005). Opportunities and challenges in antiparasitic drug discovery. *Nature reviews Drug discovery*, *4*(9), 727-740.

Saravolatz, L. D., Bern, C., Adler-Moore, J., Berenguer, J., Boelaert, M., den Boer, M., &Ritmeijer, K. (2006). Liposomal amphotericin B for the treatment of visceral leishmaniasis. *Clinical Infectious Diseases*, *43*(7), 917-924..

Sundar, S. (2001). Drug resistance in Indian visceral leishmaniasis. *Tropical Medicine & International Health*, *6*(11), 849-854.

Sundar, S., & Chatterjee, M. (2006). Visceral leishmaniasis-current therapeutic modalities. *Indian Journal of Medical Research*, *123*(3), 345.

Sundar, S., Chakravarty, J., Agarwal, D., Rai, M., & Murray, H. W. (2010). Single-dose liposomal amphotericin B for visceral leishmaniasis in India. *New England Journal of Medicine*, *362*(6), 504-512.

Svenson, S., & Tomalia, D. A. (2012). Dendrimers in biomedical applications—reflections on the field. *Advanced drug delivery reviews*, *64*, 102-115.

Tabatabaie, F., Samarghandi, N., Zarrati, S., Maleki, F., Ardestani, M. S., Elmi, T., &Mosawi, S. H. (2018). Induction of immune responses by DNA vaccines formulated with dendrimer and poly (Methyl Methacrylate)(PMMA) nano-adjuvants in BALB/c mice infected with leishmania major. *Open access Macedonian journal of medical sciences*, *6*(2), 229.

Tomalia, D. A., Baker, H., Dewald, J., Hall, M., Kallos, G., Martin, S., ...& Smith, P. (1985). A new class of polymers: starburst-dendritic macromolecules. *Polymer journal*, *17*(1), 117-132.

Tyagi, B. (2004). Cutaneous leishmaniasis in the dessert: A critical appraisal of the emerging "Sand disfiguring disease". *Advancements in Insect Biodiversity*, 313.

Volmer, A. A., Szpilman, A. M., &Carreira, E. M. (2010). Synthesis and biological evaluation of amphotericin B derivatives. *Natural product reports*, *27*(9), 1329-1349.

WHO- centre of the leishmaniasis. Report of a WHO expert committee. WHO tech Rep ser 2010:949

World health organization (2015) leishmaniasis fact sheet No- 375 http.who.int/media centre/factsheet/fs/375/em/

URINARY TRACT INFECTIONS AND PHAGE THERAPY TO TACKLE ANTIMICROBIAL RESISTANCE (AMR)

Kanika Bhargava[1], Dr G. K. Aseri[2] and Dr Neelam Jain*[3]

[1]Ph.D.Scholar, Amity Institute of Microbial Technology, Amity University Rajasthan, Jaipur 303 002, India
[2]Professor, Amity Institute of Microbial Technology, Amity University Rajasthan, Jaipur 303 002, India
[3]Professor, Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur - 303 002, India

*Corresponding Author: njain1@jpr.amity.edu

INTRODUCTION:

Urinary tract infection (UTI) is a common and distressing human disease that leads to significant health issues both in community and hospital-based settings (Angami *et al*., 2015). Its complication often leads to sepsis (Sabih and Leslie, 2020; Petrosillo *et al*., 2020) and may prove to be fatal if caused by antimicrobial-resistant organisms. Urinary tract infection is defined as an immune response to abnormal growth of the pathogen in the urothelium (Abou Heidar *et al*., 2020; Odoki *et al*., 2019). Urinary tract infection affects different parts of the urinary system where lower urinary tract infection includes urethra (urethritis), prostate in males (prostatitis) and urinary bladder (cystitis); and upper urinary tract includes ureter (ureteritis) and kidney (pyelonephritis) (Wagenlehner *et al*., 2020). It is often classified as uncomplicated UTIs and complicated UTIs. Complicated urinary tract infection is defined as infections that carries a higher risk of treatment failure, with several structural and functional abnormality of genitourinary tract, in the presence of underlying diseases and typically require a longer antibiotic course of treatment. Uncomplicated UTIs occurs and evolves in individuals without any identified risk factors and is most commonly treated in

primary health care sectors (Tan and Chlebicki, 2016; Sabih and Leslie, 2020). Urinary tract infection can also be community-acquired UTI or nosocomial UTI (Odoki *et al*., 2019). Community-acquired UTI is defined as infection taking place in a community setting or in the hospital setting of less than 48h of admission (Moyo *et al*., 2010). In contrast, nosocomial UTI is defined as infection occurring after 48 hours of hospital admission and not incubated by the patient at the time of admission or after 3 days of discharge (Lacovelli *et al*., 2014). Another category of UTI is catheter-associated urinary tract infection (CAUTI), where it is defined as a severe iatrogenic infection with the emergence of signs or symptoms in patients carrying indwelling urethral, suprapubic and/or even intermittent catheters with the presence of significant bacteria in urine (Abou Heidar *et al*., 2020).

UTI is a serious healthcare issue affecting millions per year worldwide (Sharma and Paul, 2012; Angami *et al*., 2015). Incidence and prevalence rates vary considerably based on the UTI patients' geographical location, management of the patient, patient sex and other comorbidities (Wagenlehner *et al*., 2020). Annually 150 million people suffer from UTIs globally (Odoki *et al*., 2019; Abou Heidar *et al*., 2020; Schappert and Rechtsteiner, 2011) with global treatment cost of approximately 6 billion dollars on health amenities (CDC UTI, 2015). According to the Global Prevalence Study on Infections in Urology (GPIU) estimation, 9.4% urological patients hospitalised between 2005 and 2017 resulted in the development of complicated UTI during their hospitalisation (Tandogdu *et al*., 2014).

Currently, management of urinary tract infection is empirical treatment without urine culture and susceptibility testing, hence, the growing concern of antimicrobial resistance worldwide is agitating and requires novel therapeutic and management techniques. As there has been a significant increase in antibiotic resistance globally (Yamamoto *et al*. 2010; Muratani and Matsumoto, 2006), the bacteriophages (Bacterial viruses) can be commissioned in the treatment of those bacterial infections. This process is known as

Phage therapy, where phages are unrefined predators that can be employed in the treatment of UTI.

PREVALANCE OF UTI (GENDER BASED):

UTI are the most prevalent bacterial disease in women as compared to men. This disease affects patients of all age groups and sexes, with females accounting for 87.5% of the cases and males 71.3% of cases (Odongo *et al*., 2020; Akram *et al*., 2007; Gupta *et al*., 2001; Gajdacs *et al*., 2019). It is four times more frequent in females than that of males and commonly occurs in the age of 16 to 35 years wherein general 40% of women develop an infection at some point of their life (Angami *et al*., 2015; Tan and Chlebicki, 2016; Foxman, 2013).

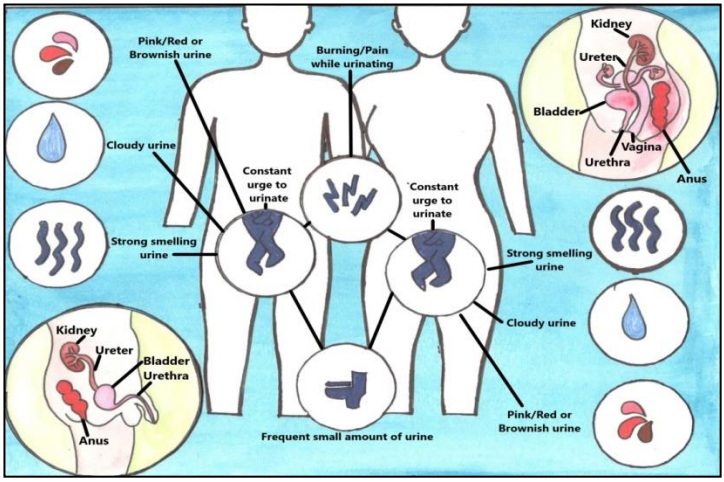


Figure 1. Urinary Tract of Male v/s Female

Bacteria can enter the urinary tract via two main routes: ascending route and haematogenous route. The most common route is the ascending route through which bacteria enter the urethra, and from there it ascends into the bladder, ureters and kidneys. In comparison

to males, the genitourinary anatomy of women makes them more prone to UTIs. As a result, the relatively short urethra and its proximity to the anus, present in the female urinary tract predisposes inherent proximal bacterial seeding (Figure 1). This anatomy thus increases the frequency of infection (Sabih and Leslie, 2020; Odongo *et al*., 2020) in females as compared to males.

Table 1. Uropathogens causing Urinary Tract Infections

Types of UTI	Microorganisms involved
Pyelonephritis	Gram-positive bacteria
	Staphylococcus aureus
	Staphylococcus saprophyticus
	Gram-negative bacteria
	Escherichia coli
	Klebsiella species
	Proteus species
	Pseudomonas aeruginosa
	Enterobacter species
Cystitis	Gram-negative bacteria
	Escherichia coli
	Klebsiella species
	Proteus species
	Gram-positive bacteria
	Staphylococcus saprophyticus
	Enterococcus species
	Staphylococcus aureus
Urethritis	*Chlamydia trachomatis*
	Neisseria gonorrhoeae
	Ureaplasma urealyticum

ISBN: 978-81-950423-8-8

UTI CAUSING AETIOLOGICAL AGENTS:

Urinary tract infection is due to urogenital tract colonisation due to perineal and rectal flora translocation or hematogenous dissemination and/ or direct contamination by medical procedures or trauma. The most common causative agent documented for urinary tract infection is *Escherichia coli* in many countries for both community and hospital-acquired UTI (Gajdacs *et al*., 2019; Terlizzi *et al*., 2017; Floege *et al*., 2010). The prevalence of uropathogenic bacterial species (Table 1) in various UTI entities (Wgenlehner *et al*., 2020; Lo and Alonto, 2011) include *Escherichia coli*-76.7%, *Proteus mirabilus*-3.5%, *Klebsiella pneumonia*- 3.5%, *Staphylococcus saprophyticus*-3.6%, *Enterococcus faecalis*-4% and other-8.7% in uncomplicated cystitis (Figure 2), *Escherichia coli*-43%, *Pseudomonas aeruginosa*-9%, *Proteus spp*.-6%, *Citrobacter spp*.-1%, *Staphylococcus aureus*-3%, *Klebsiella spp*.-13%, *Enterobacter spp*.-7%, *Acinetobacter spp*.-2%, *Enterococcus spp*.-10%, CONS-2%, Other gram-positive bacteria-1%, Other bacteria-3% and fungi-1% in complicated cystitis (Figure 3) and *Escherichia coli*-45%, *Pseudomonas aeruginosa*-8%, *Proteus spp*.-6%, *Citrobacter spp*.-2%, *Staphylococcus aureus*-2%, *Klebsiella spp*.-13%, *Enterobacter spp*.-5%, *Acinetobacter spp*.-1%, *Enterococcus spp*.-10%, CONS-1%, Other bacteria-5% and fungi-3% in pyelonephritis (Figure 4).

CURRENT TREATMENT USED FOR TREATMENT OF UTI AND AMR:

Conventionally antibiotics like Timethoprim-Sulphamethoxazole (SXT) and nitrofurantoin (Gupta *et al*., 2011) is used as front-line drug for treatment of UTI but their effectiveness has decreased in certain areas due to increasing resistance. The North American Urinary Tract Infection Collaborative Alliance (NAUTICA), ARESC and SENTRY studies reported UPEC resistance rates of 5-6%, 8% and 11%, respectively (Zhanel *et al*., 2006; Schito *et al*., 2009). Fluoroquinolones (ciprofloxacin, norfloxacin and levofloxacin) are currently approved for use as second-line agents for uncomplicated UTIs, and front-line therapy for nosocomial UTIs and pyelonephritis

(Hooton *et al*., 2010; Gupta *et al*., 2011). Alarmingly, 25% of UPEC from catheter-associated UTIs are fluoroquinolone-resistant (Hidron *et al*., 2008). From studies published between 2009 and 2014, global antibiotic resistance to uropathogens is 10 to 80% to fluoroquinolones, 10 to 70% to third-generation cephalosporins, and 5 to 35% to carbapenems (Zowawi *et al*., 2015; Wagenlehner *et al*., 2020).

NOVEL BACTERIOPHAGE THERAPY IN THE ERA OF AMR:

Before the discovery and extensive use of antibiotics, phage therapy was discovered. Although early clinical studies with bacteriophages in the United States and Western Europe were not meticulously courted; but were more frequently practised in Eastern Europe and parts of the former Soviet Union (Myelnikov, 2018). The degree of antibiotic resistance has increased due to the inefficient, inappropriate and imprudent use of antibacterial agents and has become a bane for the human healthcare sector (Aslam *et al*., 2018). Under such circumstances, the world is eager to find a substitute for antibiotics, hence, an interest in phage therapy is rekindled using bacteriophages (natural bacterial predators) which can be used methodically with the help of modern biotechnology (Haq *et al*., 2012; Benam *et al*., 2019). Earlier, there was very little understanding of the biology of phages and their bacterial interaction (Clokie *et al*., 2011). Bacteriophages replicate in two types of cycles lytic (virulent phage) and lysogenic (temperate phages). In these two groups' bacteriophages first attaches and invades susceptible specific bacterium via specific bacterial receptors (Wojewodzic, 2020) (Figure: 5 A). In the first group, they exclusively infect host cells replicates inside its synthetic machinery and produces viral genome and proteins (Figure: 5 E). Ultimately assembly and packaging of phages occur along with cell lysis with the release of new progeny that would further infect other bacterial hosts (Young, 2013) (Figure: 5 F). In the second group, biochemical machinery is hijacked where viral genetic material is integrated

(Figure: 5 B) into the host genome, and with cells division, the chromosome of the virus is transmitted to the daughter cells (Principi *et al*., 2019) (Figure: 5 C).

Phage Therapy for UTI

The effectiveness of adjunctive bacteriophage therapy was reported by Khawaldeh *et al*., (2011) for urinary tract infection under the conditions of bilateral ureteric stents and bladder ulceration and repeated antibiotic failure. A Pyo-phage cocktail was used in the concentration of 10^6 PFU/ml, which resulted in no appearance bacteriophage-resistant bacteria, and the biochemistry of urine bacteriophage and bacteria suggested that it is self-sustaining and self-limiting. A randomized, placebo-controlled, double-blind trial investigation was reported where bacteriophages were employed to treat urinary tract infection. Patients planned for transurethral resection of the prostate were screened for positive urine culture and was administered with Pyo-bacteriophage cocktail via suprapubic catheters where normalization of urine (no evidence of bacteria) culture was obtained after 7 days of intravesical treatment (Leitner *et al*., 2017). A case of a 58-year-old patient who developed a recurrent urinary tract infection in the first-month post-transplant, ultimately resulting in epididymitis underwent phage therapy. It was successfully treated with antibiotics and bacteriophage cocktail taken both orally and intravesically via intermittent catheterization (Kuipers et al., 2020). Another research conducted by Valerio *et al*., (2017) aimed to assess the possible synergistic impact of phages and antibiotics on *Escherichia coli* to monitor urinary tract infections. Phage (200 µl at 10^9 PFU/ml) and antibiotic combinations resulted in high synergistic effects in bacteria's inactivation. Letkiewicz *et al*., (2009) documented another case in which three patients with chronic bacterial prostatitis were treated via phage therapy. Previously they were inefficaciously treated with long-term antibiotics, autovaccines, and laser biostimulation. The use of phage lysates (10^7to10^9 PFU/mL) targeted against prostatic fluid-cultured bacteria produced promising results for its eradication.

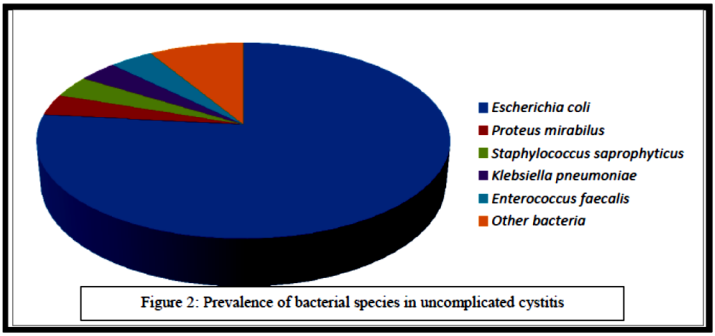


Figure 2: Prevalence of bacterial species in uncomplicated cystitis

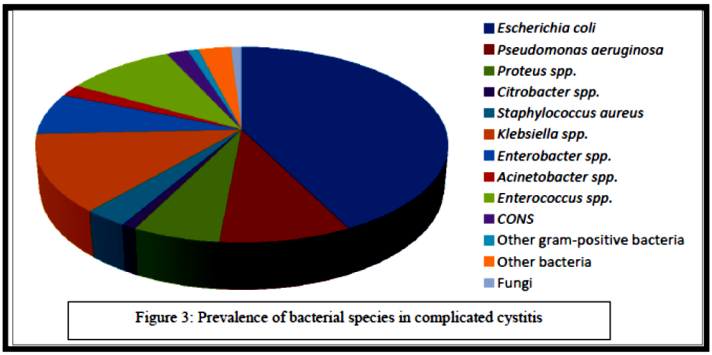


Figure 3: Prevalence of bacterial species in complicated cystitis

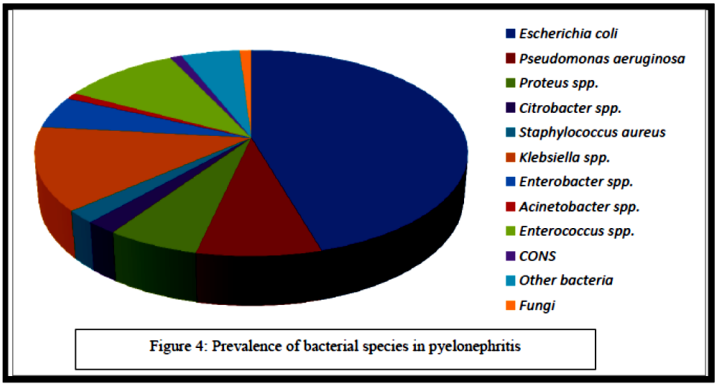


Figure 4: Prevalence of bacterial species in pyelonephritis

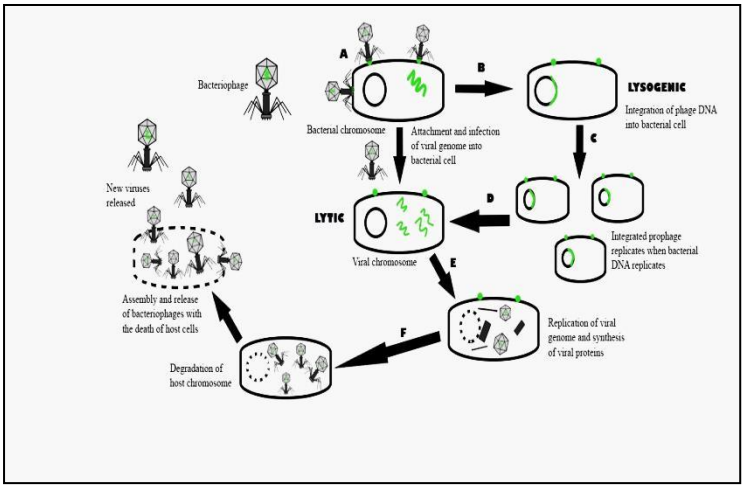


Figure 5. Lytic and Lysogenic life cycle of bacteriophages

Table 2. Phage therapy for Urinary Tract Infection

Organism	Phages used for UTI	References
Pseudomonas aeruginosa	Pyophage #051007	Khawaldeh *et al*., 2011
Escherichia coli *Proteus mirabilus* *Pseudomonas aeruginosa* *Enterococcus spp* *Staphylococcus spp*	Pyo bacteriophage	Leitner *et al.*, 2017
Klebsiella pneumoniae	Pyo bacteriophage	Kuipers *et al*., 2020
Escherichia coli	phage ECA2	Valerio *et al*., 2017
Enterococcus faecalis	Phage suspension	Letkiewicz *et al*., 2009
Staphylococcus aureus *Escherichia coli* *Enterococcus spp.* *Pseudomonas aeruginosa* *Proteus spp.*	Pyo bacteriophage	Ujmajuridze *et al*., 2018

Another research was designed to determine the feasibility, tolerability, efficacy and microbiological outcomes of phage therapy in transurethral prostate resection patients with urinary tract infection. After treatment via Pyo-bacteriophage bacteria, titre decreased, and no bacteriophage-associated adverse events were detected (Ujmajuridze *et al*., 2018). Hence, phages can also be employed for the treatment of infections caused by resistant uropathogens as mentioned in the table below (Table 2).

CONCLUSION:

Urinary tract infections are the most widespread globally and posing serious public health issues with increased morbidity and comorbidity in patients with underlying disorders. The main culprits of UTI are both Gram negative and positive bacteria and overuse and abuse of antibiotics have led to the evolution of resistance to a valuable class of medications among uropathogens. Diagnosis and management of upper and lower urinary tract infections has always been a concern for medical professionals because of their high prevalence, recurrence risk and insufficient care and the fact that antibiotic resistance has risen worldwide and requires proper antibiotic stewardship. Due to the theatrical boost and risky appearance of antibiotic-resistant bacteria on a worldwide platform, the current renaissance of using bacteriophages in phage therapy has strengthened and provides an alternative to antibiotics and an efficient management of escalating AMR in Urinary Tract Infections.

REFERENCES:

Abou Heidar, N. F., Degheili, J. A., Yacoubian, A. A., & Khauli, R. B. (2019). Management of urinary tract infection in women: A practical approach for everyday practice. *Urology Annals*, *11*(4), 339.

Akram, M., Shahid, M., & Khan, A. U. (2007). Etiology and antibiotic resistance patterns of community-acquired urinary

tract infections in JNMC Hospital Aligarh, India. *Annals of Clinical Microbiology and Antimicrobials*, *6*(1), 1-7.

Almalki, M. A., & Varghese, R. (2020). Prevalence of catheter associated biofilm producing bacteria and their antibiotic sensitivity pattern. *Journal of King Saud University-Science*, *32*(2), 1427-1433.

Angami, S., Jamir, N., Sarma, P. C., & Deka, A. C. (2015). Urinary tract infection, its causative microorganism and antibiotic susceptibility in Nagaland. *Archives of Medicine and Health Sciences*, *3*(1), 40.

Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., et al. (2018). Antibiotic resistance: a rundown of a global crisis. *Infection and Drug Resistance*, *11*, 1645-1658.

Benam, K. H., Gilchrist, S., Kleensang, A., Satz, A. B., Willett, C., & Zhang, Q. (2019). Exploring new technologies in biomedical research. *Drug Discovery Today*, *24*(6), 1242-1247.

Centres for Disease Control and Prevention (CDC). Urinary Tract Infections in the United States, 2015. CDC website. https://www.cdc.gov/antibiotic-use/community/for-patients/common-illnesses.

Clokie, M. R., Millard, A. D., Letarov, A. V., & Heaphy, S. (2011). Phages in nature. *Bacteriophage*, *1*(1), 31-45.

Floege, J., Johnson, R. J., & Feehally, J. (2010). *Comprehensive Clinical Nephrology E-Book*. Elsevier Health Sciences.

Foxman, B. (2002). Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *The American journal of medicine*, *113*(1), 5-13.

Foxman, B. (2013). Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. *Infectious disease clinics of North America*, *28*(1), 1-13.

Gajdacs, M., & Urban, E. (2019). Resistance trends and epidemiology of Citrobacter-enterobacter-Serratia in urinary tract infections of inpatients and outpatients (RECESUTI): a 10-year survey. *Medicina*, *55*(6), 285.

Gajdacs, M., Abrok, M., Lazar, A., & Burian, K. (2019). Comparative epidemiology and resistance trends of common

urinary pathogens in a tertiary-care hospital: a 10-year surveillance study. *Medicina*, *55*(7), 356.

Gunduz, S., & Altun, H. U. (2018). Antibiotic resistance patterns of urinary tract pathogens in Turkish children. *Global Health Research and Policy*, *3*(1), 1-5.

Gupta, K, Hooton, TM, Naber, KG, et al. (2011) International Clinical Practice Guidelines for the Treatment of Acute Uncomplicated Cystitis and Pyelonephritis in Women: A2010 Update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious diseases. *Clinical Infectious Diseases*. 52(5), 103-120.

Gupta, K., Sahm, D. F., Mayfield, D., & Stamm, W. E. (2001). Antimicrobial resistance among uropathogens that cause community-acquired urinary tract infections in women: a nationwide analysis. *Clinical infectious diseases*, *33*(1), 89-94.

Haq, I. U., Chaudhry, W. N., Akhtar, M. N., Andleeb, S., & Qadri, I. (2012). Bacteriophages and their implications on future biotechnology: a review. *Virology journal*, *9*(1), 1-8.

Hidron, A. I., J. R. Edwards, J. Patel, T. C. Horan, D. M. Sievert, D. A. Pollock & S. K. Fridkin, (2008). NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centres for Disease Control and Prevention, 2006-2007. *Infect Control Hosp Epidemiol* 29: 996-1011.

Hidron, A. I., J. R. Edwards, J. Patel, T. C. Horan, D. M. Sievert, D. A. Pollock & S. K. Fridkin, (2008). NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centres for Disease Control and Prevention, 2006-2007. *Infect Control Hosp Epidemiol* 29: 996-1011.

Hooton, T. M., Bradley, S. F., Cardenas, D.D., Colgan, R., Geerlings, S. E., Rice, J. C., et al. (2010). Diagnosis, prevention and treatment of catheter-associated urinary tract infection in adults; 2010 international clinical practice guidelines from the

Infectious Diseases Society of America. *Clin Infect Dis.*;50: 625–663.

Iacovelli, V., Gaziev, G., Topazio, L., Bove, P., Vespasiani, G., & Agro, E. F. (2014). Nosocomial urinary tract infections: A review. *Urologia Journal*, *81*(4), 222-227.

Kahlmeter, G., (2003) An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO.SENS Project. *J Antimicrob Chemother* 51: 69-76.

Kanj, S. S., & Kanafani, Z. A. (2011). Current Concepts in Antimicrobial Therapy Against Resistant Gram-Negative Organisms: Extended-Spectrum β-Lactamase–Producing Enterobacteriaceae, Carbapenem-Resistant Enterobacteriaceae, and Multidrug-Resistant Pseudomonas aeruginosa. In *Mayo Clin Proc*, 86(3), 250-259.

Khawaldeh, A., Morales, S., Dillon, B., Alavidze, Z., Ginn, A. N., Thomas, L., ... & Iredell, J. R. (2011). Bacteriophage therapy for refractory Pseudomonas aeruginosa urinary tract infection. *Journal of medical microbiology*, *60*(11), 1697-1700.

Kuipers, S., Ruth, M. M., Mientjes, M., de Sévaux, R. G., & van Ingen, J. (2019). A Dutch case report of successful treatment of chronic relapsing urinary tract infection with bacteriophages in a renal transplant patient. *Antimicrobial Agents and Chemotherapy*, *64*(1).

Leitner, L., Sybesma, W., Chanishvili, N., Goderdzishvili, M., Chkhotua, A., Ujmajuridze, A., et al. (2017). Bacteriophages for treating urinary tract infections in patients undergoing transurethral resection of the prostate: a randomized, placebo-controlled, double-blind clinical trial. *BMC urology*, 17(1), 90.

Letkiewicz, S., Miedzybrodzki, R., Fortuna, W., Weber-Dąbrowska, B., & Gorski, A. (2009). Eradication of Enterococcus faecalis by phage therapy in chronic bacterial prostatitis—case report. *Folia microbiologica*, *54*(5), 457-461

Lo, T. S., & Alonto, A. (2011). A Review of Uncomplicated Urinary Tract Infections. In *Urinary Tract Infections*. IntechOpen.

Moyo, S. J., Aboud, S., Kasubi, M., Lyamuya, E. F., & Maselle, S. Y. (2010). Antimicrobial resistance among producers and non-

producers of extended spectrum beta-lactamases in urinary isolates at a tertiary Hospital in Tanzania. *BMC research notes*, *3*(1), 348.

Muratani, T., & Matsumoto, T. (2006). Urinary tract infection caused by fluoroquinolone-and cephem-resistant Enterobacteriaceae. *International journal of antimicrobial agents*, 28, 10-13.

Myelnikov, D. (2018). An alternative cure: the adoption and survival of bacteriophage therapy in the USSR, 1922–1955. *Journal of the History of Medicine and Allied Sciences*, *73*(4), 385-411.

Narmada, R. V., Someshwaran, R., & Aravazhi, A. N. (2016). Prevalence of Drug Resistant Gram Negative Bacilli (DRGNB) in Patients Suffering from Symptomatic Urinary Tract Infection from a Tertiary Care Hospital in Coimbatore, India. *Int. J. Curr. Microbiol. App. Sci*, *5*(12), 95-105.

Odoki, M., Almustapha, A. A., Tibyangye, J., Nyabayo, M. J., Wampande, E., Drago, K. C., et al. (2019). Prevalence of Bacterial Urinary Tract Infections and Associated Factors among Patients Attending Hospitals in Bushenyi District, Uganda. *International journal of microbiology*, *2019*, 4246780.

Odongo, I., Ssemambo, R., & Kungu, J. M. (2020). Prevalence of Escherichia Coli and Its Antimicrobial Susceptibility Profiles among Patients with UTI at Mulago Hospital, Kampala, Uganda. *Interdisciplinary perspectives on infectious diseases*, *2020*, 8042540.

Petrosillo, N., Granata, G., Boyle, B., Doyle, M. M., Pinchera, B., & Taglietti, F. (2020). Preventing sepsis development in complicated urinary tract infections. *Expert Review of Anti-infective Therapy*, *18*(1), 47-61.

Principi, N., Silvestri, E., & Esposito, S. (2019). Advantages and limitations of bacteriophages for the treatment of bacterial infections. *Frontiers in pharmacology*, *10*, 513.

Sabih A and Leslie SW. Complicated Urinary Tract Infections. (2020). In: StatPearls [Internet]. Treasure Island (FL):

StatPearls Publishing; 2020 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK436013/

Schappert, S. M., & Rechtsteiner, E. A. (2011). Ambulatory medical care utilisation estimates for 2007. *Vital and Health Statistics. Series 13, Data from the National Health Survey*, (169), 1-38.

Schito, G. C., K. G. Naber, H. Botto, J. Palou, T. Mazzei, L. Gualco & A. Marchese, (2009). The ARESC study: an international survey on the antimicrobial resistance of pathogens involved in uncomplicated urinary tract infections. *Int J Antimicrob Agents* 34: 407-413.

Sharma, I., & Paul, D. (2012). Prevalence of community acquired urinary tract infections in silchar medical college, Assam, India and its antimicrobial susceptibility profile. *Indian journal of medical sciences*, *66*(11/12), 273-9.

Stamm, W. E., & Norrby, S. R. (2001). Urinary tract infections: disease panorama and challenges. *The Journal of infectious diseases*, *183*(Supplement_1), S1-S4.

Tan, C. W., & Chlebicki, M. P. (2016). Urinary tract infections in adults. *Singapore medical journal*, *57*(9), 485.

Tandogdu, Z., Cek, M., Wagenlehner, F., Naber, K., Tenke, P., van Ostrum, E., & Johansen, T. B. (2014). Resistance patterns of nosocomial urinary tract infections in urology departments: 8-year results of the global prevalence of infections in urology study. *World journal of urology*, *32*(3), 791-801.

Terlizzi, M. E., Gribaudo, G., & Maffei, M. E. (2017). UroPathogenic Escherichia coli (UPEC) infections: virulence factors, bladder responses, antibiotic, and non-antibiotic antimicrobial strategies. *Frontiers in microbiology*, *8*, 1566.

Ujmajuridze, A., Chanishvili, N., Goderdzishvili, M., Leitner, L., Mehnert, U., Chkhotua, A., et al. (2018). Adapted bacteriophages for treating urinary tract infections. *Frontiers in microbiology*, *9*, 1832.

Valerio, N., Oliveira, C., Jesus, V., Branco, T., Pereira, C., Moreirinha, C., & Almeida, A. (2017). Effects of single and combined use of bacteriophages and antibiotics to inactivate Escherichia coli. *Virus research*, *240*, 8-17.

Wagenlehner, F. M., Johansen, T. E. B., Cai, T., Koves, B., Kranz, J., Pilatz, A., & Tandogdu, Z. (2020). Epidemiology, definition and treatment of complicated urinary tract infections. *Nature Reviews Urology*, *17*(10), 586-600.

Wojewodzic, M. W. (2020). Bacteriophages could be a potential game changer in the trajectory of coronavirus disease (COVID-19). *PHAGE*, *1*(2), 60-65.

Yamamoto, S., Higuchi, Y. and Nojima, M. (2010) 'Current therapy of acute uncomplicated cystitis', *Int J Urol*, 17(5), 450-6.

Young, R. (2013). Phage lysis: do we have the hole story yet?. *Current opinion in microbiology*, *16*(6), 790-797.

Zhanel, G. G., T. L. Hisanaga, N. M. Laing, M. R. DeCorby, K. A. Nichol, B. Weshnoweski, J. et al. (2006) Antibiotic resistance in Escherichia coli outpatient urinary isolates: final results from the North American Urinary Tract Infection Collaborative Alliance (NAUTICA). *Int JAntimicrob Agents* 27: 468-475.

Zowawi, H. M., Harris, P. N., Roberts, M. J., Tambyah, P. A., Schembri, M. A., Pezzani, M. D., et al. (2015). The emerging threat of multidrug-resistant Gram-negative bacteria in urology. *Nature Reviews Urology*, *12*(10), 570-584.

MICROBIOME: A GENERATION OF MODERN MEDICINE

Rasanpreet Kaur[1], Parul Yadav[2]and Jagdip Singh Sohal[3*]

[1]Student, Amity Center for Mycobacterial Disease Research, Amity Institute of Microbial Technology, Amity University Rajasthan
[2]Assistant Professor, AUSIC, Amity University Rajasthan
[3]Assistant Professor, Amity Center for Mycobacterial Disease Research, Amity Institute of Microbial Technology, Amity University Rajasthan

[*]Corresponding Author: jssohal@jpr,amity.edu

INTRODUCTION

Since the dawn of the human microbiome project in 2007, there has been an immense increase in our understanding of the role of the microbiome in health and disease (Turnbaugh et al., 2007). Multicellular living beings such as humans are colonized differentially with microbes (bacteria, viruses and fungal organisms) in different ecological niches of the body. Humans maintain a commensal microbial assemblage; however, quantitative assessment of this microbial ecosystem has varied. As early as the 1970s, it was established that the number of microbial cells is far higher than human cells, with an estimated ratio of 10 microbial cells to each human cell (Luckey, 1972). It was not until 2016 that a more detailed quantification was performed, which dispelled the prior estimate, demonstrating that microbial cells and human cells actually exist in an almost 1:1 ratio, with variance between individuals (Sender et al., 2016). Irrespective of quantified ratios, the microbiome is now recognized as a significant contributor to human metabolism and physiology. This is likely due to the estimated 1:100 ratio of human to microbial genetic potential with millions of microbial genes outnumbering ~ 20,000 human genes (Turnbaugh et al., 2007). Importantly, it is the functional potential of the microbiome, encoded by these genes, that likely mediates the microbiome host drug response. It

is this association that we are only just beginning to qualify and leverage to improve medical practice.

Microbiome therapies have opened opportunities and perspectives that are clearly aligned with the directions of sustainable development. In addition, these new tools are providing a different approach for professionals such as researchers, medical doctors, clinicians and pharmaceutical experts alike. Interestingly, this angle has the potential to change the mindset of workers in the field as well as anyone involved in high-level professional development and education. Microbiome therapy bear high robustness towards interpersonal variability, have the ability to remain stable and dominant in the body environment, maintain their function in presence of native enzymes and have the potential to be used for screening of the patients based on the severity of the disease. The advantages of producing such therapeutics *in situ* include targeted drug delivery, low dose administration of the therapeutic consequently reducing the side effects, non-invasive administration, production of multiple therapeutics by the same cell upon modification of the carrier system. Moreover, the *in-situ* production is cost-effective (Mimee et al., 2016). Extensive research on the microbiome has established its application in variety of diseases and has been proved to be a predictive tool for the disease outcome including cardiovascular disease, *Clostridium difficile* infection, metabolic disorders and colorectal cancer (Vázquez-Baeza et al., 2018).

The holistic vision of single human beings being the cradle of complex communities of microorganisms is in line with the rationale of sustainability: we ought to consider humans in the same way as the planet, i.e. as hosts of delicate balance and intricate interdependence. In turn, we as human individuals depend on the proper maintenance of the 'microbiome interactome' for good health and general well-being (O'Toole & Paoli, 2017).

BENEFICIAL ASSOCIATIONS BETWEEN THE HUMAN MICROBIOME AND THERAPEUTIC AGENTS

The gut microbiota alters the chemical structure of ingested compounds, such as pharmaceutical drugs, via microbial enzymes (Chankhamjon et

al., 2019). Several examples demonstrate the potential of pharmacomicrobiomics to regulate differential drug response to enhance beneficial effects. This process of metabolic breakdown takes place in the gastrointestinal tract before drug metabolites are transferred into the liver and systemic circulation. The first evidence of a microbial role in drug metabolism was established in 1937, in which the antibacterial drug, Prontosil, which contains an azo group, was found to be activated by the bacterial enzyme, azoreductase, which removes the azo group to produce the antibacterial sulphanilamide compound required for its antimicrobial activity (Fuller, 1937).

Microbial metabolism of digoxin, a cardiovascular drug used to treat heart failure and heart rhythms, has also been linked with deactivation and reduction of efficacy of the drug. The bacterium *Eggerthella lenta* carries a cardiac glycoside reductase operon that metabolizes digoxin into inactive metabolites, making the drug ineffective (Haiser et al., 2013). Further research into this drug's metabolism led to the discovery of small pockets in the cardiac glycoside reductase operon, which is bound by digoxin (Saha et al., 1983). This opened up new opportunities for designing compounds that can bind to these pockets with higher affinity than digoxin, so that digoxin's inactivity can be prevented (Kumar et al., 2018). Similarly, other anaerobic bacteria, including *Bacteroides*, also decrease the activity of the drug omeprazole (drug used to treat stomach and esophagus-related problems, such as acid-reflux and ulcers), metabolizing it into sulfide metabolites (Watanabe et al., 1995). Host genetics also influence the gut microbiome, thus actively impacting the interaction between microbial enzymes and drug metabolism (Nichols et al., 2019). One great example is of genetic isoforms of the host-cytochrome P540 enzymes, which are known to interact with Camptothecin-11 (CPT-11) in cancer treatment (Hanioka et al., 2002). These examples highlight the role of different isoforms in regulating differential host drug metabolism and drug response.

Pharmacomicrobiomics also provide hope for alleviating life-threatening toxicities without impacting efficacy during cancer treatments (Patel & Kaufmann, 2010). Cancer is a leading cause of mortality, and despite the many medical breakthroughs, heterogeneous responses toward chemotherapy still remain a challenge in cancer treatment. Recently,

Alexender et al. proposed a TIMER framework, which covers the microbial metabolism of chemotherapeutic agents in five major processes (i.e., translocation, immunomodulation, metabolism, enzymatic degradation, and reduced diversity and ecological variation) (Alexander et al., 2017). The classic example is of the widely studied enzymatic degradation of CPT-11, which is an anticancer chemotherapy drug, also known as irinotecan (Sparreboom et al., 1998). Treatment with CPT-11 is associated with severe diarrhea, which is thought to be mediated by the bacterial enzyme beta-glucuronidase. Because the exact metabolic pathway for CPT-11 metabolism is known, researchers have developed inhibitors of beta-glucuronidases and have demonstrated in mice that these inhibitors are highly effective against the target enzyme without harming the commensal microbiota and mammalian cells (Wallace et al., 2010). It has been proposed that probiotics, prebiotics, synbiotics, postbiotics, or antibiotics could be administered as adjuvant therapy for a balanced gut microbiota, which could, in turn, increase the efficacy of the treatment (Zitvogel et al., 2015). Work by Viaud et al. have supported this by demonstrating that certain gram-positive species, including *Lactobacillus johnsonii*, *Lactobacillus murinus*, and *Enterococcus hirae*, increase the efficacy of cyclophosphamide treatment (Viaud et al., 2013). Similarly, in rats, the use of probiotic mixture VSL#3 (i.e., *Streptococcus thermophilus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei, and Lactobacillus delbrueckii subsp. bulgaricus*) can also alleviate diarrhea and weight loss; side effects of CPT-11 treatment (Bowen et al., 2007). *Lactobacillus brevis* CD2 can also mitigate oral mucositis in patients receiving the chemotherapy for head and neck cancer (Sharma et al., 2012) and Akkermansia muciniphila can increase the efficacy of anti-PD1 therapy in antibiotic-treated mice (Routy et al., 2018). Thus, understanding the interplay between cancer therapies and microbiota can promote discovery of new strategies and targets for cancer management.

FECAL MICROBIOTA TRANSPLANTS AS A THERAPY

Fecal microbiota transplantation (FMT) is an emerging therapy that has now expanded beyond the original, well-established treatment for recurrent *Clostridioides difficle* infection (rCDI). The rCDI is a well-

known issue arising from repeated, long-term antibiotic use. In a recent study, FMT from healthy donors eradicated rCDI and significantly reduced antimicrobial resistance genes in the fecal microbiota. Furthermore, the patients with rCDI in the study were dominated by proteobacteria, such as *Escherichia coli* and *Klebsiella*, but after FMT treatment, the proportion of proteobacteria was reduced and the *Bacteroidetes and Firmicutes* were increased. Such studies suggest that FMT is a promising therapy for the management of disorders associated with gut dysbiosis (Aldrich et al., 2019).

FMT has had variable success for inflammatory bowel disease (IBD) treatment. However, in a study of 122 patients with IBD (79 with ulcerative colitis, 39 with Crohn's disease, and 4 with IBD unclassified), younger patients with ulcerative colitis and patients with Crohn's disease had a remission rate of 64.1% and 60.6%, respectively, post-FMT (Colman et al., 2014). Interestingly, although much of the FMT literature has focused on the restorative element of bacteria, a recent successful resolution of *Clostridioides difficile* infection using sterile fecal filtrates suggests that nonbacterial elements and metabolites might play a more significant role than hitherto recognized. In a preliminary case series, five patients with rCDI were administered a stool solution that had been filtered to remove small particles and bacteria. The fecal filtrate still contained bacterial debris, proteins, DNA, antimicrobial compounds, metabolites and viruses. Notably, a few days post-transfer, all five patients had achieved rCDI resolution and remained symptom-free for the duration of the study (up to 6 months) (Ott et al., 2017).

The success rate and efficiency of FMT depends on the microbial diversity of the donor and has led to the concept of a "super donor." Because each individual's gut microbiota is unique, it is challenging to screen super donors to define a "healthy" microbiome. In general, high microbial diversity is associated with a healthy gut, but donors need to be screened for potential transmissible pathogens or diseases. Several studies have been conducted to identify desirable characteristics of a super donor (Vrieze et al., 2012). A consistent pattern is that the donor's microbial diversity relates to the therapeutic success of FMT, with the stool transplanted from donors with high microbial diversity resulting in better clinical response.

FMT response depends on the immune system and genetic make-up of the donor as well as the recipient, thus immune screening becomes critical prior to FMT. Approximately 5–10% of the variability in microbial composition of an individual can be explained by host genetics, and a portion of the microbiota demonstrates heritability. This heritable microbiome is mostly associated with host genes involved in innate immunity, with a bidirectional relationship (Secombe et al., 2019). The microbiome contributes to maintenance of the immune system, and the immune system, in turn, regulates the microbial composition via pro-inflammatory and anti-inflammatory pathways (Belkaid & Hand, 2014). The FDA has recently issued a warning on investigational FMT regarding the risk of transmission of multidrug-resistant organisms between the donor and the recipient. Although FMT is proving to be a new frontier in many disease models, it should be used with caution because there are unpredictable effects, for instance, fevers, chills, vomiting, diarrhea, etc. In addition to long-term adverse effects of FMT, infectious disease transfer, or acquisition of chronic disease (e.g., obesity, diabetes, atherosclerosis, etc.) is also of concern (Gupta et al., 2016) Therefore, studies with long-term follow-up are necessary to understand these risks.

BIOLOGICAL THERAPEUTICS AND PHAGEOME MEDIATED THERAPEUTIC MODULATION OF HOST-MICROBIOME

Diet can have a swift and lasting impact on the proportion and composition of bacteria present in the microbiome (Turnbaugh et al., 2006) and, therefore, dietary interventions have been investigated as a potential strategy to confer health. Dietary interventions are part of a strategy known as “biological therapeutics,” which includes prebiotics, probiotics, and postbiotics, in which dietary interventions, live biologicals, and small molecules, respectively, can be used to mediate health. Prior to microbiome studies, decades of food and nutritional research have investigated the dietary components that are beneficial or detrimental to human health. Nutritional science has been striving for a series of universal dietary guidelines to confer good health and metabolism to the wider population. Dietary interventions were often made based on studies that considered nutrient intake and the corresponding clinical outcomes. However, findings have varied widely.

With recent advancements in microbiome research, the human gut microbiome has provided an additional dimension in our understanding of nutritional intervention. Research now suggests that gut microbiota may extract energy at different levels from carbon substrates, which can result in differential host adiposity (Turnbaugh et al., 2009).

The impact of dietary modifications on the microbiome can be extremely rapid, for example, when a Western population eating a standard high-fat, high-sugar; western diet consumed a high-fiber diet for 2 weeks, they saw a significant increase in the production of the short chain fatty acid, butyrate, which is a product of microbial fermentation of fiber (O'Keefe et al., 2015). Fiber is a prebiotic that confers health benefits to the host following metabolic conversion by the host microbiome and has the potential to remediate many diseases that are now prevalent in developed societies, including immune and metabolic disorders. Dietary fiber intake can also support improved glycemic control in patients with type-2 diabetes in comparison with standard therapy (Zhao et al., 2018). The type of microbial community that results from fiber consumption can be substantially altered by the molecular structure of the fiber, the enzymatic potential of microorganisms in the community to degrade and utilize them, and their ability to tolerate the fermentation-mediated changes in the environmental conditions (such as low pH resulting from fermentation's acidic products). However, the selective growth of these species is continued as long as they are fed with favorable substrates (Bindels et al., 2015).

Probiotics, defined as live microbiological organisms that confer a health benefit when consumed in sufficient quantities, are another biological intervention that has been studied to determine the potential for mucosal colonization, interactions with the indigenous microbiome, and impact on the host. Although probiotic therapies have been practiced for more than a century, the benefit of consuming live microorganisms is still being debated, especially as establishment and effect on the native community is variable (Walter et al., 2018). However, probiotic consumption for the treatment of specific disease states and pathologies suggest that probiotics can have a statistically significant impact on disease outcomes and clinical phenotypes

(Veronese et al, 2018). Furthermore, despite the lack of evidence on probiotic efficacy, probiotic treatment consistently restores the gut microbiota post-antibiotic-induced dysbiosis, although reconstitution of the indigenous microbiome may be delayed (Suez et al., 2018). Similar to other microbial treatment strategies, unification of multiple human studies suggests inter-individual differences in response to probiotic therapies, such as resistance or permissiveness to probiotic gut mucosal colonization, may be driven by differences in the individual's microbiome.

Microbiome-based therapies, such as prebiotics and probiotics, are often aimed at proliferating specific microbial species that are known to produce beneficial metabolites (e.g., butyrate). Instead of enriching or depleting such bacterial producers, another strategy that has been developed recently is the use of postbiotics (i.e., supplements of bacteria-derived metabolites either to restore the depleted microbial pool or inhibition of specific metabolites). Experimental outcomes support these modes of action. For example, exogenous administration of short chain fatty acids improves inflammatory conditions in colitis-mouse models (Maslowski et al., 2009); similarly, supplementation of depleted flavonoids alleviates weight regain following successful dietary weight loss in animal models with recurrent obesity (Thaiss et al., 2016). Additionally, inhibitors of the microbial enzymes producing the proatherogenic metabolite, trimethylamine N-oxide from L-carnitine, reduce atherothrombotic incidents, such as stroke and myocardial infarction (Koeth et al, 2013). The evidence of success for postbiotic metabolite therapy is derived mostly from animal models; therefore, future clinical research in humans is essential to determine the intended and collateral effects and safety of such compounds.

The phageome, or the human gut virome, has emerged as an essential component of the gut microbiome and has the potential to direct bacterial behaviors of a complex community. In the last decade, advancements from viral metagenomics (i.e., deep sequencing of phage DNA present in our gut microbiome), has shed some light on its importance in human gut microbial homeostasis. For instance, screening for crAssphage (cross-assembly phage) in metagenomes from stool samples of many different donors revealed that this one phage comprises

90% of the human-associated gut virome (Yutin et al., 2018). The presence of phages in sterile fecal filtrates may have been a driver behind the successful remediation of rCDI, especially as successful treatment also associated with a significant shift in the gut virome of subjects. The gut phageome is gaining therapeutic importance due to adverse drug response or ever-increasing antibiotic resistance. Evidence suggesting their beneficial roles in overall microbiome composition and progression of gut-related disorders have been studied in cases, such as malnutrition, AIDS, and IBD (Monaco et al., 2016). The importance of considering bacteriophages during FMT and its impact on the recipient microbiome evaluating the microbial dynamics on a long-term scale highlights their role in the microbiome. However, future research investigating the structure and function of the phageome could improve our understanding of their potential application in phage-based therapeutics (Sharma et al., 2020).

An integrated approach to microbiome-based therapeutics

This approach combines lessons from probiotics, prebiotics, FMT and metabolites. While probiotics have the advantage over FMT of being comprised only of a selected highly defined number of strains, they may act differently, if at all, in each individual. As such, probiotics may only act efficiently when tailored to the individual's microbiome composition. Adding a combination of niche stabilizing metabolites to such individualized probiotic treatment may overcome colonization resistance to the newly introduced strains and enhance their long-term efficacy. Even when personally tailored, an additional important challenge to probiotics therapy is the long-term preservation of the newly introduced microorganisms, should they be featured to be of clinical importance. To this aim, dietary modifications should be considered in parallel to microbiome-based therapies as means of providing continuous support to the newly introduced or modulated microbial strains. Here too, inter-individual variability in response to dietary supplementation largely depends on person specific microbiome composition with the response to virtually any food ingredients being highly variable and affected by the microbiome (Suez & Elinav, 2017). In this scenario, also, metabolites can serve as a complimentary fast-

acting 'bridging' therapy that supports microbial stabilization while the effects of prebiotics are generated and assessed.

CONCLUDING REMARKS AND FUTURE PROSPECTS

Microbiome therapies bring about a novel approach to treatment and to the way in which we have conceived the human body. The new philosophy of our body as a collection of populations hosted by each one of us in a functional equilibrium establishes a holistic vision with repercussions on health as well as education. A strong principle distilled out of the microbiome research findings over the last decade is that of balance. All these concepts are also key pillars of the ideology of sustainability put forward by the United Nations' new 2030 Agenda (O'Toole & Paoli, 2017). It is, in fact, not possible to work towards sustainable development without embracing the need for equilibrium and balance, which are essential to the environment and Earth's resources, and also extremely relevant, as shown by microbiome research, to the functioning of the body.

The field of pharmacomicrobiomics has opened a new avenue for alleviating the effects of drugs and enhancing therapeutic benefits. The premise of pharmacomicrobiomics relies on the ability of the microbiome to metabolize the drugs and modulate the drug response. Hence, comprehensive understanding of microbiome-based pharmacokinetics and pharmacodynamics should be considered to translate microbiome therapy into the clinic. To achieve this, a combination of reductionist approaches and system-level investigations could be applied. Advanced biocomputational approaches, such as machine learning and system-level modeling, could be valuable in the validation of multidimensional -omics data for therapeutics. Using these best practices in relevant model systems with sufficient evidence on root-cause analyses and robustness could be a pragmatic way to increase our pharmacobiological understanding and to develop successful microbiome therapies, which would change the future of our healthcare industry.

REFERENCES

Aldrich, A. M., Argo, T., Koehler, T. J., & Olivero, R. (2019). Analysis of treatment outcomes for recurrent Clostridium difficile infections and fecal microbiota transplantation in a pediatric hospital. *The Pediatric Infectious Disease Journal*, *38*(1), 32-36.

Alexander, J. L., Wilson, I. D., Teare, J., Marchesi, J. R., Nicholson, J. K., & Kinross, J. M. (2017). Gut microbiota modulation of chemotherapy efficacy and toxicity. *Nature Reviews Gastroenterology & Hepatology*, *14*(6), 356-365.

Belkaid, Y., & Hand, T. W. (2014). Role of the microbiota in immunity and inflammation. *Cell*, *157*(1), 121-141.

Bindels, L. B., Delzenne, N. M., Cani, P. D., & Walter, J. (2015). Towards a more comprehensive concept for prebiotics. *Nature reviews Gastroenterology & hepatology*, *12*(5), 303-310.

Bowen, J. M., Stringer, A. M., Gibson, R. J., Yeoh, A. S., Hannam, S., & Keefe, D. M. (2007). VSL# 3 probiotic treatment reduces chemotherapy-induced diarrhoea and weight loss. *Cancer biology & therapy*, *6*(9), 1445-1450.

Butler, V. P., Neu, H. C., & Lindenbaum, J. (1983). Digoxin-inactivating bacteria: identification in human gut flora. *Science*, *220*(4594), 325-327.

Chankhamjon, P., Javdan, B., Lopez, J., Hull, R., Chatterjee, S., & Donia, M. S. (2019). Systematic mapping of drug metabolism by the human gut microbiome. *BioRxiv*.

Colman, R. J., & Rubin, D. T. (2014). Fecal microbiota transplantation as therapy for inflammatory bowel disease: a systematic review and meta-analysis. *Journal of Crohn's and Colitis*, *8*(12), 1569-1581.

Fuller, A. T. (1937). Is p-aminobenzenesulphonamide the active agent in prontosil therapy? *The Lancet*, *229*(5917), 194-198.

Gupta, S., Allen-Vercoe, E., & Petrof, E. O. (2016). Fecal microbiota transplantation: in perspective. *Therapeutic advances in gastroenterology*, *9*(2), 229-239.

Haiser, H. J., Gootenberg, D. B., Chatman, K., Sirasani, G., Balskus, E. P., & Turnbaugh, P. J. (2013). Predicting and manipulating cardiac drug inactivation by the human gut bacterium Eggerthella lenta. *Science*, *341*(6143), 295-298.

Hanioka, N., Ozawa, S., Jinno, H., Tanaka-Kagawa, T., Nishimura, T., Ando, M., & Sawada, J. I. (2002). Interaction of irinotecan (CPT-11) and its active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38) with human cytochrome P450 enzymes. *Drug metabolism and disposition*, *30*(4), 391-396.

Koeth, R. A., Wang, Z., Levison, B. S., Buffa, J. A., Org, E., Sheehy, B. T., ... & Smith, J. D. (2013). Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nature medicine*, *19*(5), 576-585.

Kumar, K., Jaiswal, S. K., Dhoke, G. V., Srivastava, G. N., Sharma, A. K., & Sharma, V. K. (2018). Mechanistic and structural insight into promiscuity-based metabolism of cardiac drug digoxin by gut microbial enzyme. *Journal of cellular biochemistry*, *119*(7), 5287-5296.

Luckey, T. D. (1972). Introduction to intestinal microecology. *The American Journal of Clinical Nutrition*, *25*(12), 1292-1294.

Maslowski, K. M., Vieira, A. T., Ng, A., Kranich, J., Sierro, F., Yu, D., ... & Xavier, R. J. (2009). Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature*, *461*(7268), 1282-1286.

Millan, B., Park, H., Hotte, N., Mathieu, O., Burguiere, P., Tompkins, T. A., ... & Madsen, K. L. (2016). Fecal microbial transplants reduce antibiotic-resistant genes in patients with recurrent Clostridium difficile infection. *Clinical Infectious Diseases*, *62*(12), 1479-1486.

Mimee, M., Citorik, R. J., & Lu, T. K. (2016). Microbiome therapeutics—advances and challenges. *Advanced drug delivery reviews*, *105*, 44-54.

Monaco, C. L., Gootenberg, D. B., Zhao, G., Handley, S. A., Ghebremichael, M. S., Lim, E. S., ... & Norman, J. M. (2016). Altered virome and bacterial microbiome in human immunodeficiency virus-associated acquired immunodeficiency syndrome. *Cell host & microbe*, *19*(3), 311-322.

Nichols, R. G., Peters, J. M., & Patterson, A. D. (2019). Interplay between the host, the human microbiome, and drug metabolism. *Human genomics*, *13*(1), 1-10.

O'Keefe, S. J., Li, J. V., Lahti, L., Ou, J., Carbonero, F., Mohammed, K., ... & Vipperla, K. (2015). Fat, fibre and cancer risk in African Americans and rural Africans. *Nature communications*, *6*(1), 1-14.

O'Toole, P. W., & Paoli, M. (2017). The contribution of microbial biotechnology to sustainable development goals: microbiome therapies. *Microbial biotechnology*, *10*(5), 1066-1069.

Ott, S. J., Waetzig, G. H., Rehman, A., Moltzau-Anderson, J., Bharti, R., Grasis, J. A., ... & Rosenstiel, P. (2017). Efficacy of sterile fecal filtrate transfer for treating patients with Clostridium difficile infection. *Gastroenterology*, *152*(4), 799-811.

Patel, A. G., & Kaufmann, S. H. (2010). Targeting bacteria to improve cancer therapy. *Science*, *330*(6005), 766-767.

Routy, B., Le Chatelier, E., Derosa, L., Duong, C. P., Alou, M. T., Daillère, R.,... & Fidelle, M. (2018). Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. *Science*, *359*(6371), 91-97.

Secombe, K. R., Coller, J. K., Gibson, R. J., Wardill, H. R., & Bowen, J. M. (2019). The bidirectional interaction of the gut

microbiome and the innate immune system: Implications for chemotherapy-induced gastrointestinal toxicity. *International journal of cancer*, *144*(10), 2365-2376.

Sender, R., Fuchs, S., & Milo, R. (2016). Revised estimates for the number of human and bacteria cells in the body. *PLoS biology*, *14*(8), e1002533.

Sharma, A., Das, P., Buschmann, M., & Gilbert, J. A. (2020). The future of microbiome-based therapeutics in clinical applications. *Clinical Pharmacology & Therapeutics*, *107*(1), 123-128.

Sharma, A., Rath, G. K., Chaudhary, S. P., Thakar, A., Mohanti, B. K., & Bahadur, S. (2012). Lactobacillus brevis CD2 lozenges reduce radiation-and chemotherapy-induced mucositis in patients with head and neck cancer: a randomized double-blind placebo-controlled study. *European journal of cancer*, *48*(6), 875-881.

Sparreboom, A., De Jonge, M. J., de Bruijn, P., Brouwer, E., Nooter, K., Loos, W. J., ... & Verweij, J. (1998). Irinotecan (CPT-11) metabolism and disposition in cancer patients. *Clinical cancer research*, *4*(11), 2747-2754.

Suez, J., Zmora, N., Zilberman-Schapira, G., Mor, U., Dori-Bachash, M., Bashiardes, S., ... & Horn, M. (2018). Post-antibiotic gut mucosal microbiome reconstitution is impaired by probiotics and improved by autologous FMT. *Cell*, *174*(6), 1406-1423.

Suez, J., & Elinav, E. (2017). The path towards microbiome-based metabolite treatment. *Nature microbiology*, *2*(6), 1-5.

Thaiss, C. A., Itav, S., Rothschild, D., Meijer, M. T., Levy, M., Moresi, C.,... & Dori-Bachash, M. (2016). Persistent microbiome alterations modulate the rate of post-dieting weight regain. *Nature*, *540*(7634), 544-551.

Turnbaugh, P. J., Ridaura, V. K., Faith, J. J., Rey, F. E., Knight, R., & Gordon, J. I. (2009). The effect of diet on the human gut

microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Science translational medicine*, *1*(6), 6ra14-6ra14.

Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., & Gordon, J. I. (2007). The human microbiome project. *Nature*, *449*(7164), 804-810.

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *nature*, *444*(7122), 1027-1031.

Vázquez-Baeza, Y., Callewaert, C., Debelius, J., Hyde, E., Marotz, C., Morton, J. T., ... & Knight, R. (2018). Impacts of the human gut microbiome on therapeutics. *Annual review of pharmacology and toxicology*, *58*, 253-270.

Veronese, N., Solmi, M., Caruso, M. G., Giannelli, G., Osella, A. R., Evangelou, E., ... & Tzoulaki, I. (2018). Dietary fiber and health outcomes: an umbrella review of systematic reviews and meta-analyses. *The American journal of clinical nutrition*, *107*(3), 436-444.

Viaud, S., Saccheri, F., Mignot, G., Yamazaki, T., Daillère, R., Hannani, D.,... & Schlitzer, A. (2013). The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *science*, *342*(6161), 971-976.

Vrieze, A., Van Nood, E., Holleman, F., Salojärvi, J., Kootte, R. S., Bartelsman, J. F., ... & Derrien, M. (2012). Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology*, *143*(4), 913-916.

Wallace, B. D., Wang, H., Lane, K. T., Scott, J. E., Orans, J., Koo, J. S., ... & Redinbo, M. R. (2010). Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. *Science*, *330*(6005), 831-835.

Walter, J., Maldonado-Gómez, M. X., & Martínez, I. (2018). To engraft or not to engraft: an ecological framework for gut microbiome modulation with live microbes. *Current opinion in biotechnology*, *49*, 129-139.

Watanabe, K., Yamashita, S., Furuno, K., Kawasaki, H., & Gomita, Y. (1995). Metabolism of omeprazole by gut flora in rats. *Journal of pharmaceutical sciences*, *84*(4), 516-517.

Yutin, N., Makarova, K. S., Gussow, A. B., Krupovic, M., Segall, A., Edwards, R. A., & Koonin, E. V. (2018). Discovery of an expansive bacteriophage family that includes the most abundant viruses from the human gut. *Nature microbiology*, *3*(1), 38-46.

Zhao, L., Zhang, F., Ding, X., Wu, G., Lam, Y. Y., Wang, X., ... & Yu, L. (2018). Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. *Science*, *359*(6380), 1151-1156.

Zitvogel, L., Galluzzi, L., Viaud, S., Vétizou, M., Daillère, R., Merad, M., & Kroemer, G. (2015). Cancer and the gut microbiota: an unexpected link. *Science translational medicine*, *7*(271), 271ps1-271ps1.

AUGMENTED NEUTROPHIL COUNT IS ASSOCIATED WITH SEVERE CORONAVIRUS DISEASE 2019 (COVID-19): AN UPDATED META-ANALYSIS

Deepanshu Sharma[1], Sanket Kaushik[2], Vijay K. Srivastava[2], Anupam Jyoti[2]*

[1]Student, Amity Institute of Biotechnology, Amity University Rajasthan

[2]Assistant Professor, Amity Institute of Biotechnology, Amity University Rajasthan, Kant Kalwar, NH-11C, Jaipur-Delhi Highway, Jaipur, India

*Corresponding Author: ajyoti@jpr.amity.edu

Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) caused an outbreak of coronavirus disease 2019 (COVID-19) in late December 2019 in Wuhan, Hubei Province, China. Till 20th May 2020, 47,89,205 cases have been detected with mortality of 2,54,045 were reported across worldwide which is increasing exponentially. The pathophysiology of SARS-CoV-2 include fever, cough, dyspnea and pneumonia and in severe case led to acute respiratory distress syndrome (ARDS) and multi-organ dysfunction syndrome (MODS) which results in mortality. Proper patient care and decision making for them to be severe is hampering due to absence of a reliable laboratory marker. Hence there is an utmost requirement to assess the severity of COVID-19 patients for timely management and intervention.

Currently, the nucleic acid amplification of critical SARS-CoV-2 genes followed by sequencing are the gold standard techniques employed for the diagnosis of COVID-19 in addition to the rapid

serological assays. Assays involving testing the immunological response of host bears the potential in the clinical judgement of disease. Neutrophils are the first immune cells to reach the site of infection/inflammation and exert their function by producing free radicals and extracellular traps (NETs). During these processes, a plethora of inflammatory mediators including cytokines (IL-1β, TNF-α, or IL-8) are produced. Augmented levels of neutrophils and its derived factors like free radicals, NETs, and cytokines have been reported to be associated with severity of disease, organ dysfunction, Acute Physiology and Chronic Health Evaluation (APACHE) and the Serial Organ Failure Assessment (SOFA) score (Kumar et al., 2019). The present meta-analysis has been designed to assess whether neutrophil count can be used as a reliable parameter to mark the difference between non-severe and severe COVID-19 patients. This will be helpful in establishing the clinical significance of neutrophil count in COVID-19.

Methods

Literature search was conducted on various electronic databases like PubMed, Scopus, Google Scholar, Science Direct, and Web of Science. Keywords such as 'COVID-19' OR 'SARS-CoV-2' OR 'novel corona virus' AND 'neutrophil' OR 'neutrophil count'. The literature search range was kept from December 2019 till to-date. Only research articles and case reports showing neutrophil count in severe and non-severe COVID-19 patients were included irrespective of location of the study. Following information were extracted and tabulated from the publications – surname of first author, number of cases, severe and non-severe cases, total neutrophil count, and neutrophil count in non-severe and severe patients. The design of included study was cohort. In the included study, following criteria were defined as having severe-type infection: (i) respiratory distress with a respiratory rate > 30 breaths per minute, (ii) oxygen saturation ≤93% in the resting state, and (iii) arterial blood oxygen partial pressure (PaO_2) /oxygen concentration (FiO_2) ≤300 mm Hg.

We used Hozo et al., 2005 published method to extrapolate the mean and standard deviation data from the median, interquartile range (IQR) and the sample size score (Hozo et al., 2005). Heterogeneity was calculated using Q test and I2 statistic. A meta-analysis was performed by using a random effects model, with calculation of WMD and 95% CI of neutrophil count in severe and non-severe COVID-19 patients. The sources of heterogeneity were evaluated by sub-group analysis. Funnel plots were constructed to assess the publication bias. Sensitivity analysis of the 14 studies was done to test the effect of each study on the pooled result. The statistical analysis was performed with MetaXL, software Version 5.3 (EpiGear International Pty Ltd., Sunrise Beach, Australia).

Results

The process of study retrieval using flow diagram is mentioned in figure 1. A total of 218 studies were retrieved on the basis of search criteria. Among these, review articles (70), encyclopedia (2), book chapters (9), conference abstracts (2), case reports (23), correspondence (37), discussion (6), editorials (5), mini reviews (2), short communications (14), and other (6) were excluded from the study. Furthermore, 42 studies (3330 COVID-19 subjects) reporting information on neutrophil count in well-defined patients of severe and non-severe COVID-19 disease were selected for our meta-analysis. All selected documents were further scrutinized on the basis of essential information required to perform meta-analysis. After the rigorous evaluation and data extraction, 14 potentially eligible studies consisting of 1473 COVID-19 patients with 544 (36.9%) severe cases were incorporated in the meta-analysis (Gao et al., 2020; Huang et al., 2020; Li et al., 2020; Liu et al., 2020; Liu et al., 2020; Qin et al., 2020; Sun et al., 2020; To et al., 2020; Wang et al., 2020; Wang et al., 2020; Xie et al., 2020; Young et al., 2020; Zheng et al., 2020; Zhu Z et al., 2020).

The detail features including study ID, total as well as number of severe and non-severe cases, neutrophil count in total, severe and non-severe cases are mentioned in table 1. The number of patients

ranges from 18 to 452 patients. The WMD in neutrophil count between severe and non-severe COVID-19 patients along with subgroup results in the 14 individual studies have been depicted in figure 2.

In sub-group analysis of low neutrophil count group, three studies (Gao et al., 2020; To et al., 2020; Young et al., 2020) (WMD -0.69 x 109/L; 95% CI, -1.28 to -0.10 x 109/L), the lower neutrophil count studies are statistically non-significant because they cross the line of null effect. Whereas, the other subgroup of high neutrophil count group, other set of eleven studies (Huang et al., 2020; Li et al., 2020; Liu et al., 2020; Liu et al., 2020; Qin et al., 2020; Sun et al., 2020; Wang et al., 2020; Wang et al., 2020; Xie et al., 2020; Zheng et al., 2020; Zhu Z et al., 2020) (WMD 2.35 x 109/L; 95% CI, 1.56 to 3.14 x 109/L), shown a statistically significant values of neutrophil level.

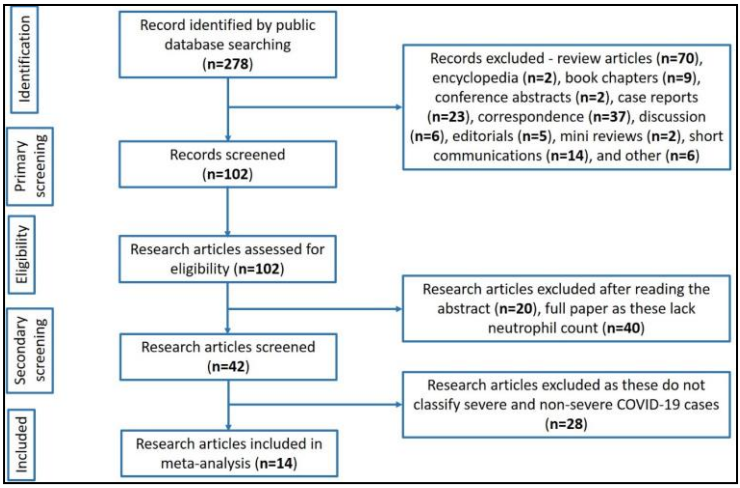


Figure 1. Flow diagram of study selection process

Table 1. Features of the included study

Study ID	Country	All cases	Severe cases	Non-severe cases	Age in years (median)	Male	Female	Neutrophil Count*: all x 10^9/L	Neutrophil Count*: Severe x 10^9/L	Neutrophil Count*: Non-Severe x 10^9/L
Gao et al., 2020	China	43	15	28	43.74	26	17	NR	2.65 (1.49)	3.43 (1.63)
Huang et al., 2020	China	41	13	28	49	30	11	5.0 (3.3-8.9)	10.6 (5.0-11.8)	4.4 (2.0-6.1)
Li et al., 2020	China	83	25	58	45.5	44	39	3.61 (2.67-5.56)	4.36 (2.87-6.48)	3.50 (2.64-4.46)
Liu et al., 2020	China	40	13	27	48·7	15	25	2.8 (1.6-4.3)	4.7 (3.6-5.8)	2.0 (1.5-2.9)
Liu et al., 2020	China	78	11	67	38	39	49	3.11 (2.25-4.82)	4.69 (2.96-7.06)	2.94 (2.20-4.60)
Qin et al., 2020	China	452	286	166	58	235	217	3.9 (2.6-5.8)	4.3 (2.9-7.0)	3.2 (2.1-4.4)
Sun et al., 2020	China	116	27	89	50	60	56	3.10 (2.33-4.30)	6.07 (3.10-7.60)	2.90 (2.15-3.80)
To et al., 2020	China	23	10	13	60.3	10	13	3.71 (1.3-9.5)	3.8 (2.0-5.2)	3.6 (1.3-9.5)
Wang et al., 2020	China	138	36	102	56	75	63	3.0 (2.0-4.9)	4.6 (2.6-7.9)	2.7 (1.9-3.9)
Wang et al., 2020	China	69	14	55	42	32	37	2.35 (1.62-367)	5.24 (2.90-6.44)	2.16 (1.60-2.70)
Xie et al., 2020	China	140	51	89	60	72	68	4.09 (2.65-6.36)	7.46 (5.01-9.60)	3.29 (2.28-4.77)
Young et al., 2020	Singapore	18	6	12	47	9	9	2.7 (0.7-4.5)	1.8 (1.2-3.7)	2.8 (0.7-4.5)
Zheng et al., 2020	China	55	21	34	60.1	24	31	3.03 (0.56–9.29)	3.46 (0.56–9.29)	2.77 (0.93–5.93)
Zhu Z et al., 2020	China	127	16	111	50.9	45	82	3.37 (2.54-4.55)	3.89 (2.25-6.57)	3.29 (2.54-4.40)

**Neutrophil Count presented as mean (SD) or median (IQR).*
NR = not reported.

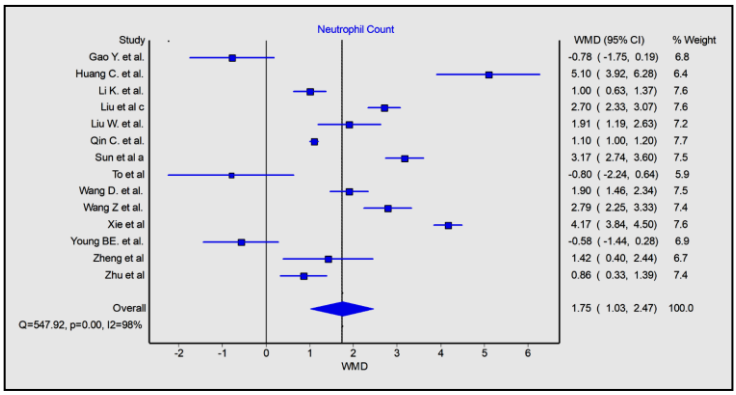


Figure 2. Forest plot of mean difference in neutrophil count between COVID-19 patients with or without severe disease

A sensitivity analysis performed by removing each individual study did not show any change (Table 2). The combined analysis revealed that neutrophil count is significantly elevated in severe COVID-19 patients (WMD 1.75 x 10^9/L; 95% CI, 1.03 to 2.47 x 10^9/L). The heterogeneity was high (I^2, 98%; $p<0.001$) and a random effects model was used. All the studies hold almost equal weight (%).

Discussion

Detection of SARS-CoV-2 at molecular level in poor resource settings is the major bottle-neck in proper clinical management of COVID-19. Moreover, a marked variation in severity of COVID-19 urges for the requirement of laboratory-based biomarker which can distinguish severe and non-severe cases of COVID-19. Hence the present study aimed to establish neutrophil count as potential biomarker to distinguish between non-severe and severe COVID-19 cases with inclusion of 14 studies with 2017 patients. Our study depicted that a high neutrophil count has direct relation with severity of COVID-19.

Table 2. Summary of sensitivity analysis of the included studies.

Study ID	Pooled WMD	I^2 %
Gao et al., 2020	1.93106418	97.7208357
Huang et al., 2020	1.521575624	97.65620097
Li et al., 2020	1.80359596	97.78123718
Liu et al., 2020	1.667464391	97.62236059
Liu et al., 2020	1.733346832	97.80458798
Qin et al., 2020	1.794869167	96.6006684
Sun et al., 2020	1.632054077	97.53468611
To et al., 2020	1.906949552	97.76994949
Wang et al., 2020	1.73167827	97.79600354
Wang et al., 2020	1.66324379	97.71688978
Xie et al., 2020	1.561981087	95.59439835
Young et al., 2020	1.921181207	97.71630195
Zheng et al., 2020	1.769870865	97.80981509
Zhu Z et al., 2020	1.815911975	97.78748214

Role of neutrophil count during severity of COVID-19 has been meta-analysed earlier using data from 8 studies (Lippi and Plebani., 2020). The outcome of our combined analysis are in agreement with the investigation from the abovementioned study. The underlying mechanism involving increased neutrophil count in coronavirus infection is well studied under different experimental models. In a mouse model, Gralinski et al have demonstrated that upon SARS-CoV infection complement signalling, as well as cytokines and chemokines (IL-5, IL-6, CXCL1, and G-CSF) gets activated that

triggered neutrophil activation and recruitment (Gralinski et al., 2018). Furthermore, the observed neutrophilia in severe as compared with non-severe COVID-19 patients could be due to induction of cytokine storm upon SARS-CoV-2 infection (Huang et al., 2020). More recently, study involving 50 patients of COVID-19 have augmented level of blood NETs, which could be responsible for inflammatory storm and thrombosis that leads to ARDS and a requirement for mechanical ventilation, hallmarks of severe SARS-CoV-2 infection (Zuo et al., 2020). Hence, increased neutrophil count may be determining factor in host hyper-inflammatory response followed by ARDS in SARS-CoV-2 infection.

The present study has some limitations. First, very limited study is available about neutrophil count and COVID-19 severity as the disease outbreak is relatively recent. Second, patient specific report is missing in both severe and non-severe COVID-19 cases, hence, appropriate cut-off value of neutrophil count could not be presented to mark the severity of disease. Further research is needed to search for the optimum cut-off value. Third, high heterogeneity among patients was observed which could be due to different definitions of disease severity and difference in the time of patient admission to the hospital. Finally, information regarding prior disease and medication which may influence neutrophil count are missing. Despite the above-mentioned limitations, our study convincingly provides the evidence that increased neutrophil count is indicative of severe COVID-19 patients.

Conclusion

Neutrophil count which is cost-effective and easily available laboratory parameter is directly associated with the severity of COVID-19. Further research is warranted to confirm our findings by including more studies as they become available.

References

Gao, Y., Li, T., Han, M., Li, X., Wu, D., Xu, Y., Zhu, Y., Liu, Y., Wang, X., Wang, L., 2020. Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. J. Med. Virol. 92, 791-796.

Gralinski, L.E., Sheahan, T.P., Morrison, T.E., Menachery, V.D., Jensen, K., Leist, S.R., Whitmore, A., Heise, M.T., Baric, R.S., 2018. Complement activation contributes to severe acute respiratory syndrome coronavirus pathogenesis. MBio. 9, e01753-18.

Hozo, S.P., Djulbegovic, B., Hozo, I., 2005. Estimating the mean and variance from the median, range, and the size of a sample. BMC Med. Res. Methodol. 5, 13.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., Cheng, Z., 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet, 395, 497-506.

Huang, L., Zhang, X., Zhang, X., Wei, Z., Zhang, L., Xu, J., Liang, P., Xu, Y., Zhang, C., Xu, A., 2020. Rapid asymptomatic transmission of COVID-19 during the incubation period demonstrating strong infectivity in a cluster of youngsters aged 16-23 years outside Wuhan and characteristics of young patients with COVID-19: a prospective contact-tracing study. J. Infection. 80:e1-e13

Kumar, S., Gupta, E., Kaushik, S., Srivastava, V.K., Saxena, J., Mehta, S., Jyoti, A., 2019. Quantification of NETs formation in neutrophil and its correlation with the severity of sepsis and organ dysfunction. Clin. Chim. Acta. 495, 606-610.

Li, K., Wu, J., Wu, F., Guo, D., Chen, L., Fang, Z., Li, C., 2020. The clinical and chest CT features associated with severe and critical COVID-19 pneumonia. Invest. Radiol. 55, 327-331.

Lippi, G., Plebani, M., 2020. Procalcitonin in patients with severe coronavirus disease 2019 (COVID-19): a meta-analysis. Clin. Chim. Acta. 505, 190-191.

Liu, J., Li, S., Liu, J., Liang, B., Wang, X., Wang, H., Li, W., Tong, Q., Yi, J., Zhao, L., Xiong, L., 2020. Longitudinal characteristics of lymphocyte responses and cytokine profiles in

the peripheral blood of SARS-CoV-2 infected patients. EBioMedicine. 55, 102763.

Liu, W., Tao, Z.W., Wang, L., Yuan, M.L., Liu, K., Zhou, L., Wei, S., Deng, Y., Liu, J., Liu, H.G., Ming, Y., 2020. Analysis of factors associated with disease outcomes in hospitalized patients with 2019 novel coronavirus disease. Chinese Med. J. 133, 1032-1038.

Qin, C., Zhou, L., Hu, Z., Zhang, S., Yang, S., Tao, Y., Xie, C., Ma, K., Shang, K., Wang, W., Tian, D.S., 2020. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clin. Infect. Dis. 71, 762-768.

Sun, S., Cai, X., Wang, H., He, G., Lin, Y., Lu, B., Chen, C., Pan, Y., Hu, X., 2020. Abnormalities of peripheral blood system in patients with COVID-19 in Wenzhou, China. Clin. Chim. Acta. 507, 174-180.

To, K.K.W., Tsang, O.T.Y., Leung, W.S., Tam, A.R., Wu, T.C., Lung, D.C., Yip, C.C.Y., Cai, J.P., Chan, J.M.C., Chik, T.S.H., Lau, D.P.L., 2020. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect. Dis. 20, 565-574.

Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., Wang, B., Xiang, H., Cheng, Z., Xiong, Y.. Zhao, Y., 2020. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. J. Am. Med. Assoc. 323, 1061-1069.

Wang, Z., Yang, B., Li, Q., Wen, L., Zhang, R., 2020. Clinical features of 69 cases with coronavirus disease 2019 in Wuhan, China. Clin. Infect. Dis. 71, 769-777.

Xie, J., Covassin, N., Fan, Z., Singh, P., Gao, W., Li, G., Kara, T., Somers, V.K., 2020, Association between hypoxemia and mortality in patients with COVID-19. Mayo Clin. Proc. 95, 1138-1147.

Young, B.E., Ong, S.W.X., Kalimuddin, S., Low, J.G., Tan, S.Y., Loh, J., Ng, O.T., Marimuthu, K., Ang, L.W., Mak, T.M. and Lau, S.K., 2020. Epidemiologic features and clinical course of

patients infected with SARS-CoV-2 in Singapore. J. Am. Med. Assoc. 323, 1488-1494.

Zheng, C., Wang, J., Guo, H., Lu, Z., Ma, Y., Zhu, Y., Xia, D., Wang, Y., He, H., Zhou, J., Wang, Y., 2020. Risk-adapted treatment strategy for covid-19 patients. Int. J. Infect. Dis. 94, 74-77.

Zhu, Z., Cai, T., Fan, L., Lou, K., Hua, X., Huang, Z., Gao, G., 2020. Clinical value of immune-inflammatory parameters to assess the severity of coronavirus disease 2019. Int. J. Infect. Dis. 95, 332-339

Zuo, Y., Yalavarthi, S., Shi, H., Gockman, K., Zuo, M., Madison, J.A., Blair, C., Weber, A., Barnes, B.J., Egeblad, M., Woods, R.J., 2020. Neutrophil extracellular traps (NETs) as markers of disease severity in COVID-19. medRxiv.

PROSPECTS *FUSARIUM* SP. AS MICROBIAL PIGMENT

Amrendra Pathak[1], Esha Dwivedi[2], Lalit Kumar Singh[3]

[1]Director, Centre of Research for Development, Parul University, Waghotia, Vadodra, Gujrat, India
[2]Ph.D.Scholar, Department of Biochemical Engineering, School of Chemical Technology Harcourt Butler Technical University Kanpur-208001 (U.P.) India
[3]Assistant Professor, Department of Biochemical Engineering, School of Chemical Technology Harcourt Butler Technical University Kanpur-208001 (U.P.) India

[*]Corresponding Author: amrendra.pathak@paruluniversity.ac.in

Introduction

Pigments are compounds with characteristics of importance to many industries. In the food industry they are used as additives, color intensifiers, antioxidants, etc. Pigments come in a wide variety of colors, some of which are water soluble. Color of a food substance is important to indicate its freshness and safety that are also indices of good aesthetic and sensorial values. In the recent years, coloring of food with pigments produced from natural sources is of worldwide interest and is gaining importance. These pigments are looked upon for their safe use as a natural food dye in replacement of synthetic ones because of undesirable market. A well textured food, rich in nutrients and flavor, cannot be eaten unless it has the right color. The demand for natural source of such compounds is increasing day by day because of the awareness of positive health benefits out of natural compound (Malik et al., 2012). Most of the bacteria and fungi are widely studied for their potential as a source of food colorants. Natural pigments possess anticancer activity, contain pro-vitamin A and have some desirable properties like stability to light, heat and pH (Joshi et al., 2003). Pigments are the chemical substances that absorb the light of visible region. The produced color is because of the chromophore, a molecule specific

structure which captures the sun energy and causes an excitation of electron from external orbital to higher orbital, where the non-absorbed energy is refracted or reflected to be captured by eye (Delgado et al., 2000).

Microorganisms have been used for a long time for production of molecules as diverse as antibiotics, enzymes, vitamins, texturizing agents and so on. There is growing interest in the food industry in the use of natural ingredients. Ingredients, such as colors, are considered natural when derived from biological sources like plants or microorganisms. Microbial colors are in use in the fish industry already, for example to enhance the pink color of farmed salmon. Further, some natural colorants have commercial potential for use as antioxidants. The industry is now able to produce some microbial pigments for applications in food, cosmetics or textiles. In nature, color rich and pigment producing microorganisms (fungi, yeasts, and bacteria) are quite common (Dufosse et al., 2009).

Table 1. Important microbial pigment and their producing microorganisms.

Microorganism(s) Bacteria	**Pigments/ Molecule**	**Color**	**Reference**
Bradyrhizobium sp.	Canthaxanthin	Dark- red	Venil et al., 2013
Flavobacterium sp.	Zeaxanthin	yellow	Berry et al., 2003
Staphyloxanthin sp.	Zeaxanthin	Golden Yellow	Liu et al., 2005
Chromobacterium violaceum	Violacein	Purple	Blosser et al., 2000
Hematococcus sp.	Canthaxanthin	Yellow-orange-red	Christaki et al., 2013
Pacilomyces farinosus	Anthraquinone	Red	Velmurugan et al., 2010
Saccharomyces neoformans var. *phaffiarhodozyma*	Astaxanthin	Pink-red	Bjerkeng et al., 2007
Yarrowia lipolytica	Melanin	Brown	Carreira et al., 2001

Microbial pigments

Like plants, filamentous fungi also play an important role in ecosystem. They synthesize natural products because they have an ecological function and are of value to the producer (Firn et al., 2003). Pigments are derived from natural sources such as plants, insects, and microorganism (Table 1). There has been much interest in the development of new natural colorants for use in the food industry owing to strong consumer demand for more natural products and others.

Naturally occurring microbial pigments

Pigments produced by bioorganisms as recollection of its secondary metabolism are commonly known as biopigments. These biopigments have many synthetic and commercial application (Shirata *et al.*, 2000). Biological pigments can be categorized based on their structural and natural occurrence. Some examples of naturally occurring microbial pigments are:

Beta-carotene

*Phycomyces*and *Mucorcircinelloides* (wild type) are a prospective source of beta-carotene. The European Union Committee considers that beta-carotene obtained by fermentation of *Blakesleatrispora*is equivalent to the chemically synthesized material used as food colorant and is therefore acceptable for use as a coloring agent in foodstuffs (Dufossé, 2006).

Canthaxanthin

It is produced as the major carotenoid pigment by orange and dark pinkpigmented bacteriochlorophyll containing *Bradyrhizobium* (photosynthetic) strains isolated from stem nodules of *Aeschynomene* species and *Halobacterium* sp (Asker and Ohta, 1999). Canthaxanthins are effective antioxidants and inhibit the oxidation of lipids in liposomes (Woodall *et al.*, 1997).

Carotenoids

These are yellow to orange-red pigments that are ubiquitous in nature. Several numbers of microorganisms produce this pigment such as *Serratia* and *Streptomyces.* Carotenoids are potent antioxidants and are widely used as food colorants. Majority of 26 microbes investigated produce carotenoids belonging to *Myxococcus*, *Streptomyces*, *Mycobacterium*, *Agrobacterium* and *Sulfolobus* (Malik *et al.*, 2012).

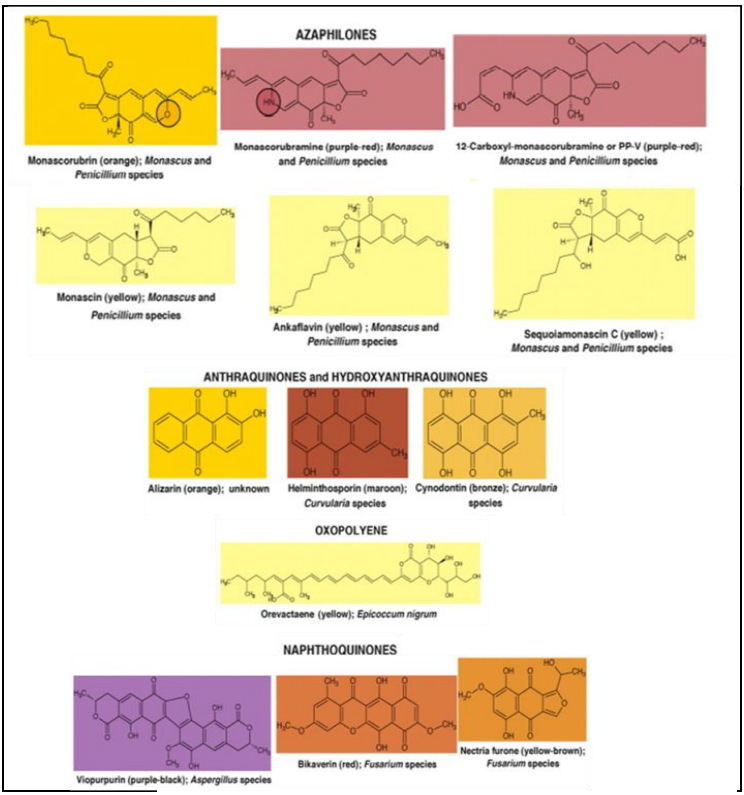


Figure: 1. Example of classes of fungal polyketide pigments exhibiting the color

Prodigiosin

It is a multipurpose red pigment, produced by a number of microorganisms such as *Serratiamarcescens, Vibrio psychoerythrus, Rugamonasrubra,* actinomycetes, such as *Streptoverticillium rubrireticuli* and other eubacteria. It is known to have anti-malarial, antibacterial, antineoplastic and antibiotic activity.

Phycocyanin

It is a blue pigment, produced by cyanobacteria which contain chlorophyll *a*. The blue colorant is known by the name spirulina (blue green alga), and is used as a dietary supplement which is rich in proteins. Here the supplement consists of dried cyanobacteria.

Violacein

It is a versatile pigment from a bacterium *Chromobacterium violaceum* that exhibits numerous biological activities. It has gained immense importance in industrial markets, such as in medicine, cosmetics, food and textiles.

Astaxanthin

Chemically it is 3, 3'-dihydroxy-b, b-carotene-4, 4'- dione and is an orange - red pigment and produced by microorganisms such as red *Basidiomycetous* yeast *Xanthophyllomyces dendrorhous*, green algae *Heamatococcus pluvialis* and *Agrobacterium aurantiacum* (Malik et al., 2012).

Anthraquinones

Anthraquinones and their derivatives constitute a large group of quinoid compounds with about 700 molecules described. They are using as substance for different industries as clothes dyeing and food colorants. Their positive and/or negative effect(s) are because of due to its 9, 10-anthracenedione structure which influence the effects on human health. A marine microorganism recently appeared as producers of an astonishing variety of structurally unique secondary metabolites (Fouillaud et al., 2016).

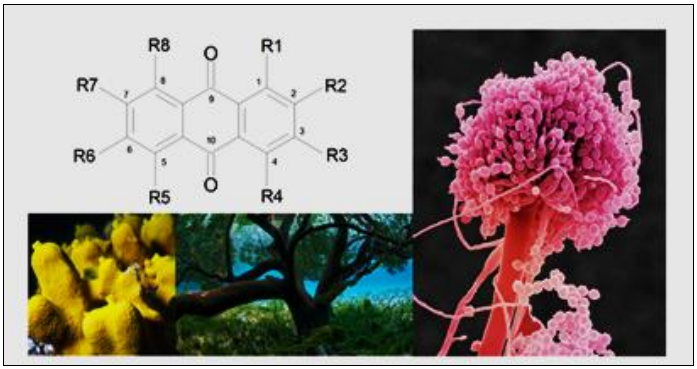


Figure 2. Anthraquinone pigment from marine derived fungi

Difference between Dyes and Pigment

The dyestuff industry is suffering from increase in costs of feedstock and energy for dye synthesis, and they are under increasing pressure to minimize the damage to the environment. The industries are continuously looking for cheaper, and more environmental friendly routes to existing dyes. Since then synthetics dyes have almost completely replaced natural dyes. The wide range of available colors, greater reproducibility, improved quality of dyeing, and economic benefits of synthetic dyes are highly desirable. However, it is well known that some of the synthetic dyes are environmental unfriendly and process negative impact on ecosystems. Recent studies reported that 10–35% of these dyes are lost in wastewater during the dyeing process (Rai et al., 2005). Natural dyes or pigments are an important alternative to potentially harmful synthetics. The more ecological interesting source of pigments is fungi, since some fungal species are rich in stable colorants such as anthraquinone and others.

The main difference between dyes and pigment are dyes are water soluble substances and have at least one salt-forming group. The most common salt forming is the sulfonic acid group; however carboxylic acid residues can also be used. These dyes are generally

isolated as sodium salts. They have colored anions and are well-known as anionic dyes. The other dyes containing basic groups, like $–NH_2$, $-NH-CH_3$, or $–N(CH_3)_2$, from water –soluble salts with acids. These are the cationic dyes and have positively charged colored ion. If both acidic and basic groups are present there, an internal salt is usually formed. Whereas pigments are the particulate solids disperse into a medium without significant solution or their interactions. Pigments are soluble in water or in solvent colorants lack with salt-forming groups. They occupy a major place in our daily life. Pigments are used in food, cosmetics, paints, pharmaceuticals, glass, textiles etc. The most primitive known pigments were natural minerals. Natural iron oxides, anhydrous Fe_2O_3, charcoal and so on are several well-known pigments since prehistoric times (Stafsnes et al., 2010).

Fusarium sp.

Classification and morphology of *Fusarium*

The genus *Fusarium* belongs to the *Ascomycota* phylum, *Ascomycetes* class, *Hypocreales* order (Leslie et al., 1995) while the teleomorphs of *Fusarium* species are mostly classified in the genus *Gibberella*, and for a smaller number of species, *Hemanectria* and *Albonectria* genera. The main approach for the *Fusarium* classification is still morphology, and the primary trait for species to be placed in *Fusarium* genus is the occurrence of the asexual spores, the distinctive banana-shaped macroconidia. *Fusarium* species produce three types of spores i.e macroconidia, microconidia and chlamydospores. Two separated macroconidia can be produced on monophialides and polyphialides in the aerial mycelium, but also on short monophialides in specialized structures called sporodochia. (Leslie et al., 2006).

Identification of *Fusarium* species

The genus *Fusarium* comprises a diverse array of fungi, members of which are phytopathogenic to a wide range of plants under diverse environmental conditions. Phytopathogenic *Fusarium* fungi cause several diseases of small-grain cereals, including seedling blight and

foot rot, fusarium head blight (FHB) (also known as 'scab' or ear blight) and ear rot of maize (Parry *et al.*,1995). Three *Fusarium* species were isolated from infected maize: *F. graminearum, F. moniliforme* (syn. *F. verticillioides*, teleomorph *G. fujikuroi* mating population A) and *F. subglutinans* (teleomorph *G. fujikuroi*mating population E). Other species responsible for ear rot of maize include *F. culmorum*, *F. proliferatum* (teleomorph *G. fujikuroi* mating population D) and *F. equiseti*. Canadian Researcher WL Gordon had published many articles on *Fusarium* Genus between 1930 and 1960.

Traditional methods for identification of *Fusarium* sp. requires specialized growth media for growth. They require one or two weeks of growth before identifications can be established. *F. verticillioides* and other fumonisin producing infections in maize tissues (Grimm and Geisen, 1998). Therefore, improved and quicker methods for identifying fumonisin forming fungi from maize tissues has become important, especially since fumonisins are now being implicated in diseases and cancer of animals. Molecular methods such as polymerase chain reaction (PCR) are now widely used in fungal taxonomy.

Fusarium moniliform

F. moniliform (teleomophrph *Gibberella moniliformis*) is a phytopathogenic filamentous fungus which has a composite distribution on wide range of host plants producing serious diseases in a number of agriculturally important plant species. *Fusarium verticillioides*is the most commonly reported fungal species infecting maize. *Fusarium moniliforme* produces many toxins that have potential toxicity for humans and animals. The main toxins produced by *F. verticillioides* are the fumonisins. The presence of fumonisins in asymptomatic grain means that a better understanding of the asymptomatic endophytic portion of the life cycle of *F. verticillioides* is badly needed. *Fusarium* sp. is one of the most important genera of plant pathogenic fungi with a record of devastating infections in many different kinds of economically important plants.

Nutrient Media

To select suitable media for maximum antimicrobial metabolite production, selected strain was grown in various semisynthetic, synthetic and its modified variant. During the fermentation process, cell growth and the yield of antimicrobial metabolites by the strain and other data have to be collected. The medium in which the strain exhibits optimum levels of antimicrobial metabolites in terms of antimicrobial and biomass production was selected for further studies.

Raw materials used for pigment production

Cellulases are an important class of enzymes which are used for the hydrolysis of cellulosic materials for the production of glucose, alcohol, cellulose acetate oligosaccharides etc. The raw materials containing glucose in any form can be used as raw material for production of pigment i.e. rice, wheat, corn, bagasses, husk etc. They also have applications in various industries like fuel, textile, paper, starch processing, feeds, fruits, vegetables, etc. (Zaldivar et al., 2001). Lignocellulosic materials mainly consist of cellulose which is available abundantly in nature. Hydrolysis of lignocellulosic wastes is of prime importance for its conversion into important industrial products. β-glucosidase is an important class of cellulolytic complex that completely breaks down various ligocellulosic wastes/materials by cleaving the β-1, 4-glycosidic bond.

Fermentation

Fermentation is a metabolic process that converts sugar to acids, gases and/or alcohol and usually occurs in fungi, yeast and bacteria. The production of microbial pigments by fermentation is an interesting area and a lot of attention is now paid to this biotechnological approach. This technique can be of solid or submerged fermentation for pigment production and also for other purposes.

Submerged fermentation

Submerged fermentation utilizes free flowing liquid substrates, such as molasses and broths. The bioactive molecules are secreted into the fermentation broth. The substrates are utilized rapidly; hence need to be constantly replaced or supplemented with nutrients. This fermentation technique is best suited for microorganisms such as bacteria that require high moisture content. An added advantage of this technique is that purification of products is easier (Dhale, 2007). For example *Monascus* has been successfully cultured submerged condition for pigment production and versatile substrates like breads, rice and other amylaceous (starch, dextrins, glucose, maltose and fructose) materials for high productivity of red pigments which occurs due to glucose and maltose utilization. Carotenoid production by *Aspergillus* sp. melanin type pigment by *Aspergillus niger* (Jorgensen, *et al.*, 2011), water soluble red pigment by *Penicillium purpurogenum* (Méndez et al., 2011) etc are some specific features of fungi has been recorded.

Solid- state fermentation

Solid state fermentation (SSF) is defined as any fermentation process performed on a nonsoluble substance that acts both as physical support and source of nutrients in absence of free flowing liquid (Pandey et al., 1999). SSF utilizes solid numerous versatile substrates, like bran, bagasse, and paper pulp etc. The main advantage of using these substrates is that nutrient-rich waste materials can be easily recycled as substrates (Couto et al., 2006). In this fermentation technique, the substrates are utilized very slowly and steadily, so the same substrate can be used for long fermentation periods. Hence, this technique supports controlled release of nutrients. SSF is best suited for fermentation techniques involving fungi and microorganisms that require less moisture content. However, it cannot be used in fermentation processes involving organisms that require high aw (water activity), such as bacteria. The quantity and quality of the pigments can be improved by changing the various culturing parameters like carbon source, nitrogen source, pH and temperature etc, associated with the

submerged fermentation. Hence SMF is more suitable for the industrial application.

Effect of different parameters

An ideal pigment producing microorganism should be capable of using a wide range of C and N sources, tolerant to pH, temperature, and minerals. The nontoxic and nonpathogenic nature coupled with easy separation from cell biomass is also preferred qualities. Microbial pigments have many advantages over artificial and inorganic colors. One relates this to fermentation, which is an inherently faster and more productive process as compared to other chemical processes. The other enduring strength of microbes is their relatively large and easily manipulated strands of genes. Besides, pigment production from microorganisms is independent of weather conditions, which produce different color shades and grow on cheap substrates (Joshi et al., 2003). The nitrogen sources like peptone have been observed to be the most expensive medium constituent. Keeping in view of this, experimentation has been done to prepare peptone from waste chicken feathers through acid hydrolysis and to investigate the usability of this peptone as substrate for biomass and carotenoid production by Rho-dotorulaglutinis (Taskin et al., 2011).

Temperature

Varying the temperature in a simple model ecosystem produces changes in the community structure of *Fusarium* sp. that mimic those found along climatic temperature and rainfall gradients (Saremi et al., 2011). The temperature optima for growth of *Fusarium* sp. appears to be dependent on a_w. It was found that the optimal growth temperature for European isolates of *F. graminearum* (24–28°C) increased slightly when lower water potentials prevailed. *Fusarium graminearum* grew optimally at −10 to −20 bars and *F. culmorum* at −8 to −14 bars. Increasing a_w (>0.925) favored growth of *F. moniliforme* and *F. proliferatum* on sterile layers of maize at 30°C. Fumonisins and moniliformin are commonly produced in maize infected by *F. moniliforme* and *F. proliferatum*, species which tend to grow better at higher

temperatures. Microconidia of *F. moniliforme* germinated optimally at 25–37^{o}C and 0.96–0.98 a_w, but at 30^{o}C when the a_w was 0.90–0.94, with intra isolate variation (Etcheverry *et al*., 2002).

Production of nepthoquinone

The production of naphthoquinone metabolite by *Fusarium* strain was regulated by many factors in the culture media. The levels of induction, repression or even inhibition depends on different types and amount of carbon source, nitrogen source, mineral salt and pH for the culture media which has been prepared for purpose. *F. verticillioides* was able to utilize a variety of sugars as carbon source whereas peptone and yeast extract were good nitrogen sources for naphthoquinone pigment production. There might be a great advantage in adding K^+ metal ion to the medium for enhancing the pigment production in different ways. Mycelial growth favored the acidic pH range and reached maximum yield at pH 5 while the pigment production was suitable for the alkali pH range. From our results, the highest naphthoquinone production of *F. verticillioides* was achieved when cultured in the modified PDB (20 g/l glucose; 2.5 g/l yeast extract containing 5 mg/l of K^+ and adjusted the initial medium pH to 8). This pigment production was two times higher than those cultured in the common PDB medium (Boonyapranai et al., 2008).

Conclusion

Fungi have the ability to produce pigments of different colour. The biosynthesis of pigment is directly related to cultural conditions that also include biomass production. The main outcome of this work is that we have screened pigments using different raw materials containing glucose, and nitrogen sources, mineral salts, amino acids supplement to the culture broth strongly influenced the growth and biosynthesis of pigment by *Fusarium moniliforme*. The microbial pigment produced is natural, cost effective and easily degradable and without production of recalcitrant intermediates when they enter the ecosystem.

References

Berry, Alan, et al., 2003. *Paracoccuszea xanthinifaciens* sp. nov., a zeaxanthin-producing bacterium. *International Journal of systematic and Evolutionary Microbiology* 53.1: 231-238.

Blosser, R. S., and Kendall M. G., 2000. Extraction of violacein from *Chromobacterium violaceum* provides a new quantitative bioassay for N-acyl homoserine lactone autoinducers. *Journal of microbiological methods* 40.1: 47-55.

Bjerkeng, B., et al., 2007. Digestibility and muscle retention of astaxanthin in Atlantic salmon, Salmosalar, fed diets with the red yeast Phaffiarhodozyma in comparison with synthetic formulated astaxanthin. *Aquaculture* 269.1: 476-489.

Boonyapranai K.R, Tung P, Lhieochaiphant S, Phutrakul S. , 2008. Optimization of submerged culture for the production of naphthoquinones pigment by *Fusariumverticillioides*. Chiang Mai J Sci; 35: 457–466.

Carreira, A., Luísa M. Ferreira, and Virgílio L., 2001. Brown pigments produced by *Yarrowia lipolytica* result from extracellular accumulation of homogentisic acid. *Applied and environmental microbiology*67, no. 8: 3463-3468.

Christaki, Efterpi, et al. 2013. Functional properties of carotenoids originating from algae. *Journal of the Science of Food and Agriculture* 93.1: 5-11.

Couto, Susana Rodríguez, and Ma Angeles Sanromán, 2006. Application of solid-state fermentation to food industry—a review. *Journal of Food Engineering*76, no. 3: 291-302.

Dufosse L. 2009. Pigments, Microbial. Encyclopedia Microbiol, 4: 457-471.

Etcheverry, M., et al., 2002. In vitro control of growth and fumonisin production by *Fusarium verticillioides* and *F. proliferatum* using antioxidants under different water availability and temperature regimes. *Journal of Applied Microbiology* 92, 4: 624-632.

Delgado-Vargas, et al., 2000. Natural pigments: carotenoids, anthocyanins, and betalains—characteristics, biosynthesis, processing, and stability. Critical Reviews in Food Science and Nutrition, 40: 173-289.

Firn, Richard D., and Clive G. Jones, 2003. Natural products–a simple model to explain chemical diversity. *Natural product reports* 20, 4: 382-391.

Fouillaud, Mireille, MekalaVenkatachalam, Emmanuelle Girard-Valenciennes, Yanis Caro, and Laurent Dufossé, 2016. Anthraquinones and Derivatives from Marine-Derived Fungi: Structural Diversity and Selected Biological Activities. *Marine drugs* 14, 4: 64.

Grimm, C., and R. Geisen, 1998. A PCR-ELISA for the detection of potential fumonisin producing Fusarium species." *Letters in applied microbiology* 26, 6: 456-462.

Joshi, V.K., Attri, D., Bala, A. and Bhushan, S. 2003. Microbial Pigments. Indian J. Biotech., 2: 362-369.

Jorgensen, Thomas R., Kristian F. Nielsen, Mark Arentshorst, JooHae Park, Cees A. van den Hondel, Jens C. Frisvad, and Arthur F. Ram, 2011. Submerged conidiation and product formation by *Aspergillus niger* at low specific growth rates are affected in aerial developmental mutants. *Applied and environmental microbiology* 77,15: 5270-5277

Leslie, John F., and Brett A. Summerell, 2006. Techniques and methods. *Fusarium laboratory manual. Blackwell Publishing Ltd., Oxford, UK* (2006): 7-8.

Liu, George Y., et al., 2005. *Staphylococcus aureus* golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity, *The Journal of experimental medicine* 202, 2: 209-215.

Malik, Kamla, Jayanti Tokkas, and SnehGoyal, 2012. Microbial pigments: a review. *Int J Microbial Res Technol* 1, 4: 361-365.

Méndez, Alejandro, Catalina Pérez, Julio Cesar Montañéz, Gabriela Martínez, and CristóbalNoé Aguilar, 2011. Red pigment production by *Penicillium purpurogenum* GH2 is influenced by pH and temperature." *Journal of Zhejiang University Science B* 12, 12: 961-968.

Parry, D. W., P. Jenkinson, and L. McLeod, 1995. *Fusarium earblight* (scab) in small grain cereals—a review. *Plant pathology* 44, 2: 207-238.

Pandey, Ashok, W. Azmi, J. Singh, and U. C. Banerjee, 1999. Types of fermentation and factors affecting it. *Biotechnology: Food Fermentation* 1: 383-425.

Rai, Harpreet Singh, et al., 2005. Removal of dyes from the effluent of textile and dyestuff manufacturing industry: a review of emerging techniques with reference to biological treatment. *Critical reviews in environmental science and technology* 35, 3: 219-238.

Shirata, Akira, Takanori Tsukamoto, Hiroe Yasui, Tamako Hata, Shoji Hayasaka, Atushi Kojima, and Hiroshi Kato, 2000. Isolation of bacteria producing bluish-purple pigment and use for dyeing. *Japan Agricultural Research Quarterly* 34, 2: 131-140.

Stafsnes, Marit H., et al. 2010. Isolation and characterization of marine pigmented bacteria from Norwegian coastal waters and screening for carotenoids with UVA-blue light absorbing properties. *The Journal of Microbiology* 48, 1: 16-23.

Stahmann, K-P., J. L. Revuelta, and H. Seulberger, 2000. Three biotechnical processes using *Ashbyagos sypii*, *Candida famata*, or *Bacillus subtilis* compete with chemical riboflavin production. *Applied Microbiology and Biotechnology* 53,5: 509-516.

Taskin, Mesut, Turgay Sisman, Serkan Erdal, and Esabi Basaran Kurbanoglu. Use of waste chicken feathers as peptone for production of carotenoids in submerged culture of *Rhodotorulaglutinis* MT-5. 2011. *European Food Research and Technology* 233, 4: 657-665.

Venil, Chidambaram Kulandaisamy, Zainul Akmar Zakaria, and Wan Azlina Ahmad, 2013. Bacterial pigments and their applications. *Process Biochemistry* 48, 7: 1065-1079.

Velmurugan, Palanivel, et al. 2010. Natural pigment extraction from five filamentous fungi for industrial applications and dyeing of leather. *Carbohydrate Polymers* 79,2: 262-268.

Woodall, Alan A., George Britton, and Malcolm J. Jackson, 1997. Carotenoids and protection of phospholipids in solution or in liposomes against oxidation by peroxyl radicals: relationship between carotenoid structure and protective ability. *Biochimicaet BiophysicaActa (BBA)-General Subjects* 1336, 3: 575-586.

Zaldivar, Jesus, Jens Nielsen, and Lisbeth Olsson, 2001. Fuel ethanol production from lignocellulose: a challenge for metabolic engineering and process integration. *Applied microbiology and biotechnology* 56.1-2: 17-34.

BIOETHANOL: PRODUCTION AND APPLICATION

Sudarshan Singh Lakhawat*[1] and Sunil Kumar[1]

[1]Assistant Professor, Amity Institute of Biotechnology, Kant-Kalwar, NH11C, RIICO Industrial Area, Jaipur, Rajasthan 303007, India

*Corresponding Author: sslakhawat@jpr.amity.edu

Introduction

The sudden surge in the demands of fuel caused by population expansion has led to the immediate search of alternative source of fuels (Souza et al., 2017; Chandel et al., 2020). Excessive consumption of the conventional fuels has resulted in major challenges (IRENA, 2013). While the fossil fuel reserves are inadequate and estimated to be exhausted in next 40-50 years (Li et al., 2011). The world's present economy is highly dependent on various fossil energy sources such as oil, coal, natural gas, etc. The various organisms have been used in the production of Bioethanol viz. Saccharomyces cerevisiae and others in combination of different substrates with promising results (Yadav et al., 2011,Suriyachi et al., 2013, Hanly & Henson, 2012, Hanly & Henson, 2013, Unrean & Khajeeram, 2015,) In comparison Fossil fuels are being used to produce fuel, electricity and other goods(Sarkar et al. 2012). Presently, more than 80% of the total global energy demand is obtained from fossil fuels, of which 58% alone is spent by transport sector (Escobar et al. 2009). The non-renewable fuels are being exhausted at a very fast rate in order to fulfil the increasing demand of all kinds of fossil fuels stemmed due to motorization and industrialization of the world (Agarwal & Kumar, 2007).

Growing consumption of fossil fuels contribute to the emission of the greenhouse gases and global warming subsequent in the upsurge of sea levels, loss of diversity and urban pollution (Singh et al., 2007). Political crisis has led us to an imbalance in oil demand and supply resulting in import of fossil fuels. This has led us to a re-think of our dependence on fossil fuels, since such a crisis is unsettling to the energy sector of both the developed and the developing country (Ogbonna et al., 2001). Therefore, it is essential to find out a substitute of fossil fuels for our industrial economies and consumer societies. The substitute should be renewable, efficient and cost-effective with lesser emission of greenhouse gases (Zabed et al., 2016). Gasoline and petroleum have been the dominant transportation fuel for a century with a few noticeable deleterious effects. Biofuels are extensively seen as alternative to fossil fuels to offset forthcoming decline of oil production and to mitigate the nascent increase in GHG emissions. Ethanol can be a sustainable replacement of these conventional fuels because of its ease of production and lesser toxicity. Ethanol as a fuel gained importance in the 1970's, when the cost of production dropped in line with the gasoline and oxygen addition to the fuel was required by the air quality regulations (US dry-mill ethanol industry). Ethanol production capacity has increased to 109% since 2000 and is growing at a rate of 3.5 billion gallons per year (gpy).

Despite of this, the ethanol production by volume is only 2% of gasoline and 1.7% of petroleum in US (Annual Energy Outlook, 2003). Currently, there are five fuel ethanol producers across the country, producing 1.52 million tons of fuel ethanol annually from starch-based feedstock including corn, wheat and cassava. Ethanol, also called ethyl alcohol, is an organic compound and the simplest primary alcohol. 170 million gpy of industrial ethanol is produced via synthetic route in US which represents over 60% of ethanol used for manufacturing (Bomtempo & Alves, 2014). Ethanol can either be used as a fuel or as a blend with other fuels like gasoline. Without mutilation, unmodified engines can resist up to 30% ethanol blended in gasoline, but rubber and plastic components of

engine can deteriorate over time at higher concentrations of ethanol in gasoline.

Ethanol has a low energy density when used in combustion because it is 30% oxygen by weight. Pure ethanol has an octane rating of 113 thereby increasing the horsepower and prevents knocking. Ethanol is added to fuels to add oxygen to the fuel because oxygenated fuel burns cleaner, producing lesser carbon mono oxide and particulates. Pipelines cannot be used for ethanol distribution because ethanol, being hydroscopic, can get contaminated with latent water. To avoid this problem, ethanol is splash blended in tankers before transport to service stations (Hodge, 2002).

Ethanol has been shown to have potential of reducing many air pollutants like particulates and benzene (Whitten & Smog, 2004). Ethanol in blend with gasoline is traded at two different grades: E10 and E85. The former, E10, is required at locations where oxygenated fuel is needed and sold as reformulated gasoline. It has been considered to be ozone neutral because the fuel reduces carbon mono oxide emissions. E85 is 85% ethanol in gasoline and this can be used in "Flexible Fuel Vehicles" (FFVs) (Brodt, 2006). E85 blend is hard to find and hence most FFVs run on 0 to 10% ethanol blends without any damage. Ethanol has many advantages as a fuel over other conventional fuels and its production is sustainable at levels that demand widespread adoption. Bioethanol cannot be simply regarded as a renewable source of energy but many thermodynamic analyses has provided a comprehensive indication of energy related sustainability of the biofuel techno-system (Liao et al., 2011).

FEEDSTOCKS FOR ETHANOL PRODUCTION

The process of ethanol production was fully explained till the mid-18th century, however, the method of fermentation and distillation to produce ethanol has been used for thousands of years. The process of production of ethanol by biological route at industrial scale have been optimized in decades of research.

Anaerobic breakdown of sugars by yeast or bacteria to produce ethanol and carbon dioxide is called fermentation and represented by the reaction:

$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$ ..... (1) (ΔG° = -227 kcal/mol)

Fermentation must proceed in the lack of oxygen because the respiration of glucose releases much more energy and hence preferred by the organism.

$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ H_2O + 6\ CO_2$ ..... (2) (ΔG° = -686 kcal/mol)

The form of energy that is used by a cell is ATP (Adenosine Triphosphate), and only 15 kcal of energy released during fermentation is used by the cell as ATP (showing an efficiency of 6.6% as compared to 39.4% in respiration). Ethanol is produced by fermentation of sugars which can be obtained from biomass. Feed-stocks containing carbohydrates can be broken down into free fermentable sugars for the production of ethanol. These feedstocks can be divided into 3 major groups: Sugar (containing sugar crops, like sugarcane, and by-products of sugar refineries), Starchy crops (like corn) and Lignocellulosic biomass (2nd generation ethanol). The abovementioned groups of feedstocks differ from each other significantly in terms of retrieval of sugar solutions (Zabed et al., 2016).

OVERALL PROCESS FOR BIOETHANOL PRODUCTION

Ethanol production is a multi-step process that depends on the raw material being used for the production. The three major steps involved in the production of ethanol can be summarized as follows:

1. Obtainment of fermentable sugars in a solution.
2. Fermentation – production of ethanol from sugars.
3. Purification of the ethanol obtained.

One or more of the above-mentioned steps can be combined in the process of ethanol production depending upon the type of raw

material (feedstock) being used and the technology for conversion. Biomass is stored in warehouses in the ethanol plant where it is conditioned so that bacterial contamination can be avoided, and early fermentation does not occur. Depending on the biomass, an additional step called pretreatment is used to extract the carbohydrates and make them more accessible to fermentation. The hydrolysate (liquid obtained after the saccharification or hydrolysis of the carbohydrates and containing the sugars) is subjected to fermentation along with the yeast and other nutrients for the growth of yeast.

The mode of fermentation can be either batch, fed-batch or continuous (in a very few cases). The cell densities can be made high by recycling or immobilizing the yeasts in order to enhance the fermentation productivity and make the process of ethanol production efficient (Wyman, 2004).

Depending on the species of the yeast, composition of hydrolysate, cell density and other parameters, the fermentation lasts from 6 h to 72 h at temperatures between 25.8 to 30.8 degrees Celsius. At higher concentrations of ethanol (above 8-14% in broth) the activity of the yeast is inhibited. The last step after the production is distillation which is used to purify and concentrate the ethanol. In this step, anhydrous ethanol containing 99.6% alcohol and 0.4% water is obtained from hydrated azeotropic mixture of 95.5% alcohol and 4.5% water. The remaining flow from the distillation is called vinasse, or stillage. The co-products obtained from the column may include products for feeding animals, fertilizers, etc. (Walker & Graeme, 2010).

Sugar can be directly extracted from sugarcane, and the residual bagasse is used as a boiler fuel to provide much of the energy for the extraction and ethanol production and recovery operations. In a corn dry mill, corn is ground, and enzymes and heat are added to

hydrolyze starch to sugars for conversion to ethanol, while the oil, protein, and fiber in corn are recovered after fermentation as an animal feed known as DDGS. Wet mills first fractionate corn to separate corn oil, corn gluten meal (CGM), and corn gluten feed (CGF) to capture value for food and animal feed, and the starch can then be hydrolyzed to sugars for fermentation to ethanol. For cellulosic biomass, heat and acids or enzymes hydrolyze the hemicellulose and cellulose portions to release sugars that can be fermented to ethanol, and the lignin and other remaining fractions can be burned to provide all the process heat and electricity for the conversion step with excess electricity left to export(Wyman, 2004).

ETHANOL FROM VARIOUS SOURCES

Ethanol is produced from raw materials that contain carbohydrates or other complex polysaccharides containing sugar. The agricultural raw material can be classified in different generations:

First Generation (1G) Bioethanol

When a raw material containing sugar (and starch) is used for ethanol production, it falls under the category of first-generation biofuel. Sugars directly yield ethanol when subjected to fermentation. First generation raw material includes sugarcane, molasses, sugar beet, and fruits. These category of biofuels does not require processes like milling, pretreatment, hydrolysis and detoxification. Starchy materials like corn, wheat, cassava, etc. are also used as a raw material for the production of 1G ethanol.

Second Generation (2G) Bioethanol

Second generation of biofuels are produced by using agricultural raw material that contains complex polysaccharides like cellulose, hemi-cellulose which can be further broken down into simple sugars thereby yielding ethanol on fermentation. For the production of 2G ethanol, the raw material needs to undergo processes like milling, pretreatment, and hydrolysis are required. Sometimes an additional

step of detoxification might be required, when a toxic substrate is fed into the bioreactors or a toxin is produced during the reaction. The step preceding fermentation process is the main difference between the production processes of different generations of ethanol from simple sugar, starch or lignocellulosic material (Mussatto et al.,2010).

ETHANOL FROM SUGARS

Main feedstocks for ethanol production from sugars are sugar cane, sugar beet and molasses (by-product of sugar mills). In Brazil, almost about 79% of the ethanol is produced from fresh sugar cane juice and the rest of it is produced by using the cane molasses (Wilkie, 2000). Presently in India, sugar cane juice is not being used for ethanol production. Instead sugar cane molasses is majorly used as a raw material (Ghosh & Ghose, 2003).

Saccharomyces cerevisiae has high capability to hydrolyze the cane sugar (sucrose) into glucose and fructose which can be further fermented to ethanol. Hence, *S. cerevisiae* is the most employed organism for ethanol fermentation. Although saccharomyces could grow under anaerobic conditions, aeration is an important factor for growth of the organism and ethanol production. Under anaerobic conditions, the salts and other compounds can pose a negative influence on the fermentation. The molasses is conditioned so as to neutralize the inhibitory effects of the medium component on fermentation. The fermentation media should be supplemented with nutritional factors that promote the growth of yeast. The conversion of non-fermentable substances into assimilable compounds for improving the alcoholic fermentation can be achieved with the addition of some commercial enzymatic complex of amylases, cellulases and amylo-pectinases. On the other hand, according to Maye (Maye et al., 1996), addition of a minimum inhibitory concentration of hop acids to molasses will stop bacteria growth and avoid the need of antibiotics along with the enhanced yield of ethanol.

APPLICATIONS OF BIOETHANOL

The major application of Bioethanol is its use as a fuel for running vehicles. The few developed countries viz. Germany and Brazil which are coincidentally the major producers of Corn and Sugarcane respectively are using bioethanol only as a fuel for running hundred percent of cars. The India is also focusing on the utilization of Bioethanol as a fuel but following the limitations of conventional five stroke and four stroke engines, currently blending of petrol with 10 percent of bioethanol is preferred. Recently Punjab and Maharashtra states has seen rise in the blending of bioethanol and petrol as a good alternative for running the vehicles. In future the Bioethanol would be an obvious fuel in comparison to conventional fuels.

REFERENCES

Agarwal, Avinash Kumar. 2007. 'Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines', *Progress in Energy and Combustion Science*, 33: 233-271.

Bomtempo, J.V. and Alves, F.C., 2014. Innovation dynamics in the biobased industry. *Chemical and Biological Technologies in Agriculture*, *1*(1), p.19.

Brodt-Giles, D. *Clean Cities and Alternative Fuels Data Center Quarterly Report: 1stQuarter FY 2006 (Milestone Report)*. No. NREL/MP-540-39539. National Renewable Energy Laboratory (NREL), Golden, CO., 2006.

Chandel, A.K., Garlapati, V.K., Jeevan Kumar, S., Hans, M., Singh, A.K., Kumar, S., 2020. The role of renewable chemicals and biofuels in building a bioeconomy. Biofuels, Bioprod. Biorefin. 14(4), 830-844.

Escobar, José C., Electo S. Lora, Osvaldo J. Venturini, Edgar E. Yáñez, Edgar F. Castillo, and Oscar Almazan. 2009. 'Biofuels: Environment, technology and food security', *Renewable and Sustainable Energy Reviews*, 13: 1275-87.

Ghosh, P. and Ghose, T., 2003. Bioethanol in India: recent past and emerging future. *Biotechnology in India II*, pp.1-27.

Hanly, T.J., Henson, M.A., 2013. Dynamic metabolic modeling of a microaerobic yeast co-culture: predicting and optimizing ethanol production from glucose/xylose mixtures. Biotechnol. Biofuels 6.

Hanly, T.J., Urello, M., Henson, M.A., 2012. Dynamic flux balance modeling of S. cerevisiae and E. coli co-cultures for efficient consumption of glucose/xylose mixtures. Appl. Microbiol. Biotechnol. 93, 2529–2541.

Hodge, Cal. "Ethanol use in US gasoline should be banned, not expanded." *Oil & GasJournal* 100.37 (2002): 20-20.

IRENA, 2013. Road Transport: The Cost of Renewable Solutions. UAE.

Li, Y., Park, J., Shiroma, R., Tokuyasu, K., 2011. Bioethanol Production from Rice Straw by a Sequential Use of Saccharomyces cerevisiae and Pichia stipitis with Heat Inactivation of Saccharomyces cerevisiae Cells Prior to Xylose Fermentation. J. Biosci. Bioeng. 111, 682–686.

Liao, Wenjie, Reinout Heijungs, and Gjalt Huppes. 2011. 'Is bioethanol a sustainable energy source? An energy-, exergy-, and energy-based thermodynamic system analysis", *Renewable Energy*, 36: 3479-87.

Maye, J.P. and Weis, S.W., Maye, John P., Weis and Scot W., 1996. *Solid salts of hopacids*. U.S. Patent 5,583,262.

Mussatto, Solange I., Giuliano Dragone, Pedro M. R. Guimarães, João Paulo A. Silva, Lívia M. Carneiro, Inês C. Roberto, António Vicente, Lucília Domingues, and José A. Teixeira. 2010. 'Technological trends, global market, and challenges of bio-ethanol production', *Biotechnology Advances*, 28: 817-30.

Ogbonna, J.C., Mashima, H. and Tanaka, H., 2001. Scale up of fuel ethanol production from sugar beet juice using loofa sponge immobilized bioreactor. *Bioresource Technology*, *76*(1), pp.1-8.

Sarkar, Nibedita, Sumanta Kumar Ghosh, Satarupa Bannerjee, and Kaustav Aikat. 2012. 'Bioethanol production from agricultural wastes: An overview', *Renewable Energy*, 37: 19-27.

Singh, Anoop, Deepak Pant, Nicholas E. Korres, Abdul-Sattar Nizami, Shiv Prasad, and Jerry D. Murphy. 2010. 'Key issues in life cycle assessment of ethanol production from lignocellulosic biomass: Challenges and perspectives', *Bioresource Technology*, 101: 5003-12.

Souza, G.M., Ballester, M.V.R., de Brito Cruz, C.H., Chum, H., Dale, B., Dale, V.H., Fernandes, E.C., Foust, T., Karp, A., Lynd, L., 2017. The role of bioenergy in a climate-changing world. Environ. Dev. 23, 57-64.

Suriyachi, N., Weerasaia, K., Laosiripojana, N., Champreda, V., Unrean, P., 2013. Optimized simultaneous saccharification and co-fermentation of rice straw for ethanol production by Saccharomyces cerevisiae and Scheffersomyces stipitis co-culture using design of experiments. Bioresour. Technol. 142, 171–178.

The US dry-mill ethanol industry. Biobased products and Bioenergy Initiative Success Stories. DoE, U. S. "Annual Energy Outlook, 2003." *US Department of Energy* (September 2002).

Unrean, P., Khajeeram, S., 2015. Model-based Optimization of Scheffersomyces stipitis and Saccharomyces cerevisiae Co-culture for Efficient Lignocellulosic Ethanol Production. Bioresour. Bioprocess. 2, 1–11.

Walker, Graeme M. *Bioethanol: Science and technology of fuel alcohol*. 2010. 1st Edition: 32-56. ISBN 978-87-7681-681-0.

Whitten, Gary Z., and Smog Reyes. "Air quality and ethanol in gasoline." *9th AnnualNational Ethanol Conference: Policy & Marketing*. 2004.

Wilkie, A.C., Riedesel, K.J. and Owens, J.M., 2000. Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks. *Biomass and Bioenergy*, *19*(2), pp.63-102.

Wyman, Charles E. "Ethanol fuel." *Encyclopedia of energy* 2 (2004): 541-555.

Yadav, K.S., Naseeruddin, S., Prashanthi, G.S., Sateesh, L., Rao, L.V., 2011. Bioethanol fermentation of concentrated rice straw hydrolysate using co-culture of Saccharomyces cerevisiae and Pichia stipitis. Bioresour. Technol. 102, 6473–6478.

Zabed, H., G. Faruq, J. N. Sahu, A. N. Boyce, and P. Ganesan. 2016. 'A comparative study on normal and high sugary corn genotypes for evaluating enzyme consumption during dry-grind ethanol production', *Chemical Engineering Journal*, 287: 691-703.

BIOTECHNOLOGICAL APPROACHES FOR SUSTAINABILITY OF HANDMADE PAPER INDUSTRIES

Shweta Kulshreshtha*

Associate Professor, Amity Institute of Biotechnology, Amity University Rajasthan, NH 11C, RIICO industrial area, Kant Kalwar, Jaipur -303006

*Corresponding Author: skulshrestha@jpr.amity.edu

INTRODUCTION

With the development of human civilization, the different commodities were also modified according to increase demand and maintain sustainability. Similar developments can be seen in the development of paper. The most ancient form of the paper called "papyrus" and developed by Egyptians, Greeks and Romans. Later, paper from wood was developed by China and the art of making paper spread in the whole world. Now, in the whole world there are many paper industries which are fulfilling the demand of paper as paper is an important commodity in every field like knowledge or information transfer, official work, education purpose. Here, in this chapter, the focus is on handmade paper and the waste generated by these industries. We have also focused on biotechnological approaches to make handmade paper and treat the waste generated by them.

Handmade paper is an especial type of paper made from agrowastes and cotton or linen clothes. The cuttings from linen and cotton clothes industries sale their waste to these industries for making paper. Therefore, these are eco-friendly industries which not only recycle the waste cotton or linen cuttings but also produce useful products. The type of handmade paper and paper products which are in general demand in Indian and foreign markets are water colour

drawing paper, Bond paper, Decorative and fancy paper, Unsized mottled art paper, Gift wrapping paper, Marble paper and blended fibre paper, insulation paper and filter paper (Saakshi et al., 2015). Perfume boxes and carry bags, Invitation cards, Greeting cards, Hotel menu cards etc., Stationary items like pads, envelops, price tags, diary, note books, table covers, wall posters etc (Figure 1.2).

Handmade paper industries are following traditional art of making paper using hand or simple machineries which is still found in many parts of India. The quality of the paper is superior to the other types of paper as it lacks lignin which makes the paper yellow and weak after some time. Handmade paper has greater tensile, bursting, tearing and double fold strength compared to mill made paper (paper from wood). Therefore, it is an excellent material for writing as well as printing. According to KVIC experiences "Handmade paper product is a real treasure with full of creative, innovative and thought provoking ideas and the industry got an excellent future because of value addition and export oriented prospective".

It is difficult to remove completely from the wood based paper, however; it is not a problem with handmade paper. It is lignin free and remains white for a longer time and maintains its strength and therefore, it is good to preserve important literature on this paper. Handmade paper is also known for its beautiful products. These industries are now known as commercial venture due to the growing demand in international market and are the source of foreign currency.

This craft of handmade paper making spread in India in 11th Century and thereafter, it passed through generations to generations (Hubbe, 2009; Saakshi et al., 2015). These artisans are known as "Kagzis" and this term is derived from the word "Kagaz" which means paper. This craft is still present in many part of India. One of the major hubs of these Kagzis is in Jaipur, Rajasthan, India.

Handmade paper industries are now having 2,970 units in India which are producing over 54.57 crore worth of handmade paper and its products. These are also providing employment to more than 7,000 to 10,000 persons (Singh and Chauhan, 2000). Handmade

paper is produced due to recycling of waste rags, and paper cuttings and thought to be free from pollution. There are many reasons to support its eco-friendly nature like non-utilization of wood for its manufacturing, no use of chemicals and use of sunlight for its drying, and its biodegradability.

Eco-friendly handmade paper

Handmade paper is 100% lignin free, wood free, chemical free, cellulose-rich paper which makes it eco-friendly. However, modification in the paper quality and paper making process leads to the use of glazing agents, chemicals, and dyes which can put a question mark on the sustainable technology of making paper.
462 Bamboo or 277 Eucalyptus trees are required for making 1 ton of paper. However, 1 lac greeting cards on handmade paper can save approximately 500 trees. Nowadays, many new upgrades are going on in the technology of making paper. New and different pulp and paper fibres are researched for paper making technology more viable and environmentally sustainable.

National and International Status of Handmade paper

In India, handmade paper is not very popular due to high making cost. However, it is having a great popularity in International market due to its eco-friendly nature. Indian handmade paper industries are fulfilling international demand of handmade paper and its products.

Though the share of handmade paper industry in the national production of paper and board is insignificant, the case of handmade paper stands out as having vast potentials, especially suited for Indian economy, for example-

1. Handmade paper is the best technology for rural area as agricultural waste can also converted into handmade through biopulping process. Agricultural wastes will be evergreen material for making handmade paper.
2. In the paper manufacturing process, simple machineries and tools are used. There is no use of sophisticated equipments or process automation for making handmade paper.

3. It is based on decentralized and micro and cottage small scale production. It can cater demand of varieties of paper and can easily switch to make handmade paper.
4. It provides employment to more people with less investment as compared to the industries in the medium and large scale sector. This is beneficial for the rural people for providing them employment and foreign currency.
5. As paper is made by using few types of machinery, its energy requirement for making is relatively lower than the mill made paper.
6. It is eco-friendly because it is not only removing the waste from the environment but also producing useful marketable product.

The Indian handmade paper industry produces a variety of paper and paper products mainly by using waste materials such as cotton rags, tailor cuttings, hosiery cuttings and small quantities of waste paper. Other agro and bast fibres available in the North Eastern region like jute, sabai grass, banana, straw etc. are also used to blend with the primary fibres for mottling effects and to manufacture special varieties of thin paper. Availability of raw materials and existing infrastructural facilities offer good scope for development of special varieties of handmade paper in the North East. It has also an added advantage to earn from world market. In India, Khadi and Village Industrial Commission (KVIC) has undertaken the project of handmade paper industries to strengthen these industries. The aim of this centre is to improve the quality and marketability of handmade paper by developing and transferring the technology. It also facilitates the participation of women in rural based industries and contributes to social development. It also provides consultancy services and training courses.

Handmade paper making technology

Raw materials and their sources

Handmade paper technology relies on the availability of wastes from different industries. For eg. cotton and hosiery rags from textile industries and different types of straws, jute, bagasse, cotton stalks, grasses from agro-industrial sectors. Their regular supply to

the industries in abundant amount is mentioned by several bulk sellers.

Equipments / tools used for handmade paper making

According to the type of waste raw material for making handmade paper, different equipments are required such as rag chopper, beaters, pulp tanks, calendar machine, agitator, hydraulic press, cylinder mould, vat power drives machine, Iron box etc. The different raw materials are processed in different ways to make paper. For instance, agricultural waste or fibers can be converted into paper however, requires different processing approach from the cotton or linen rags processing. The reason is cotton or linen rags do not possess lignin, however, it is present in agricultural waste. Therefore, there is need of different processing approaches, machines and tools. These tools, machines and equipments are available in India and can be easily procured for establishing new industry.

Methodology and process of manufacturing Handmade Paper

Raw material for making paper is collected and examined for its purity. The mixed fibres are sorted prior to use. Raw materials are sorted out to remove any non-fibrous, mixed fibrous content and for any artefacts or debris.

Cutting

Fibrous raw material is cut into small pieces using chaff cutter for further processing.

Dusting/ washing

Dust and dirt may interfere with the making of uniform sheet and therefore, need to be removed. After cutting the raw material, pieces are dusted and washed to remove dust and dirt particles. The dusting

can be done by mechanical duster or by beating the material by hand.

Digestion

The agricultural wastes need to be cooked with caustic soda in the steel vessel for the removal of lignin. Thereafter, pulp is washed thoroughly for the removal of traces of alkali and dissolved content of fibres. In contrast, this step is not required for the production of paper from cotton or linen rags.

Pulping

After cutting or chopping of rags, these can be used directly for making pulp. However, pulp of agro-wastes need to be pre-treated in the aforesaid ways for the removal of lignin. There are several ways of pulping i.e. biopulping, chemical pulping, and enzymatic pulping. Bio-pulping is based on the use of microbes for pulping process. In enzymatic pulping, microbial enzymes are used for pulping process while in chemical pulping chemicals are used for pulping process. After making pulp, desired colour, rosin or alum can be added to the pulp.

Vat processing

The pulp mixture is spread on the net frame to drain the water from the pulp and binding the pulp fibres together to form the sheet. It is pressed tightly on the layer of the sheet on muslin cloth. These sheets are collected in the form of the heap.

Cylinder Mould Processing

After forming the pulp, it is spread on the wire mesh to form the sheet. However, in this case machine is used for spreading purpose. Thereafter, the spread sheet is transferred to rotating drum where it takes the form of smooth sheet.

Sheet separation and sunlight drying

When a pile of quite good number of sheets is made, it is put in hydraulic press for removing excessive water. The muslin cloth, present in between two sheets, and sheets are separated out and allowed to dry in the sunlight.

Calendering

After sun-drying process, sheets are passed through the press to make smooth sheets. As per the demand, coating with starch, polishing with glazed chemicals can be done.

Cutting

This is last step of making marketable paper. The sheets are cut to make smooth margins and in the required size. The left over trimmed cuttings are called as mill broke. These cuttings can be recycled again in the same industry.

BIOTECHNOLOGICAL APPROACHES FOR HANDMADE PAPER INDUSTRIES

BIOBLEACHING

Pulp can be bleached by using microbes or their enzymes and this process is called biobleaching. Banana pulp was bleached using xylanase enzyme for making handmade paper which saved 25% of the bleach chemical requirement, cost and provided a good quality pulp (Chauhan and Pant, 2005). There are many microbial sources of xylanases like *Rhodothermus marinus* (Abou Hachem et al., 2000), *Cellulomonas Xavigena* (Alejandro et al., 2007), *Aspergillus versicolor* (Andrade et al., 2004) *etc*. Paper mulberry (*Broussentia papyrifera*) pulp was treated with laccase enzyme for biobleaching and results revealed the increase in brightness up to 10 points (Chauhan et al., 2016). *Bacillus coagulans* was used for biopulping of wheat and rice straw pulp and was reported to provide brightness at pH 8.5 in the range of 4-5 points compared to the control pulp.

BIOPULPING

Biopulping is the fungal pretreatment of agricultural waste prior to make pulp (El Enshasy et al., 2016). This step is not required for making paper from pure cotton or linen rags. In contrast to this, handmade paper developed from agricultural wastes, requires the removal of lignin from the pulp. As mentioned earlier, there is the method of chemical pulping for making pulp from agricultural fibres however, biopulping through microbes and enzymatic pulping through microbial enzymes are preferable methods. In an experiment, *Calotrophis procera* was used as raw material for making handmade paper which was treated with *Schizophyllum commune*, *Perenniporia tephropora* and *Ganoderma lucidum* (MTCC 1039) for deriving biopulp. It was reported that *S.commune* reduced the lignin content to a level of 4.34% from 12.98% which is a great decrease without any adverse effect on cellulosic content of pulp. It was suitable for making biopulping for handmade paper industries (Aswal et al., 2020). Banana pulp was treated with *Trichoderma* sp. and *Pythium* sp. for 3 to 5 days in order to obtain biopulp (Muraleedharan and Perumal, 2010). When oil palm empty fruit bunches were treated with *Marasmius* sp., lignin removed up to 35.94% along with improvement in paper properties (Risdianto and Sugesty, 2015). Banana pulp can be extracted by using pectinases (Chauhan and Sharma, 2014).

Biopulping not only saves water, energy or Chemicals but also provide a good quality paper. In search of new improved quality paper, KNHPI developed a good quality paper from the shredded currency waste. The quality of shredded currency based handmade paper was superior to handmade paper developed from office waste (Chauhan et al., 2009).

Bioretting or Enzymatic retting

Bioretting is the process to treat the plant parts or stems in order to obtain good quality pulp fibres. It is an important process for handmade paper industries to cope up with the increasing demand of

cotton rags. There are many methods of retting. Traditional way of water retting depends on the use of anaerobic bacteria like *Clostridium* spp. Another type of retting is dew-retting which is based on the use of plant degrading aerobic fungi. This is used to degrade flax stems however, it results in the low and inconsistent quality and not preferred. Nowadays, enzymatic retting is emerging as a new method for making a better quality fiber (Bajpai, 2012).

The efforts of bast fibres extraction from *Calotropis procera* using pectinases was done at Kumarrappa National Handmade Paper Institute. The extracted fibres have potential of replacing the demand of cotton or linen rags (Chauhan et al. 2013). Further, it reduced the time to extract more fibres by using enzymes as compare to the conventional retting. The quality of fibre is also equivalent to that of banana fibre. Moreover, blending of fibres with the banana fibres increases the strength. It also provides the benefit of reduced water use and enzyme recyclability. (Jain et al. 2009; Chauhan et al., 2013).

Biotechnology in waste treatment & Role of microbes in effluent treatment

Pulp and paper making processes of handmade paper industries are water intensive processes which discharges huge amount of effluent to the drainage system. Generally, cotton pulp based industries discharges coloured effluent to the drain. In contrast to this, effluent released from agricultural industries is coloured and toxic due to use of chemicals. When this effluent mixed with other effluents, it becomes toxic and untreatable. Therefore, it is of utmost importance that water must be treated at industrial level. The effluent of handmade paper industries was reported to possess genotoxicity (Kulshreshtha et al. 2011). In a study, Chauhan et al. (2015) reported the method of isolation and purification of white rot fungal culture for the effluent treatment. These white rot fungi decolourized the effluent significantly. In another study, Kulshreshtha et al., (2012) reported the use of white rot fungi in dye decolourization and mutagenicity reduction in the absence or presence of nutrients. The study reported the best dye decolourization and mutagenicity

reduction efficiency in *Phanerochaete chrysosporium* without nutrient supplementation.

Role of microbes in solid waste treatment

The unused fibres from the pulping machine released with the effluent and accumulate in the drain and block the drain. These fibres can be collected and used for the cultivation of mushroom. When waste was used for the cultivation of mushroom, satisfactory mushroom bodies were not obtained. However, the combination of waste in 1:1 ration with Wheat straw provided the best results in terms of yield, growth, biological efficiency (Kulshreshtha et al. 2010; Kulshreshtha et al. 2013). Besides, there was no mutagenicity in the fruiting bodies. This revealed the successful use of mushroom for solid waste treatment.

Conclusion

Handmade paper industries are eco-friendly industries based on the recycling of cotton and linen cloth fibres; and agricultural residues. The pulping and bleaching of fibres can be made more eco-friendly by using microbe or their enzymes. Further, waste can be treated by using different fungi in ecofriendly way. Therefore, the ecofriendly credentials of handmade paper industries can be maintained by biotechnological approaches.

References

Abou Hachem, M., Karlsson, E.N., Bartonek-Roxa, E., Raghothama, S., Simpson, P.J., Gilbert, H.J., Williamson, M.P., Holst, O. 2000. Carbohydrate-binding modules from a thermostable *Rhodothermus marinus* xylanase: Cloning, expression and binding studies. Biochem. J., 345, 53-60.

Alejandro, S.H., Jesús, V.E., del María, C.M.H., María, E.H.L., 2007. Purifcation and characterization of two sugarcane bagasse-absorbable thermophilic xylanases from the mesophilic *Cellulomonas Xavigena*. Microbiol. Biotechnol. 34, 331-338.

Andrade, S.V., Polizeli, M.L.T.M., Terenzi, H.F., Jorge, J.A., 2004. Effect of carbon source on the biochemical properties of β-xylosidases produced by *Aspergillus versicolor*. Process Biochem. 39, 931-938.

Aswal, S., Chauhan, S. Bhatnagar, P. 2020. Identifying efficient isolates of white rot fungi for lignin degradation of *Calotropis procera* fibre in handmade papermaking. J. Sci. Res. 64(2).

Bajpai, P., 2012. Bioretting. In: Biotechnology for Pulp and Paper Processing. Springer, Boston, MA. https://doi.org/10.1007/978-1-4614-1409-4_6.

Chauhan, S., Choudhury, B., Singh, S. N., Ghosh, P. (2006). Application of xylanase enzyme of *Bacillus coagulans* as a prebleaching agent on non-woody pulps. Process Biochem., 41(1), 226-231. doi:10.1016/j.procbio.2005.06.003.

Chauhan, S., Khan, M.E., Sharma, A.K., Jain, R.K., Hussain, G. 2009. Cost-effective production of handmade paper through recycling of shredded currency waste of reserve Bank of India-An enzymatic Route. IPPTA J. 21(3), 11-117. 2009.

Chauhan, S., Medicherla, K.M., Bhatnagar, P., 2016. Biobleaching of paper mulberry (*Broussentia papyrifera*) pulp using laccase mediator system. Int. J. Adv. Biotechnol. Res. 7 (4), 2067-2077.

Chauhan, S., Pant, R., 2005. Biobleaching: A new tool to maintain eco-friendly credentials of handmade paper industry, Inpaper International. 19-27.

Chauhan, S., Sharma, A.K., 2014. Utilization of pectinases for fiber extraction from banana plant's waste. Int. J. water Resources 4(162), 2

Chauhan, S., Sharma, A.K., Jain, R.K., 2013. Enzymatic Retting: A revolution in the handmade papermaking from *Calotropis procera*. In book: Biotechnology for Environmental Management and Resource Recovery. doi: 10.1007/978-81-322-0876-1_5.

El Enshasy, H.A., Kandiyil, S.K., Malek, R., Othman, N.Z., 2016. Microbial xylanases: sources, types, and their applications. In: Gupta V. (eds) Microbial enzymes in bioconversions of biomass. Biofuel and Biorefinery Technologies, vol 3. Springer, Cham. https://doi.org/10.1007/978-3-319-43679-1_7.

Hubbe, M., 2009. A peep into handmade paper industry. Bioresources 4(4), DOI: 10.15376/biores.4.4.1736-1792. IPPTA J., 12(4), 67-76.

Jain, R.K., Sharma A.K., Chauhan, S. 2009. Bioretting- in the context of handmade paper industry. In book: 'Handmade Paper: From Rags to Riches. Das L. (ed.) Bhartiya Gramodyog Mahasangh and Concept Publishing Company, New Delhi.

Kulshreshtha, S., Mathur, N., Bhatnagar, P. 2012. Aerobic treatment of handmade paper industrial effluents by white rot fungi. J Bioremed. Biodeg. 3, 151. doi: 10.4172/2155-6199.1000151.

Kulshreshtha, S., Mathur, N., Bhatnagar, P., 2011. Pros and cons of *P.florida* cultivation for managing waste of handmade paper and cardboard industries. The IIOAB Journal, spl. Issue, 2(1), 45-48.

Kulshreshtha, S., Mathur, N., Bhatnagar, P., Jain, B.L., 2010. Bioremediation of industrial wastes through mushroom cultivation. J. Environ. Biol. 31, 441-444.

Kulshreshtha, S., Mathur, N., Bhatnagar, P., Kulshreshtha, S. 2013. Cultivation of *Pleurotus citrinopileatus* on handmade paper and cardboard industrial wastes. Industrial crops and products, 41: 340-346.

Muraleedharan H. and Perumal K. 2010. Booklet on Eco-friendly handmade paper making, accessed from Eco-friendlyhandmade. pdf (amm-mcrc.org) on 17th Dec 2020.

Risdianto, H., Sugesty, S. 2015. Pretreatment of *Marasmius* sp. on biopulping of oil palm empty fruit bunches. Mod. Appl. Sci. 2015; 9:1.

Saakshy, Sunita, Khan, M.E., Kumar, A., Sharma, A.K., 2015. Potential of industrial grades of handmade paper- insulation paper & filter paper. Paperex, paper and products souvenir.

Singh, S.N., Chauhan, S., 2000. Handmade paper industry in the context of green, clean and closed loop system. Journal of the Indian Pulp and Paper Technical Association. 12(4), 67-76

MICROBIAL ENZYME ENGINEERING: METHODS AND APPLICATIONS

Veerendra Singh Nagoria[1*], Pradeep Kumar Singh[2]

[1, 2] Assistant Professor, School of Life Sciences, Rai University, Ahmedabad, Gujarat

[*]Corresponding Author: veerendra.nagoria@gmail.com

Introduction

Microbial enzymes as a metabolic catalyst have been crucial in the development of various industries. At present, a lot of product are being commercially produced using enzymes. The enzymes have been preferred over chemical for catalysis as they are sustainable and efficient. The term Enzyme got first mention by the German Physiologist Professor Wilhelm Kűhne while describing the production of alcohol from sugar using Yeast in 1878. Though the term was coined in 1878, the use of microorganism and cell free extracts was well known to the mankind since centuries. Initially enzymes were linked to specific reactions of interest such as brewing, bread making etc.

It has been reported that microbial enzymes are currently being used to produce more than 500 industrial products. (Gurung, Ray, Bose & Rai, 2013) Some of the Industrial application of enzymes are listed in Table 1. (Ray, Pramanik & Bera, 2016). The industrial production takes place in a very diverse conditions as reaction chemistries, substrate concentrations, product concentration, process temperature and pH in any Industrial process vary from normal physiological processes. Such variations results in reduced enzymatic stability, activity and efficiency for catalysis. As the industries now rely heavily on enzymes for products there is a constant need of microbial enzymes with novel characteristics and improved performance (Table 1). One of the important feature usually industries look for is thermo-stable enzymes with improved efficiency and biochemical properties to meet the demands of

ISBN: 978-81-950423-8-8

economic and environment friendly processes. Initially such enzymes were looked from thermophilic, mesophilic or psychrophilic organisms for industrial applications. (Krüger, Schäfers, Schröder & Antranikian, 2018). However, often enzymes obtained from such habitat was not always ideal candidate for industrial application as not all the conditions were fulfilled by such enzymes. Also, possibility of finding a novel enzyme with desired function from any new source is very less.

Table 1: Application of Microbial enzymes

Industry	Application	Enzymes
Animal feedstock	Fortification of nutrients	Phytase
Beverages	Degradation of Starch and Proteins into Sugar	Amylase, glucanase, protease, acetolactate decarboxylase
Bioremediation	Degradation of toxic pesticides and pollutants	Hydrolases, oxidoreductases
Dairy	Cheese production, protein hydrolysis	Lipase, lactase, renin
Detergents and cleaning industry	Stain removal	Proteases, lipases, amylases
Food Industry	Starch degradation, protein degradation, Meat tenderising	Amylases, proteases, and papain
Fruit Juices	Clarify fruit juice	Cellulases, pectinases
Paper Industry	Pulp bleaching	Xylanases

As the existing methods pose limitations in finding a suitable enzyme for industrial applications, Scientists begin to look for improving the existing enzymes. Various strategies have been put forward by scientists to generate suitable enzyme having a higher performance compared to the existing variant. (Adrio & Demain, 2014). Protein engineering by modifying existing gene sequence or through synthetic gene sequence, is a promising technique for the

design and development of proteins with the desired properties. In this chapter we will discuss briefly the available tools of protein engineering. It will help us to understand the ongoing research and recent technological development in the field of protein engineering. (Kolmar, 2019) (Glover, Xu & Clark, 2019).

Protein Engineering Methods

In general the major strategies employed in protein engineering includes rational design, directed evolution and *De novo* design. The methods listed here vary with respect to practical requirements and depends mainly on structural consideration and selection method or screening system. In many of the cases the methods are not exclusive in nature and are being employed in tandem to achieve the desired modification in a gene sequence in order to obtain a protein with desired functional capacity.

Directed evolution

Directed evolution is one of the most basic, simple yet complicated method used for engineering the existing protein. The method was employed initially to modify subtilisin E. Random mutagenesis was employed to improve the stability and activity of enzyme in polar organic media (Chen and Arnold, 1991). As the name suggests, directed evolution mimics natural evolution. Natural evolution is very slow process of mutations, genetic rearrangements and modifications selected over a long period of time. Directed evolution employs the similar principal of generating mutations followed by multiple round of screening and selection of desired mutants. Repeated round of screening and selection makes it slow process while the methods available for generating mutations results in creation of large no. of libraries for screening and selection, thereby requiring high throughput techniques for improving screening. One of the major advantage of the method lies in its ability to work on those proteins as well, who have been either discovered recently, having *De novo* design or for whom structural or sequential information is not available. Directed evolution relies on its ability of random mutagenesis.

The various methods available for random generation of mutation can be categorised into two categories as recombinant and non-recombinant strategies. Recombination based random mutagenesis strategies include method based on DNA shuffling approach. The recombination based approach includes Random fragmentation and reassembly (Stemmer, 1994), Random recombination based on combinatorial method of overlapping gene fragments Incremental Truncation for the Creation of Hybrid enzYmes (ITCHY) (Ostermeier, Nixon, Shim & Benkovic, 1999), *in vitro* recombination method RAndom CHImeragenesis on Transient Templates" (RACHITT) (Coco et al., 2001), Degenerate oligonucleotide gene shuffling (DOGS) (Gibbs, Nevalainen & Bergquist, 2001) and Design based fragment shuffling (Bei et al., 2009). Non-Recombination based strategies includes method based on PCR including Error Prone PCR (Leung et al., 1989, (Cadwell & Joyce, 1992, 1994), Mutagenic Oligonucleotide directed – PCR (Mod-PCR) (Chiang, Kovari & Howe, 1993) and Sequence Saturation Mutagenesis (Wong et al., 2004). Comparing the available methods and selecting the best among them is not going to be an easy task. Development in the protein engineering could improve the rate and cost effectiveness of mutation generation.

We have seen that in order to obtain the desired level of enzyme function, repeated evolution process needs to be completed and a large scale screening need to be carried out for selecting the desired mutants having required set of function. To help understand the complexity and screening requirement, let us take an example of protein of 100 amino acid.

Rational design

Rational design as the name suggests the method utilises rational thinking using the available set of data about protein, its structure, active sites, homologous counterparts from extremophiles and a proposed change of amino acid for obtaining desired engineered variant with improved functionality, stability or activity. Firstly the method relies on our existing knowledge of sequence and structure and has been greatly supported by the improved method in studying

protein structure, development of Databases, improvements in available bioinformatics methods and use of artificial intelligence and machine learning approach in designing proteins and Secondly the method has been immensely contributed by the development in techniques to introduce specific mutation in gene sequences like Site Specific Mutagenesis to CRISPR-Cas9 . Rational design has been the earliest method of development of protein engineering. (Llorens et al. 1980). The three major underlying conditions on which the rational design works include (1) amino acid sequence, (2) Three dimensional (3D) structure, and (3) catalytic mechanism of the protein. A total of 172650 structure information is available in Protein Data Bank database, providing a lot of data to start with for rational design of proteins. *In silico* methods for integration of structural information, catalytic function and available functional data are used to predict the possible sites of modification thereby reducing the number of variants for further screening and selection using focused approach. (Li, Zhang & Song, 2012). Some of the *in silico* approach includes (1) Working with Evolutionary information available and using Multiple Sequence Alignment to focus on conserved sequence to finding out active sites and Homology modelling to look for genes with similar catalytic activities. (Pei & Grishin, 2013) (2) Screening enzyme design alternative using statistics based data driven approach. The method can take leverage of existing information about sequence information, structure related information and utilising expanding information from scoring function and combinatorial search tools. Various software tools have been developed to work on this principle including Rosetta Design (Liu & Kuhlman, 2006), Tinker (Rackers et al., 2018) and IPRO. (Pantazes, Grisewood, Li, Gifford & Maranas, 2014). (3) One of the approach which is based on *in silico* approach includes structure based data to screen variants for protein engineering. The method is based on existing protein being converted to binary profiles and collecting data about different parameters like dihedral angle, orientation of amino acid, arrangement of amino acids– hydrophobic and hydrophilic, solvent exposure etc. The method uses Interaction and overall stability score to design proteins on Enzyme substrate affinity and modelling conditions. The approach has been widely

utilised as the computational tools have been developed to work on these parameters. Some of the tools developed to work on such data includes Amber, CHARMM and GROMOS etc. (Li, Zhang & Song, 2012) (Chowdhury & Maranas, 2019).

De novo Design

De novo design as the name suggests is the method of designing a protein which is non-existent in nature. It relies on all the information available related to protein from sequence, to structure, to catalysis and the exponential growth in Computational techniques, database and development of tools based on databases, Artificial intelligence and Machine learning to application of designing a protein from scratch. It does not rely only on the information available with respect to protein but also depends on the advancements in the field of DNA technologies. As the protein sequence is being predicted using available resources, it become interestingly important to create a DNA sequence which will eventually code for such protein sequence and fold into the required conformation to achieve the desired function. The increasing capabilities in computing and synthetic biology is immensely contributing the development of protein design and helping in *De novo* design of proteins.

De novo design has been put into practice since 1970s. It was Gutte, who initially designed the half-length variant of bovine ribonuclease in 1975 (Gutte, 1975). Later in 1979, taking clues from secondary structure prediction rules and model building, a 34-residue polypeptide was produced (Gutte, Däumigen & Wittschieber, 1979). The initial experiments paved the way to look beyond introduction of modification to existing enzymes to look for one created artificially using available data set. Various innovative studies for artificial, novel functional *De novo* designed enzymes have been conducted, taking enzyme stability, folding and activity into account. The approaches involve assembling short peptides fragments forming backbone, using mathematical equations to specify the geometry etc. Further, such conformation need to be tested for identification of one with lowest-energy sequence and

further subjected to check using *Ab initio* structure prediction to check for structure for the lowest energy state of the sequence.

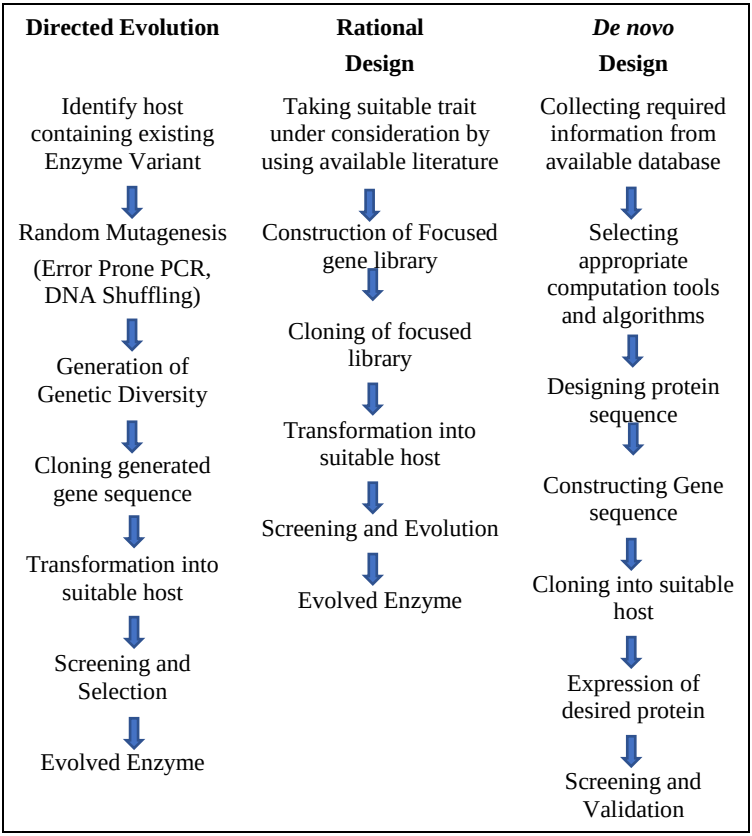


Figure 1. Schematic Representation of protein engineering strategies

De novo design also requires to provide proper assembly of sequence into desired structure. A lot of progress has been made in computational design of protein assemblies with cyclical, helical, cubic and lattice symmetries by the development of symmetry aware algorithms (Norn & André, 2016). Several tools and algorithms are being developed for computational protein design to look into

various factors and design proteins based on available data. Some of the tool which has been found useful in computational protein design includes Rosetta (Kuhlman, 2019) WISDOM (Smadbeck, Peterson, Khoury, Taylor & Floudas, 2013).

Industrial Applications

Enzymes are used commercially in various industrial processes including detergent, paper, chemical, biofuels, alcohol, textile etc. Native enzymes have been part of such industries from a very long time. Various limitations faced by industries with native enzymes have been studied and possible solutions are being looked using enzyme engineering. Enzyme engineering have been put into use for the enhancement of enzyme stability to high temperature, alkaline conditions, acidic environment etc. Subtilisin, a serine protease enzyme for detergent industry, has been subjected to various studies for modification of catalytic mechanism, folding process, stability under harsh environment and activity under oxidizing activity. Subtilisin was subjected to amino acid substitution from methionine to alanine, near catalytic serine, resulting in improved stability towards oxygen bleach. It was also subjected to removal of calcium binding site thereby increasing the activity of enzyme 1000 fold under chelating conditions. (Bryan, 2000) (Vojcic et al., 2015). *Rhizopus chinensis lipase* was subjected to improvements in half-life by introducing disulphide bonds, which resulted in 11-fold increased half-life. (Yu, Tan, Xiao & Xu, 2012). It was also subjected to random mutagenesis using PCR and DNA shuffling and further screened and selected for thermo-stable variant with Tm 22^0C higher. The variant was also tested for stability and found 46X and 23X time longer half-lives at 60^0C and 65^0C respectively. (Yu, Wang, Zhang, Xu & Xiao, 2012).

Amylase is another enzyme which has found immense application in diverse industries. α-amylase is one of the key enzyme in the maltose formation. It has been subjected to improvement using site saturation mutagenesis of histidine 286. It was found that it resulted in increased optimum temperature, increased half-life and improved stability under acidic pH condition. (Li, Yang, Tang & Chen, 2018).

In another study, thermal stability of α-amylase was improved by removing calcium binding site. The optimum temperature was increase by 9^0C and half-life was improved by 4 fold. (Ghollasi, Ghanbari-Safari & Khajeh, 2013).

Conclusion and Future Perspectives

Protein engineering can be looked for providing solutions to modern day industrial problems either by redesigning approach or by de novo synthesis (Figure 1) . Redesigning holds a lot of clues from naturally occurring counterparts which can be accessed, studied and compiled into one enzyme system. Nature presents so many variations that can be studied to look for solution to existing problem but requires a lot of work to introduce such changes into industrially acceptable enzyme system. While *De novo* design holds promising future, it relies heavily on existing data sets. The studies from naturally existing proteins to understand the existing structure, sequence relationship and enzymatic activities will provide the basic data for *De novo* designing. *De novo* designing requires inputs in terms of computing ability and development of algorithms also. The field has opened plethora of opportunity to work in the field of Biochemistry, Genetic Engineering, Computational Biology and Biophysics. Protein engineering holds promising future ahead for providing novel solutions which not only reduces our dependency on harsh chemicals but also ensures that enzymes are not degraded with process and can be further utilized for many cycles with improved efficiency.

References

Adrio, J., & Demain, A. (2014). Microbial Enzymes: Tools for Biotechnological Processes. Biomolecules, 4(1), 117-139. doi: 10.3390/biom4010117

Bei, J., Chen, Z., Fu, J., Jiang, Z., Wang, J., & Wang, X. (2009). Structure-based fragment shuffling of two fungal phytases for combination of desirable properties. Journal Of Biotechnology, 139(2), 186-193. doi: 10.1016/j.jbiotec.2008.08.011

Bryan, P. (2000). Protein engineering of subtilisin. Biochimica Et Biophysica Acta (BBA) - Protein Structure And Molecular

Enzymology, 1543(2), 203-222. doi: 10.1016/s0167-4838(00)00235-1

Cadwell, R., & Joyce, G. (1992). Randomization of genes by PCR mutagenesis. Genome Research, 2(1), 28-33. doi: 10.1101/gr.2.1.28

Cadwell, R., & Joyce, G. (1994). Mutagenic PCR. Genome Research, 3(6), S136-S140. doi: 10.1101/gr.3.6.s136

Chen, K., & Arnold, F. (1991). Enzyme Engineering for Nonaqueous Solvents: Random Mutagenesis to Enhance Activity of Subtilisin E in Polar Organic Media. Bio/Technology, 9(11), 1073-1077. doi: 10.1038/nbt1191-1073

Chiang, L., Kovari, I., & Howe, M. (1993). Mutagenic oligonucleotide-directed PCR amplification (Mod-PCR): an efficient method for generating random base substitution mutations in a DNA sequence element. Genome Research, 2(3), 210-217. doi: 10.1101/gr.2.3.210

Chowdhury, R., & Maranas, C. (2019). From directed evolution to computational enzyme engineering—A review. Aiche Journal, 66(3). doi: 10.1002/aic.16847

Coco, W., Levinson, W., Crist, M., Hektor, H., Darzins, A., & Pienkos, P. et al. (2001). DNA shuffling method for generating highly recombined genes and evolved enzymes. Nature Biotechnology, 19(4), 354-359. doi: 10.1038/86744

Coluzza, I. (2017). Computational protein design: a review. Journal Of Physics: Condensed Matter, 29(14), 143001. doi: 10.1088/1361-648x/aa5c76

Gainza, P., Nisonoff, H., & Donald, B. (2016). Algorithms for protein design. Current Opinion In Structural Biology, 39, 16-26. doi: 10.1016/j.sbi.2016.03.006

Ghollasi, M., Ghanbari-Safari, M., & Khajeh, K. (2013). Improvement of thermal stability of a mutagenised α-amylase by manipulation of the calcium-binding site. Enzyme And Microbial Technology, 53(6-7), 406-413. doi: 10.1016/j.enzmictec.2013.09.001

Gibbs, M., Nevalainen, K., & Bergquist, P. (2001). Degenerate oligonucleotide gene shuffling (DOGS): a method for enhancing the frequency of recombination with family shuffling. Gene, 271(1), 13-20. doi: 10.1016/s0378-1119(01)00506-6

Glover, D., Xu, D., & Clark, D. (2019). Shaping the Future of Protein Engineering. Biochemistry, 58(8), 1019-1021. doi: 10.1021/acs.biochem.8b01322

Gurung, N., Ray, S., Bose, S., & Rai, V. (2013). A Broader View: Microbial Enzymes and Their Relevance in Industries, Medicine, and Beyond. Biomed Research International, 2013, 1-18. doi: 10.1155/2013/329121

Gutte B. (1975). A synthetic 70-amino acid residue analog of ribonuclease S-protein with enzymic activity. The Journal of biological chemistry, 250(3), 889–904.

Gutte, B., Däumigen, M., & Wittschieber, E. (1979). Design, synthesis and characterisation of a 34-residue polypeptide that interacts with nucleic acids. Nature, 281(5733), 650-655. doi: 10.1038/281650a0.

Huang, P., Boyken, S., & Baker, D. (2016). The coming of age of de novo protein design. Nature, 537(7620), 320-327. doi: 10.1038/nature19946.

Kolmar, H. (2019). Protein engineering comes of age. Biological Chemistry, 400(3), 255-256. doi: 10.1515/hsz-2019-0108

Krüger, A., Schäfers, C., Schröder, C., & Antranikian, G. (2018). Towards a sustainable biobased industry – Highlighting the impact of extremophiles. New Biotechnology, 40, 144-153. doi: 10.1016/j.nbt.2017.05.002

Kuhlman, B. (2019). Designing protein structures and complexes with the molecular modeling program Rosetta. Journal Of Biological Chemistry, 294(50), 19436-19443. doi: 10.1074/jbc.aw119.008144

Leung, D. W., Chen, E., and Goeddel, D. V. (1989) A method for random mutagenesis of a defined DNA segment using a modified polymerase chain reaction. Technique 1, 11–15.

Li, S., Yang, Q., Tang, B., & Chen, A. (2018). Improvement of enzymatic properties of Rhizopus oryzae α-amylase by site-saturation mutagenesis of histidine 286. Enzyme And Microbial Technology, 117, 96-102. doi: 10.1016/j.enzmictec.2018.06.012

Li, X., Zhang, Z., & Song, J. (2012). Computational enzyme design approaches with significant biological outcomes: progress and

challenges. Computational And Structural Biotechnology Journal, 2(3), e201209007. doi: 10.5936/csbj.201209007

Liu, Q., Xun, G., & Feng, Y. (2018). The state-of-the-art strategies of protein engineering for enzyme stabilization. Biotechnology Advances, 37(4), 530-537. doi: 10.1016/j.biotechadv.2018.10.011

Liu, Y., & Kuhlman, B. (2006). RosettaDesign server for protein design. Nucleic Acids Research, 34(Web Server), W235-W238. doi: 10.1093/nar/gkl163

Llorens, C., Gacel, G., Swerts, J. P., Perdrisot, R., Fournie-Zaluski, M. C., Schwartz, J. C., & Roques, B. P. (1980). Rational design of enkephalinase inhibitors: substrate specificity of enkephalinase studied from inhibitory potency of various dipeptides.Biochemical and biophysical research communications, 96(4), 1710–1716. https://doi.org/10.1016/0006-291x(80)91371-6

Norn, C., & André, I. (2016). Computational design of protein self-assembly. Current Opinion In Structural Biology, 39, 39-45. doi: 10.1016/j.sbi.2016.04.002

Ostermeier, M., Nixon, A., Shim, J., & Benkovic, S. (1999). Combinatorial protein engineering by incremental truncation. Proceedings Of The National Academy Of Sciences, 96(7), 3562-3567. doi: 10.1073/pnas.96.7.3562

Pantazes, R., Grisewood, M., Li, T., Gifford, N., & Maranas, C. (2014). The Iterative Protein Redesign and Optimization (IPRO) suite of programs. Journal Of Computational Chemistry, 36(4), 251-263. doi: 10.1002/jcc.23796

Porter, J., Rusli, R., & Ollis, D. (2015). Directed Evolution of Enzymes for Industrial Biocatalysis. Chembiochem, 17(3), 197-203. doi: 10.1002/cbic.201500280

Rackers, J., Wang, Z., Lu, C., Laury, M., Lagardère, L., & Schnieders, M. et al. (2018). Tinker 8: Software Tools for Molecular Design. Journal Of Chemical Theory And Computation, 14(10), 5273-5289. doi: 10.1021/acs.jctc.8b00529

Ray, L., Pramanik, S., & Bera, D. (2016). Enzymes- An Existing and Promising Tool of Food Processing Industry. Recent

Patents On Biotechnology, 10(1), 58-71. doi: 10.2174/1872208310666160727150153

Ribeiro, L., Ribeiro, L., Barreto, M., & Ward, R. (2018). Protein Engineering Strategies to Expand CRISPR-Cas9 Applications. International Journal Of Genomics, 2018, 1-12. doi: 10.1155/2018/1652567

Smadbeck, J., Peterson, M., Khoury, G., Taylor, M., & Floudas, C. (2013). Protein WISDOM: a workbench for in silico de novo design of biomolecules. Journal Of Visualized Experiments, (77). doi: 10.3791/50476

Stemmer, W. (1994). DNA shuffling by random fragmentation and reassembly: in vitro recombination for molecular evolution. Proceedings Of The National Academy Of Sciences, 91(22), 10747-10751. doi: 10.1073/pnas.91.22.10747

Tuck Seng Wong, Kang Lan Tee, Berhard Hauer, Ulrich Schwaneberg, Sequence saturation mutagenesis (SeSaM): a novel method for directed evolution, Nucleic Acids Research, Volume 32, Issue 3, 1 February 2004, Page e26, https://doi.org/10.1093/nar/gnh028

Vojcic, L., Pitzler, C., Körfer, G., Jakob, F., Ronny Martinez, Maurer, K., & Schwaneberg, U. (2015). Advances in protease engineering for laundry detergents. New Biotechnology, 32(6), 629-634. doi: 10.1016/j.nbt.2014.12.010

Woolfson, D., Bartlett, G., Burton, A., Heal, J., Niitsu, A., Thomson, A., & Wood, C. (2015). De novo protein design: how do we expand into the universe of possible protein structures?. Current Opinion In Structural Biology, 33, 16-26. doi: 10.1016/j.sbi.2015.05.009

Yu, X., Tan, N., Xiao, R., & Xu, Y. (2012). Engineering a Disulfide Bond in the Lid Hinge Region of Rhizopus chinensis Lipase: Increased Thermostability and Altered Acyl Chain Length Specificity. Plos ONE, 7(10), e46388. doi: 10.1371/journal.pone.0046388

Yu, X., Wang, R., Zhang, M., Xu, Y., & Xiao, R. (2012). Enhanced thermostability of a Rhizopus chinensis lipase by in vivo recombination in Pichia pastoris. Microbial Cell Factories, 11(1), 102. doi: 10.1186/1475-2859-11-102

EXPLOITING THE POTENTIAL OF HAIRY ROOTS - A BIOTECHNOLOGICAL TOOL WITH VARIOUS INDUSTRIAL APPLICATIONS

Keya Patel[1], Smriti Yadav[1] and Neeraj Khare[2]*

[1]Ph.D.Scholar, Amity Institute of Microbial Technology, Amity University Rajasthan, NH-11C, Kant-Kalwar, Jaipur-303002, Rajasthan
[2]Assistant Professor, Amity Institute of Microbial Technology, Amity University Rajasthan, NH-11C, Kant-Kalwar, Jaipur-303002, Rajasthan

*Corresponding Author: nkhare@jpr.amity.edu

Introduction

Earlier, hairy roots were considered a sign of pathogen invasion or disease in plants. Until the 1970s- 1980s, *Agrobacterium rhizogenes* was identified as the bacterial agent that induces hairy roots in plants by gene transfer of bacterial root- inducing plasmid (Gelvin, 2009). Hairy roots arise from the site where the plant is damaged or is altered physiologically, through infection caused by *A. rhizogenes*, which is now renamed as *Rhizobium rhizogenes*. Hairy root infection takes place on specific bacterial DNA fragments like T-DNA from root-inducing plasmid into plant cells. Even when the plant responds to the infection by releasing defense related proteins to reduce the impact of infection, the bacteria, *R. rhizogenes* takes advantage of the proteins released and in turn breaks the defense mechanism of the host (Mauro et al., 2017).

Production of therapeutic proteins using modified plants offer several different benefits like safety i.e., no risk of humans and low costs. Hairy root complexes present many advantages over cell suspensions like stability in terms of genotype and phenotype and

possible extracellular secretion of proteins which is also known as rhizosecretion, thus offering an easy way to procure desired proteins. Hairy root complexes are able to produce complex compounds and it increases the productivity. According to this, the making of recombinant proteins has been considered an important use of hairy root complexes. This allows the making of recombinant proteins by hairy roots grown in an artificial environment like a bioreactor and their secretion in controlled conditions.

Secondary or specialized metabolites produced by the transformation of *R. rhizogenes* have a high demand at pharmaceutical and cosmetic levels. Lately, *R. rhizogenes* transformation is used to explain different biosynthetic pathways and several physiological processes for the generation of plant derived molecules. Due to new evolving advancements of *R. rhizogenes*, the development of hairy root cultures has gained a lot of interest by researchers, biotechnology companies and pharmaceutical industries (Häkkinen et al., 2018).

Production of recombinant proteins and specialized metabolites

R. rhizogenes regulated modification can be done, once a molecular assembly is made for the addition of the gene of interest in the plant without disturbing the gene structure of the host which will in return allow the production of desired protein. The technique consists of changing the *R. rhizogenes* strain by adding a different gene of interest in bacteria through standard expression vector with known T-DNA sequence with the desired gene is used as a classical method before infecting the host. By this, the desired gene is procured. Due to the random addition the plant, it is important to know the most stable clone compared to other clones on the basis of its capacity to grow and produce (Huet et al. 2014).

Another technique consists in directly infecting a modified plant already expressing the desired protein using a wild strain of *R. rhizogenes*. As mentioned above, the recombinant proteins can be released from the cells or can be found in the media (Cardon et al.,

2019). The secretion and/or retention are affected by the charge as well as the nature of the protein. Stability can be achieved by adding stabilizing agents in the media. Hairy root complexes are more preferred as these are genetically more stable and produce more amount of biomass and are efficient. Hairy root complexes are viable for a longer duration (Häkkinen et al., 2016).

Usually non- transgenic hairy root complexes are used for the production of secondary or specialized metabolites but even if transgenic hairy root complexes are used just by monitoring the growth conditions the production can be improved. The expression of specialized metabolites in hairy root complexes, as in all plant-based production systems, requires the identification of the most appropriate pathogen signal metabolites and administration scheme. There is substantial literature exemplifying the pathogen signal metabolites that can be used to produce specialized metabolites using *in vitro* plant tissue culture system (O' Kennedy et al., 2016).

Conclusions and future perspectives

Hairy root complexes are procured from every species of plants. Hence, these complexes can be used to conserve the environment. Hairy root complexes are also important for recombinant protein or specialized or secondary metabolite production. Using recent tools, new devloped types or strains can be made which have great productivity and functions efficiently. This can be used in different types of industries and sectors like phamaceutical, food and cosmetics.

The increase of hairy root complex requires developing strong cost effective good manufacturing practices. The strong advancement in the process in terms of productivity and the ability to modify hairy root complexes to produce desired molecules creates great oppurtunity and an important future tool.

Table 1: Specialized proteins and metabolite by hairy root culture technique

Plant species	Recombinant protein / metabolite	References
Nicotiana tabacum	Monoclonal antibody M12	Madeira et al., 2016
Brassica rapa	Green fluroscent protein	Huet et al., 2014
Cucumis melo	Humman tissue plasmogen activator (t- PA)	Abdoli Nasab et al., 2016
Nicotiana tabacum	MAP30	Moghadam et al., 2016
Nicotiana benthamiana	Tumor- targeting monoclonal antibody mAb H10	Lonoce et al., 2016
Brassica rapa	Human gastric lipase	Ele Ekouna et al., 2017
Nicotiana tabacum	Recombinant human erythropoietin	Gurusamy et al., 2017
Nicotiana tabacum	Recombinant Jg CSMV-derived VLPs	Alemzadeh et al., 2017
Nicotiana benthamiana	Anti-CD20 scFv-Fc antibody	Lonoce et al., 2019
Brassica rapa	Alpha-L-iduronidase (IDUA)	Cardon et al., 2019
Arachis hypogaea	Resveratrol and Piceatannol	Yang et al., 2015
Astragalus membranaceus	Isoflavonoid	Gai et al., 2016
Echinacea purpurea	Caffeic	Abbasi et al., 2012
Fagopyrum tataricum	Rutin, quercetin	Huang et al., 2016
Papavar orientale	Morphine	Hasemi and Naghavi 2016
Plumbago indica	Plumbagin	Gangopadhyay et al., 2011
Psoralea corylifolia	Daidzin	Zaheer et al., 2016
Salvia castanea	Tanshinone	Li et al., 2016
Salvia miltirrhiza	Tanshinone	Hao et al., 2015
Solanum khasianum	Alkaloids	Srivastava et al., 2016

References

Abbasi, B. H., Stiles, A. R., Saxena, P. K., and Liu, C. Z. (2012). Gibberellic acidincreases secondary metabolite production in Echinacea purpurea hairy roots.Appl. Bio chem. Biotechnol. 168, 2057–2066.

AbdoliNasab, M., JalaliJavaran, M., Cusido, R. M., and Palazon, J. (2016). Purification of recombinant tissue plasminogen activator (rtPA) protein from transplastomic tobacco plants. Plant Physiol. Bio chem. 108, 139–144.

Alemzadeh, E., Izadpanah, K., and Ahmadi, F. (2017). Generation of recombinantprotein shells of Johnson grass chlorotic stripemosaic virus in tobacco plantsand their use as drug carrier. J. Virol. Methods 248, 148–153.

Cardon, F., Pallisse, R., Bardor, M., Caron, A., Vanier, J., EleEkouna, J. P., et al. (2019).Brassicarapa hairy root based expression system leads to the production of highly homogenous and reproducible profiles of recombinant human alpha-Liduronidase. Plant Biotechnol. J. 17, 505–516.

Ele Ekouna, J.-P., Boitel-Conti, M., Lerouge, P., Bardor, M., and Guerineau, F. (2017). Enhanced production of recombinant human gastric lipase in turniphairy roots. Plant Cell Tissue Organ Cult. 131, 601–610.

Gai, Q. Y., Jiao, J., Luo, M., Wang, W., Gu, C. B., Fu, Y. J., et al. (2016). Tremendous enhancements of iso flavonoid biosynthesis, associated gene expression and antioxidant capacity in Astragalus membranaceus hairy root cultures elicited by methyl jasmonate. Process Bio chem. 51, 642–649

Gangopadhyay, M., Dewanjee, S., and Bhattacharya, S. (2011). Enhancedplumbagin production in elicited Plumbago indica hairy root cultures. J.Biosci. Bioeng. 111, 706–710.

Gelvin, S. B. (2009). Agrobacterium in the genomics age. Plant Physiol. 150, 1665–1676.

Gurusamy, P. D., Schäfer, H., Ramamoorthy, S., and Wink, M. (2017). Biologicallyactive recombinant human erythropoietin expressed in hairy root cultures and regenerated plantlets of Nicotiana tabacum L. PloS One 12 (8), e0182367.

Häkkinen, S. T., and Oksman- Caldentey, K.-M. (2018). *"*Progress and prospects ofhairy root research,*"* in Hairy Roots: An Effective Tool of Plant Biotechnology.Eds. V. Srivastava, S. Mehrotra and S. Mishra (Singapore: Springer), 3–19.

Häkkinen, S. T., Moyano, E., Cusidó, R. M., and Oksman-Caldentey, K.-M. (2016). Exploring the metabolic stability of engineered hairy roots after 16 yearsmaintenance. Front. Plant Sci.7,1486.

Hao, X., Shi, M., Cui, L., Xu, C., Zhang, Y., and Kai, G. (2015). Effects of methyl jasmonate and salicylic acid on tanshinone production and biosynthetic geneexpression in transgenic Salvia miltiorrhiza hairy roots. Biotechnol. Appl. Bio chem. 62, 24–31.

Hashemi, S. M., and Naghavi, M. R. (2016). Production and gene expression ofmorphinan alkaloids in hairy root culture of Papaver orientale L. using abioticelicitors. Plant Cell Tissue Organ Cult. 125, 31–41.

Huang, X., Yao, J., Zhao, Y., Xie, D., Jiang, X., and Xu, Z. (2016). Efficient rutin and quercetin biosynthesis through flavonoids-related gene expression in Fagopyrum tataricumgaertn. Hairy root cultures with UV-B irradiation. Front. Plant Sci. 7, 63.

Huet, Y., Ele, J.-P., Aurore, E., Katiba, C., Michèle Boitel-Conti, M., and Guerineau, F. (2014). Production and secretion of a heterologous protein byturnip hairy roots with superiority over tobacco hairy roots. Biotechnol. Lett.36, 181–190.

Li, B., Wang, B., Li, H., Peng, L., Ru, M., Liang, Z., et al. (2016). Establishment of Salviacastanea Diels f. tomentosaStib. hairy root cultures and the promotion of tanshinone accumulation and gene expression with Ag+, methyl jasmonate, and yeast extract elicitation. Protoplasma253, 87–100.

Lonoce, C., Salem, R., Marusic, C., Jutras, P. V., Scaloni, A., Salzano, A. M., et al. (2016). Production of a tumour-targeting antibody with a human-compatibleglycosylation profile in N. benthamiana hairy root cultures.Biotechnol. J. 11,1209–1220.

Lonoce, C., Marusic, C., Morrocchi, E., Salzano, A. M., Scaloni, A., Novelli, F., et al. (2019). Enhancing the secretion of a glyco-engineered anti-CD20 scFv- Fc antibody in hairy root cultures. Biotechnol. J. 14, 1800081.

Madeira, L. M., Szeto, T. H., Henquet, M., Raven, N., Runions, J., Huddleston, J., Garrard I and Ma, J. K. C. (2016). High-yield production of a human monoclonal IgG by rhizosecretion in hydroponic tobacco cultures. Plant biotechnology journal, 14(2), 615-624.

Mauro, M. L., Costantino, P., and Bettini, P. P. (2017). The never ending story of rol genes: a century after. Plant Cell Tissue Organ Cult. 131, 201–212.

Moghadam, A., Niazi, A., Afsharifar, A., and Taghavi, S. M. (2016). Expression of a recombinant anti-HIV and anti-tumor protein, MAP30, in Nicotiana tobacum hairy roots: A pH-stable and thermophilic antimicrobial protein. PloS One 11, e0159653.

O' Kennedy, R., Murphy, C., and Devine, T. (2016). Technology advancements inantibody purification. Antib. Technol. J. 6, 17–32.

Srivastava, M., Sharma, S., and Misra, P. (2016). Elicitation based enhancement of secondary metabolites in Rauwolfia serpentina and Solanum khasianum hairy root cultures. Pharmacogn. Mag. 12, S315–S320.

Yang, T., Fang, L., Nopo-Olazabal, C., Condori, J., Nopo-Olazabal, L., Balmaceda, C., et al. (2015). Enhanced production of resveratrol, piceatannol, arachidin-1, andarachidin-3 in hairy root cultures of peanut co-treated with methyl jasmonateandcyclodextrin. J. Agric. Food Chem. 63, 3942–3950.

Zaheer, M., Reddy, V. D., and Giri, C. C. (2016). Enhanced daidzin production from jasmonic and acetyl salicylic acid elicited hairy root cultures of Psoralea corylifolia L. (Fabaceae). Nat. Prod. Res. 30, 1542–1547.

MICROBIAL FUEL CELLS: SOURCES TO SUSTAINABLE SOLUTIONS

Rajal Patel[1], Krati Khandelwal[1], Nishtha Gaur[1], Neelam Jain[*2]

[1] Student, Amity Institute of Biotechnology, Kant-Kalwar, NH11C, RIICO Industrial Area, Jaipur, Rajasthan 303007, India
[2] Professor, Amity Institute of Biotechnology, Kant-Kalwar, NH11C, RIICO Industrial Area, Jaipur, Rajasthan 303007, India

[*]Corresponding Author: njain1@jpr.amity.edu

Introduction

Today the world is looking for alternative sustainable solutions for the production of energy since the increasing use of non-renewable fossil fuels led to climate change and environmental pollution. With the changing lifestyle and rapid urbanization, the amount of generation of food waste from different sources also increased. Food waste is rich in organic nutrients; a traditional method of disposing it into landfills and incineration releases toxic gases that cause a severe environmental and human health hazard. But food waste can be used as a source for energy production by using Microbial Fuel Cells (MFCs). MFCs are bio-electrochemical devices, which generate electricity from the degradation of organic matter by releasing protons and electrons (Pandit and Das, 2018). It can directly produce electricity by converting chemical energy into electrical energy by utilizing microorganisms from organic waste through bio-electrochemical reactions. However, MFCs can better run at ambient temperature and atmospheric pressure and can also be useful in locations that lack electricity facilities in contrast to conventional fuel cells.

Bioenergy is a renewable energy that plays an indispensable role in meeting today's ever increasing energy needs. Unlike biofuels, microbial fuel cells (MFCs) convert energy harvested from redox reactions directly into bioelectricity. MFCs have great potential in wastewater treatment, power generation and Biosensor devices.

Microbial fuel cells have the prospective to convert organic waste to usable energy and generate renewable and carbon-neutral energy. Along with wastewater treatment and bioelectricity generation, this concept of Microbial fuel cells can also aid other treatment processes like anaerobic digestion. It also deals with the wastewater treatment. MFCs can be used in distant areas with lagging electrical infrastructure, dealing with the reliability of external power source (Trapero et al., 2017).

Basic Principles of MFCs

It works on a conversation of chemical energy of organic matter to electrical energy by tracking the efficiency of electron transfer from anode to cathode.

Construction and Working mechanism of MFCs

For the construction of MFC, we generally take bioanode and biocathode in separate chambers that must have two openings. One is to attach both the camber and the other to put or remove samples. Then the chambers must be attached with a salt bridge and joints must be covered to prevent leakage. Graphite electrodes are used for their construction. Then anode and cathode are attached with wires which are further connected to some voltage (Fig. 1). The electrons produced during oxidation are sent to the electrode and electron flux is moved to the cathode. Mostly in MFC organic electron donors are used and oxygen is widely credited as the electron acceptor of the system. Organic matter is considered as the feed of the cell. MFC works well in mild conditions from 20°C to 40°C and around a pH of 7. The current generated from them is directly proportional to the organic matter content of the wastewater used as fuel. Here anode chamber is anaerobic and the cathode chamber is aerobic. Oxidation is carried out inside the bacteria in the anode chamber. The bacteria present in there break bonds into electrons. The important part of the whole system is the separation of cathode and anode chambers by a membrane. This membrane should be permeable to chemicals such as oxygen or ions and should have long term stability.

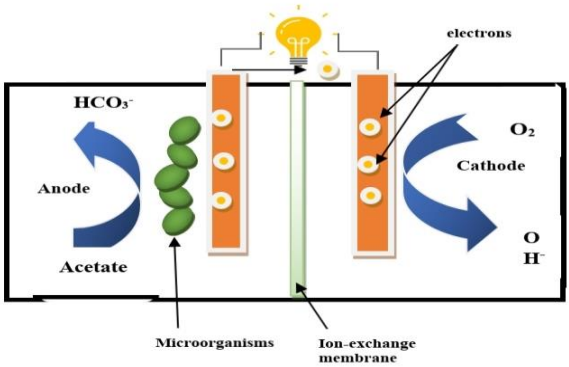


Fig. 1. A typical two-chamber microbial fuel cells (Adapted from : Shah et al., 2019)

The following equations show the processes of MFCs occurring at anode and cathode (Shah et al., 2019):

Anodic compartment:

$$H_3C - \underset{\underset{\textbf{O}}{|}}{C} = O + 2\,(O - H - O) \longrightarrow O = C = O + H^+ + e^-$$

Cathodic compartment:

$$2\,(O = O) + 8H^+ + 8e^- \longrightarrow 4\,(O - H - O)$$

Classification of MFC

The MFC can be classified based on the microbe used, configuration, mode of nutrition, mediators (Ng et al., 2017) and

usage of membrane. The different types of MFC are represented in Fig. 2.

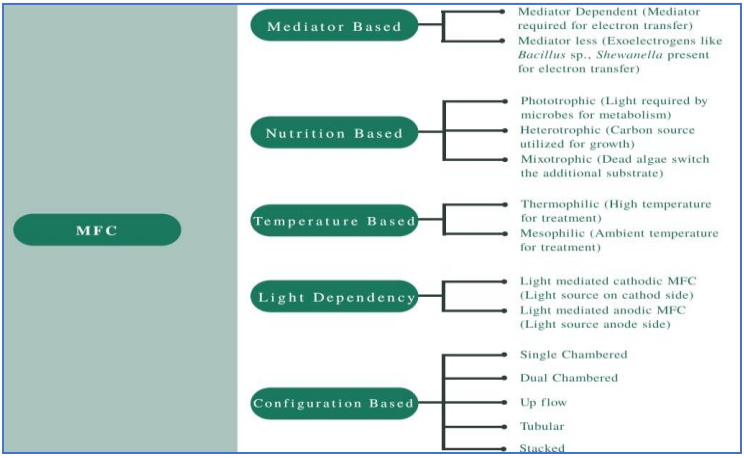


Fig 2. Classification of MFC (Adapted from Bruno et al., 2018)

Based on Mediator

The mechanism of electron transfer in the anode compartment tends to divide MFC into mediator-dependent MFC and mediator-less MFC. Electrons can shuttle to anode in three different ways:

1. The soluble exogenous mediators accept the electron from microbe and transfer it to anode (Xu et al., 2014) (e.g. methylene blue, neutral red, etc.)
2. The soluble mediators produced by the microbe shuttle the electron from microbe membrane to anode (e.g. flavin, pyocyanin, etc.) (Rollefson et al., 2011)
3. Direct transfer of electron from microbe to electrode by nanowires ((Zhang et al. 2018) e.g. c-Cyts, pili)

Based on Dependency of Microbial Nutrition

The type and mode of nutrition tend to classify the MFC into three following classifications.

1. Phototrophic MFC

The phototrophic MFC largely depends on light for the metabolic processes of microbes. These photosynthetic microbes exploit the light energy for the generation of chemical energy rich compound which in turn is converted into electric energy. The phototrophic microalgae MFC utilize carbon dioxide to produce electrons in the presence of light. This property is used in cost effective treatment of wastewater, biofuel and electricity generation. (Lakaniemi et al. 2012)

2. Heterotrophic MFC

In nutritional-dependent MFC heterotrophic microorganisms are implied for the production of electricity. In fact, most of the MFC studies are sustained by heterotrophic bacteria which utilises various organic carbon substrates for their growth (Liang et al. 2009) and are thus useful in faster removal of nitrogen, phosphorus and organic content in the wastewater, and electricity production.

3. Mixotrophic MFC

The mixotrophic MFC is uses algae with bacteria. In mixotrophic mode of operation, the requirement of additional substrate to the bacteria (electrogens) is substituted by the dead algae (Lakaniemi et al., 2012). The use of algae with bacteria has simultaneous benefits of energy supply, oxygen release, COD reduction and CO_2 utilization.

Based on Dependency of Light

Since the amalgamation of phototrophic microorganism in MFC, the phototrophic organism either could be used as an electron donor or electron acceptor. The microalgae grown on the anode compartment act as an electron donor, while if they are grown in the cathodic compartment, then they act as an electron acceptor. This makes them to classify MFC into two types:

1. Light-mediated anodic MFC
2. Light-mediated cathodic MFC

In light-mediated cathodic MFC, either phototrophic or mixotrophic microorganismis used in the cathode compartment. In this type the light source is placed on the cathode side and is vice-versa for light-

mediated anodic MFC. The assembly of MFC diverges from single-chamber (Wu et al., 2013) to two-chamber configuration as in for the microalgae *Rhodobacter sphaeroides* (Rosenbaum et al., 2005).

Based on Dependency of Temperature

Thermophilic microbes have unique features making them more advangeous over mesophiles. Recently studies are also focusing on use of thermophiles in MFC for the generation of electricity and wastewater treatment. Therefore on basis of temperature requirements MFC can be categorized into thermophilic and mesophilic MFC. The mesophilic MFC can function in ambient temperature while the thermophilic MFC requires high temperature for metabolism of integrated thermophiles (Dai et al., 2017)

Based on Configuration

On the basis of configuration, MFC can be segregated into majorly five types for bioenergy production and wastewater treatment:

1. Single-chambered MFC
2. Dual-chambered MFC
3. Upflow MFC
4. Tubular MFC
5. Stacked MFC

The single-chambered MFC has is cost effective, membrane-less, with little internal resistance, high power output and simple in construction (Wu et al., 2013). The dual-chambered MFC has benefits over the single-chambered one such as exclusion of oxygen diffusion by employing alternating membrane between anode and cathode and also reduced internal resistance thus enhancing its output efficiency (Rozendal et al., 2006). The concurrent advantage of bioenergy and wastewater treatment is improved by upflow and tubular MFC but increases the need for space and also the cost of production due to requirement of external assistance (Tee et al. 2016). In stacked MFC, a series of individual models are stacked in parallel or in vertical position thus reducing the internal resistance due to using of small set-up and increasing the overall power output (Zhou et al. 2013).

Role of microorganisms in MFCs as energy producers

Microbial cells are largely non-conductive due to presence of non-conductive materials such as lipids, polysaccharides, and peptidoglycans in their cell membranes. Those microbes that are able to oxidise organic compounds completely and transfer the electrons with augmented rates to the anode can serve as key players for power generation. Electron transfer between microbes and electrodes rely on two mechanisms, namely direct electron transfer (DET) and mediated electron transfer (MET). Table 1 shows a list of some electrogenic microbes reported in the literature for MFC applications. The diversity of microbes from different phylogenetic groups have been stated to generate bioelectricity in MFCs without using a mediator. These mostly include five classes of Proteobacteria, Firmicutes and Acidobacteria phyla with great potential for generation of electrical current. Other microbes like Yeast, moulds and some microalgae, have also been reported in MFCs, being used as substrate or assist the anode or the cathode.

Microorganisms that transfer electrons exogenously to the anode without using any artificial mediator include exoelectrogens, electrogenic microbes, electrochemically active bacteria, anodophiles, anode-respiring bacteria and electricigens (Cao et al., 2019). The microorganisms that bestow electrons to the anode in MFCs are referred to as electrode reducers, while those that accept electrons are referred to as electrode oxidisers (Logan 2010). Widespread studies have been steered on exoelectrogens like dissimilatory iron-reducing *Geobacter sp.*, *Burkholderia sp.*, *Shewanella sp.*, and *Rhodoferax ferrireducens*, to understand the electrogenesis process, to recognize the ecology and diversity of exoelectrogens in the biofilm (Feng et al., 2014). The other bacterial species known to produce electricity in MFCs include *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Clostridium butyricum* and *Enterococcus gallinarum*.

Many yeast like *Saccharomyces cerevisiae*, *Hansenula anomala and Candida melibiosica* can also act as the electricigen in a mediator-free MFC with efficient current generation through direct

electron transfer owing to the presence of redox proteins in cell membranes (Prasad et al., 2007; Hubenova and Mitov, 2010). *Arxula adeninivorans*, a non-conventional yeast can also act as MFC catalyst as it could transfer electrons to the anode through the secretion of reducing molecules (Haslett et al., 2011). Alternatively, microalgae have been used as a substrate or biocathode in MFC (Wang et al., 2012). Cyanobacteria are photosynthetic microorganisms and eco-friendly sources for bioenergy production and use as photosynthetic MFCs (PMFCs), which uses light as the power source and generate electricity through the light-driven oxidation of water. Different species of cyanobacteria including *Synechocystis* sp (Ma et al., 2012), *Spirulina platensis* (Fu et al., 2010) and Nostoc sp have been evaluated as the electricigens in PMFCs (Sekar et al., 2014). Eukaryotic algal biomass also serves as the substrates for electricigens in MFCs (Kondaveeti et al., 2014). Moreover, algae like Chlamydomonas reinhardtii, Chlorella sp., and Scenedesmus obliquus can be used as both electron donors in the anode and acceptors in the cathode.

Studies indicate that the biofilms of microbial consortia can produce higher current density than the biofilms of pure cultures due to the direct electron transfer between the microbes and the surface of the anode. The co-culture system of *Geobacter. sulfurreducens* and *E. coli* improved the performance of MFC as compared to the pure culture of *G. sulfurreducens* (Qu et al., 2012). Similarly the co-culture system of *P. aeruginosa* and *Klebsiella variicola* resulted in three times higher current density making MFC more efficient than either of these two strains alone (Islam et al., 2018). Even the co-culture of *S. cerevisiae* and *S. oneidensis* consortium was designed as glucose-fed MFC (Lin et al., 2017) and a high power density of 123.4 mW/m^2 was attained.

The bacteria capable of dissimilatory metal reduction can effectively produce electricity in a mediatorless MFC by electron transfer through direct contact via outer membrane cytochromes, by expelling electron shuttles or by synthesising appendages

known as microbial nanowires capable of transferring electrical current (Kumar et al., 2017).

Table1: Micro-organisms and diverse substrates used in MFCs for bioelectricity generation

Microorganisms	Substrates	Current density
Single culture		
Rhodoferax ferrireducens	Glucose, xylose, sucrose, maltose	158 mW/m^2
Pseudomonas aeruginosa	Pyocyanin	4310 mW/m^2
Saccharomyces cerevisisae	Glucose	16 mW/m^2
Pseudomonas sp.	Peptone	979 mA/cm^2
Klebsiella pneumoniae strain L1	Glucose	34.77 mW/m^2
Mixed culture		
Thermophilic effluent from anaerobic digestion of brewery wastewater	Acetate	1030 mA/cm^2
Gammoproteo and *Shewanella affinis* (KMM3586)	Cyctenin	36 mW/m^2
Desulphobulbus and *Clostridium*	Rice straw hydrolysate	137.6 mA/cm^2
Fly ash leachate	Fermentation effluent	85.07 mA/cm^2

Source: (Shah et al., 2019)

In a study, a bacterium *Pelotomaculum thermopropionicum* was found connected to the methanogen *Methanothermobacter thermautotrophics* by an electrically conductive appendage, promoting the interspecies electron transfer (Xia et al., 2018). Multiple studies suggest that quorum-sensing chemicals (e.g. fatty acyl-homoserine lactones) play an important role in the communication between the bacteria of different species within

the biofilm (Rollefson et al., 2011). *Pseudomonas aeruginosa* produces pyocyanin that acts as an electron shuttle and signalling molecule to upreg-ulate the transcription of quorum sensing genes (Rabaey et al., 2005).

Applications of MFCs

Certain progress has been made over the past few decades in MFCs apart from the generation of electricity. MFCs have also been worked for various applications such as bioremediation, removal of heavy metals, biosensors, etc (Fig.3).

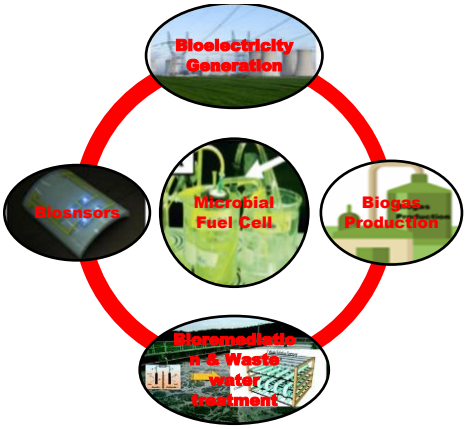


Fig. 3. Applications of MFCs

1. Bioremediation

Bioremediation is a treatment process of removing toxins or contaminants from any media including water, soil by using microorganisms. It is a very sustainable process, that if we replace it with conventional methods, it can show a reduction in energy demands and operational costs. In addition to this, it has the potential to treat recalcitrant wastewater (wastes that comprise dyes, pesticides, polyalcohol, and heterocyclic compounds produced by industry). Presently, the discharge of wastewaters containing

pollutants has become an environmental issue of concern as the discharge blocks the light penetration and oxygen transfer into the water, thus affecting the aquatic life. Detection of toxicity in water is a vital parameter to determine necessary actions for providing safe water within the appropriate degree of quality for consumption by humans, animals, and crops. Therefore, any alteration in the toxicants in fluent water can be easily sensed by monitoring the perturbations in the electric current generated by MFCs (Stein et al., 2012) thus reducing the time and costs compared to conservative methods.

Heavy metals being non-biodegradable and toxic, this causes severe environmental pollution and serious public health problems. Conventional methods to treat heavy metals are energy-intensive and become impotent if metal concentrations are below 1-100 mg/L (Pandit and Das, 2018). Currently, this MFCs technology is looking for state-of-the-art features and environmental welfare to a great extent.

2. Water Desalination

We, humans, cannot drink saline water directly. But by a process called water desalination (which removes salts and minerals from saline water), we can easily consume it. Microbial desalination cells (MDCs) is a novel, energy-sustainable technique for desalination of water that uses organic matter as an energy source. In this method, Electrochemically Active Bacteria (EABs), at anode create a negative potential gradient that allows cation and anion transfer through ion-exchange membranes to balance the electro-neutrality (Pandit and Das, 2018).

3. Secondary fuel production

MFCs can be used to produce secondary fuels such as hydrogen (H_2) as an alternative to electricity. Researchers have currently reported the production of H_2 and methane with the help of microbial electrolytic cells using modified MFC with increased external potential at the cathode terminal (Choi and Ahn, 2015).

4. Biosensors

Biosensors are analytical devices that convert a biological response into an electrical signal. There are different types of biosensors like enzyme-based, DNA biosensors, immunosensors which have essential applications in various fields. MFCs can be used as a BOD (Biochemical Oxygen Demand) sensor or BOD biosensor (Zeng et al, 2012). However, conventional methods are time-consuming and require great skill for profitable production. In MFCs, bacteria at the anode can easily utilize biodegradable substrate and convert it into electricity in a single step process called electrogenesis. Due to rapid monitoring of environmental factors such as pH, temperature, composition, and concentration of organic matter, and other parameters related to the quality of water effluents through MFC-based biosensors have made them the next generation of biosensing technology (Yang et al., 2015).

Challenges and Future of Microbial Fuel Cell Market

The main challenge of MFC is to scale up the energy output concerning to volume of wastewater being treated (Pandit and Das, 2018). A very few pilot-scale studies have been accomplished on MFCs to date. However, there're many challenges which need to be considered to the utilization of MFCs in various application. Power output, economic feasibility, shape, and size of the reactors, electrodes, and suitable microorganisms raise queries on the ability of MFCs for various applications. An understanding of the microbiology of the present producing process for limiting the growth of non-electrogenic microbes is required before further advances in power output.

Due to increasing urbanization there is increase in demand for power & electricity by industries and all the stakeholders which in turn, is expected to drive the propagation of the microbial fuel cell market in the mere fututre. The report presented by Market Research

Future (MRFR) indicates that the global microbial fuel cell market is supposed to augment at 9% CAGR over the forecast period from 2020-2023. MFC can have four major market segmentation. On the basis of types, they are mediators and non-mediator. Based on the applications, they are Power Generation, Wastewater Treatment, Biosensor. Coming to the End-Users, they are Agriculture, F&B, Healthcare, Government & Municipal and on the basis of Regions.

Conclusion

Microbial fuel cells influence microbial flora for generating electrical energy. MFC is sustainable approach for wastewater treatment but still, several challenges stand which need to overcome for commercialization. The critical demand of alternate energy sources, pollution issues related to the conservative energy sources had paved the path to find a novel technology without any environmental effects. MFC has immense potential for electricity generation without any emission of carbon. MFC is a promising technology for harvesting energy and can be advantageously combined with various applications, such as bioremediation, sensors and powering electronic moni-toring devices. The market benefits of MFC could be seen increasing in the future due to the huge electricity demand and it's eco-friendly approach.

References

Bruno, L. B., Jothinathan, D., & Rajkumar, M. (2018). Microbial fuel cells: fundamentals, types, significance and limitations. In *Microbial Fuel Cell Technology for Bioelectricity* (pp. 23-48). Springer, Cham.

Cao, Y., Mu, H., Liu, W. *et al.* (2019). Electricigens in the anode of microbial fuel cells: pure cultures versus mixed communities. *Microb Cell Fact* 18, 39

Choi, J. and Ahn, Y. (2015). Enhanced bioelectricity harvesting in microbial fuel cells treating food waste leachate produced from biohydrogen fermentation, *Bioresource Technology*, 183, 53-60.

Dai K, Wena JL, Zhang F, Maa XW, Cui XY, Zhang Q, Zhao TJ, Zeng RJ (2017) Electricity production and microbial

characterization of thermophilic microbial fuel cells. Bioresour Technol 243:512–519

Daud SM, Daud WRW Feng, C., Li, J., Qin, D., Chen, L., Zhao, F., Chen, S., Hu, H., & Yu, C. (2014). Characterization of exoelectrogenic bacteria enterobacter strains isolated from a microbial fuel cell exposed to copper shock load. *PLoS One*, *9*, e113379.

Fu CC, Hung TC, Wu WT, Wen TC, Su CH. (2010). Current and voltage responses in instant photosynthetic microbial cells with *Spirulina platensis*. *Biochem Eng J*. 52:175–80.

Haslett ND, Rawson FJ, Barrière F, Kunze G, Pasco N, Gooneratne R, Baronian KHR. (2011). Characterisation of yeast microbial fuel cell with the yeast *Arxula adeninivorans* as the biocatalyst. *Biosens Bioelectron.* 26:3742-7.

Hubenova Y, Mitov M. (2010). Potential application of *Candida melibiosica* in biofuel cells. *Bioelectrochemistry*.78:57-61

Islam MA, Ethiraj B, Cheng CK, Yousuf A, Khan MMR. (2018). An insight of synergy between *Pseudomonas aeruginosa* and *Klebsiella variicola* in a microbial fuel cell. *ACS Sustain Chem Eng*.; 6:4130-7.

Kondaveeti S, Choi KS, Kakarla R, Min B.(2014). Microalgae *Scenedesmus obliquus* as renewable biomass feedstock for electricity generation in microbial fuel cells (MFCs). *Front Environ Sci Eng.* 8:784-91.

Kumar, R., Singh, L., & Zularisam, A. (2016). Exoelectrogens: Recent advances in molecular drivers involved in extracellular electron transfer and strategies used to improve it for microbial fuel cell applications. *Renewable and Sustainable Energy Reviews*, *56*, 1322–1336.

Lakaniemi AM, Tuovinen OH, Puhakka JA (2012) Production of electricity and butanol from microalgal biomass in microbial fuel cells. *Bioenergy Res* 5:481–491

Liang Y, Sarkany N, Cui Y (2009) Biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic growth conditions. *Biotechnol Lett* 31:1043–1049

Lin T, Bai X, Hu Y, Li B, Yuan YJ, Song H, Yang Y, Wang J. (2017). Synthetic *Saccharomyces cerevisiae -Shewanella*

oneidensis consortium enables glucose-fed high-performance microbial fuel cell. *AIChE J*.;63:1830–8.

Logan, B. E. (2010). Scaling up microbial fuel cells and other bioelectrochemical systems. *Applied Microbiology and Biotechnology*, *85*, 1665–1671. https://doi.org/10.1007/s00253-009-2378-9.

Ma M, Cao L, Ying X, Deng Z. (2012). Study on the performance of photosynthetic microbial fuel cells powered by *Synechocystis* PCC-6803. *Renew Energy Resour*. 30:42–6

Mohan, S. V., Raghavulu, S. V., & Sarma, P. N. (2008). Biochemical evaluation of bioelectricity production process from anaerobic wastewater treatment in a single chambered microbial fuel cell (MFC) employing glass wool membrane. *Biosensors and Bioelectronics*, *23*(9), 1326-1332.

Ng, I. S., Hsueh, C. C., & Chen, B. Y. (2017). Electron transport phenomena of electroactive bacteria in microbial fuel cells: a review of Proteus hauseri. *Bioresources and Bioprocessing* 4: 53.

Pandit S., Das D. (2018) Principles of Microbial Fuel Cell for the Power Generation. In: Das D. (eds) Microbial Fuel Cell. Springer, Cham. https://doi.org/10.1007/978-3-319-66793-5_2

Prasad D, Arun S, Murugesan M, Padmanaban S, Satyanarayanan RS, Berchmans S, Yegnaraman V. (2007). Direct electron transfer with yeast cells and construction of a mediatorless microbial fuel cell. *Biosens Bioelectron*. 22:2604–10.

Qu Y, Feng Y, Wang X, Logan BE. (2012). Use of a coculture to enable current production by *Geobacter sulfurreducens*. *Appl Environ Microbiol*. 78: 3484-7.

Rabaey, K., Boon, N., Höfte, M., & Verstraete, W. (2005). Microbial phenazine production enhances electron transfer in biofuel cells. *Environmental Science & Technology*, *39*, 3401–3408. https://doi.org/10.1021/es048563o.

Rollefson JB, Stephen CS, Tien M, Bond DR (2011) Identification of an extracellular polysaccharide network essential for cytochrome anchoring and biofilm formation in *Geobacter sulfurreducens*.J Bacteriol 193:1023–1033

Rosenbaum M, Schröder U, Scholz F (2005) In situ electrooxidation of photobiological hydrogen in a photobioelectrochemical fuel cell based on *Rhodobacter sphaeroides*. *Environ Sci Technol* 39:6328–6333

Rozendal RA, Hamelers HVM, Buisman CJN (2006) Effects of membrane cation transport on pH and microbial fuel cell performance. *Environ Sci Technol* 40:5206–5211

Sekar N, Umasankar Y, Ramasamy RP. (2014). Photocurrent generation by immobilized cyanobacteria via direct electron transport in photo-bioelectrochemical cells. *Phys Chem Chem Phys.* 16:7862-71.

Shah, S., Venkatramanan, V., & Prasad, R. (2019). Microbial fuel cell: Sustainable green technology for bioelectricity generation and wastewater treatment. In *Sustainable Green Technologies for Environmental Management* (pp. 199-218). Springer, Singapore.

Sharma, P., Gaur, V. K., Kim, S. H., & Pandey, A. (2020). Microbial strategies for bio-transforming food waste into resources. *Bioresource Technology*, *299*, 122580.

Stein, N.E.; Hamelers, H.M.V.; van Straten, G.; Keesman, K.J. (2012). On-line detection of toxic components using a microbial fuel cell-based biosensor. *J. Process Control*, *22*, 1755–1761.

Tee PF, Abdullah MO, Tan IAW, Amin MAM, Nolasco-Hipolito C, Bujang K (2016) Performance evaluation of a hybrid system for efficient palm oil mill effluent treatment via an air-cathode, tubular upflow microbial fuel cell coupled with a granular activated carbon adsorption. *Bioresour Technol* 216:478–485

Trapero, J. R., Horcajada, L., Linares, J. J., & Lobato, J. (2017). Is microbial fuel cell technology ready? An economic answer towards industrial commercialization. *Applied energy*, *185*, 698-707.

Velasquez-Orta, S. B., Head, I. M., Curtis, T. P., & Scott, K. (2011). Factors affecting current production in microbial fuel cells using different industrial wastewaters. *Bioresource technology*, *102*(8), 5105-5112.

Wu XY, Song TS, Zhu XJ, Wei P, Zhou CC (2013) Construction and operation of microbial fuel cell with *Chlorella vulgaris*

biocathode for electricity generation. *Appl Biochem Biotechnol* 171:2082–2092

Xia, C., Zhang, D., Pedrycz, W., Zhu, Y., & Guo, Y. (2018). Models for microbial fuel cells: A critical review. *Journal of Power Sources*, *373*, 119–131.

Xu B, Chen BY, Hsueh CC, Lj Q, Chang CT (2014) Deciphering characteristics of bicyclic aromatics – mediators for reductive decolorization and bioelectricity generation. *Bioresour Technol* 163:280–286

Yang, H.J.; Zhou, M.H.; Liu, M.M.; Yang, W.L.; Gu, T.Y. (2015). Microbial fuel cells for biosensor applications. *Biotechnol. Lett.*, *37*, 2357–2364.

Zhang, Y.F.; Angelidaki, I. (2012). A simple and rapid method for monitoring dissolved oxygen in water with a submersible microbial fuel cell (SBMFC). *Biosens.Bioelectron. 38*, 189-194.

Zhang Y, Jiang J, Zhao Q, Wang K, Yu H (2018). Analysis of functional genomes from metagenomes: revealing the accelerated electron transfer in microbial fuel cell with rhamnolipid addition. *Bioelectrochemistry* 119:59–67

Zhou M, Wang H, Hassett JD, Gu T (2013) Recent advances in microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) for wastewater treatment, bioenergy and bioproducts. *J Chem Technol Biotechnol* 88:508–518

ADVANCES IN APPLIED MICROBIOLOGY FOR SUSTAINABLE DEVELOPMENT

ABOUT EDITORS

Prof. Neelam Jain is presently working as Secretary, Industry Advisory Council at Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur. She has 20 years of teaching experience in Microbiology and Biotechnology. Her research interest focuses on 'Health for all' as reflected from her publications in frontier areas of Agricultural and Environmental microbiology, Natural antimicrobials based on Herbal and Microbial products like Bacteriophages and Bacteriocins in combating AMR and treating Infectious diseases of Humans and animals. She has published more than 40 Research papers in Journals and Books of National and International repute and presented around 45 Papers at National and International forums through various Conferences and Symposia. Prof. Jain has supervised many PG & UG dissertations and 2 Ph.D. degrees. She is actively engaged in teaching Advanced Microbial Technology, Food Microbiology & Biotechnology, Environmental Biotechnology, Bio fertilizers & Bio pesticides, Food & Nutrition to UG & PG students. Prof. Neelam is member of many prestigious societies like Asian PGPR Society of Sustainable Agriculture, Alabama, USA, International Society for Infectious Diseases (ISID), ISCA (Indian Science Congress Association), Association of Microbiologists of India (AMI), AISTI (Association of Food Scientists & Technologists India), Medicinal and Aromatic Plants Association of India (MAPAI) & The Science Advisory Board (SAB). She is an active member of Editorial Board and Reviewer of many National and International Journals. She has also organized International/ National Conferences, Symposia and DST -INSPIRE program for School students. Prof. Neelam Jain has received TWAS Young Scientist Travel Award for Paper presentation in Alexandria, Egypt.

Prof. G. K. Aseri is currently working as Provost, Dean, Academics & Director, Amity Institute of Microbial Technology (AIMT) & Internal Quality Assurance Cell (IQAC) of Amity University Rajasthan. He is having 20 years of experience in academic administration in higher education. As subject expertise in Microbiology, he is heading Amity Institute of Microbial Technology (AIMT), running Ph.D, UG & PG programs and research is well recognized by DST, DBT-BIRAC, ICAR, ICMR, MoFPI, and Ministry of Agriculture-Govt. of India. He has initiated academic reforms as Chairman of University NAAC Steering Committee. He has been awarded with his doctorate degree from ICAR- Central Arid Zone Research Institute. His area of research includes wide spread area of Microbiology with more emphasis on soil microbial ecology, Microbial products, Biofertilizers and Biocontrol agents. He has supervised many PG & UG dissertations, 6 Ph.D. degrees and has published two patents. His research credentials include more than 70 research papers/presentations in International & National Journals and Conferences and book chapters. He has also organized International / National conferences/Symposia in his subject arena and DST-INSPIRE program for School students. Prof. Aseri is member of Editorial Board and Reviewer of several Journals of International repute. He is member of many prestigious societies like International Society for Infectious Diseases (ISID), Epicore, ISCA (Indian Science Congress Association), Association of Microbiologists of India (AMI), Medicinal and Aromatic Plants Association of India (MAPAI). His work has been internationally recognized and has been invited to present his research work at Germany, Italy, Switzerland, Turkey, Egypt, Singapore & Nepal.

9 788195 042388

MRP : ₹ 500

Published By:

ESN PUBLICATIONS,

3/151 - A,Muthuramalingapuram, Kalloorani Post,Aruppukottai Taluk,

Virudhunagar District, Tamilnadu, India, Pincode - 626 105.

+918838173189 esnpublications@gmail.com www.esnpublications.com www.esnresearch.com

ESN PUBLICATIONS | ADVANCES IN APPLIED MICROBIOLOGY FOR SUSTAINABLE DEVELOPMENT