Isolation of potent zinc and phosphate solubilizing bacterial isolates from garden soil

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Abstract

Human population is continuously increasing; as a result there is a rapid demand of food throughout the world. More yield and increase in crop production as well as increase in soil fertility without effecting the environment are major concern of today's world. Agriculture contributes most to the increasing amount of chemical pollutants via excessive use of synthetic chemical fertilizers and pesticides, which causes environmental damage with potential risks to human health. The whole world is shifting to an organic based sustainable agriculture. It is necessary to preserve the nature's wealth for the future generation. The population of world is increasing rapidly and is creating pressure on the existing land area for fiber, fuel, food and raw materials. The above said problem may be resolved by application of plant growth promoting rhizobacteria capable of solubilizing zinc and phosphate in the soil. Thus, the aim of this study was isolate potent bacterial strains from the soil sample having capacity of zinc and phosphate solubilization.

Keywords: PGPR; Soil; Zinc; Phosphate; Biofertilizer

1. Introduction

Agriculture contributes in the major part of the economy of the country in many developing countries and plays a vital role. It ensures the food security and employment [1]. There is a large competition for producing more crop yield through adopting more and more advanced, improved and intensive agronomic practices. Use of fertilizers in large amount for crop yield is adversely affecting the health of soil and soil is continuously degrading. Sustainable agriculture practice is important to meet the demand of today's world without polluting

the environment. By mean of conventional method of agriculture, we can't meet the demand of future. Plant vigor and fertility of soil can be increased by solubilizing various micronutrients with help of bacterial strains. In the last few decades, the agriculture policy in India has undergone a major change through diversification and emphasis on sustainable production system [2]. Zinc is required for growth as well as metabolism of various micro-organisms and plants. It is required in small but critical concentration to allow several key plant physiological pathways to function normally. These pathways have important roles in photosynthesis, sugar formation, protein synthesis, fertility and seed production, growth regulation and defense against disease [3]. Deficiency of zinc affects the morphological function of plants and adversely affects health and production. It also leads to lower yielding of crops or even crop failure and frequently in poor quality crop production. Zinc has its role in nutrition and physiology of both prokaryotic and eukaryotic organisms. Availability of the zinc in soil and aquatic environment can affect the productivity and diversity of ecosystem. Zinc is also present in enzyme system and also found as a co-factor and metal activator of many enzymes. Zinc deficiency in fungi and bacteria is accompanied by impairment of the formation of pigments such as melanin, chrisogenin, prodigiosin, subtilin and others. Many bacterial enzymes contain zinc in its structure and the active sites [4]. Zinc is found in several forms in soil such as sulphate, olivine, augite, biotite etc. This form of zinc has its role in conversion of such unavailable sources into available one. Microorganism such as *Rhizobacteria* plays an important role in such conversion. Zinc solubilizing bacteria are those which are capable of solubilizing the insoluble zinc compound or minerals in agar plate as well as in soil [5]. Phosphorus is an essential element for plant growth. It is available in limited quantity in soluble form. Phosphorus often limits the growth and development of plants. Generally, very high amount of phosphorus is present in soil. However, most of the phosphorus is insoluble and not available to plants for its growth. Several *Rhizospheric* bacteria are capable of transforming soil phosphorus to the form easily available to plants. Several bacterial genuses like *Pseudomonas, Bacillus, Rhizobium,* and *Enterobacter* are phosphate solubiliser. *Pseudomonas fluorescens* is relatively new bacterial strain which solubilizes phosphorus. Organic matter

derived from dead and decaying plant debris is rich in organic sources of phosphorus. However, plants are able to utilize phosphorus from soil only in the free available from. Soil phosphates are rendered available either by plant roots or by soil microorganism. Therefore, phosphate – dissolving soil organisms play a part in correcting phosphorus deficiency of crop plants [6]. Excessive use of chemical fertilizers have adverse effects on soil microorganism, it affects the fertility status of soil and also pollutes environment [7]. The application of these fertilizers often leads to reduction in pH and thus making the zinc and phosphate unavailable to crops and thus leads to reduction in crop yield. Besides being costly, the production of chemical fertilizers depletes non-renewable resources, the oil and natural gas used to produce these fertilizers, and poses human and environmental hazards [2]. Plant growth promoting Rhizobacteria (PGPR) plays an important role in the sustainable agriculture. The increasing demand for crop production with a significant reduction of synthetic chemical fertilizers and pesticides use is a big challenge nowadays. The use of PGPR has been proven to be an environmentally sound way of increasing crop yields by facilitating plant growth through either a direct or indirect mechanism. The mechanisms of PGPR include regulating hormonal and nutritional balance, inducing resistance against plant pathogens, and solubilizing nutrients for easy uptake by plants. In addition, PGPR show synergistic and antagonistic interactions with microorganisms within the rhizosphere and beyond in bulk soil, thus indirectly boosts plant growth rate. There are many bacteria species that act as PGPR.

2. Materials and Methods

2.1. Sample collection

Soil sample was collected from Rhizospheric soil of plants planted in kitchen garden following the procedure prescribed by Rai et al. 2014. Soil samples were serial diluted and dilution of 10^{-5} – 10^{-7} was plated on Nutrient agar medium.

2.2. Isolation and purification of isolates

Isolation and purification of bacterial strain was performed by sub culturing the obtained colonies on Nutrient agar media (NAM) having composition (g/L) Peptone 05.0 gm; Beef extract 03.0 gm; Sodium chloride 05.0 gm; Agar15.0 gm; Distilled water 1000 ml and the pH was maintained to 7.

2.3. Morphological characterization

Morphological characterization of the isolates was done using the standard

techniques of Microscopic observation based on colony morphology (Size, shape, colour, elevation, texture, opacity and margin).

2.4. Microscopic observation

For cell morphology and differentiating in bacterial strain, isolated bacterial strains were studied microscopically by Gram staining procedure. The prepared slide was observed under oil immersion (100x) for bacterial morphology, shape and mode of arrangements.

2.5. Zinc solubilization test:

The zinc solubilizing activity of each *isolate was tested* in liquid broth as well as on solid agar plate. Medium used was Pikoviskaya's medium (Yeast Extract 0.050 g; Dextrose 10 g; Calcium Phosphate 0.5 g; Ammonium Sulphate 0.50 g ;Potassium Chloride 0.20 g; Magnessium Sulphate 0.10 g; Magnese Sulphate 0.0001 g; Ferrous Sulphate 0.0001 g in g/L). Quantative study of zinc solubilization was studied in 500 ml of conical flask containing 250 ml Pikoviskaya's broth medium. In broth assay method first we have to prepare a Pikoviskaya's broth media. The Broth was inoculated with loopful culture of overnight grow bacterial inoculum. Then incubated for 5 days at 160 rpm in a shaker incubator at 28^{o}C. After incubation, broth culture were centrifuged at 8000 rpm for 10 min and filtered through Whatman No.42 filter paper. In plate assay method, first Pikoviskaya's Agar media was prepared. Then sterilization and plating were done. Freshly grown bacterial cultures were spot inoculated into the agar plate. The spotted plates were incubated at 28^{o}C for 48 hrs in incubator. The clearing zone or haloes around colonies were formed; the diameter of the haloes around the colony and colony diameter were measured. Subsequently the plates were flooded with methyl red solution to observe acid production by bacteria. The change of clear zone to red is an indication of acid production.

2.6. Phosphate Solubilization:

To detect the phosphate solubilisation potential of isolated bacterial strain, first a Pikovaskaya's agar media is prepared. Then sterilization and poring were done. Bacterial cultures were spot inoculated on the Pikovaskaya's agar medium and incubate at 28^{o}C for 2-3 days. Clear zone formed around bacterial colony after the incubation. Clear zone formation indicated the positive results. Diameter of the halo zones around the colony and the colony diameter were measured.

3. Results

3.1 Isolation of bacteria

Bacterial isolates were obtained from the rhizospheric soil by 10^{-5}, 10^{-6} and 10^{-7} serial dilution. Among the bacterial isolates, five Isolates showed characteristics of ***Pseudomonas*** species on Nutrient agar media.

3.2. Microscopic identification

All the isolates were identified macroscopically by studying colony morphology based on their appearance on individual Nutrient agar medium plate.

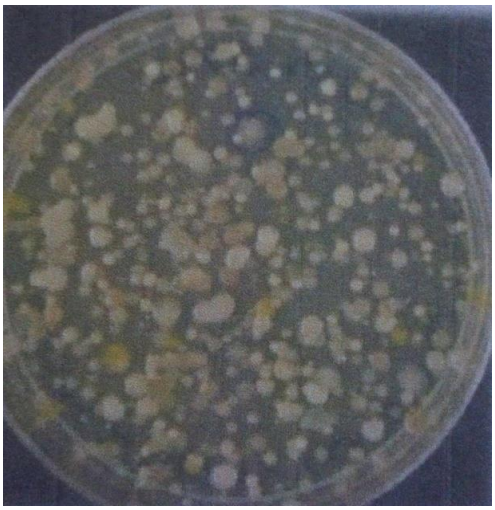
Table 1- Colony charaterization of the isolates on zinc supplemented Nutrient agar medium.

Isolate No	No. of Col.	Size (mm)	Shape Of colony	Colour	Eleva-tion	Texture	Margin	Opacity
1	71	10	lrregular	Greenish	Flat	Smooth	Undulate	Translucent
2	63	7	lrregular	Greenish	Flat	Smooth	Undulate	Translucent
3	52	6	lrregular	Greenish	Flat	Smooth	Undulate	Translucent

Table 2- Colony characheteristics of isolates on phosphate supplemented Nutrient agar medium.

Isolate No.	No.of Col.	Size (mm)	Shape of colony	Colour	Eleva tion	Texture	Margin	Opacity
1	7	5	lrregular	Orengus	Flat	Smooth	Undulate	Translucent
2	12	3	lrregular	Orengus	Flat	Smooth	Undulate	Translucent
3	5	7	lrregular	Orengus	Flat	Smooth	Undulate	Translucent

A



B

Fig. 1 – Colony characteristics of isolates on zinc and phosphate supplemented nutrient agar medium.

3.3. Microscopic identification of bacterial isolation

Microscopic examination was done on the basis of gram staining. Isolates were found Gram negative and short rods.

3.4. Phosphate and Zinc solublization potential

Two metods is used for the determination of zinc solubilization potential. These two methods are Broth assay methods and Agar assay methods. For the Quantative study of zinc and phosphate Pikoviskaya,s Broth and Pikoviskaya,s Agar is used as solid medium respectively in the experiment. The zinc solubilizing activity of each *Psedumonas fluorescence* isolate in liquid broth was determined quantitatively. The concentration of zinc in supernatant was estimated in atomic absorption spectrophotometer at 600 nm for OD. The OD of the supernatant is measured and the weight of the biomass was observed to detrmined the zinc solubilizing potential.

The clearing zone or haloes around colonies were formed on a solid medium of bacterial plate. The diameter of the haloes around the colony and colony diameter were measured. Subsequently the plates were flooded with methyl red solution to observe the acid production by bacteria. The change of clear zone to red is an indication of acid production. The zinc solubilization ability is measured by calculating the diameter of colonies and the halo zones.

Table 3. Zinc solubilization potential of isolates

Isolate	**Diameter of Halozone**	**Diameter of Culture**
1	**1.3 cm**	**0.6 cm**
2	**1.3 cm**	**0.6 cm**
3	**1.4 cm**	**0.7 cm**
4	**2.2 cm**	

		1.9 cm

Table 4. Phosphate solubilization potential of isolates

1.5 cm	**0.8 cm**
1.4 cm	**0.6 cm**
1.2 cm	**0.7 cm**
1.2 cm	**1.9 cm**

4. Discussion

Plant growth promoting and biocontrol activities of rhizobacteria have been reported by numerous studies in last three decades (3;8). However, the isolates have seldom been applied to elevate the growth and yield of host pants. The application has also been limited due to inconsistency in results of laboratory ,greenhouse and field studies (Mishustin and Naumova, 1962). Production of indole acetic acid (IAA) and soluble phosphate are the mostcommon mechanisms of action implicated in PGPR andindeed microbes demonstrating these attributes arewidespread in rhizosphere. The two isolates found potent in this study were found gram negative, short rods, and fluorescent green in appearance showed the presence of pseudomonas species, were potent for significant amount of IAA, phosphate solubilization and siderophore production. Production of ammonia, phosphate solubilization, siderophore production, and HCN production was most frequently encountered by all the isolates. Another important trait of PGPR, that may indirectly influence the plant growth, is the production of siderophores. They bind to the available form of iron Fe^{3+} in the rhizosphere, thus making it unavailable to the phyto-pathogens and protecting the plant health. In the present, investigation, isolates of *Pseudomonas* spp. showed multiple PGP activities. Several studies have demonstrated that production of siderophores, other secondary metabolites and lytic enzymes by *Pseudomonas* strains was most effective in controlling the plant root pathogens further studies on the performance of these isolates and their mutants on the growth of plant will uncover the mechanism and potential of these PGPR exhibiting multiple plant growth promoting traits.

5. Conclusion

The current study aims to exploit phosphate solubilizing bacterial communities in the plant growth promotion. Since there are several factors, inhibiting the growth and development of plants, plant growth promoting bacteria with different attributes facilitating the root and shoot growth. Plant growth promotion by PGPR is a well known phenomenon and this growth enhancement is due to certain rhizobacteria. Here in this report two bacterial strains *P.*

aeruginosa and *P. fluorescens* were successfully isolated and have the capability of Phosphate and zinc solubilization. The isolate *P. fluorescens* was found more potent for PGPR activity and further large scale mass production of this isolate may be carried for its commercialization purpose.

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