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ISOLATION OF PHOSPHATE SOLUBILIZING BACTERIA FROM RHIZOSPHERE SOIL OF ORANGE PLANTS AND THEIR ROLE IN PLANTS GROWTH PROMOTION

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ABSTRACT
Plant growth promoting rhizobacteria (PGPR) mediate the soil processes such as decomposition, nutrient mobilization, mineralization, solubilization, nitrogen fixation and growth hormone production. Microorganisms having the phosphate solubilizing capacity can covert the insoluble phosphates into soluble forms through the production of organic acids. Isolation of phosphate solubilizing bacteria for the effective plant growth promotion broadens the spectrum of phosphate solubilizers available for field application. Improvement of solubilizing capacity is a important step for commercial purposes. Microbial phosphate solubilization can also improve the effectivity of mineral phosphate fertilization. There are many biofertilizers available containing phosphate solubilizing bacteria, but not all the biofertilizers can give good results in all kind of environment and in all areas. Sometimes, by using superior quality of biofertilizers there is no much gain or yield because the bacterial strain used in that biofertilizers cannot adjusted in that particular environment of soil. Like other plants rhizosphere of the orange tree is great source of phosphate solubilizing bacteria, and there is vast scope for increasing the productivity of orange tree. To increase the crop productivity and soil fertility, the phosphate solubilizing bacteria are very useful. There is no literature available on isolation of phosphate solubilizing bacteria from rhizosphere of orange tree. So, our aim is to isolate the phosphate solubilizing bacteria from the rhizosphere of the orange tree from Amravati district. During this study, the species obtained showing the maximum zone of solubilization can be further recommended for the preparation of biofertilizers which can give best results in this particular environment.

KEYWORDS: Phosphate solubilizing bacteria, rhizospheric bacteria, PGPR, rhizosphere.
Abbreviation: PGPR- Plant Growth Promoting Rhizobacteria, PSB- Phosphate Solubilizing Bacteria.

INTRODUCTION

The soil environment surrounding plant roots is the zone of intense microbial activity. The existence of soil microbes capable of transforming soil phosphorus and fixing nitrogen from the atmosphere to forms available to the plants has been recorded by many investigators. It has been observed that a high proportion of phosphate solubilizing microorganisms are concerned in the rhizosphere of plants.(Ramachandran *et al.*,2007). Soil microorganisms are responsible to intervene the soil processes such as decomposition, nutrient mobilization, mineralization, solubilization, nitrogen fixation and growth hormone production. (Akhtar *et al.*, 2010).

Phosphorus is essential for growth and productivity of plants. It plays an important role in plants in many physical activities such as cell division, photosynthesis and development of good root system and utilization of carbohydrate. Plants acquire phosphorus from soil solution as phosphate anion. It is the least mobile element in plants and soil contrary to other macronutrients. In plants, phosphorus incease the strength of cereal straw, promotes flower formation and fruit production stimulates root development and also essential for seed formation. (Sharma *et al.*,2011).
In particular, soil microorganisms are effective in releasing P from inorganic and organic pools of total soil P through solubilization and mineralization. Currently, the main purpose in managing soil phosphorus is to optimize crop production and minimize P loss from soil. Plant growth promoting rhizobacteria (PGPR) are soil and rhizosphere bacteria that can benefit plant growth by different mechanisms and P-solubilization ability of the microorganisms is considered to be one of the most important traits associated with plant P nutrition. (Chen *et al*,2005). It is well known that a considerable number of bacterial species, mostly those associated with the plant rhizosphere, this group of bacteria has been termed 'plant growth promoting rhizobacteria'(PGPR) and among them are strains from genera such as *Bacillus*, *Pseudomonas, Enterobacter, Azospirillum, Rhizobium, Erwinia, Serratia, Alcaligenes, Arthrobacter, Burkholderia, Acinetobacter and Flavobacterium.*(Rodriguez and Fraga, 1999).

Rhizospheric bacteria can improve plant growth by utilizing diverse mechanisms including phosphate solubilization, phytohormone production, nitrogen fixation and bio-control of plant pathogens.(Tahir *et al.*, 2013). Microorganisms exerted beneficial effect on plant growth and development through different means are termed as Plant Growth

Promoting Rhizobacteria (PGPR). PGPR influenced the plant growth by direct or indirect modes. Direct modes are production of growth stimulators, improvement in plant nutrient status, released of phosphates and micronutrients from insoluble sources, lowering of the ethylene level in plant, and induction of systemic resistance. Indirect modes of action are production of antibiotics, production of biocontrol angents and degradation of xenobiotics. (Qureshi *et al .,* 2012). Rhizosphere is the soil zone surrounding the plant roots while rhizoplane is directly in contact with roots. Kennedy (2005). Biofertilizers are ready to use live formulates of such beneficial microorganisms which on application to seed, root or soil mobilizes the availability of nutrients by their biological activity and help to build up the micro flora, thus increases the soil fertility and in turn the crop yield.

From long time the chemical fertilizers were in use but due to the hazardous effect of chemical fertilizers on soil, the biofertilizers are widely used now-a-days. Orange plants or citrus trees (*Citrus seninensis*) possess greater adaptability to variety of climatic and soil conditions and are grown successfully in tropical, subtropical and even in some of the favourable and mild parts of temperature regions. In India these are grown in almost all the states and occupy third largest area among the fruit crops next to mango and banana. Maharashtra is one of leading state in the cultivation of orange plants. The objective of this study was to isolate phosphate solubilizing bacteria from rhizosphere soil of Orange plants.

MATERIALS AND METHODS

Collection of soil sample: Total 40-45 soil sample were collected from rhizosphere soil of orange plant.These samples were collected in the plastics bags.
Preparation of Medium:

Table 1. Composion of Pikovskaya Medium

Ingredients	Gram /L
Yeast extract	**0.50**
Dextrose	**10.00**
Calcium phosphate	**5.00**
Ammonium sulphate	**0.50**
Potassium chloride	**0.20**
Magnesium sulphate	**0.10**
Manganese sulphate	**0.0001**
Ferrous sulphate	**0.0001**
Agar	**15.00**

Isolation of phosphate solubilizing bacteria: Phosphate solubilizing bacteria were isolated from eash sample by serial dilution and spread plate methad on Pikovskaya's agar and incubated at 37^0C for 3-4 days. Colonies showing halo zones were picked and were inoculated on another plate of pikovskaya's agar medium by point inoculation for measuring the zones and studying colony morphology. (Sharma *et al.*, 2011).

Morphological Characterization: Morphological characteristics of isolates viz. shape, size, elevation, surface texture, margins and colour were observed for their characterization.

Method for testing the efficiency of isolated phosphate solubilizing bacteria:
In vitro Experiment: - For testing the efficiency of isolated phosphate solubilizing bacteria, it's effect was observed on growth of wheat plant in vitro. The broth culture of each isolated was made in Pikovskaya's broth medium. After making the broth culture, the equal numbers of seeds of wheat were soaked in the each broth culture separately for some time. Also the soil was made sterile by autoclaving. Then the equal number of seeds was potted in the sterile soil

in small pots providing optimum condition for growth. Also one pot was kept as control in which the seeds were potted in sterile soil without soaking in any culture of phosphate solubilizing bacteria. Then all these pots were observed for germination of seeds, growth of plants and root length after regular interval of time.

RESULTS

Phosphate solubilizing bacteria were isolated from the rhizospheric soil samples from orange plant. It was found that out of 40-45 samples, only 6 samples showed the presence of phosphate solubilizing bacteria.

On the basis of cultural characteristics, biochemical characteristics and morphological characteristics, phosphate solubilizing bacteria were identified via. *Bacillus spp. (*PSB-I), *Pseudomonas spp.*(PSB-II), *Enterobacter spp.*(PSB-III), *Pseudomonas spp.*(PSB-IV), *Acinetobacter spp.*(PSB-V), *Bacillus spp.*(PSB-VI).

On Pikovskaya's agar, strain PSB-I, PSB-II, PSB-III, PSB-IV, PSB-V, PSB-VI showed distinct very large, clear and transparent zone of solubilization and highest percentage of phosphate solubilization in Pikovskaya's broth.

Out of six isolates, *Bacillus spp*. have more efficiency of phosphate solubilization as compare to other five isolated phosphate solubilizing bacteria, that is 280 and *Enterobacter spp.* have efficiency of phosphate solubilization was less as compare to other five isolated phosphate solubilizing bacteria 118.

Determination of efficiency of phosphate solubilization:

% of efficiency of phosphate solubilization was calculated by using following formula (Table 2).

$$Efficiency\ of\ phosphate\ solubilization = \frac{Solubilization\ diameter}{Diameter\ of\ colony}\ x\ 100$$

Table 2. Efficiency of Phosphate Solubilization

SN	PSB strain	Colony diameter	Solubilization diameter	% Efficiency 48 hr
1	*Bacillus spp.*	0.6	1.6	266
2	*Pseudomonas spp*	0.5	1.2	240
3	*Enterobacter spp.*	1.1	1.3	118
4	*Pseudomonas spp*	0.6	1.5	250
5	*Acinetobacter spp*	0.7	1.1	150
6	*Bacillus spp.*	0.5	1.4	280

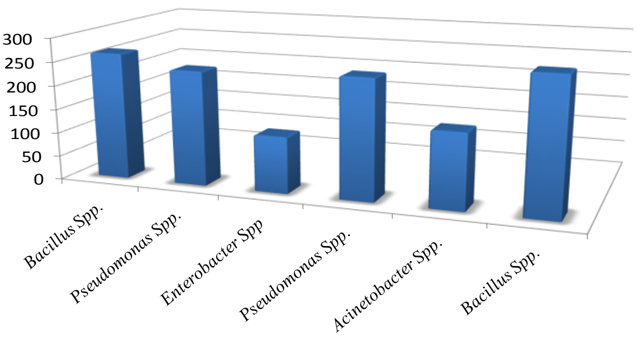

Figure 1. Graphical representation of efficiency of phosphate solubilization

Figure 2. Effect of PSB on the growth of Wheat Seed

DISSCUSSION

Growth promotion: - The maximum and significantly high, shoot length was observed on PSB treatment (Figure 2). The inoculation of PSB has also increased the height of shoot. The control plants on the potting media have recorded the lowest shoot length. Katznelson and Bose (1959) found that rhizosphere bacteria has greater metabolic activity and suggested that they might contribute significantly to the phosphate economy of the plant.

In the study, inoculation of P solubilizer was evaluated for the growth promotion of wheat. Results demonstated that phosphate solubilizing bacterial species enhanced the yield components of wheat compared to uninoculated control. PGPR having the potential of phosphate solubilization enhanced the growth hormone production availability of phosphorus and rate of nitrogen fixation. (Ponmurugan and Gopi, 2006). Inoculation of PSB enhanced the seed wheat yield, plant height, NP contents in wheat leaves and available P status of soil. Rodriguez and Fraga (1999) found that, the use of phosphate solubilizing bacteria as inoculants simultaneously increases P uptake by the plant and crop yield. Strains from the genera *Pseudomonas, Bacillus* and *Rhizobium* are among the most powerful phosphate solubilizers. Ramachandran *et al.,* (2007), found that bacterial isolate *Pseudomonas spp.* from the rhizosphere soil and root cuttings of bush pepper (*Piper nigrum)* exhibiting high phosphate solubilizing ability in vitro and the highest P solubilization efficiency.

Tahir *et al.,* (2013) reported that three phosphate solubilizing bacterial strains viz, *Azospirillum, Bacillus* and *Enterobacter* were isolated by serial dilution method from the rhizosphere of wheat grown under wheat-cotton and wheat-rice crop rotation and their effects on plant growth promotion. These results indicated that bacterial isolates having plant beneficial traits like phosphate solubilization and IAA (Indol-3-acetic acid) production and capable of improving growth of wheat used as inoculants qualify for production of biofertilizer for wheat for wheat crop.

CONCLUSION

It is concluded from the present study that all isolated phosphate solubilizing bacteria are very useful for increasing crop productivity. Phosphate solubilizing bacteria play an important role in plant nutrition through the increase in phosphate as well as nitrogen uptake by plant and their use as PGPR is an important contribution to biofertilization of agricultural crops. Biofertilizer are considered more efficient and safe as compare to chemical fertilizers. The biofertilizers are eco-friendly and economically important to farmers as compared to chemical fertilizers. Thus, the strains isolated from Amravati region are symbiotically associated with orange plants rhizosphere and can be used for making the biofertilizers and are well adjusted in this particular environment.

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