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23. Screening for Phosphate Solubilizing Bacteria (PSB) from Rhizospheric Soil

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Abstract

In present study, soil samples from rhizospheric niches of Tur (*Cajanus cajan*), Soyabean (*Glycine max*), Neem (*Azedirachta indica*), and Bavachi (*Psoralia corylifolia*) were collected and brought to the laboratory. All rhizospheric soil samples were screened for phosphate solubilizing bacteria on Pikovskaya agar by serial dilution method. Among the soil samples screened, rhizospheric niches from the Soyabean showed highest phosphate solubilizing bacteria, 114 than the other rhizospheric soil samples.The rhizospheric niches of Tur, Neem and Bavachi showed 47, 07, and 02 phosphate solubilizing bacteria, respectively. Out of total 170 Phosphate solubilizing rhizospheric bacteria, SMD6, SMD22, SMD28 and SMD30, presented highest phosphate solubilization index on Pikovskaya Agar.

Key Word: Rhizospheric Soil, Pikovskaya Agar, Phosphate Solubilizing Bacteria.

1.0 Introduction

Phosphorus is an essential macronutrient for growth and development of plants involved in important metabolic pathways like photosynthesis, biological oxidation, nutrient uptake and cell division (Illmer and Schinner 1992, Gupta *et al.*, 2012). Worldwide soils are supplemented with inorganic P as chemical fertilizers to support crop production, but repeated use of fertilizers deteriorates soil quality (Tewari *et al.*,2004). Present scenario is shifting towards a more sustainable agriculture by using Phosphate Solubilizing Bacteria.

Natural solubilization of mineral phosphates is an important mechanism exhibited by different microorganisms, known as phosphate solubilizing microorganisms (PSM). Bacteria are the predominant microorganisms that solubilize mineral phosphate in nature, as compared to other microorganisms (Yin, 1988, Paul and Sinha, 2017). Phosphate solubilizing bacteria (PSB) play an importantrole in biogeochemical phosphorus cycling in both terrestrial and aquatic environments (Das *et al.*, 2007). Application of phosphate solubilizing bacteria increases soil fertility due to their ability to convert insoluble P to soluble P by releasing organic acids, chelation and ion exchange (Omar, 1998; Narula *et al.*,2000; Whitelaw,2000).

The present investigation mainly focuses on the isolation of high Phosphate Solubilizing Bacteria from rhizospheric niches of different plants.

2.0 Materials and Methods

2.1 Collection of Soil sample from Rhizospheric Niches

Soil samples were collected from the rhizospheric niches of four crop plants *viz.*, Tur (*Cajanus cajan*), Soyabean (*Glycine max*), Neem (*Azedirachta indica*), and Bavachi (*Psoralia corylifolia*) grown in the farmer fields, near Purna city. For this purpose, the plants were uprooted carefully, shoots were cut off and roots along with rhizosphere soils were brought to the laboratory in polythene bags. The soil samples were processed immediately and stored at 4-8 °C for the isolation of Phosphate solubilizing microorganisms.

2.2 Isolation of Phosphate Solubilizing Bacteria (PSB)

Phosphate Solubilizing Bacteria (PSB) were isolated from the rhizospheric soil samples by dilution plate technique using Pikovskaya's medium (Pikovskaya 1948) containing tri-calcium phosphate (TCP) (Gupta *et al.*, 2012, Kaur, 2014). Appropriate soil dilutions were plated on Pikovskaya's agar medium by spread plate technique and incubated at 30 ± 1 °C for 2-3 days. The colonies forming halo zone of clearance (Pikovskaya's medium) around them were counted as P-solubilizers. All the bacterial colonies exhibiting halo. zones were selected, purified and maintained on nutrient agar slants for further studies.

2.3 Estimation of phosphate solubilization efficiency

Pure cultures of phosphate solubilizing bacteria were spot inoculated on the plates containing Pikovskaya's medium. The plates were incubated at 28 ± 1°C and halozone around colonies were recorded at regular intervals upto 10 days. The abilities of the isolated phosphate solubilizing bacterium to solubilize TCP on Pikovskaya'sagar media was determined in terms of solubilization index (SI). Phosphate solubilization index was calculated by measuring the colony diameter and the halo zone diameterand the colony diameter, using the following formula of Edi-Premono *et al.*,(1996).

$$\text{Phosphate Solubilization Index (SI)} = \frac{\text{(Colony diameter + Halo zone diameter)}}{\text{Colony diameter}}$$

3.0 Result and Discussion

3.1 Isolation of Phosphate Solubilizing Bacteria (PSB)

In present study, 114 phosphate solubilizing bacteria (PSB) were isolated on Pikovskaya Agar from the Soyabean rhizospheric niches. by using dilution technique, which were far greater than the other rhizospheric niches samples. Similarly, from the rhizospheric niches of Tur, Neem and Bavachi 47, 07, and 02 phosphate solubilizing bacteria were isolated, respectively. Use of Pikovskaya's agar medium for isolation of Phosphate Solubilizing Bacteria (PSB) was a simple way to detect PSB through formation of halo zone on agar plate containing tri-calcium phosphate as a sole Phosphorous source (Kaur, 2014). These rhizospheric isolates were tentatively named as SMD 1 to SMD 170.

These reports support the fact that phosphate solubilizing bacteria can be isolated from rhizospheric niches. In this study, rhizospheric niches of Soyabean showed greater amount of phosphate solubilizing bacteria. Kaur (2014) isolated 1270 bacteria was isolated on Pikovskaya's agar plate by serial dilution method at 10^{-5}dilutions. Out of these 1270 bacterial isolates only 169 bacteria isolates were observed to be formed a halo zone around the colonies.

The role of microorganisms in solubilizing insoluble phosphates in soil and making it available to plant is well known (Kundu and Gaur,1981). Phosphate solubilizing microorganisms include several bacteria, fungi, actinomycetes, yeast and Cyanobacteria (Gerretsin, 1948; Banik and Dey 1982 and Illmer and Schinner 1992). The phosphate solubilizing microorganisms were isolatedfrom different sources such as soil (Gupta *et al.*,1986; Kapoor *et al.*, 1989),rhizosphere (Sardina*et al.*, 1986; Singh and Kapoor, 1994), root nodules (Suranga and Kumar, 1993),compost (Gupta *et al.*, 1993), and rock phosphates (Gaur *et al.*, 1973).

3.2 Estimation of phosphate solubilization efficiency

Qualitative screening of 170 phosphate solubilizing bacterial isolates revealed variations in phosphate solubilization efficiency. In total of 170 phosphate solubilizing bacterial isolates from different niches, 4 isolates, SMD6, SMD22, SMD28 and SMD30, were found to be fare more than 5 mm zone of solubilization on Pikovskaya's agar plates. The Phosphate solubilization activity of these isolates of PKV agar plates was ranged between 2.0 to 2.6 (Table 4.1). The phosphate solubilization activity of these isolate is shown in photo plate 4.1 the results were found slightly better than Kaur (2014), who stated that 169 phosphate solubilizing bacteria isolated from different rhizospheric niches revealed phosphate solubilization index in range between 1.36 to 3.17.

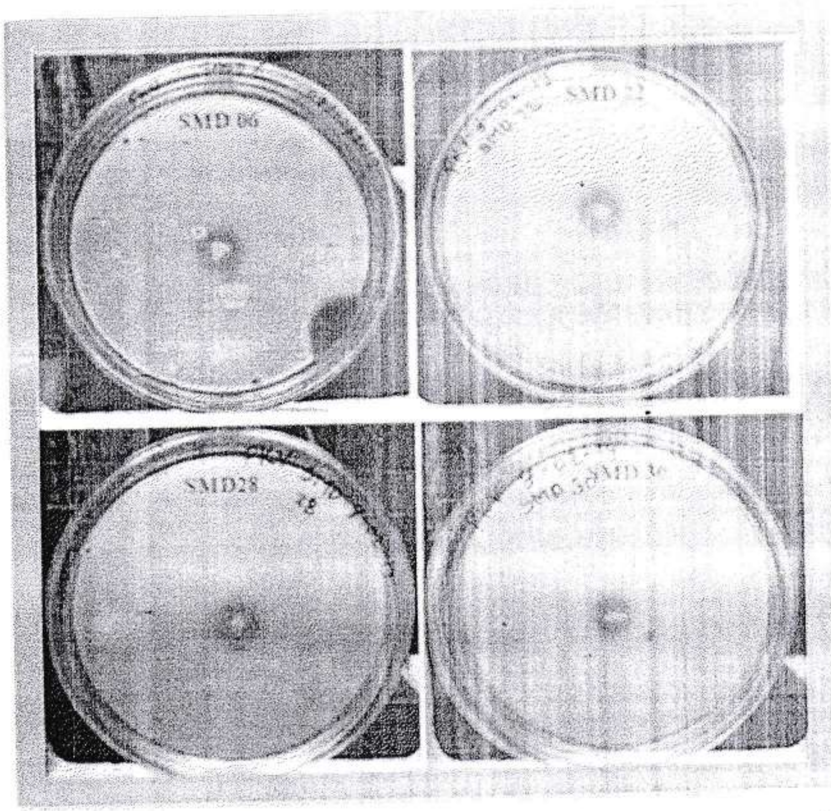

Photo Plate 4.1: Phosphate Solubilization of Isolated Bacteria from Rhizospheric Niches

Table 4.1: Phosphate Solubilization Index of Selected Rhizospheric Isolates

Rhizospheric Isolates	**Diameter of Colony + Halo zone (mm)**	Diameter of Colony (mm)	Diameter Halo zone (mm)	Phosphate Solubilization Index
SMD 06	10	5	5	2.00
SMD 22	9	4	5	2.25
SMD 28	8	3	5	2.66

SMD 30	8	3	5	2.66

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