Research Article

Isolation and Characterisation of Phosphate Solubilising Bacteria from Different Soil

S.Anbuselvi*, L. Jeyanthi Rebecca, Guddu Kumar, Jitendra Kumar
Department of Industrial Biotechnology,Bharath University, Chennai, Tamil Nadu, India.
***Corresponding author's E-mail:** anbuselvichennai@yahoo.com

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ABSTRACT

Soil microbes play a significant role in maintaining the nitrogen and phosphorus in soil. The phosphate content was found to be low in soil and get phosphates from rocks and decaying matter. In order to improve the phosphate in soil, is applying phosphor bacteria as inoculants to get sufficient amount of phosphorus in soil. Deficiency of phosphate leads to restrict the plant growth. The phosphate solubilising bacteria was isolated from different types of soil and the solubility characteristics of phosphate solubilising microbes were analyzed. Plants were grown with PSB Inoculants and morphological features were studied.

Keywords: PSB, phosphate, inoculants, biofertilizer

INTRODUCTION

Phosphorus is important for growth and maintenance of plants. It shows many physiological functions such as cell division, photosynthesis and development of root system[1]. Plants absorb phosphate only in soluble form. The transfer of insoluble phosphate into soluble form is carried out by variety of microbes in the soil. Microbes can dissolve insoluble inorganic phosphates present in the soil and make them available to the plants[2].

Plants require phosphorus from the soil solution as phosphate anion. It increases the strength of plants, promotes flower formation and fruit production. It also increases their resistance to diseases and adverse conditions[3]. Phosphorus deficiency is found to be common in soil and phosphorus fertilizers are required to solve it and maintain the crop production. Only small portion is utilized by plants and remaining is converted into insoluble phosphate[4].

Phosphorus can supply to the plants through biological ways. Phosphate solubilising microbes have been reported in conversion of insoluble phosphate to soluble primary and secondary orthophosphate ions [5]. This phosphate bio fertilizer helps to increase the accessibility of accumulated phosphates for plant growth[6,7]. PSM improved phosphate uptake by plants and activated phosphorus in crop plants[8].

PSM are best to be present more abundant in the rhizosphere of soil. One gram of fertile soil contains thousands of bacteria and Fungi. They can be divided into phosphate solubilising bacteria and phosphate solubilising fungi[9]. The solubilisation of phosphates takes place trough processes or mechanisms of organic acid production and proton extrusion[10].

Phosphate solubilising bacteria are found to be more common in fertile soil. Soil bacteria are in cocci, rod or spiral shape. Bacillus and Pseudomonas secrete organic acids and lower the pH in their vicinity to bring about the dissolution of bound phosphate in soil. The present study was aimed at the isolation and characterisation of phosphate solubilising bacteria from different types of soil and its impact on the growth of plants.

MATERIALS AND METHODS

Soil samples were collected from different areas. Samples were air dried powdered and check the pH and NPK content of the soil.

10gm of soil sample was dissolved in 100ml distilled water sterilized water and mix the sample well and considered the diluted the soil sample in sterilized distilled water up to 10^{-7} dilution (each test tube containing 9ml of sterilized distilled water) then 10^{-5} 10^{-6} 10^{-7} dilutions taken for spread plate technique. Sterilized nutrient agar prepared and poured into petri dishes after solidification of the medium 0.1MLsample was poured into agar medium plate by using L-Rod spread the sample evenly over the agar surface and then incubated at 37°C f or 24 hours.

Isolation of PSB

Pikovskaya's agar medium was found to be as selective media for the isolation of phosphate solubilising bacteria. The composition of pikovskaya medium was maintained in[11]. The sterilized pikovskaya medium was prepared and poured into Petri dishes .After solidification of the medium, 0.1ml sample was poured into agar medium plate by using L-Rod spread the sample evenly over the agar surface and then incubated at 37°C f or 24 hours[3].

Detection of PSB

0.1 ml of PSB were isolated from each sample was subjected into pikovskaya agar medium containing insoluble tri calcium phosphate and incubated at 27-30°C for 7 days. Insoluble tri calcium phosphate is present in

the medium used for halo zone formation at 37°C in two weeks.

The morphological features of isolated bacteria via shape, size elevation, surface margins, surface texture and colour were observed[12] and characterized by gram staining.

Solubilisation Index

10 ml of each PSB culture was preserved in sterile distilled water. This was placed in pikovskaya agar medium at 28°C for seven days. Solubilisation index was measured by Edi-premono[13].

Quantitative analysis of phosphorus solubilisation by PSB

The determination of available phosphorus in sample was determined by phosphor molybdate method[14].

Preparation of liquid inoculants

The pikovskaya 's broth incubated with water in 250ml conical flask . It was allowed to multiply by incubating at 32°C in a incubator cum shaker at 100 rpm for 72 hours. The broth containing approximately $25x10^{11}$ cfu/ml was used as a starter culture for the production of liquid inoculants. This can be used as nutrient enhancer for growth of plants.

RESULTS AND DISCUSSION

The isolation of phosphate solubilising bacteria from different types of soil were carried out and exhibited halozone formation (Figure 1).

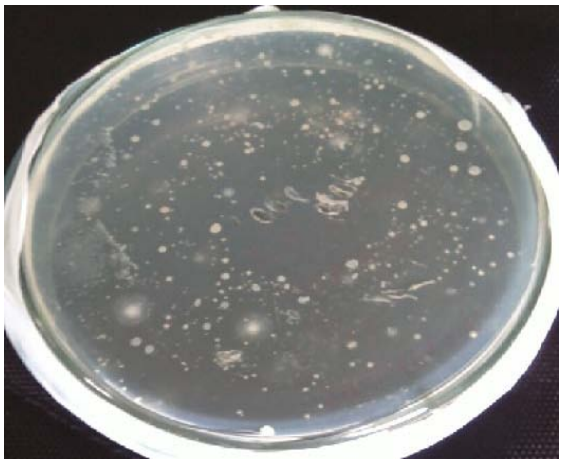

Figure 1: Clear zone formation from garden soil

It showed the capability of PSB secrete organic acids. The isolate showed colorless colonies which donot produce pigment. Cells were gram-negative, rod shaped and identified based on biochemical tests. The isolate were identified as *Bacillus, Bacillus substilis, Diphtheroids* and *arthobacteia and Actinomyces*.

High density of *bacillus and bacillus substitis* were found in fertile soil with the range of $2x10^7$ and $3x10^6$ cfu/g when compared with garden soil. *Bacillus* and actinomyces were observed in garbage soil. In non fertile soil, *bacillus* was found to be very less in the concentration of $1x10^2$ with 3 zone of clearance. The Arthobacter and Diphtheroids were also found (Table1). Six zone of clearance was found in *bacillus* of fertile soil. The maximum solubilisation of phosphate was observed in 5% of TCP (Table 2). Similar results showed that solubilisation index of *bacillus* ranged from 3.0 to 4.5[15].

Table 1: Different kinds of phosphate solubilising bacteria in soil samples

S. No	Soil sample	Microbes	Colony Count (cfu/g)	Average zone of clearance
1	Fertile soil	*Bacillus*	$2x10^7$	10
		Bacillus substilis	$3x10^6$	9
2	Garden soil	*Bacillus*	$4x10^6$	6
		Bacillus substilis	$3x10^5$	5
3	Garbage soil	*Bacillus*	$2x10^5$	5
		Actinomyces	$3x10^6$	4
4	Non fertile soil	Arthobacter	$2x10^4$	3
		Diphtheroids	$7x10^4$	0

Table 2: Solubilisation zone of inhibition (mm)of different PSB

S. No	Organism	2.5% TCP	3%TCP	5%TCP	7%TCP
1	*Bacillus*	45	10.8	5.5	3.2
2	*Bacillus substilis*	80	10.6	10.2	2.5
3	Actinomyces	3.0	0.2	1.0	-

The amount of phosphate solubilisation activity f soil sample ranged from 16.3 µg to150 µg/ml. According to samiran, Bacillus TRSB16 showed high rate of solubilisation of calcium phosphate (144µg /ml) and Arthobacter showed low amount of phosphate solubilisation[16].

The PSB based inoculants were used as nutrient enriched form of biofertilizer for the growth of green gram plant. The rapid growth was observed in inoculants treated plants than normal plants(Figure 2).

Figure 2: Morphological features of green gram plant (control and PSB treated)

CONCLUSION

The isolation and characterization of phosphate solubilising bacteria were identified in different range of

soil. The morphological features of PSB were analyzed based on degree of phosphate solubilisation and phosphorus uptake was measured.

Thus phosphate solubilising bacteria are predominantly in fertile and garden soil. This phospho bacteria can be used as biofertilizer for improving the yield of plants.

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