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Isolation, Identification of Phosphate Solubilizing bacteria(PSB) isolated from Onion fields

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ABSTRACT
Soil microbes in different forms can solubilize the fixed form of P so that plants can use it. These organisms are known as bacteria that solubilize phosphate (PSB).Phosphate solubilizing bacteria (PSB) are being used as biofertilizers, which increase the plant uptake of phosphorus thereby improving crop yields in a number of crop species. In vitro conditions were used to characterize the isolated strains. The onion rhizosphere soil had a higher PSB population dynamic. Studies were conducted using production in the solid medium that was chosen based on the solubilization zone. The chosen PSB strains underwent in vitro screening. Five strains of Bacillus megaterium, three strains of Pseudomonas putida, and two strains of B. polymyxa were identified among the ten PSB isolates.

Key words: PSB, Onion,Isolation,Identification ,Bacillus megaterium,Pseudomonas putidaand B. polymyxa

I. INTRODUCTION

An element that is necessary for plant growth and development is phosphorus(P). P is taken up by plants as phosphate anions from the soil solution. Phosphate anions, on the other hand, are highly reactive and, depending on the specific characteristics of a soil, can be immobilized by precipitation with cations like $Ca^{2+,}$ Mg^{2+}, Fe^{3+}, and Al^{3+}. P is largely insoluble in these forms and inaccessible to plants. By causing favorable changes in soil reaction and soil microenvironment that lead to the solubilization of inorganic phosphate sources, phosphate solubilizing microorganisms (PSM) play a crucial role in making phosphorus available to plants. It is possible for certain microorganisms connected to various pant rhizospheres to solubilize inorganic insoluble P salts. According to Reyes et al., two significant genera of soil bacteria with promising phosphate solubilization activities are Pseudomonas and Bacillus.

II. MATERIALS AND METHODS

Isolation and Identification of PSB

From onion fields, soil samples were taken from the rhizosphere of the onion plant plot wise. Soil samples were put on blotting paper and stored at 25°C. Ten grams of the dry soil were placed in 250 ml Erlen Meyer flasks, and then 90 ml of sterile physiological saline solution (0.85%) was added to each flask. In a shaker, the erlenmeyers were shaken for thirty minutes. 10 ml tube vials were filled with half a milliliter of suspension and 4.5 ml of pure physiological saline solution. The vials were then shaken for a minute. A serial dilution method was used to achieve dilutions up to 10−7.A 100 µl portion of this mixture was applied to plates that had solid NBRIP growth medium with (g l-1): 20 g of agar, 0.2 g of KCl, 0.1 g of (NH4)2SO4, 5 g of MgCl2·6H2O, 0.25 g of MgSO4·7H2O, and 10 g of glucose (Nautiyal, 1999). P solubilizing strain colonies with a clear zone were deemed positive for phosphate solubilization after the dishes were incubated for one week at 25°C. The following formula was used to calculate the index of phosphate solubilization (Premonoet al., 1996).

SI (Solubilisation Index) = Total diameter (colony + halo zone) / Colony diameter

In NBRIP broth medium ((g l-1): 10.0 g of glucose, 10.0 g of tricalcium phosphate (TCP), 5.0 g of MgCl2.6H2O, 0.25 g of MgSO4.7H2O, 0.2 g of KCl, and 0.1 g of (NH4)2SO4), these phosphate-solubilizing strains were simultaneously tested for their in vitro phosphate-solubilizing properties. Phosphate solubilization was quantified using 15 ml test tubes that held 10 ml of freshly inoculated NBRIP growth medium containing 0.1 ml of each PSB strain. For seven days, the sample was incubated at 27°C in an incubator shaker set to 125 rpm. After centrifuging the tubes for 15

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minutes at 10,000 rpm, the supernatant of each culture was measured using Barton's method to determine the phosphate concentration in ppm. (1948).

III. EXPERIMENTAL RESULTS AND DISCUSSION

Identification of PSB

The biochemical and morphological tests allowed for the genus-level identification of PSB. Five strains of Bacillus megaterium, three strains of Pseudomonas putida, and two strains of B. polymyxa were identified among the ten PSB strains.

Pseudomonas putida: All pseudomonas lacked spores and were motile, gram-negative, strictly aerobic growers. P. putida showed positive reactions to argininedihydrolase, catalase, oxidase, glucose acid, and growth on citrate agar; however, they showed negative reactions to denitrification, gelatin hydrolysis, starch hydrolysis, and growth at 41ºC.

B. polymyxa: This pathogen, which is Gram positive, opposes oomycetic pathogens. P. polymyxa is a potentially valuable biocontrol agent for commercial use because of its wide host range, capacity to form endospores, and capacity to produce a variety of antibiotics. Thus far, the majority of research on P's biocontrol ability.

Bacillus megaterium: Endospores were produced and the growth was strictly aerobic. The cells were motile and Gram positive. In glucose agar, the growth was mucoid. In the rhizosphere of an onion field, Frietas and Germida (1990) isolated the phosphate-solubilizing microorganisms Pseudomonas aeruginosa, P. cepacia, P. fluoresence, and P. putida. Phosphobacteria were discovered to have gram-negative, rod-shaped, non-motile morphological and biochemical characteristics. The organism passed the catalase, methyl red, and indole production tests (Amuthaet al. 2014).

Table :1Designation of Phosphobacteria isolates obtained from onion rhizospheres oil samplesin Trichy District

Sl.No.	Location	Isolatedesignation
1.	P.K. Agaram	OPB-1
2.	M.R.Palayam	OPB-2
3.	Sanamangalam	OPB-3
4.	Edhumalai	OPB-4
5.	Thirupattur	OPB-5
6.	Padalur	OPB-6
7.	Siruganur	OPB-7
8.	Konalai	OPB-8
9.	Perakambi	OPB-9
10.	Pulivalam	OPB-10

Table.2 Identificationof PSB

PSBStrains	IdentifiedPSBstrains
OPB-1	B.megaterium
OPB-2	B.megaterium
OPB-3	B.polymyxa
OPB-4	B.polymyxa
OPB-5	B.megaterium
OPB-6	Pseudomonas
OPB-7	B.megaterium
OPB-8	Pseudomonas
OPB-9	Pseudomonas
OPB-10	B.megaterium

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Estimation of phosphatase activity:

The method outlined by Eivazi&Tabatabai (1977) was used to estimate the activity of phosphatase. After centrifuging the culture broth for ten minutes at 10,000 rpm, the pellet was suspended in five milliliters of sterile distilled water. A 50 ml conical flask was filled with 1 ml of the sample, 0.25 ml of toluene, 4 ml of modified universal buffer, and 1 ml of p-nitrophenyl phosphate solution. The flask was then allowed to sit at room temperature for one hour. Following the incubation period, filter the contents through filter paper and add 1 milliliter of 0.5 M calcium chloride and 4 milliliters of 0.5 M sodium hydroxide. The absorbance was measured at 660 nm. Peptase activity was measured in terms of µ moles of PNP released per milliliter of filtrate per hour.

Table 3.Estimation of phosphatase activity

PSB strains	Solubilization zone (mm)	pHReduction	Organic acid (0.1 N NaOH Consumed)	Phosphatase activity (µ mole/ml/hr)	Available P (ppm)
OPB-1	2	5.6	3.1	20.8	43.00
OPB-2	4	5.1	3.7	22.3	44.80
OPB-3	2	4.7	6.1	20.9	45.00
OPB-4	2	4.7	6.7	21.0	45.50
OPB-5	4	4.6	8.3	22.9	45.40
OPB-6	5	4.5	8.8	25.1	45.96
OPB-7	2	4.7	7.0	19.6	44.68
OPB-8	2	4.7	7.4	19.3	44.36
OPB-9	3	4.5	7.0	20.2	42.00
OPB-10	2	4.2	7.1	22.0	43.00

Plate 1.SolubilizationzoneproducedbyPSB

P solubilization and phosphatase activityby PSB:

The zone in the KNB solid medium was measured to estimate the solubilization of P by PSB. In vitro, OPB-6 exhibited the largest solubilization zone in comparison to other strains. Every PSB strain lowered the liquid culture medium's pH.Tricalcium phosphate (TCP) was the source of phosphate in OPB-1, where the greatest pH reduction was observed. Using an estimation of the amount of available phosphorus in the culture medium, the P solubilization potential of specific PSB strains was examined in vitro. The outcomes showed that the ability of various strains in PSB to solubilize phosphate varied greatly. Of the ten strains, OPB-6 (45.96 ppm) and OPB-4 (45.50 ppm) released the most phosphorus into the medium.

IV. \DISCUSSION:

PSB colonize at the roots of plants and, using a wide range of techniques, have beneficial effects on plant growth and development. Although the precise mechanism by which PGPR promotes plant growth is unclear, several theories, including the generation of IAA, the suppression of harmful organisms, the solubilization of phosphates, and an increase in mineral uptake, are generally accepted to be connected (Ludueñaet al., 2018). One of the essential nutrients for plants is P. According to Richardson (2001), the majority of the P in the soil is insoluble and cannot be utilized by plants. The effects of PSB strains on onion growth were assessed in this study.

On NBRIP agar medium, phosphate solubilizing strains create a sizable zone. On an agar plate, some of the strains did not, however, form sizable halo zones. This could be as a result of the PSB secreting a variety of organic acids with varying diffusion proportions. Therefore, in order to determine their phosphate solubilizing efficiency, the PSBs were also screened in NBRIP broth medium. As a result, it was simple to determine which microorganisms were phosphate solubilizing. Phosphate solubilization of microorganisms varies for isolated and distinct locations, according to reports similar to this one. Plant growth promoting mechanisms were recently characterized after endophytic and epiphytic plant

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growth promoting bacteria (PGPB) were isolated from healthy almond trees.

Both solid and liquid media were used in the investigation, and microorganisms' capacity to solubilize phosphate was found to range from 80 to 100 ppm (Audipudiet al., 2012). In a different investigation, Pikovskaya's agar was used to isolate PSB. In order to assess the P solubilization index (PSI), ten strains were inoculated on Pikovskaya's agar plates in this investigation. The SI of PSB strains with a range of 1.8 to 5.0 was determined by researchers (Alia et al., 2013). It has been officially documented that PSBs are beneficial in supplying adequate amounts of mineral nutrients, especially P, for crop production. The PSB Enterobacter, Pantoea, Azotobacter, and Burkholderia were reported on by Vermaet al. (2015).

Under greenhouse conditions, they found that five inoculated bacterial strains with PSB and other PGPR traits increased the biomass of the plants by 20–40%. According to Suri and Choudhary (2013), the use of PSB increased soybean (Glycine max) productivity, protein content, and nutrient uptake.According to Teleket al. (2019), rhizobacteria were also found to have positive effects on fruit-related characteristics like root length, fruit wet weight, fresh weight, dry weight, and fruit flesh. According to Biswas et al. (2018), mung bean (Vignaradiata) seeds were sterilized by the PSB strains Bacillus megaterium Staphylococcus haemolyticus and Bacillus licheniformis, and the results showed a higher growth and germination ratio in bacterium-enriched environments.

In order to determine the antagonistic potentials (such as siderophore and ammonia productions) and plant growth-promoting traits (like IAA and phosphate solubilization) of endophytic plant growth-promoting bacteria (PGPB) from healthy bean plants growing in various regions of Turkey against the bacterial halo blight disease agent Pseudomonas syringaepv. phaseoli in vitro, Duman and Soylu (2019) recently conducted a study. Compared to the control plants, PSB injection increased leaf length by 39.5%, root length by 31.1%, and total number of leaves by 48.1%. It was discovered in this study that PSB strains are efficient phosphate solubilizers. When P is a limiting factor for plant growth, PSB strains' capacity to solubilize otherwise insoluble P and transform it into a form that plants can use is a crucial feature (Zhang et al., 2012).

V. CONCLUSION

The study's findings unequivocally showed how P solubilizers may affect the growth of onion plants. To fully comprehend the precise mechanisms underlying PSB's beneficial effects on onion growth in field settings, more research is necessary.

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