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ORIGINAL ARTICLE **OPEN ACCESS**

Isolation, Characterization of Phosphate Solubilizing and Phytohormone Producing Bacteria from Soil

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ABSTRACT

Soil is the hub of macronutrients among which phosphate is one of the main nutritive component. Soil contains nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur which serve as macronutrients by actively participating in development of plants. Phosphorus is one of the macronutrient that is present in insoluble form in the soil. There are a group of microorganisms called Phosphate solubilizing microbes(PSM) or Phosphate solubilizing bacteria(PSB) that solubilize the insoluble phosphate and makes it available for the plant for its growth and development. In the present work, 3 Phosphate solubilizing bacterial colonies were isolated on the Pikovskaya's agar medium which contain insoluble tricalcium phosphate(TCP). Colonies that showed halozones around the growth indicated phosphate solubilization. Out of the 3 isolates isolate-3 showed maximum solubilization efficiency of 75%. Later, the isolates were screened for Indole Acetic Acid (IAA) production (Loper and Schroth, 1986). It was found that all the isolates produced IAA among which production of IAA of isolate-1was maximum i.e.1400 µg/mL. Subsequently, the characterization of isolates was done on the basis of morphological and cultural characteristics and identified as Bacillus subtilis, Pseudomonas aeruginosa and Bacillus megaterium.

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INTRODUCTION

Soil contains many microorganisms which contributes in maintenance of ecological balance by actively participating in various cycles like Nitrogen, Phosphorous, Sulphur, Carbon in nature [1]. Soil contains various macronutrients which help in growth and development of plant among which phosphorous is one of them. Phosphorous is an important component of "ATP" which is the energy unit in plants. It participates in many other physiological activities such as cell division, photosynthesis, and development of good root system and utilization of carbohydrate. Thus, phosphorous is essential for general health and vigor of the plants. It was found that 98% of Indian soils lack the required amount of phosphorous. The availability of P is low in soils because of its fixation as insoluble phosphates of iron, aluminum and calcium[1]. Phosphorous is not directly available for the plants as it is present in insoluble form. Hence, to overcome this problem and for optimum yield of the plants phosphate fertilizers can be used. Inspite of the use of P-fertilizer, the plants have very less capacity for uptake of phosphorous. Secondly, when we add large portion of fertilizer it becomes unavailable for the plants as P becomes insoluble and immobilized when added in large amounts [2]. Additionally, fertilizer when used at a high amount have many hazardous effects among which pollution and bad effect on human healthare the main problem. To maintain the crop production the phosphorous must be supplied in ample amount. The insoluble phosphate compounds can be solubilized by using various phosphatase enzymes which have the ability to dissolve phosphate produced by plants and microorganisms [3]. The phosphate solubilizing microorganisms can be isolated from soil using Pikovaskaya's media. IAA is important phytohormone needed for plant growth promotion and microorganisms have potential to produce it[4].
In the present study, we isolated 3 isolates namely *B.subtilis, P.aeruginosa and B.megaterium* were found to be promising for the production of IAA.

MATERIAL AND METHODS

Collection of Soil Samples

Rhizospheric soil samples were collected from Betel wine plant from Vadgaon-haveli, in Satara district (Maharashtra, India).

Isolation of Phosphate Solubilizing Microbes

PSM were isolated from all the samples by using serial dilution technique and spread plate method. 1g of soil sample was dispersed in 10mLof sterile distilled water and it was thoroughly mixed. 1mL of the above solution was transferred to 9 mL of sterile distilled water which formed 10^{-2} dilution. Similarly 10^{-3}, 10^{-4},10^{-5},10^{-6}, 10^{-7} and 10^{-8} dilutions were made for each soil sample. 0.1 mL of each dilution was spread on Pikovaskaya's agar medium (PVK) which contains insoluble Tricalcium phosphate and was incubated at 27-30^{o}C for 7 days. Colonies showing halozones were picked and purified by subculturing on Pikovaskaya's (PVK) agar medium for studying colony morphology.

Colony Characterization

Colony characteristics of isolates viz. shape, size, elevation, surface form, margins and surface texture, color were observed for their characterization.

Gram Staining

Gram nature of the isolates were studied by following the standard protocol.

Biochemical Characteristics

Biochemical characteristics like Catalase test, Oxidase test, Sugar fermentation tests, Starch hydrolysis test, Gelatin hydrolysis test, Urease test, Indole test, Methyl red test, Voges-Proskauer test, Citrate Utilization were studied.

Estimation of Phytohormones Produced by PSB:

Assay for IAA:

IAA was determined by following the method [5]. All the test organisms were screened for IAA production [4]. Briefly, test bacterial culture was inoculated in the Pikovaskaya's broth with tryptophan (0.1 g/L) and without tryptophan and incubated at 30^{o}C. Cultures were centrifuged at 3000 rpm for 30 min. 2mL of the supernatant was mixed with 2 drops of orthophosphoric acid and 4mL of Salkowski reagent. Salkowski reagent is a mixture of 0.5M ferric chloride ($FeCl_3$)and 35% perchloric acid ($HClO_4$) which upon reaction with IAA gives pinkcolour which appears due to formation of complex with reduction of Fe^{3+}[6].

RESULTS AND DISCUSSION

Cultural and Morphological Characteristics

Isolates were studied for their morphological, cultural and biochemical characterization.

The colony characteristics of the isolates are as per the following table 1:

From the results of morphological and cultural characteristics, staining properties and biochemical characteristics with reference to Bergey's manual of systematic Bacteriology Volume-I & II, the organisms were tentatively identified as *Bacillus subtilis* (PSB1), *Pseudomonas aeruginosa*(PSB2) and *Bacillus megaterium* (PSB3).

Results for Solubilization Efficiency

Calculations

$$\text{Solubilization efficiency (SE)(\%)} = (Z-C)/C \times 100$$

Where, Z is solubilization (Halo) zone and C is the colony diameter.

The colony diameter of B.subtilis was 3mm and its zone diameter was 5mm and the calculated solubilization efficiency was 66%. The colony diameter of P.aeruginosa was 5mm and its zone diameter was 8mm and calculated solubilization efficiency was 60%. The colony diameter of B.megaterium was 4mm and its zone diameter was 7mm and calculated solubilization efficiency was 75%. Hence, B.megaterium showed maximum solubilization efficiency i.e. 75% when compared to other isolates.

Estimation of IAA

The production of IAA was detected by the formation of pink color. All the isolates were capable of developing pink color, which showed the production of IAA.

From the graph (fig.1) it was determined that isolate Bacillus subtilis(PSB1) produces 1400 µg/mL, Pseudomonas aeroginosa(PSB2) produces 380 µg/mL, B.megaterium(PSB3) produces 890 µg/mL of IAA.

Table1: Colony characteristics of the isolates on Pikovaskaya media at 30^{o}C FOR 48-h

Isolate name	Size	Shape	Color	Margin	Opacity	Elevation	Consistency
PSB 1	2mm	Circular	White	Irregular	Opaque	Convex	Moist
PSB 2	3mm	Circular	Yellowish	Irregular	Opaque	Flat	Moist
PSB 3	1mm	Circular	White	Irregular	Opaque	Flat	Moist

Table 2: Gram nature, motility and Spore properties of the isolates

Isolate Name	Gram nature and morphology	Motility	Endospore staining
PSB 1	Gram positive rods	Motile	Pink colored spores
PSB 2	Gram negative rods	Motile	No spores
PSB 3	Gram positive rods	Motile	Pink colored spores

Table 3: Biochemical characteristics of the isolates

Test	Isolates		
	PSB 1	**PSB 2**	**PSB 3**
Starch hydrolysis	+	-	+
Gelatin hydrolysis	+	+	+
Urease production	+	-	+
Oxidase production	-	+	-
Catalase production	+	+	+
Glucose fermentation	+	+	+
Sucrose fermentation	+	+	+
Lactose fermentation	-	-	+
Mannitol fermentation	-	-	+
Indole production test	+	-	+
Methyl red test	-	-	+
Voges Proskauer test	+	-	+
Citrate utilization test	+	-	+

Table 4: Solubilization efficiency of the isolates

Isolate	Colony diameter	Zone diameter	SE (%)
Bacillus subtilis	3mm	5mm	66%
Pseudomonas aeruginosa	5mm	8mm	60%
B.megaterium	4mm	7mm	75%

Table 5: IAA production by the isolates (standard graph and result of isolates)

Amount of standard solution(µg/mL) of IAA	O.D. at 540nm	IAA(µg/mL)
0	0.0	-
200	0.08	-
400	0.10	-
600	0.12	-
800	0.15	-
1000	0.19	-
1200	0.22	-
1400	0.25	-
1600	0.28	-
Isolate PSB1	0.23	1400
Isolate PSB2	0.09	380
Isolate PSB3	0.16	890

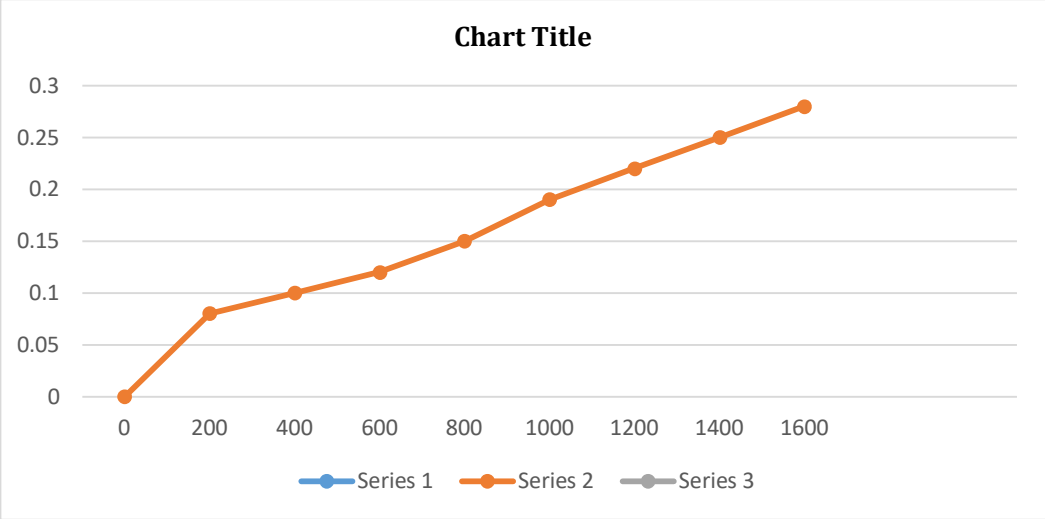
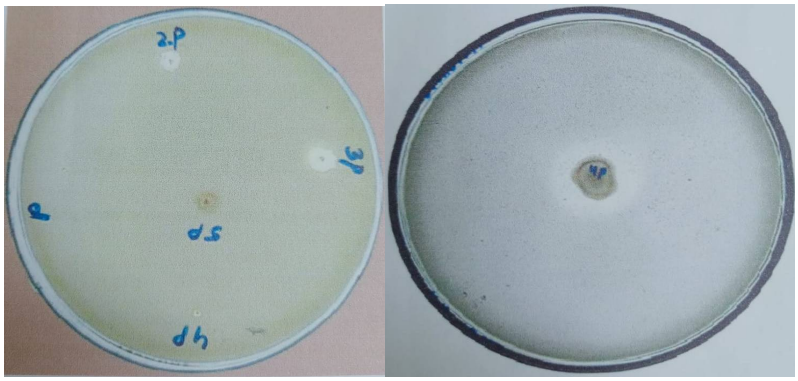
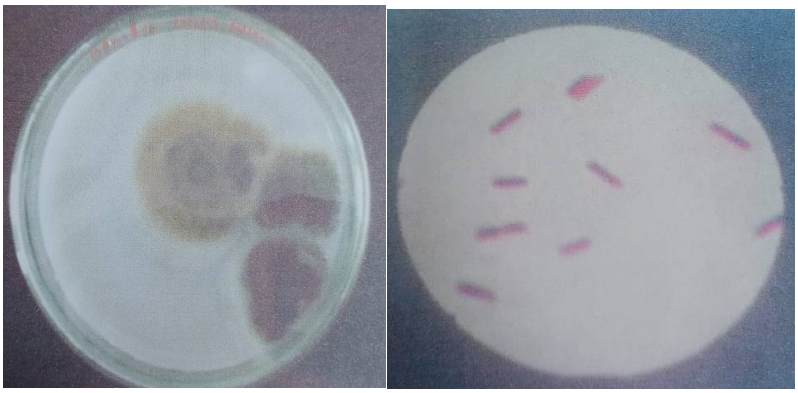


Fig.1: IAA production by the isolates



(i) (ii)

Photoplate 1 and 2-Colonies showing clear zones of hydrolysis on Pikovaskaya's agar plate.

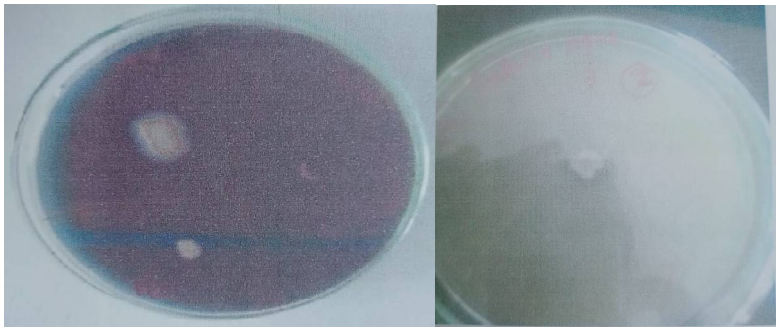


(iii) (iv)

Photoplate 3-Fungal isolate showing clear zone on Pikovaskaya's agar plate:

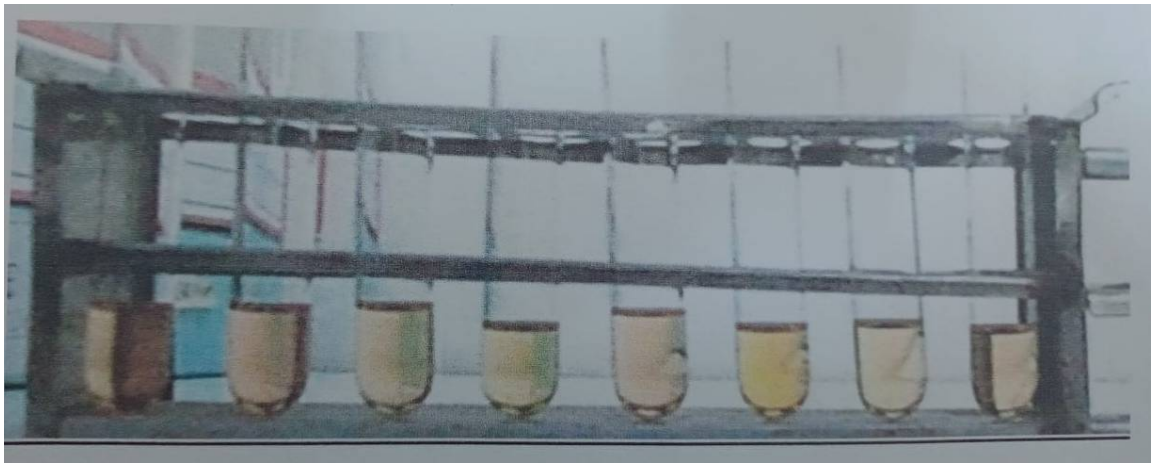
Photoplate 4- Microscopic examination of the isolate:

Biochemical characteristics



(v) (vi)

Photoplate 5-Starch hydrolysis: **Photoplate 6-** Gelatin hydrolysis:



Photoplate 7-Results for IAA production:

DISCUSSION

Three isolates were obtained and were later tentatively identified as Bacillus subtilis, Pseudomonas aeruginosa and Bacillus megaterium on the basis of morphological and cultural and biochemical characteristics. All the 3 isolates showed potential for the production of IAA. The solubilization efficiency of the isolates were calculated and *B. megaterium* showed maximum solubilization efficiency i.e. 75%. IAA production by the isolates were calculated and B. subtilis showed maximum production of IAA i.e. 1400 µg/mL [1] have reported isolation of 37 isolates of phosphate solubilizing bacteria and identification of 3 of them. [7] have reported isolation of a phosphate solubilizing strain from rhizosphere of Chinese cabbage plants grown in Yangling.

CONCLUSION

Phosphate solubilizing bacteria has a wide range of applications to make available the insoluble phosphorous present in the soil to plants. Isolation of phosphate solubilizing bacteria was done from rhizospheric soil. The obtained isolates were investigated for their ability to produce IAA by Salkowski method. The results provided valuable information about the phosphate solubilizing bacteria and production of growth hormone by them which can be used for plant growth promotion.

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