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SCREENING AND ISOLATION OF PHOSPHATE SOLUBILISING MICROORGANISMS FROM SOIL SAMPLES COLLECTED FROM AURANGABAD FARM LANDS

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ABSTRACT
Phosphorus deficiency results in the leaves turning brown accompanied by small leaves, weak stem and slow development. In present study soil samples were collected from different locations of Aurangabad. The serially diluted soil samples were inoculated in Pikovaskaya's medium by four plate method and inoculated at 28^{0}cfor 48 hours.12 bacterial and 05 fungal isolates was estimated in Pikovaskaya's broth in mg/lit. Amongst the bacterial isolates, PSB -4 (187mg/lit), PSB-1 (156.3 mg/lit) and PSB-19(137 mg/lit) were found to be most active solubilisers. Even on solid Pikovaskaya's medium these bacterial isolates were found to give greater zone of solubilisation. Amongst the fungal isolates, PSF-2 (148mg/lit), PSF-1 (140mg/lit) and PSF-4 (107mg/lit) were found to be most active solubilisers. Even on solid Pikovaskaya's medium these fungal isolates were found to give greater zone of solubilisation. Cultural, morphological and biochemical characteristics of the bacterial isolates were studied.

KEYWORDS: PSB, Zone of solubilisation, Pikovaskaya's medium.

INTRODUCTION
Next to nitrogen, phosphorus is the second major and essential growth limiting macronutrients required in optimum quantity for plant growth and productivity. Plants take phosphorus from the soil in the form of soluble orthophosphate anions. phosphorus plays an important role in many physiological activities and metabolic pathways. Thus phosphorus is called the "Key of Life".

Indian soils are characterized by poor and medium quantity of available phosphorus (Baby 2002; Li et Al. 2003; Ramanathan et al 2004). As compared to other nutrients. As compared to other nutrients, the concentration of phosphorus in the soil is generally low.

Most tropical soils are acidic, rich in Iron and deficient in soluble forms of phosphorus(Baby 2002; Khan et al 2010; Singh and Reddy 2011). Phosphorus deficient soils cannot give high yields. The use of microbial inoculants (biofertilizers) possessing Phosphate solubilising activities in soils is an environment friendly alternative to chemical based Phosphate fertilizers.

MATERIAL AND METHODS
1. Sample collection—The samples for screening Phosphate solubilising bacteria were collectsd from agriculture soil, rhizosphere soil, and garden soilfrom Paithan, Dr.Babasaheb ambedkar Marathwada university, Aurangabad, Padegaon, Itkheda, Satara. Collection of soil samples was made at a depth of 6-15cms from five different points within the area.

All collected soil samples were stored in polythene bags and transported to laboratory aseptically and maintained and stored at 4^{0}c prior to be analysed. These samples were air dried and ground to pass through 2mm sieve before microbial analysis.

2. Physico- chemical analysis odf soil – The physical –properties of soil samples from the study area were studied laboratory.

a. Estimation of p^H
b. Soil moisture
c. Estimation of available phosphorus

3. Analysis of rhizosphere and non rhizosphere microbial population from soil sample.

Soil samples were collected from rhizosphere and non rhizosphere region during the cropping season from farmers field in Aurangabad. Five plants were pulled out and soil attached with the complete intact roots was collected and kept separately in polythene bags. Non rhizosphere soil was collected in between space of two rows of plants.

Total bacterial and fungal count of rhizosphere and Non rhizosphere soil samples were estimated. Serial dilution of $1:10^3$ was prepared in sterilized water and plated on potato dextrose agar medium for fungi. Serial dilution of $1:10^4$ was prepared in sterilized water and plated on soil extract agar medium for bacteria. 0.5ml of soil suspension placed in each sterilized Petri plate and 20ml of cooled melted medium was poured in the same plate and gently rotated horizontally to get uniform distribution in medium. These plates were incubated at $28+-1^0$c for 48 hours for bacteria and 72 hours for fungi in three replication. Total bacterial and fungal count of rhizosphere and non rhizosphere was calculated.

Screening of phosphate solubilising bacteria from soil samples

soil samples were collected from different locations of Aurangabad. The soil samples were air dried under shade and used for screening and isolation of phosphate solubilising bacteria. Screening of phosphate solubilising bacteria was carried out by dilution plate technique using Pikovaskaya's medium. The serially diluted soil samples were inoculated in Pikovaskaya's agar medium by pour plate method and incubated at $28+-1^0$c for 48 hrs. After incubation phosphate solubilising bacterial (PSB)colonies were visually identified by the formation of clear zone around the bacterial colony. Simillarly, the total colony count of PSB was taken on Pikovaskaya's agar medium. The colonies showing maximum diameter of phosphate solubilisation were further selected. These colonies were sub-cultured and maintained on Pikovaskaya's agar slants for further study.

RESULTS AND DISCUSSION

Rhizosphere and non rhizosphere soil samples were collected from different locations of farm land around Aurangabad. Table 1,2,3

Table 1: Locations of soil samples collected.

Sr. No.	Locations
1	Farm of Paithan
2	Farm of Padegaon
3	Farm of Itkheda
4	Farm of Satara
5	Garden of Dr. Babasaheb Ambedkar MarathwadaUniversity

Table 2: Physico- Chemical analysis of the soil samples.

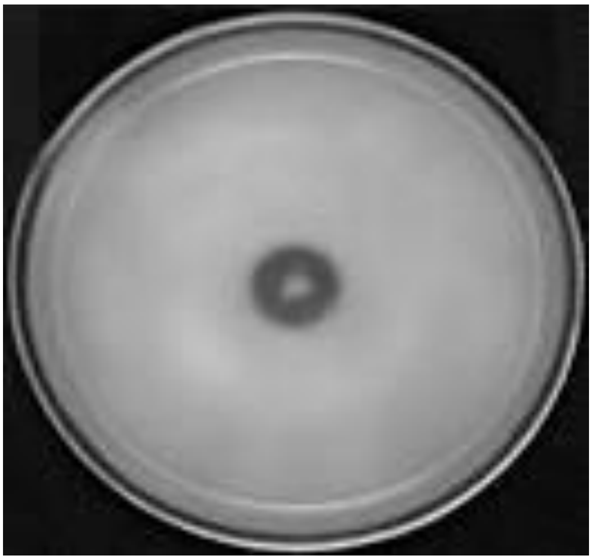
Sr. No.	Soil sample	Moisture %	pH
1	Farm of Paithan	55	7.6
2	Farm of Padegaon	49	6.7
3	Farm of Itkheda	53	6.9
4	Farm of Satara	56	7.2
5	Garden of Dr. Babasaheb Ambedkar Marathwada University	54	7.4

Soil samples were tested for their p^H values, moisture and available phosphorus content. The results are shown in table 2 and 3

Table 3: Estimation of available phosphorus in soil.

Sr. No.	Soil sample	Available Phosphorus in Kg/ha
1	Farm of Paithan	35.1
2	Farm of Padegaon	30.8
3	Farm of Itkheda	32.2
4	Farm of Satara	38.0
5	Garden of Dr. Babasaheb Ambedkar Marathwada University	40.4

Formation of clear zone around the bacterial colony., of PSB on Pikovaskaya's agar




Examination of the soil samples show that the values of pH of the soil samples range from 6.7 to 7.6. This shows that the pH of the soil samples range from weakly acidic to weakly alkaline. P^H can affect the availability of nutrients in the soil. The moisture content of the samples studied varied from 49 to 56%. This moisture content indicates the soil is well aerated. The available phosphorus in soil samples studied ranges from 30.8 Kg/ha to 40.4 Kg/ha, which is comparatively low. Application of phosphorus is necessary for maintaining a balance between the other plant nutrients and ensuring the normal growth of the crops. Low phosphorus availability can also constrain nitrogen fixation.

Microbiological analysis of the soil samples

Rhizosphere as well as Non- rhizosphere soil samples were analyzed for their total bacterial and fungal count. Table 4,5.

Table 4: Total bacterial count on soil extract agar medium.

Sr. No.	Soil sample	No. of organisms/gram of Non-rhizospheresoil	No. of organisms/gramof Rhizosphere soil
1	Farm of Paithan	1.01×10^7	8.1×10^7
2	Farm of Padegaon	1.48×10^7	7.8×10^7
3	Farm of Itkheda	1.23×10^7	9.7×10^7
4	Farm of Satara	1.89×10^7	7.2×10^7
5	Garden of Dr. Babasaheb Ambedkar Marathwada University	1.33×10^7	7.9×10^7

Table 5: Total fungal count on Potato dextrose agar medium.

Sr. No.	Soil sample	No. of organisms/gramof Non-rhizosphere soil	No of organisms/gramof Rhizosphere soil
1	Farm of Paithan	1.4×10^5	5.9×10^5
2	Farm of Padegaon	1.7×10^5	5.1×10^5
3	Farm of Itkheda	1.9×10^5	9.7×10^5
4	Farm of Satara	1.3×10^5	2.1×10^5
5	Garden of Dr.BabasahebAmbedkar MarathwadaUniversity	1.1×10^5	1.8×10^5

Itkheda soil sample showed highest total count for both, bacteria as well as in rhizosphere and non-rhizosphere soil.

Screening of phosphate solubilisers from soil

Screening of phosphate solubilisers from collected soil samples was performed on Pikovaskaya medium. The identification of the phosphate solubilizers was based on the development of clear zone of phosphate solubilization around their colonies on Pikovaskaya medium.

Total count of bacterial and fungal phosphate solubilisers was also recorded.

Table 6: Total count of phosphate solubilizing bacteria and fungi present in thesoil samples.

Sr. No.	Soil sample	No. of phosphate solubilizing bacteria/g of soil	No. of Phosphate solubilizing fungi/g of soil
1	Farm of Paithan	$1.2 X 10^4$	$1.0 X 10^3$
2	Farm of Padegaon	$1.5 X 10^4$	$1.2 X 10^3$
3	Farm of Itkheda	$1.7 X 10^4$	$1.3 X 10^3$
4	Farm of Satara	$1.1 X 10^4$	$1.1 X 10^3$
5	Garden of Dr. BabasahebAmbedkar Marathwada University	$1.1 X 10^4$	$1.0 X 10^3$

A total of 59 isolates of phosphate solubilizing microorganisms were screened from the different soil samples as shown in Table 7.

Table 7: Screening of phosphate solubilisers from soil on Pikovaskaya medium.

Sample No.	Location of Rhizosphere soil	Number of isolates
1	Farm soil and Paithan	13
2	Farm of Padegaon	09
3	Farm of Itkheda	16
4	Farm of Satara	07
5	Garden of Dr. Babasaheb Ambedkar Marathwada University	14

These isolates were subjected to *in vitro* phosphate solubilizing ability on Pikovaskaya medium. Selection of phosphate solubilizers for further study was based on higher solubilization activity as measured in terms of diameter of zone of solubilization. Accordingly higher solubilization was exhibited by 12 bacterial and 05 fungal isolates. Twelve bacterial isolates were labeled serially from PSB-1 to PSB-12. Similarly 05 fungal isolates were labeled PSF -1 to PSF-5.

***In vitro* test for phosphate solubilization**

Table 8: Phosphate solubilizing microbial Isolates showing zone of solubilizationon Pikovaskaya medium.

S. No.	Isolates code	Zone of Solubilization(mm)
1	PSB 1	12.0
2	PSB 2	8.0
3	PSB 3	8.0
4	PSB 4	17.0
5	PSB 5	6.0
6	PSB 6	6.0
7	PSB 7	7.0
8	PSB 8	9.0
9	PSB 9	11.0
10	PSB 10	7.0
11	PSB 11	8.0
12	PSB 12	9.0
1	PSF 1	11.0
2	PSF 2	12.0
3	PSF 3	8.0
4	PSF 4	9.0
5	PSF 5	6.0

On the basis of zone of solubilization on Pikovaskaya agar plates, total of 12 bacterial and 05 fungal strains were selected for further studies.

Clear zone of phosphate solubilization was found in 12 bacterial isolates ranging from 6 mm to 17 mm. Higher solubilization activity, as measured in terms of diameter of zone of solubilization, was exhibited by PSB-4(17 mm), PSB-1(12 mm) and PSB-9(11mm). Amongst them the highest zone of solubilization was shown by the isolate PSB-4(17 mm)

Amongst the fungal phosphate solubilizing isolates, higher zone of solubilization were shown by isolates

PSF-2 (12mm), PSF-1 (11mm) and PSF-4 (9mm). Highest zone of solubilization was shown by PSF-2(12mm). Table 8

Solubilization of Phosphate in Pikovaskaya's broth

Table 9: Phosphate solubilizing microbial Isolates showing solubilization of phosphate in Pikovaskaya's broth.

S. No.	Isolates	Phosphate solubilization in mg/lit.
1	PSB 1	156.3
2	PSB 2	119.8
3	PSB 3	120.0
4	PSB 4	187.0
5	PSB 5	78.0
6	PSB 6	60.0
7	PSB 7	110.0
8	PSB 8	130.0
9	PSB 9	137.0
10	PSB 10	108.0
11	PSB 11	92.0
12	PSB 12	98.0
1	PSF 1	140.0
2	PSF 2	148.0
3	PSF 3	98.0
4	PSF 4	107.0
5	PSF 5	58.0

The amount of phosphate released in the Pikovaskaya's broth by each of the 12 bacterial isolates was quantitatively measured using chlorostannous reduced molybdophosphoric acid blue method as described by Jackson in 1973. In quantitative estimation, the amount of tri calcium phosphate solubilized was found between 58mg/lit. to 187mg/lit. as shown in table 9.

Highest amount of phosphate was solubilized by PSB-4 (187mg/lit.), followed by PSB-1 (156.3mg/lit.) and PSB-9 (137.0 mg/lit.).

With fungal phosphate solubilizing isolates, highest phosphate solubilization was shown by PSF2 (148mg/lit.), followed by PSF-1 (140 mg/lit.) and PSF-4(107.0 mg/lit.).

Table 9: Morphological characteristics of phosphate solubilizing microbial isolates.

Microscopic observation of bacterial isolates was done to study their shape, arrangement, sporulation, cyst formation and motility. Gram's nature was also recorded.

Table 10 Morphological characterization of phosphate solubilizing bacterial isolates.
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Table 10: Morphological characterization of phosphate solubilizing bacterial isolates.

Sr.No	Phosphate solubilizing bacterial isolates	Gram'snature	Shape	Spore formation	Cyst formation	Motility
1	PSB 1	Positive	Big rods in chains	Spore	Non-cyst former	Motile
2	PSB 2	Positive	Big rods in chains	Spore	Non-cyst former	Motile
3	PSB 3	Negative	Short rods	Non spore	Non-cyst former	Motile
4	PSB 4	Negative	Short rods	Non spore	Non-cyst former	Motile
5	PSB 5	Negative	Short rods	Non spore	Cysts formed	Motile
6	PSB 6	Positive	Big rods in chains	Spore	Non-cyst former	Motile
7	PSB 7	Positive	Big rods in chains	Spore	Non-cyst former	Motile
8	PSB 8	Negative	Short rods	Non spore	Non-cyst former	Motile
9	PSB 9	Positive	Big rods in chains	Spore	Non-cyst former	Motile
10	PSB 10	Negative	Short rods	Non spore	Cysts formed	Motile
11	PSB 11	Positive	Big rods in chains	Spore	Non-cyst former	Motile
12	PSB 12	Positive	Big rods in chains	Spore	Non-cyst former	Motile

Various biochemical activities of the bacterial isolates were studied.

Table 11: Biochemicalcharacteristics of the phosphate solubilizing bacterial isolates.

Sr. No.	Characteristics	PSB1	PSB2	PSB 3	PSB 4	PSB 5	PSB 6	PSB7	PSB8	PSB9	PSB10	PSB1 1	PSB12
1	Sucrose Fermentation	+	+	+	+	+	+	+	+	+	+	+	+
2	Lactose fermentation	+	+	-	-	+	+	+	-	+	+	+	+
3	Indole production	-	-	-	-	+	-	-	-	-	+	-	-
4	Methyl redtest	-	-	-	-	+	-	-	-	-	+	-	-
5	Voges Proskauer	+	+	-	-	+	+	+	-	+	+	+	+
6	Citrate utilization	+	+	+	+	+	+	+	+	+	+	+	+
7	Starch hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+
8	Gelatin hydrolysis	+	+	-	-	-	+	+	-	+	-	+	+
9	Oxidasetest	+	+	+	+	-	+	+	+	+	-	+	+
10	Catalase test	+	+	+	+	+	+	+	+	+	+	+	+
11	H_2S production	-	-	+	+	-	-	-	+	-	-	-	-
12	Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+
13	Production of NH_3 from arginine	-	-	+	+	-	-	-	+	-	-	-	-
14	Growth in N_2 free mannitolbroth	-	-	-	-	+	-	-	-	-	+	-	-
15	Pigment production	-	-	Green	Green	Brown	-	-	Green	-	Brown	-	-

Positive (+) sign indicates ability to bring about the biochemical change **Negative (-)** signindicates inability to carry out the biochemical change

The cultural, morphological and biochemical characteristic of these isolates are shown in the Table 10 and 11. The results of morphological and biochemical studies show that the isolates PSB-1,PSB-2,PSB-6,PSB-7,PSB-9,PSB-11 and PSB12 are *Bacillus* spp, PSB-3, PSB-4 and PSB-8 are *Pseudomonas* spp, PSB-5 and PSB-10 are *Azotobacter* spp.

The isolate PSB-4(*Pseudomonas* spp) has maximum solubilizing activity (187mg/lit) followed by PSB-1 *Bacillus* spp (156.3mg/lit) and PSB-9 *Bacillus spp*. (137mg/lit)

The fungal phosphate solubilizing isolates were identified by the characteristics of their sexual spores and mycelia. Isolate PSF-2, PSF-3 and PSF-4 was *Aspergillus* spp and PSF-1 and PSF-5 was *Penicillium* spp

On wet mounting with Lacto phenol cotton blue, Isolates PSF-2, PSF-3 and PSF-4 identified as *Aspergillus* spp had septate branching mycelia. The conidiophores arise from the foot cell. At the apex of conidiophores swollen vesicle was seen. That in turn gave rise to sterigmata. Conidia arise from the sterigmata and are borne in chains

On wet mounting with Lacto phenol cotton blue, the isolates PSF-1 and PSF-5 identified as *Penicillium* spp had septate vegetative mycelia. On aerial hyphae conidiophores developed. Conidiophores were branched and had brush like heads bearing spores. Cluster of sterigmata were in one place and from each a chain of conidia was seen.

The isolate PSF-2 *Aspergillus* spp has maximum solubilizing activity (148mg/lit) followed by PSF-1*Penicillium* spp (140mg/lit) and PSF-4 *Aspergillus* spp (107mg/lit)

DISCUSSION

A total of 59 isolates of phosphate solubilising microorganisms were screened from the collected soilsamples. These isolates weresubjected to in vitro phosphate solubilising ability on Pikovaskaya's medium. Selection of phosphate solubilisers for further study was based on higher solubilisation activity as measured in forms of diameter of zone of solubilisation. Accordingly higher solubilisation was exhibitedby 12 bacterial and 5 fungal isolates. 12 bacterial isolates were labeled serially from PSB-1 to PSB-12. Similarlly 5 fungal isolates were labeled as PSF-1 to PSF-5.

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