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Occurrencence of phosphate solubilizing *Pseudomonas* species from rhizosphere and non rhirosphere soils of Bhavnagar, India

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ABSTRACT

Thirty six phosphate solubilizing *Pseudomonas* sp. were isolated from rhizosphere and non-rhizosphere soils of Bhavnagar, India. 21 were fluorescent type of Pseudomonas sp. while remaining were non-fluorescent type of *Pseudomonas* sp. The size of the phosphate solubilization zone was highest with fluorescent *Pseudomonads* sp. followed by green pigmented *Pseudomonas* sp. while no phosphate solubilizing zone was observed with other pigmented *Pseudomonas* sp. In Pikovskaya's broth, P - solubilization by fluorescent *Pseudomonas* sp. ranged from 28.6 to 163.1 mg % P_2O_5 while for green pigmented *Pseudomonas* sp. ranged from 26.6 to 87.0 mg % P_2O_5. Rhizospheric fluorescent *Pseudomonas* sp. were better performers in terms of P solubilization than non rhizospheric fluorescent *Pseudomonas* sp. Five promising isolates were selected based on their phosphate solubilizing activity for subsequent studies. The drop in pH was noted in all the cases.

Key words : Soil microflora, *Pseudomonas* species, Phosphate solubilization,Tricalcium phosphate**.**

INTRODUCTION

Phosphorus (P) is second only to nitrogen as mineral nutrient required by both, plants and microorganisms. About 98% of Indian soils have inadequate supply of available P (Gaur, 1987). In Indian soils, P is predominantly inorganic chiefly locked as tricalcium phosphate (TCP) (Narsian and Patel 2006, Gaur 1990, Gaur and Gaind 1983). On monitoring the available P status of Gujarat soils in 1984, the State Department of Agriculture termed Bhavnagar soil as "medium"type. It is however, encouraging that the fixed phosphate present in soil can be made available by their solubilization through some microbes. Soil microflora plays a significant role in mineralization / solubilization of bond phosphate, either in the form of organic or inorganic form of phosphatic compounds, and makes available to plant (Narsian and Patel 2006, Kundu *et al*. 2002, Krishna Raj *et al*. 1999, Bangar & Mishra 1990, Kundu and Gaur 1981). Phosphate solubilizing microorganisms (PSMs) include bacteria, fungi, yeast and cyanobacteria can be isolated from rhizosphere soils, non-rhizosphere soils, rhizoplane, areas where phosphate deposited and marine environment (Gaur 1990). However, the response is not always consistent because soil, environment and plant influence the phosphate solubilizing (PS) activity of microorganisms (Kundu *et al*. 2002). Katznelson & Bose (1959) reported that the origin zones / sites of bacterial isolates have direct effect upon their PS activity. Isolates from rhizosplane, rhizosphere and non-rhizosphere have highest, intermediate and least PS activity, respectively. Among bacteria most efficient phosphate solubilizers belong to genera *Bacillus* and *Pseudomonas* (Ostwal and Bhide 1972, Baradiya and Gaur 1974, Venkateshvarlu *et al*. (1984), Gaur 1985, Gaur 1990,). The genus *Pseudomonas* has been reported as best phosphate solubilizer among bacteria by Arora & Gaur (1979), Gaur (1985), Ostwal and Bhide (1972), Venkateshvarlu *et al*., (1984), Gaur (1987), Gaur (1990). The present study deals with isolation, identification and screening of *Pseudomonas* sp. from rhizosphere and non rhizosphere soils of Bhavnagar District for their PS activity.

MATERIALS AND METHHODS

Source of Organisms:

Pseudomonas sp. were isolated from rhizosphere and non-rhizosphere soils. Sampling of the rhizosphere soil was done by uprooting the plants gently and soil particles were collected by shaking the roots and stored in sterile wide mouth glass-stoppered bottles. The non-rhizosphere soils from different places of Bhavnagar district were collected up to the depth of 20 cm. in sterile wide mouth glass stoppered bottle

Medium for isolation, identification and screening of PS Pseudomonas sp. :

Nutrient agar and selective media i.e. King's A, King's B and Cetrimide agar (Krieg & Holt, 1984) were used to isolate and identified *Pseudomonas* sp. Pikovskaya's medium (Pikovskaya 1948) was used for screening of phosphate solubilizing *Pseudomonas* sp.

Isolation & Identification:

Pseudomonas sp. were isolated by serial dilution plate technique using Nutrient agar and selective media. (Collins, 1967). The plates were incubated at 30°C for 48h. Isolated pigmented colonies were picked up and purified further by repeated subculturing on the same media and pure cultures were maintained on Nutrient agar slants. The cultures were identified up to genus level on the basis of cell morphology, cultural characteristics and biochemical reactions as described by the Bergey's manual of systematic Bacteriology (Krieg & Holt, 1984)

Screening of Pseudomonas species for TCP solubilization:

The isolates were screened for their TCP solubilization activity on Pikovskaya's agar plates and in Pikovskaya's broth medium.

On solid medium:

Each isolate was spot inoculated under aseptic condition, on modified Pikovskaya's agar medium containing bromothymol blue, in the centre of the individual plates. The plates were incubated at 28° ± 0.2°C for 6 d and observed for the presence of phosphate solubilizing zone around the colonies.

In liquid medium:

A set of 36 Erlenmeyer flasks (250 ml) containing 100 ml Pikovskaya's broth were inoculated aseptically with 1.0 ml inoculum (6.56 x 10^7 cells / ml) in each. Uninoculated medium served as control and all flasks were incubated at 28° ± 0.2°C for 21 d, under static condition and were shaken at 12h intervals. 10.0 ml medium was withdrawn on every third day and centrifuged at 10,000 R. P. M. for 20 minutes. The supernatant was analysed for its water soluble -P content by chlorostannous reduced molybdophosphoric acid blue method (Jackson 1973). The final pH of the medium was measured using 'Elico' pH meter against standard buffer.

REESULTS AND DISCUSSION

Isolation and Identification:

Table1 lists the 36 *Pseudomonas* sp. which were isolated using Nutrient agar medium and selective media from different rhizosphere and non rhizosphere soil. Among all isolates, 18 were rhizosphere isolates while remaining were non rhizosphere isolates. The pigment production was observed on Nutrient agar medium and on selective media.

Among all these Isolates, 12 isolates (P_1, P_2, P_6, P_{10}, P_{12}, P_{18}, P_{21}, P_{23}, P_{25}, P_{26}, P_{29} and P_{30}) though did not show any pigment on Nutrient agar, produced pigment either on King's A or King's B or on both media. 21 isolates produce fluorescent pigment on Cetrimide agar, among which 12 were non rhizosphere isolates while 9 were rhizosphere isolates. The remaining isolates showed growth on the cetrimide agar but did not produce pigment on the same. .Non-fluorescent type of *Pseudomonas* sp. produced either green, yellow, bluish green or yellowish green pigment on King's A or King's B media. Thus, the *Pseudomonas* species were distinguished by pigment production on selective media.

Screening of phosphate solubilizing *Pseudomonas* species for TCP solubilization on solid and in liquid media:

Out of 36 *Pseudomonas* sp. only 13 produced clear zone of TCP solubilization around colonies after 6 d. Table2 showed that 13 isolates which produced zone of phosphate dissolution, include 10 fluorescent and 3 non fluorescent types of *Pseudomonas* sp. Non fluorescent *Pseudomonas* sp., were rhizospheric isolates, but amongst 10 fluorescent *Pseudomonas* sp. 5 were rhizospheric isolates while 5 were non-rhizospheric isolates. The results also revealed that phosphate solubilization zone was highest with fluorescent *Pseudomonas* sp. (1-9 mm). Maximum size of zone was 5.0 mm in case of non fluorescent type *Pseudomonas* sp. (P_{23}) and 9.0 mm in case of fluorescent type *Pseudomonas* sp. (P_{35}). This suggests that fluorescent pigment might be playing some role in enhancing PS activity.

The remaining 23 species out of 36 though did not show zone of phosphate solubilization on Pikovskaya's agar medium, showed PS activity in the Pikovskaya's broth (Table3).

In the liquid medium P solubilization by fluorescent *Pseudomonas* sp. ranged from 28.6 to 163.1 mg % P_2O_5 while for green pigmented *Pseudomonas* sp. ranged from 26.6 to 87.0 mg % P_2O_5. The organisms can be categorized on the basis of day on which maximum TCP solubilization occurred as follows:

Day	Isolates	Range (mg%P_2O_5)
9th	P_5, P_6, P_{35}	45.40 –163.12
12th	P_7, P_8, P_{11}, P_{14}, P_{15}, P_{16}, P_{21}, P_{24}, P_{26}, P_{27}, P_{28}, P_{29}, P_{31}, P_{33}, P_{36}	44.70 – 110.19
15th	P_1, P_{10}, P_{22}, P_{30}	47.87 – 54.43
18th	P_2, P_3, P_{12}, P_{13}	26.61 - 81.84
21st	P_4, P_9, P_{17}, P_{18}, P_{19}, P_{20}, P_{23}, P_{25}, P_{32}, P_{34}	38.89 – 150.93

Maximum phosphate solubilization in liquid medium occurred not earlier than 9th d which showed longer lag phase followed by active release of phosphorus. This is in confirmation with the observation of Venkateswarlu *et*

Table1: Pigment producing pseudomonas spp. from rhizosphere and non-rhizosphere soils of Bhavnagar District

Isolate	Non Rhizosphere Culture Media				Isolate	Rhizosphere Culture Media			
	N-agar	King's A	King's B	Cetrimide		N-agar	King's A	King's B	Cetrimide
P_1	NP	NP	F	F,+	P_4	G	G	G	+
P_2	NP	NP	F	F,+	P_5	G	G	G	+
P_3	BG	G	F	F,+	P_7	G	G	F	F,+
P_6	NP	G	G	+	P_{11}	G	NP	F	F,+
P_8	BG	NP	F	F,+	P_{13}	BG	BG	BG	+
P_9	Y	NP	Y	+	P_{14}	YG	G	F	F,+
P_{10}	NP	G	F	F,+	P_{15}	Y	G	G	+
P_{12}	NP	BG	G	+	P_{16}	Y	YG	F	F,+
P_{18}	NP	NP	F	F,+	P_{17}	BG	F	F	F,+
P_{19}	YG	G	G	+	P_{22}	G	G	NP	+
P_{20}	BG	G	G	F,+	P_{23}	NP	G	NP	+
P_{21}	NP	NP	F	F,+	P_{26}	NP	G	NP	+
P_{24}	G	G	G	+	P_{28}	Y	YG	F	F,+
P_{25}	NP	G	F	F,+	P_{30}	NP	G	G	+
P_{27}	G	G	F	F,+	P_{32}	G	G	G	+
P_{29}	NP	G	NP	+	P_{34}	YG	YG	F	F,+
P_{31}	BG	G	F	F,+	P_{35}	YG	YG	F	F,+
P_{33}	YG	YG	F	F,+	P_{36}	YG	YG	F	F,+

NP = no pigment, G = green pigment, F = fluorescent pigment,
Y = yellow pigment, B = blue pigment, + = growth on cetrimide.

al. (1984) with phosphate solubilizing *P. fluorescens*.

Out of 36 phosphate solubilizing isolates those solubilizing TCP maximally on the 12th d were highest in number than on other days. Although the number of isolates that solubilized TCP maximally on the 9th d was lowest, the range of PS activity was highest (45.40 - 163.12 mg % P_2O_5) than on other days. Five isolates (P_{16}, P_{18}, P_{33}, P_{34}, P_{35}) showing maximum zone size and highest PS activity in the liquid medium can be rated further in the following decreasing order.

	P_{35} >	P_{34} >	P_{33} >	P_{16} >	P_{18}
% Solubilization	(72.5)	(55.0)	(48.8)	(44.3)	(44.0)

Statistical analysis showed that there was a moderate positive correlation between the phosphate solubilization zone on agar medium and amount of phosphate solubilized in the liquid medium.

Rhizospheric fluorescent *Pseudomonas* sp. were better performers in terms of P solubilization than non rhizospheric fluorescent *Pseudomonas* sp. The results in Table 3 indicate a fall in pH of the medium accompanied

Table2 : Phosphate solubilizing zone by different pigmented Pseudomonas spp. On modified Pikovskaya's agar medium after 6 days.

Isolates	Size of Phosphate solubilization zone (mm)	Pigmentation	Sources
P_{13}	1.0	Bluish Green	RS
P_{16}	8.0	Fluorescent	RS
P_{17}	5.0	Fluorescent	RS
P_{18}	7.0	Fluorescent	NRS
P_{20}	7.0	Fluorescent	NRS
P_{23}	5.0	Green	RS
P_{25}	7.0	Fluorescent	NRS
P_{26}	2.0	Green	RS
P_{27}	1.0	Fluorescent	NRS
P_{33}	7.0	Fluorescent	NRS
P_{34}	7.0	Fluorescent	RS
P_{35}	9.0	Fluorescent	RS
P_{36}	3.0	Fluorescent	RS

RS = rhizosphere soil, NRS = non-rhizosphere soil

Table3: Screening of Pseudomonas spp. For TCP solubilization in Pikovskaya's broth medium

Pigmentation & Isolate No.	Maximum *p* solubilization (mg % P_2O_5)		Final pH of the medium
Fluorescent P_1	47.90	(15)	4.8
P_2	54.40	(18)	4.8
P_3	28.60	(18)	5.0
P_7	44.70	(12)	4.0
P_8	74.00	(12)	4.1
P_{10}	51.00	(15)	4.2
P_{11}	51.00	(12)	4.0
P_{14}	65.80	(12)	4.0
P_{16}	99.60	(12)	3.9
P_{17}	57.90	(21)	4.3
P_{18}	98.60	(21)	3.9
P_{20}	38.80	(21)	4.8
P_{21}	57.90	(12)	4.2
P_{25}	44.60	(21)	4.7
P_{27}	80.20	(12)	4.2
P_{28}	77.30	(12)	4.6
P_{31}	62.30	(12)	4.4
P_{33}	110.00	(12)	4.1
P_{34}	150.90	(21)	3.9
P_{35}	163.10	(09)	4.0
P_{36}	77.30	(12)	4.7
Green P_4	57.90	(21)	4.6
P_5	45.40	(09)	4.3
P_6	66.60	(09)	4.3
P_{12}	26.60	(18)	4.3
P_{15}	54.40	(12)	4.1
P_{19}	65.80	(21)	4.4
P_{22}	54.40	(15)	4.5
P_{23}	76.80	(21)	4.0
P_{24}	69.90	(12)	4.0
P_{26}	47.40	(12)	4.2
P_{29}	74.40	(12)	4.5
P_{30}	47.80	(15)	4.6
P_{32}	87.10	(21)	4.6
Yellow P_9	47.90	(21)	4.8
Blue Green P_{13}	81.80	(18)	4.5

Figures in parenthesis show day on which maximum solubilization occurred

phosphate solubilization which may be due to the production of organic acid.

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