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Potential Biofertilizers, *Rhizobium,* and Phosphate Solubilizers from Salinity affected villages of Purna River Basin of Vidharbha (Maharashtra State, India)

D.H.Tambekar*, V.H.Ajane, S.R.Samdani, M.S.Zagare, S.D.Tambekar

P.G. Department of Microbiology, S.G.B. Amravati University, Amravati 444 602 (INDIA)

E-mail : diliptambekar@yahoo.com

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ABSTRACT

The soil in the identified saline area of Akola and Buldhana district (India) was investigated for the study of nitrogen fixation and phosphate solubilization by bacteria. In the present study 133 samples were analysed from saline affected area of Purna River, among these 22 samples showed the high ability to solubilize the inorganic insoluble phosphate. A total 95 samples showed the fast growing large colonies of *Rhizobium* strains. From the study it was observed that *Rhizobium* species have predominant and efficient fast growing large colonies. Also *Pseudomonas* and *Bacillus* species have more solubilizing ability of inorganic insoluble phosphate. Application these the isolated strains of *Rhizobium* species which are fast grower and efficient strains of PSB can be used as biofertilizer as they have local ecology and can tolerate high salt concentration and alkalinity of soil of Akola and Buldhana district of Maharashtra state, India.

KEYWORDS

Biofertilizer;
Symbiotic nitrogen fixer;
Phosphate solubilizing bacteria.

INTRODUCTION

Nitrogen and phosphorus are the major plant nutrients, which are referred to as the master key element in crop production. However a greater part of soil phosphorus (approximately 95-99%) is present in the form of insoluble form; dominant in alkaline soil and unable to utilized by the plants[2]. Phosphate solubilizing microorganisms are capable of solubilizing calcium, aluminium and iron phosphates as well as rock phosphates and mineralizing organic phosphorus making the phosphorus present in the soil available to the crops[8]. The bound form of phosphate is made available by soil microorganisms like bacteria and fungi, which solubilize the bound form and make it available to the plants[4]. Nitrogen is also most abundant element in atmosphere (approximately 78.08%) but plants are unable to use it as such. It can be made available through chemical or biological means but chemical nitrogen fertilizers are expensive[11]. To increase the availability of phosphorus and nitrogen for plants, large amounts of fertilizers are used on a regular basis but after applications, a large proportion of fertilizer phosphorus is quickly transferred to the insoluble form and very little (about 20-25%) of the applied phosphorus is useful[3,5]. Symbiotic nitrogen fixer and phosphate solubilizing microorganisms play an important role in supplementing nitrogen and phosphorus to the plant allowing a sustainable use of nitrogen and phosphate fertilizer. Use of these microbes as fertilizers in the field has been reported to increase crop yield. This is especially important for developing countries where farming will continue to be in hand of small farmers[6]. The most efficient and dominant phosphates solubilizers belong to bacterial groups are *Bacillus* species and *Pseudomonas* species as PSB and *Rhizobium* species as nitrogen fixer, which are grown in field, is adopted for a particular environmental factor and soil texture.

Environmental stresses are one of the most limiting factors in agricultural productivity. The growth and physiological activity of microbes may also be affected with the change in the soil condition such as salinity, pH, moisture, and carbon and nitrogen sources. Salinization is responsible for low fertility status of soils[1]. The commercial biofertilizers are unable to grow and give optimum benefits on the region other than from where they are isolated[10]. Hence efforts are made to isolate local salinity and ecologically adopted strains of *Rhizobium* and phosphate solubilizing bacteria from saline belt of Akola and Buldhana district. So the strains that are adapted to such environmental condition in saline belt can help to derive maximum benefits in agricultural production.

MATERIALS AND METHODS

Collection of samples

A total of 133 soil samples were collected from depth of 5-7 cm from different agricultural land of 133 villages of saline affected villages in Purna river of Akola and Buldhana district of Maharashtra in July – December 2007. Samples were collected from rhizosphere of plants like Tur, Mung, Soyabean, and Cotton in sterilized polythene bags and transfered to laboratory for screening of *Rhizobium* and phosphate solubilizing bacteria.

Isolation of PSM and *Rhizobium*

A 1g of each soil samples were dissolved in 10 mL sterile distilled water, and mixed thoroughly. The supernant of these suspensions was used for isolation of Rhizobium species and PSM. From these prepared soil suspension, loopful of soil suspension was inoculated on *Rhizobium* agar medium by streak plate technique and point inoculated on Pikovskaya's agar medium. The *Rhizobium* plate was incubated at 25^0C for 24h and Pikovskaya's plate was incubated at 37^0C for 24h. For the isolation of *Bacillus* species as PSM, soil suspension was heated at 80^0C for 10 minutes to kill the vegetative cells and then inoculated on Pikovskaya's agar medium. Such isolates were further reconfirmed as PSM and *Rhizobium* species. Isolated predominant, morphologically distinct colonies were selected from Rhizobium agar and Pikovskaya's medium. Isolates were identified by their colony characteristics and microscopic observation.

RESULTS AND DISCUSSION

In the present study 133 soil samples were collected from different villages of Purna alluvial soil of Akola and Buldhana district for the isolation of native locally adapted strains of *Rhizobium* and Phosphate solubilizing bacteria. A total of 67 isolates were identified as *Rhizobium*, 43 from Akola district and 24 from Buldhana district. Out of these 67 PSB isolates, 28 each were *R. japonicum* (18 from Akola district and 8 from Buldhana district) and *R. leguminosarum* (17 from Akola district and 11 from Buldhana district), 7 isolates of *R. trifoli* (6 from Akola district and 1 from Buldhana district) and *4* isolates (2 each from Akola district and Buldhana district) of *R. meliloti* (TABLE 1). Out of 67 Rhizobium spp isolates, 11 isolates were fast growers (Photo 1). Surange *et al*.,[7] also isolated *Rhizobium* strains from alkaline soils, which were salt tolerant. In the adverse condition of pH, the organisms isolated from salinity-affected soil that indicates high salinity tolerance, which can be used for preparation of biofertilizers. The isolates are adapted to soil environmental factors such as pH, temperature, and moisture content in saline belt. The isolated strains of *Rhizobium* can be used for preparation of biofertilizers, which will be more effective in saline belt soil. This biofertilizers used for adding the nitrogen in soil, and increase the crop yield and fertility of soil.

A total of 101 isolates were identified as PSB, 54 from Akola district and 47 from Buldhana district. Out of these 101 PSB isolates, 49 were *Pseudomonas aeruginosa* and 52 were *Bacillus subtilis* (TABLE 1). Out of these 6 isolates of *P.aeruginosa* and 5 isolates of *B. subtilis* from Akola district and 9 isolates of *P.aeruginosa* and 19 isolates of *B. subtilis* from

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TABLE 1 : Nitrogen fixer and PSB from soil of Akola and Buldhana district villages from Purna River basin

Sr.No.	Villages in Akola District	Nitrogen fixer *Rhizobium* spp	*Pseudomonas aeruginosa*				*Bacillus subtilis*				Sr.No.	Villages in Buldhana district	Nitrogen fixer *Rhizobium* spp	*Pseudomonas aeruginosa*				*Bacillus subtilis*			
			PSB	Zon in 24h	Zone in 48h	Oxidase	PSB	Zone in 24h	Zone in 48h	Catalase				PSB	Zone in 24h	Zone in 48h	Oxidase	PSB	Zone in 24h	Zone in 48h	Catalase
1	Hingna Tamaswadi	A	+	6	10	+	+	10	12	+	1	Bhon	A	+	9	14	+	+	7	9	+
2	Malwada	A	+	8	12	+	+	7	10	+	2	Dadegaon	-	+	9	11	+	+	8	12	+
3	Brahmapuri	B	+	10	12	+	-	-	-	-	3	Hingna Gavhan	B	+	9	13	+	+	8	24	+
4	Chandpur	A	+	12	14	+	-	-	-	-	4	Isarpur	-	+	14	16	+	+	7	11	+
5	Dahihavda	-	+	14	18	+	-	-	-	-	5	Mandwa	B	+	8	11	+	+	6	10	+
6	Gopalkhed	C	+	6	10	+	-	-	-	-	6	Mominabad	B	+	9	13	+	+	9	18	+
7	Gotra	A	+	7	10	+	-	-	-	-	7	Tiwadi	B	+	10	15	+	+	10	14	+
8	Hatla	A	+	10	12	+	-	-	-	-	8	Gandhinagar	-	+	12	14	+	-	-	-	-
9	Kawsa	-	+	16	18	+	-	-	-	-	9	H. Dadegaon	-	+	10	11	+	-	-	-	-
10	Lonagra	A	+	12	14	+	-	-	-	-	10	Khiroda	-	+	10	14	+	-	-	-	-
11	Malwada	A	+	8	10	+	-	-	-	-	11	Manegaon	+	+	10	13	+	-	-	-	-
12	Rohna	A	+	16	18	+	-	-	-	-	12	Takalie khasa	+	+	8	15	+	-	-	-	-
13	Wadad	C	+	12	14	+	-	-	-	-	13	Chinchol	-	-	-	-	-	+	16	21	+
14	Akhatwada	A	-	-	-	-	+	10	14	+	14	Golegaon	B	-	-	-	-	+	10	12	+
15	Ambikapur	B	-	-	-	-	+	12	16	+	15	Hingana Nagpur	B	-	-	-	-	+	14	21	+
16	Apoti	A	-	-	-	-	+	14	16	+	16	Jigaon-1	B	-	-	-	-	+	17	28	+
17	Dhorel	-	-	-	-	-	+	11	13	+	17	Jigaon-2	A	-	-	-	-	+	12	20	+
18	Donwada	-	-	-	-	-	+	10	14	+	18	Kalegaon	A	-	-	-	-	+	7	19	+
19	Ghusarwadi	A	-	-	-	-	+	10	12	+	19	Khelgaon	-	-	-	-	-	+	8	18	+
20	Jagalbad	B	-	-	-	-	+	7	12	+	20	Natwel	A	-	-	-	-	+	12	20	+
21	Kapileshwar	B	-	-	-	-	+	7	12	+	21	Pimprikoli	B	-	-	-	-	+	22	26	+
22	Karodi	-	-	-	-	-	+	9	12	+	22	Sawargaon	D	-	-	-	-	+	18	21	+
23	Khanapur	C	-	-	-	-	+	12	16	+	23	Takdivatfal	A	-	-	-	-	+	10	19	+
24	Majalpur	B	-	-	-	-	+	13	18	+	24	Vallabhnagar	-	-	-	-	-	+	18	18	+
25	Mhatodi	-	-	-	-	-	+	9	12	+	25	Bhendwall	-	+	5	6	-	-	-	-	-
26	Nawthala	B	-	-	-	-	+	12	13	+	26	Gadegaon (Kh)	-	+	4	5	-	-	-	-	-
27	Ugwa	-	-	-	-	-	+	10	12	+	27	Mamalwadi	C	+	5	7	-	-	-	-	-
28	Apatapa	B	+	2	5	-	-	-	-	-	28	Pahurpurna	-	+	7	8	-	-	-	-	-
29	Apoti	A	+	4	5	-	-	-	-	-	29	Belad	A	-		-	-	+	7	10	-
30	Dhamna	C	+	3	5	-	-	-	-	-	30	Dolarkhed	D	-	-	-	-	+	10	11	-
31	Dudhala	A	+	7	8	-	-	-	-	-	31	Gaulkhed	-	-	-	-	-	+	7	12	-
32	Enapur	B	+	4	6	-	-	-	-	-	32	H. Balapur	A	-	-	-	-	+	7	11	-
33	Ghusar	D	+	5	7	-	-	-	-	-	33	H. Bhoba	-	-	-	-	-	+	5	9	-
34	Hingni	B	+	6	8	-	-	-	-	-	34	Kathora	-	-	-	-	-	+	7	12	-
35	Jagalbad	B	+	4	6	-	-	-	-	-	35	Manasgaon	B	-	-	-	-	+	6	8	-
36	Jaulkhed	A	+	5	6	-	-	-	-	-	36	Mhaisang	-	-	-	-	-	-	7	12	-
37	Kapilkhed	B	+	8	9	-	-	-	-	-	37	Nimkrad	A	-	-	-	-	+	5	9	-
38	Keliwedi	C	+	5	7	-	-	-	-	-	38	Roti	-	-	-	-	-	+	3	5	-
39	Khambora	A	+	2	4	-	-	-	-	-	39	Sawali	B	-	-	-	-	+	5	9	-
40	Lokhanda	-	+	6	8	-	-	-	-	-	40	Yerali	B	-	-	-	-	+	7	18	-
41	Nimbhora	C	+	5	6	-	-	-	-	-											
42	Pastul	B	+	6	8	-	-	-	-	-											
43	Pilakwadi	B	+	4	6	-	-	-	-	-											
44	Ramgaon	D	+	7	9	-	-	-	-	-											
45	Sambad	B	+	6	8	-	-	-	-	-											
46	Taroda	B	+	4	6	-	-	-	-	-											
47	Yeklara	-	+	4	7	-	-	-	-	-											
48	Kasali -1	A	-	-	-	-	+	7	10	-											
49	Kasali -2	B	-	-	-	-	+	8	10	-											
50	Katyar	B	-	-	-	-	+	8	10	-											
51	Sangwi	A	-	-	-	-	+	8	11	-											
52	Tarapur	A	-	-	-	-	+	8	11	-											

(A) R. japonicum, (B) R. leguminosarum, (C) R. trifoli, (D) R. meliloti

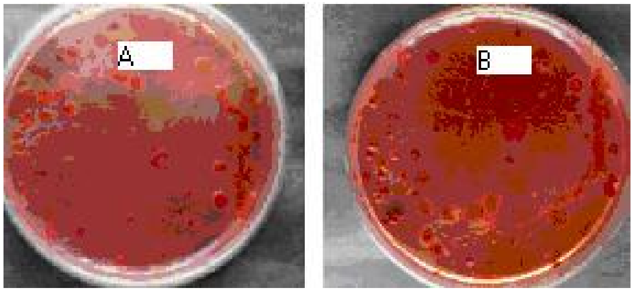

Photo 1: Isolated colonies of (A) *Rhizobium leguminosarum* and (B) *Rhizobium japonicum*

Photo 2: Phosphate solubilization by (A) *Bacillus subtilis* and (B) *Pseudomonas aeruginosa*

Buldhana district were highly efficient (Photo 2). These efficient PSB strains with local ecology can be used for the preparation of Biofertilizer. Tambekar *et al.*,[10] had also isolated fifty strains phosphate solubilizing *Bacillus subtilis* from saline tract of Vidarbha, which are currently used as biofertilizers by local farmers. Tambekar and Bhokre[9] and Rajankar *et al*.,[5] also reported similar type of phosphate solubilizing activity in isolated PSB and fungi. The application of the biofertilizers prepared by these bacteria can be helpful in salinity-affected area of soil.

In conclusion, the isolated strains of *Rhizobium* species which are fast grower and effeiceint strains of PSB can be used as biofertilizer as they have local ecology and can tolerate high salt concentration and alkalinity of soil of Akola and Buldhana district of Maharashtra state, India.

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