African Journal of Microbiology Research Vol. 6(17), pp. 3924-3931, 9 May, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR12.224
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Potential microbial candidate strains for management of nutrient requirements of crops

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Accepted 30 March, 2012

Zinc (Zn) and phosphorus (Pi) are essential for optimum plant growth. Upon application, they get fixed in the soil matrix. Application of more than one microbe for each nutrient is often difficult. *Azotobacter* (31), *Azospirillum* (38), *Bacillus* (19) and *Pseudomonas* (82) strains were isolated from diverse crop production systems and were evaluated for solubilization of 'Zn' and 'Pi' *in vitro* from insoluble zinc (ZnO, $ZnCO_3$) and phosphorus [tricalcium phosphate (TCP)], respectively. After 15 days of incubation, 15 strains solubilized zinc and produced >50 cm^2 solubilization zone on solid media. In broth culture, with ZnO as zinc source, B116 could release maximum available Zn (13.12 ppm) and with $ZnCO_3$, B118 could release highest available Zn (16.3 ppm). *Pseudomonas* strain PIII-105 released highest available 'Pi' (14.8 ppm) and solubilization of Zn and Pi corresponded with fall in pH of the medium except in case of B116. Two (2) strains of *Azospirillum*, 6 strains each of *Bacillus* and *Pseudomonas* showed a clearance zone area of >50 cm^2 with both Zn sources. Similarly, 2 strains each of *Azospirillum* and *Bacillus* and 3 strains of *Pseudomonas* solubilized TCP. *Azospirillum* strains As-20, As-22; *Bacillus* strains B113, B118; and *Pseudomonas* strains P17, P33 and PIII 105 solubilized both Zn and P sources showing their ability to supplement both essential nutrients to plants. Interestingly, *Azospirillum* is already known to supplement nitrogen also.

Key words: Multi-nutrient management, zinc carbonate, zinc oxide, tricalcium phosphate, *Pseudomonas, Bacillus, Azospirillum*.

INTRODUCTION

Plants need several macro- and micro-nutrients for their growth and reproduction. These nutrients supplemented through inorganic or organic forms are taken up by the plant roots along with water. Bacteria also play an important role in mobilizing nutrients required by the plants to some extent. Zinc (Zn) is one of the essential micronutrients required for optimum plant growth and plays a vital role in metabolism (Hughes and Poole, 1989). Its deficiency is displayed as a remarkable reduction in plant height and plants develop whitish-brown patches that turn necrotic subsequently. Zn deficiency results in reduced membrane integrity, reduction in synthesis of carbohydrates, cytochromes, nucleotides, auxins, chlorophyll and increased susceptibility to heat stress (Bhupinder et al., 2005). About 96 to 99% of the exogenously applied Zn is converted into different insoluble forms depending upon the soil types, physico-chemical reactions within 7 days of application (Venkatakrishnan et al., 2003). The solubility of Zn is highly dependent upon soil pH and moisture and hence, arid and semi-arid areas of Indian agro-ecosystems are often zinc-deficient. Zn occurs in soil as sphalerite, olivine, hornblende, augite and biotite. However, availability of Zn from these sources is guided by many factors among which biochemical actions of rhizomicroorganisms play an important role in converting such unavailable

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sources into available ones (Bhupinder et al., 2005). Di Simine et al. (1998) reported a Zn solubilizing strain of *Pseudomonas fluorescens* from forest soil. Hutchins et al. (1986) reported that *Thiobacillus thioxidans, Thiobacillus ferroxidans* and facultative thermophilic iron oxidizers solubilized Zn from sulphide ore (sphalerite).

Among macronutrients, apart from nitrogen, plants need phosphorus and potassium for their growth. Phosphorus is a major plant growth limiting nutrient despite being abundant in soils in both organic and inorganic forms (Gyaneshwar et al., 2002). 'Pi' plays an important role in root development, flower, seed formation, N-fixation in legumes, resistance to plant diseases. Soil microbiota enhances 'Pi' availability to plants by mineralizing organic phosphorus in soil and by solubilizing precipitated phosphates. The solubilization of 'Pi' in the rhizosphere is the most common mode of action implicated in plant growth promoting rhizobacteria (PGPR) that increase nutrient availability to host plants (Richardson, 2001). Cattelan et al. (1999) found only 2 of the 5 rhizospheric strains positive for 'Pi' solubilization that had a positive effect on soybean seedling growth. The 'Pi'-releasing fungi produced more organic acids and the solubilization has been attributed to the release of succinic, citric, maleic, fumaric, glyoxalic and gluconic acids (Gaur, 1990). In culture media, 'Pi' solubilising ability is generally related to the degree of acidification of the medium (Venkateswarlu and Wani, 1984). The importance of root growth and architecture for the efficient capture of 'Pi', well documented and in many cases, is a specific response of plants to 'Pi' deficiency (Lynch, 2005; Richardson et al., 2009). Under controlled growth conditions, various studies have demonstrated enhanced growth and 'Pi' nutrition of plants inoculated with phosphate solubilizing microorganisms (PSM) (Gyaneshwar et al., 2002; Rodriguez and Fraga, 1999).

Individual strains of microorganisms have been reported to solubilize either phosphorus or zinc. Such situation warrants for compatibility between such strains for formulating consortia and use in the field. However, if strains that possess ability to mobilize multi-nutrients could be a boon as they could be deployed directly with minimum efforts without going through the formulation of consortia. In the present study, probably for the first time, we report microorganisms that can solubilize both phosphorus and zinc and thus, could be used for mobilization of both nutrients required for plant growth.

MATERIALS AND METHODS

Bacterial cultures

Rhizosphere soil samples from diverse agro-ecological sub regions of India were obtained and *Azotobacter, Azospirillum, Bacillus* and *Pseudomonas* cultures were isolated by serial dilution of soil samples and plating on Jensen's agar (Jensen, 1940), N-free bromothymol blue media (Doebereiner et al., 1976), nutrient agar and King's B (King et al., 1954) media, respectively. All the bacterial strains were identified using standard biochemical tests to genus level.

Qualitative and quantitative assay for zinc solubilization

All the bacterial strains were screened for their ability to solubilize zinc in mineral salts agar medium (Glucose-10 g, $(NH_4)_2SO_4$-1.0 g, KCl-0.2 g, K_2HPO_4-0.1 g, $MgSO_4$-0.2 g and H_2O-1000 ml with pH 7.0) amended with 0.1% of either insoluble zinc oxide (ZnO) or zinc carbonate ($ZnCO_3$). The actively growing cultures (5 µl) were spot inoculated onto the medium, incubated at 28°C and solubilization zone was measured 15 days after inoculation and clearing zone was expressed as area in cm^2.

All the strains were further evaluated for Zn solubilization efficiency. The cultures were grown in mineral salts broth (100 ml) amended with 0.1% of insoluble zinc salts (ZnO or $ZnCO_3$). Flasks containing 150 ml of sterile broth were inoculated with 100 µl of 4 h old actively growing test bacterial cultures and incubated at 28°C for 10 days with a shaking at 140 rpm. Three replications were maintained for each strain along with an un-inoculated control. The samples were drawn on 3rd, 6th and 9th days after inoculation and centrifuged at 10000 rpm for 10 min to remove cell debris. The pH of the supernatant was measured. Available Zn in the supernatant was assessed by atomic absorption spectrophotometry (AAS-GBC, Australia).

Qualitative and quantitative assay for phosphorus solubilization

For qualitative assessment of phosphate solubilizing ability of test strains, Pikovskaya's medium amended with tricalcium phosphate (TCP) was used (Pikovskaya, 1948). Petri dishes containing sterilized medium were spot-inoculated with 10 µL of overnight grown bacterial cultures and incubated at 28°C. The solubilization zone size was measured after 7 and 14 days of inoculation and clearing zone was expressed as area in cm^2.

For quantification of phosphate solubilization, the method described by Olsen and Sommers (1982) was adapted which is described briefly here. Pikovskaya's broth was prepared with 1 mg/mL of TCP (or rock phosphate) as phosphorus source. Flasks containing 150 ml of sterile broth were inoculated with 100 µl of 4 h old actively growing test bacterial cultures and incubated at 28°C for 10 days with a shaking at 140 rpm. For each treatment, three replicates were maintained along with an un-inoculated control. The broth was centrifuged and pH of the supernatant was measured. One (1) mL aliquot from the supernatant was taken to which 5 ml of sulphomolybdic acid containing ascorbic acid was added. Final volume of the solution was made up to 50 ml. The absorbance of the reaction mixture was measured at 660 nm and amount of soluble phosphorus was determined from the standard curve of KH_2PO_4.

RESULTS

Isolation of plant growth promoting bacteria

In all, 31 strains of *Azotobacter* spp. (Az), 38 strains of *Azospirillum* spp. (As) spp., 82 fluorescent *Pseudomonas* spp. and 19 *Bacillus* spp. were isolated from rhizosphere soils of different cropping systems across different agro-climatic regions of India (Figure 1). As shown in Figure 1, it is evident that the collection of strains was done

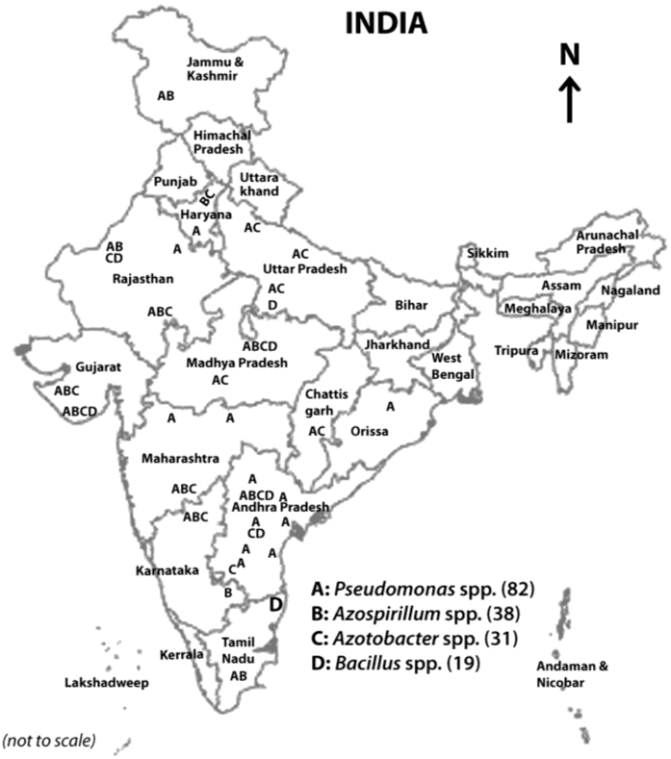

Figure 1. India map showing soil sampling locations used for the isolation of test strains of rhizobacteria. Figures in parentheses indicate number of isolates.

Table 1. Solubilization area (cm^2) produced on mineral salts agar medium amended with zinc oxide (ZnO).

Bacterial isolate	Solubilization zone of ZnO		
	Weak (>7.5 cm^2)	Moderate (7.6-50.0 cm^2)	High (>50.0 cm^2)
Azospirillum	As-3, 5, 8, 10, 21, 23, 26, 27, 35	As-9, 11, 22, 24, 25, 29, 30, 36, 37, 38	As-20, 22
Azotobacter	Az-29	-	-
Bacillus	B-51	B-41, 117	B-40, 61, 113, 114, 116,118
Pseudomonas	P-47, 48, 56, 70	P-18, 20, 22, 23, 25, 28, 30, 31, 37, 38, 40, 42, 43, 45, 52-55, 57-59, 62, 64-69, 71-76, 78, II33, III94	P-17, 21, 24, 29, 33, III105

encompassing many states to cover diverse regions spread over different agro-climatic regions.

Plate assay

Test strains differed in their ability to solubilize the insoluble forms of Zn and P in agar plates after 15 days of incubation. Based on their solubilising ability, the strains were classified into strong (>50 cm^2 area), moderate (7.6 to 50 cm^2 area) and weak (3.8 to 7.5 cm^2 area) solubilizers. All strains showing either less than 3.8 cm^2 or no clearing zone were considered as very weak or non-solubilizers and hence, not considered for further studies.

The strains differed for their ability to solubilize either ZnO or $ZnCO_3$. Two (2) strains of *Azospirillum* viz. As-20 and As-22, 4 strains of *Bacillus* viz. B-40, B-113, B-114 and B-116, and 6 strains of *Pseudomonas* viz. P-17, P-20, P-24, P-29, P-33 and PIII-105 were categorized as strong strains with an area of clearing zone of >50 cm^2 (Table 1). Ten (10) strains of *Azospirillum*, 3 strains of

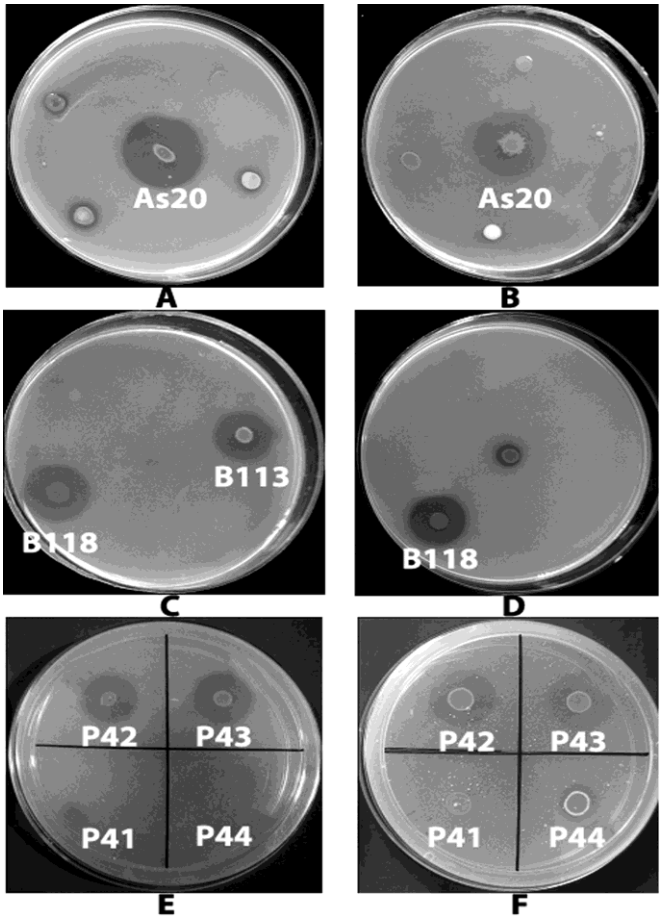

Figure 2. Solubilization of ZnO (A, B, C) and $ZnCO_3$ (D, E, F) by *Azospirillum* (A, D), *Bacillus* (B, E) and *Pseudomonas* (C, F) isolates on solid medium after 15 days.

Bacillus and 22 strains of *Pseudomonas* were classified as moderate solubilizers. Nine (9) strains of *Azospirillum*, 1 isolate of *Bacillus*, 4 strains of *Pseudomonas* and 1 isolate of *Azotobacter* were categorized weak solubilizers after 15 days of incubation on ZnO amended medium (Table 1 and Figure 2). Rest of the strains were either very weak or non-solubilizers.

When $ZnCO_3$ was used as Zn source, similar trends were observed. Two (2) strains of *Azospirillum* viz. As-20 and As-22, 6 strains of *Bacillus* (B-40, 61, 113, 114, 116 and 118) and 5 strains of *Pseudomonas* (P-31, P-33, P-52, P-74 and P-III-105) were classified as strong solubilizers whereas 2 strains of *Bacillus* and 34 strains *Pseudomonas* were classified as moderate and 1 isolate each of *Bacillus* and *Azotobacter*, 7 strains of *Pseudomonas* were classified as weak solubilizers after 15 days of incubation (Table 2 and Figure 2).

Bacterial strains varied in their ability to solubilize TCP after 15 days of incubation. Two (2) strains each of *Azospirillum* (As-20 and As22) and *Bacillus* (B113 and B118) and *Pseudomonas* (P33 and PIII-105) were identified as strong solubilizers. Five (5) strains of *Azospirillum*, 2 strains each of *Azotobacter* and *Pseudomonas* and 1 isolate of *Bacillus* were classified as moderate whereas 2 strains each of *Azospirillum*, *Azotobacter* and *Pseudomonas* and 1 isolate of *Bacillus* were classified weak solubilizers (Table 5 and Figure 3).

Liquid assay

Maximum solubilization of Zn with both the sources was observed on the 9th day. The available Zn concentration in the treatments ranged between 5.6 and 13.1 ppm. Among the strains, B116 showed highest solubilization (13.12 ppm available zinc) followed by B118 (11.8 ppm) and As-20 (11.13 ppm). B61 showed lowest solubilization (5.6 ppm available Zn) in ZnO amended medium (Table 3). On the other hand in $ZnCO_3$ amended medium, B118 showed maximum available Zn (16.3 ppm) followed by B114 (14.7 ppm) and the lowest available Zn was recorded in B40 (5.82 ppm). Among *Pseudomonas*

Table 2. Solubilization area (cm^2) produced on mineral salts agar medium amended with zinc carbonate ($ZnCO_3$).

Bacterial isolate	Solubilization zone of $ZnCO_3$		
	Weak (>7.5 cm^2)	Moderate (7.6-50.0 cm^2)	High (>50.0 cm^2)
Azospirillum	-	-	As-20, 22
Azotobacter	Az-29	-	-
Bacillus	B-117	B-41, 51	B-40, 61, 113, 114, 116,118
Pseudomonas	P-37, 56, 67, 68, 73, I76, P-II33	P-18, 20, 22, 23, 25, 28, 29, 30, 40, 42, 43, 47, 53, 54, 57, 58, 59, 62, 64, 66, 69, 70, 71, 72, 75, 78, P III94	P-17, 21, 24, 29, 33, III105

Table 3. Solubilization of zinc oxide (ZnO) by rhizobacterial isolates in liquid medium.

Bacterial isolate	Solubilization of ZnO on:					
	3rd day		6th Day		9th Day	
	Zn (ppm)	pH	Zn (ppm)	pH	Zn (ppm)	pH
As 20	8.29 (±0.60)	4.53	9.63 (±0.88)	4.44	11.13 (±1.03)	3.97
As 22	4.97 (±0.79)	7.21	7.25 (±0.66)	7.02	8.49 (±0.78)	6.94
B40	5.02 (±2.05)	6.96	7.68 (±0.70)	6.83	8.54 (±0.79)	6.45
B61	5.34 (±0.92)	6.95	5.50 (±0.50)	6.84	5.66 (±0.52)	6.41
B113	3.93 (±3.86)	7.18	5.00 (±0.46)	7.14	5.57 (±0.51)	7.08
B114	1.82 (±6.22)	6.99	9.46 (±0.87)	6.88	9.68 (±0.89)	6.44
B116	3.74 (±1.32)	7.06	8.40 (±0.77)	6.89	13.12 (±1.21)	6.44
B118	2.50 (±1.66)	7.19	9.06 (±0.83)	7.18	11.80 (±1.09)	7.13
P17	3.02 (±0.84)	6.73	5.46 (±0.50)	4.04	10.00 (±0.92)	4.15
P21	6.73 (±0.82)	4.52	9.67 (±0.89)	3.72	10.53 (±0.97)	3.58
P24	4.56 (±1.48)	7.02	5.66 (±0.52)	6.74	10.32 (±0.95)	6.78
P29	8.54 (±0.75)	6.88	9.33 (±0.86)	4.23	9.46 (±0.87)	4.19
P33	8.47 (±1.06)	4.16	9.27 (±0.85)	4.01	10.47 (±0.97)	4.08
PIII105	8.06 (±0.73)	6.65	9.84 (±0.90)	4.48	11.30 (±1.04)	4.40

Values in the columns are means two independent experiments with threevalues every time. Values in parentheses indicate ±SD. As, *Azospirillum* sp.; B, *Bacillus* sp.; P, *Pseudomonas* sp.

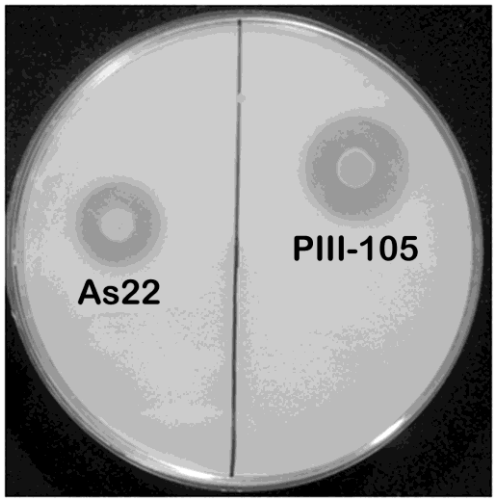

Figure 3. Solubilization of tricalcium by *Azospirillum* and *Pseudomonas* isolates on Pikovskaya's agar medium.

Table 4. Solubilization of zinc carbonate ($ZnCO_3$) by rhizobacterial isolates in liquid medium.

Bacterial isolates	Solubilization of $ZnCO_3$ on:					
	3rd day		6th day		9th day	
	Zn (ppm)	pH	Zn (ppm)	pH	Zn (ppm)	pH
As 20	9.10 (±0.84)	4.45	9.47 (±0.87)	4.06	10.2 (±0.947)	3.80
As 22	8.72 (±0.80)	6.00	9.36 (±0.86)	4.61	10.3 (±0.949)	3.69
B40	1.72 (±0.16)	7.02	3.98 (±0.37)	6.89	5.82 (±0.53)	6.74
B61	10.86 (±1.0)	7.05	11.8 (±1.1)	6.93	12.7 (±1.17)	6.81
B113	2.53 (±0.23)	7.43	8.08 (±0.74)	5.95	9.96 (±0.91)	5.76
B114	2.44 (±0.22)	7.05	10.4 (±0.96)	6.97	14.7 (±1.35)	6.76
B116	9.10 (0.84)	6.95	11.0 (±1.02)	4.73	11.6 (±1.07)	4.59
B118	13.3 (±1.2)	7.09	13.4 (±1.24)	5.64	16.3 (±1.50)	5.41
P17	9.44 (±0.87)	4.01	10.8 (±1.00)	4.00	10.9 (±1.01)	3.98
P21	12.3 (±1.1)	4.37	13.5 (±1.24)	4.27	13.9 (±1.28)	4.10
P24	9.30 (±0.86)	7.00	6.87 (±0.63)	6.88	9.58 (±0.883)	4.78
P29	8.24 (±0.76)	4.51	9.46 (±0.87)	4.41	9.62 (±0.887)	4.10
P33	8.22 (±0.76)	4.63	9.47 (±0.87)	4.33	10.34 (±0.95)	4.11
PIII105	8.69 (±0.80)	7.01	9.45 (±0.87)	4.28	10.16 (0.93)	3.69

Values in the columns are means two independent experiments with three values every time. Values in parentheses indicate ±SD. As, *Azospirillum* sp.; B, *Bacillus* sp.; P, *Pseudomonas* sp.

Table 5. Solubilization zone diameter (mm) produced on Pikovskaya's agar medium.

Bacterial isolate	Solubilization zone of TCP		
	Weak (<10 mm)	Moderate (11-22 mm)	High (>25 mm)
Azospirillum	As-15, 21,	As-9, 24, 25, 37, 38,	As-20, 22
Azotobacter	Az-19, 20	Az-29, 16	-
Bacillus	B-41	B-40, 51	B-113, 118
Pseudomonas	P-63, 66	P-24, 46	P-17, 33, III105

strains, release of available Zn ranged from 9.5 to 13.96 across strains with highest being by P21 (Table 4).

Mostly, decrease in pH of culture medium progressed with increase in incubation time. At the end of 9th day, pH of the medium ranged between 3.58 and 7.35. The lowest pH of 3.58 was observed in supernatant of P21. There was a negative correlation between incubation time except in case of B116 where despite highest solubilization of ZnO, the pH of the medium was only 6.44 (Table 3). Like in ZnO, decrease in pH of the culture medium progressed with increase in incubation time. The pH of the medium ranged between 3.69 and 6.76. However, B118 showed highest release from $ZnCO_3$ amended medium (16.3 ppm) though pH of the medium remained at 5.41 (Table 4).

Estimation of available 'P' in the culture broth of bacteria showed that solubilisation increased from 3rd to 9th day of incubation. Available 'P' in the medium ranged between 7.2 and 14.8 ppm on 9th day. Among the strains, PIII-105 showed highest solubilisation of 14.8 ppm 'Pi' followed by As-22 (14.42 ppm) and P33 (14.13 ppm). The spent medium of PIII-105 that showed highest solubilization recorded a pH of 3.80 (Table 6).

DISCUSSION

In this preliminary study, to identify promising microorganisms with a potential to solubilize more than one nutrient was successful as we could find strains of *Pseudomonas*, *Azospirillum* and *Bacillus* that can solubilize both Zn and Pi. From the data, it is obvious that the strains varied in their ability to solubilize different forms of insoluble zinc. But all strains of *Pseudomonas*, *Azospirillum* and *Bacillus* categorized as strong are both ZnO and $ZnCO_3$ which makes it obvious that these strains possessed very strong mechanisms of solubilization and hence, could use both substrates. Since, we got promising strains across three genera, there is a possibility to find such strains across genera of microorganisms. However, in moderate and weak categories, there were some differences suggesting that

Table 6. Solubilization of tricalcium phosphate by rhizobacterial isolates in liquid medium.

Bacterial isolate	Solubilization of TCP on:					
	3rd day		6th day		9th day	
	'P' (ppm)	pH	'P' (ppm)	pH	'P' (ppm)	pH
As20	11.36 (1.04)	4.9	12.23 (1.12)	4.6	12.62 (1.16)	4.3
As22	11.47 (1.05)	5.4	11.55 (1.06)	5.1	13.67 (1.26)	4.5
Az29	11.82 (1.08)	5.7	12.11 (1.11)	5.1	13.54 (1.24)	4.9
B113	7.68 (0.70)	4.2	9.37 (0.86)	4.1	9.70 (0.89)	4.0
B118	3.19 (0.29)	4.4	6.44 (0.59)	4.2	12.38 (1.14)	4.0
P17	11.31 (1.04)	6.9	10.81 (0.99)	6.8	9.02 (1.83)	5.9
P33	10.86 (1.0)	5.7	13.32 (1.22)	4.7	13.59 (0.25)	4.4
P46	11.31 (1.04)	5.0	11.77 (1.08)	4.6	8.31 (0.76)	4.3
PIII105	2.84 (0.26)	5.5	7.82 (0.72)	5.1	14.18 (1.30)	5.0

Values in the columns are means two independent experiments with three values every time. Values in parentheses indicate ±SD. As, *Azospirillum* sp.; Az, *Azotobacter* sp.; B, *Bacillus* sp.; P, *Pseudomonas* sp.

these organisms could have limited modes of solubilization methods. Out of 130 strains, we could identify 14 strong Zn solubilizers. In the past also, efforts were made to identify zinc solubilizers with varying abilities. The demonstrated variation in the ability of solubilising given zinc sources could be due to metabolic activity of a given strain which is in agreement with observations of Sadaf and Nuzhat (2008). There are different mechanisms of solubilization which have been identified including proton excretion, production of organic acids and other chelating metabolites (Agnihorti, 1970). Organic acid production by microbial strains has been reported to be a major mechanism of solubilization (Nguyen et al., 1992; Fasim et al., 2002). The zinc solubilization in our studies could be due to production of organic acids, like gluconic acids that is augmented by the fall in pH of culture media noted in all cases. The transformation of glucose to gluconic acid by the glucose oxidative external pathway in *Pseudomonas* spp. and other bacteria has been interpreted as a competitive strategy by microorganisms (Whiting et al., 1976). Zinc phosphate solubilization by a strain of *P. fluorescens* was investigated by Di Simine et al. (1998) and they observed that supplementing medium with zinc sulphate resulted in more gluconic acid production. In the present study, the acidic pH showed by all the bacterial strains attributed due to production of organic acids. It can be correlated that the higher availability of Zn is directly proportional to acidic pH of the culture broth. The ability to dissolve appreciable amount of zinc phosphate is not common feature amongst the culturable bacteria on the soil surface (Di Simine et al., 1998). However, in some potent strains, the pH did not fall drastically suggesting that in these strains other mechanisms may be active and this aspect is being emphasized. Among 14 promising zinc solubilizing bacteria (ZSB) (Tables 3 and 4), 6 strains were obtained from alfisols of Deccan plateau agro-ecosystem, other 6 were from vertisols of Deccan plateau and eastern ghats agro-ecosystems, where high zinc deficiency is noted. This could be the possible reason for those strains showing such high quantity of zinc solubilization. The nativity of strains gives a preliminary idea for the purpose of their prevalence in specific areas.

Mostly, solubilization of zinc sources on solid media can be correlated to solubilization in liquid media. However, solubilization of $ZnCO_3$ by some bacterial strains (PIII-105) was high in liquid medium though they did not show distinct solubilization in solid media (Tables 2 and 4). This could be due to easy availability of nutrients and aeration in liquid medium. All these variations can be mapped to the diversity of strains in physiology, nutritional requirements, location of isolation etc. as reported by Venkatakrishnan et al. (2003).

Studies have reported the isolation and characterization of phosphobacteria from rhizosphere (Chung et al., 2005; Reyes et al., 2006; Turan et al., 2006). In the present study, bacteria when characterized by Zn and Pi solubilization was observed that some strains have high potential to solubilize the said essential nutrients. The potential of a given rhizobacteria to promote nutrient uptake and plant growth could be due to synergistic action of the growth promoting traits than individual effect (Glick et al., 1999). The solubilization of TCP was possible by simple acidification of the medium and acidity contributed to its solubilization by the release of carboxylic acids (Puente et al., 2004; Rodriguez et al., 2006). Application of phosphate solubilizing bacteria to improve plant growth by solubilizing sparingly soluble inorganic phosphates in soil was demonstrated by Rodriguez and Fraga (1999). Diverse factors (Salts, pH and temperature) can affect the capacity of phosphobacteria to solubilize 'P' (White et al., 1997; Johri et al., 1999). It is presumed that the secretion of organic acids as evidenced by a fall in pH of the culture medium

would also help in solubilizing phytates in soil. Phosphate solubilizing PGPR and their plant growth promoting effect on maize was demonstrated by Hameeda et al. (2006).

In the present study, Pseudomonas isolate P33, Azospirillum As-22 showed TCP solubilization, and were also able to solubilize insoluble Zn sources. Interestingly, Bacillus isolate B-116 that could solubilize maximum Zn could not show any Pi solubilization. Interestingly, *Azospirillum* strains are already known as free-living nitrogen fixers and in this study, we could find 2 strains of *Azospirillum* that could solubilize both Zn and 'Pi'. There are some PGPR that can fix nitrogen, solubilize mineral nutrients and mineralize organic compounds (Martinez-Viveros et al., 2010).

Most of the soils are rich in total Zn and P. However, their availability to the plants when needed is very limited. Zn and P in soils tend to change their forms and convert into unavailable form constantly. Recurrent use of inorganic Zn and phosphate fertilizers to alleviate deficiency in soils is not only a costly practice but also may lead to excessive deposition of these nutrients over time. Strains of *Azospirillum*, already proven to fix nitrogen are able to solubilize P and Zn and thus, offer best possible nutrient recycling mechanism at a low input cost where expensive inorganic fertilizers are becoming unaffordable by small and marginal farmers.

ACKNOWLEDGEMENTS

Financial support by Indian Council of Agricultural Research (ICAR), New Delhi under a network project on Application of Microorganisms in Agriculture and Allied Sectors-Nutrient Management and PGPR (AMAAS) is gratefully acknowledged.

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