Advanced Journal of Microbiology Research Vol. 2016
Available online at http://internationalscholarsjournals.org/journal/ajmr

Full Length Research Paper

Evaluation of *in vitro* solubilization potential of phosphate solubilising *Streptomyces* isolated from phyllosphere of *Heritiera fomes* (mangrove)

Nibha Gupta[1]*, Dolly Sahoo[1] and Uday Chand Basak[2]

[1]Department of Microbiology, Division of Biotechnology, Regional Plant Resource, Centre, Bhubaneswar -751 015 Orissa, India.
[2]Division of Taxonomy and Conservation, Regional Plant Resource Centre, Bhubaneswar -751 015 Orissa, India.

Accepted 12 November, 2015

Phosphate solubilising ability of five *Streptomyces* isolated from the phyllosphere of *Heritiera fomes* grown in Bhitarkanika mangrove ecosystem was evaluated using tricalcium phosphate (TCP) in both plates and broth culture conditions as well as with and without NaCl. *Streptomyces* ST24 showed highest solubilization of TCP, with 50.8 and 48.0 mm of halo zone in the plate assay done at pH 7.2 and temperatures 30 and 37°C. *Streptomyces* ST21, ST24 and ST26 showed good solubilization of TCP in culture medium with 52.15, 50.77 and 52.07 µg/ml, respectively. The requirement of NaCl for better solubilization of TCP was observed in all *Streptomyces*. However, ST23 and ST24 showed solubilization activity without addition of NaCl. Thus the solubilization potential varies among different isolates of *Streptomyces*. It also differed according to incubation period. Over all, the best solubilization ability of all test *Streptomyces* could be observed in the presence of 0.2% NaCl. The solubilization might be due to production of acids by the culture, since the pH of the culture broth was changed from initial pH of 7.2 and 9.0 to lower pH values.

Key words: Phosphate solubilization, *Streptomyces*, mangrove, salt, NaCl.

INTRODUCTION

Occurrence and distribution of bacteria and fungi have been well studied in the mangrove and marine environment (Vazquez et. al., 2000). However, studies on actinomycetes in the mangrove area have been less reported. *Streptomyces* is one of the special group among them and has been reported to be found in mangrove environment. Mangrove ecosystem is known to be highly rich due to high amount of dissolved and particulate organic matter attributed to various microbial activities. Microbes from this area play important roles in the biodegradation of plant material through their various enzymatic and metabolic activities. Several bacteria and fungi are reported as solubiliser of phosphate of inorganic and rock sources (Narsian and Patel, 2000) . But very few reports were available on marine microbes (Seshadri et. al., 2002). *Arbuscular mycorrhiza* a phosphate solubilizing bacteria of the rhizosphere of the mangrove ecosystem of Great Nicobar island of India was recently reported (Kothamsi et al., 2006). The phosphate solubilizing potential of the rhizosphere microbial community in mangroves was demonstrated when culture media supplemented with insoluble tribasic calcium phosphate, and incubated with roots of black *Avicennia germinans* and white *Laguncularia racemosa* (L.) Gaertn. Mangrove became transparent after few days of incubation (Vazquez et. al., 2000). Degradation of phosphonate by *Streptomyces* was also reported (Obojska and Leiczak, 1999). In mangrove ecosystem, phosphorus is present in insoluble inorganic forms. However, mangrove plants do not exhibit phosphorus deficiency due to the presence of phosphate solubilisers in rhizosphere. *Streptomyces* was collected from Bhitarkanika mangrove ecosystem that has not been properly studied in this regard. Thus, the present study

*Corresponding author. E-mail: nguc2003@yahoo.co.in.

Abbreviations: TCP; Tricalcium phosphate, **PVK;** Pikovskaya medium, **ISP;** international *Streptomyces* project, **UV;** ultraviolet light.

on phosphate solubilization potential will definitely be an important input to the lack of knowledge in microbial diversity in mangrove ecosystem.

MATERIALS AND METHODS

Source of streptomyces

The study was carried out on *Streptomyces* isolated from samples obtained from mangrove forest of Bhitarkanika (20°4' - 20°8' N; 86° 45' - 87° 50' E) situated at the east coast of Orissa, India. The Bhitarkanika is a delta plain around the Bay of Bengal and formed with the tributaries of the river Mahanadi (the Brahmani, the Baitarini, the Patshala, the Dhamara and the Devi river).

Methodology

Isolation of Streptomyces: Phyllosphere *Streptomyces* were isolated by dilution plate method using different ISP media stipulated for specific isolation (Aneja, 1993; Booth, 1978).

Solid plate assay: The screening for phosphate solubilization was done on Pikovskaya (PVK) medium at pH 7.2 (Srivastav et al., 2004). The colony forming halo zone was considered as positive and selected for further studies.

Effect of culture conditions on phosphate solubilization potential: Standardization and evaluation of phosphate solubilization was done according to Dave and Patel (2003). These organisms were grown in liquid PVK medium of pH 4.5, 7.2, 9.0 and 10.5. Incubation was carried out for seven days at 30 and 37°C. Halo zone formation around colony was measured and recorded. Due to the source of *Streptomyces*, acidic and alkaline range of pH was considered for plate tests. Since *Streptomyces* are fungal, culture plates were grown at 30 and 37°C.

Evaluation of tricalcium phosphate (TCP) solubilization in liquid culture: The selected organisms were grown in liquid PVK medium at selected pH and temperature for the analysis of released phosphate content in the culture filtrate (Bhargava and Raghupathi, 1993). Three experimental sets were prepared (i) PK media to which 25 ml of 0.5% TCP was added (ii) media plus 0.5 g tricalcium phosphate and 0.2% NaCl and, (iii) media plus 0.5 g tricalcium phosphate and 3% NaCl and inoculated with fresh culture of selected organisms in triplicate. These sets were incubated for 7, 15 and 30 days. The total phosphate content available in 25 ml of culture filtrate (prepared by filtration through Whatman no. 1 paper and then centrifuge at 3000 rpm for 15 min) was measured by UV using spectrophotometer at 420 nm and expressed in µg/ml. In each experimental setup, the final pH of culture filtrate was also measured at different interval of incubation period.

RESULTS AND DISCUSSION

A total of 12 isolates were obtained from the phyllosphere of *Heritiera fomes* used for the isolation. Morphological characteristics confirmed them as *Streptomyces*. Of the 12 isolates, 5 were found to be positive for the solubilization of TCP on PVK agar plates and selected.

Screening of microbial isolates for phosphate solubilization revealed the variation among different groups of organisms. All 5 selected isolates of *Streptomyces* were evaluated for their performance in different cultural condition (pH and temperature) (Figure 1). All *Streptomyces* showed halo zones at pH 7.2 and 9.0 and temperatures 30 and 37°C. Most of the isolates carried out good phosphate solubilization at pH 7.2, whereas a few of them performed better at pH 9. Only ST23 and ST26 were able to carry out phosphate solubilization at pH 4.5. *Streptomyces* ST23 exhibited solubilization zone at both temperature and in all three pH (4.5, 7.2 and 9.0). The three isolates of *Streptomyces* ST21, ST23 and ST24 were also able to solubilize TCP on PK agar plate at pH 10.5. However, the maximum zone of solubilization was observed in *Streptomyces* ST24 (50.8 mm and 48.1 mm at 30 and 37°C in PVK medium of pH 7.2).

There were variations in the phosphate solubilization potential of 5 isolates of *Streptomyces*. Very poor phosphate solubilization by ST21 was observed without NaCl and gradually decreased due to extension of incubation period (Table 1). This organism required the addition of 0.2% NaCl into broth for better solubilization of TCP and released 52.15 µg/ml of phosphorus after 21 days of incubation at 37ºC and pH 7.2. The enhancement in solubilization potential along with the incubation days was observed. The tolerance against 3% NaCl for solubilization by this organims was also observed for 7 days incubation. Similarly, it was observed that ST22 exhibited better solubilization of TCP in liquid culture upon addition of 0.2% NaCl when compared to non saline condition. This isolates showed optimum solubilization at 15 days of incubation which gradually declined upon extension to 21 days (Table 2). The addition of 3% NaCl also made this isolate reluctant for solubilization in both pH and temperature. However, ST23 and ST24 performed considerably well under this condition. Both isolates exhibited good solubilization ability in culture medium with or without NaCl but still with a preference of 0.2% NaCl for better phosphate solubilization but behaved differently with respect to the pH of the medium. ST23 showed best solubilization at pH 4.5 whereas ST24 preferred pH 7.2 (Tables 3 and 4). The presence of 3% NaCl caused drastic change in phosphate solubilization activity of ST23 at temperatures 30 and 37°C and pH 9.0. Here phosphorus content was shifted from 10.74 to 25.60 µg/ml and from 1.87 to 35.07 µg/ml, respectively (Table 3). The solubilization activity of ST26 was better in the presence of 0.2% NaCl and at pH 4.5 and 7.2. This was gradually enhanced due to incubation at 37°C for 21 days (Table 4).

The data recorded on changes in pH during solubilization of TCP by *Streptomyces* are shown in Table 5. Almost all *Streptomyces* isolates used in this study were producers of acid in the medium. A decline in the final pH of culture filtrate was observed. In the control (without phosphate), the pH was decreased towards acidic condition (Table 6). Similarly, in the test group, pH also declined as compared to initial pH. The pH of the culture filtrate was not affected much in the absence of NaCl as well as in the addition of 3% NaCl.

In the present study, occurrence of phosphate

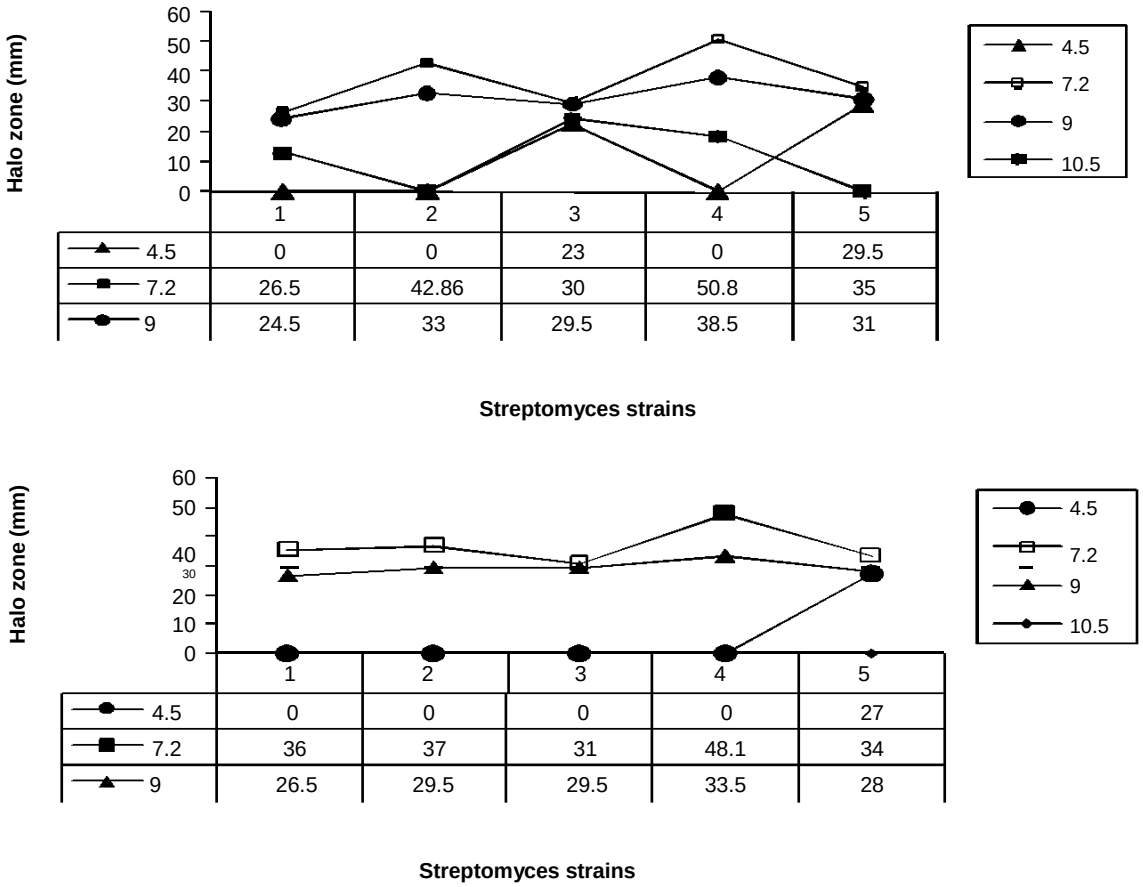

Figure 1. Halo zone formation (mm) by *Streptomyces* grown under different pH at 30°C in Pikovaskaya medium containing tricalcium phosphate. 1 = Streptomyces ST21; 2 = Streptomyces ST22; 3 = Streptomyces ST23; 4 = Streptomyces ST24; 5 = Streptomyces ST26.

Table 1. Phosphate solubilization by *Streptomyces* (ST21) in broth culture at different temperature and pH of the medium.

NaCl (%)	Experimental conditions		Incubation period (days)/ P content ug/ml		
	Temperature (ºC)	pH	7 days	15 days	21 days
0	30	7.2	10.76 ±1.80	0.26 ± 0.33	0.13 ± 0.21
		9	0.00	0.00	0.00
		10.5	0.00	0.00	0.00
	37	7.2	0.00	0.00	0.00
		9	0.00	0.00	0.00
0.2	30	7.2	34.60 ±1.73	36.73 ± 2.20	46.33± 2.30
		9	11.10 ±1.71	19.43 ±1.31	23.00 ± 2.16
		10.5	0.00	16.1 ± 0.50	12.66 ± 0.69
	37	7.2	34.70 ±1.94	49.83 ±1.06	52.15 ± 3.86
		9	10.95 ±1.14	27.00 ± 2.28	30.20 ±1.51
3	30	7.2	24.56 ± 1.11	0.00	0.00
		9	0.00	0.00	0.13 ± 0.24
		10.5	0.283 ± 0.18	0.00	0.00
	37	7.2	27.93 ± 2.94	0.00	0.00
		9	0.00	0.00	0.00

SD (±) = Standard Deviation of 6 replications.

Table 2. Phosphate solubilization by *Streptomyces* (ST22) in broth culture at different temperature and pH of the medium.

NaCl (%)	Experimental conditions		Incubation period (days)/ P content ug/ml		
	Temperature (ºC)	pH	7 days	15 days	21 days
0	30	7.2	0.00	0.00	1.36±0.14
		9	0.00	0.00	0.00
	37	7.2	0.00	0.00	0.00
		9	0.00	0.00	0.00
0.2	30	7.2	30.16 ± 3.51	42.13 ± 1.17	37.60 ± 3.22
		9	0.00	24.93 ± 0.68	28.34 ± 0.81
	37	7.2	18.60 ± 4.84	38.00 ± 1.56	35.7 ± 2.46
		9	8.466 ± 1.21	26.56 ± 3.4	23.23 ± 1.26
3	30	7.2	0.00	0.00	0.83 ± 0.096
		9	0.00	0.00	0.13 ± 0.024
	37	7.2	0.00	1.46±1.61	2.03 ± 0.15
		9	0.266 ± 0.04	0.00	1.3 ± 0.0.18

SD (±) = Standard Deviation of 6 replications.

Table 3. Phosphate solubilization by *Streptomyces* (ST23) in broth culture at different temperature and pH of the medium.

NaCl (%)	Experimental conditions		Incubation period (days) / P content (ug/ml)		
	Temperature (ºC)	pH	7 days	15 days	21 days
0	30	4.5	24.1 ± 1.12	2.1 ± 0.70	0.00
		7.2	22.9 ± 1.40	0.00	0.00
		9	13.033 ± 1.59	0.00	0.00
		10.5	9.06 ± 1.76	0.00	0.00
	37	4.5	40.93 ± 2.86	1.53± 1.35	13.8 ± 0.42
		7.2	37.23 ± 1.65	3.33± 1.80	4.16 ± 1.15
		9	7.53 ± 0.52	5.93± 2.10	0.00
0.2	30	4.5	46.27 ± 1.06	42.5± 1.11	44.37 ± 1.19
		7.2	30.136 ± 1.28	45.83 ± 3.02	43.73 ± 0.09
		9	10.74 ± 3.58	29.54 ± 1.66	28.37 ± 0.84
		10.5	6.23 ±1.87	24.60 ± 1.90	27.2 ± 0.22
	37	4.5	46.14 ± 2.07	38.343 ± 3.46	41.54 ± 0.30
		7.2	25.4 ± 1.30	39.63 ± 1.34	42.07 ± 0.87
		9	1.86 ± 0.55	20.80 ± 3.70	24.23 ± 0.84
3	30	4.5	26.53 ± 3.91	0.00	0.00
		7.2	31.7 ± 2.45	1.3± 1.1	0.00
		9	25.6 ±1.17	0.00	0.00
		10.5	10.13 ± 0.99	0.00	0.00
	37	4.5	37.2 ±1.26	0.23± 0.02	10.13 ± 0.39
		7.2	37.16 ± 1.80	7 ± 1.24	2.96 ± 0.37
		9	35.06 ±2 .26	15.05 ± 2.89	2.1 ± 0.52

SD (±) = Standard deviation of 6 replications.

solubilising organisms useful for tricalcium phosphate solubilization was confirmed. All 12 isolates of *Streptomyces* screened for this purpose were not able to solubilize TCP in solid culture state. However, the degree of phosphate solubilization varied with the organism involved (Srivastav et al., 2004) . A varied solubilization potential was found among the *Streptomyces* isolates. This might be related to differences in their metabolic set

Table 4. Phosphate solubilization by *Streptomyces* (ST24) in broth culture at different temperature and pH of the medium.

NaCl (%)	Experimental conditions		Incubation period (days) / P content (ug/ml)		
			7 days	15 days	21 days
	Temperature (ºC)	pH	Mean	Mean	Mean
0	30	7.2	24.83 ± 1.02	0.00	0.00
		9	15.5 ± 0.94	0.00	0.00
		10.5	3.43 ± 2.68	0.00	0.00
	37	7.2	27.33 ± 1.69	0.26±0.03	0.00
		9	6.06 ± 1.33	0.00	0.00
0.2	30	7.2	45.3 ± 3.01	37.80 ± 2.67	40.43 ± 2.62
		9	36.00 ± 0.69	23.80 ± 2.47	30.9 ± 1.55
		10.5	12.80 ± 1.95	17.13 ± 2.31	25.08 ± 1.66
	37	7.2	50.76 ± 0.70	41.0 ± 5.54	42.06 ± 1.35
		9	42.40 ± 1.73	25.66 ± 2.14	35.46 ± 1.47
3	30	7.2	27.06 ± 0.45	0.00	0.00
		9	38.23 ± 2.49	0.00	0.00
		10.5	5.3 ± 2.63	0.00	0.00
	37	7.2	26.6 ± 4.53	0.00	0.00
		9	33.4 ± 1.046	0.00	0.00

SD (±) = Standard Deviation of 6 replications.

Table 5. Phosphate solubilization by *Streptomyces* ST 26in in broth culture at different temperature and pH of the medium.

NaCl (%)	Experimental conditions		Incubation period (days)/ P content (ug/ml)		
	Temperature (ºC)	pH	7 days	15 days	21 Days
0	30	4.5	0.00	0.00	0.00
		7.2	0.00	0.00	0.33 ± 0.064
		9	0.00	0.00	0.00
	37	4.5	0.00	6.13 ± 1.20	0.00
		7.2	25.06 ± 2.5	0.00	3.03 ±1 .40
		9	0.00	0.00	0.00
0.2	30	4.5	47.87 ± 3.60	43.24 ± 1.64	34.97 ± 1.46
		7.2	25.90 ± 4.68	38.03 ± 1.59	41.03 ± 3.14
		9	1.47 ± 0.62	27.66 ± 1.22	27.4 ± 1.85
	37	4.5	47.61 ± 0.58	47.24 ± 2.93	50.54 ± 5.59
		7.2	41.06 ± 2.65	50.24 ± 4.46	52.06 ± 3.01
		9	0.00	32.86 ± 0.76	30.03 ± 6.45
0.3	30	4.5	0.33 ± 0.27	0.00	0.00
		7.2	0.00	0.00	0.33 ± 0.064
		9	0.00	0.00	0.00
	37	4.5	0.26 ± 0.03	2.2 ± 0.55	0.00
		7.2	1.46 ± 0.187	0.00	0.7 ± 0.08
		9	1.03 ± 0.10	0.00	0.00

SD (±) = Standard Deviation of 6 replications

up and physiology at the species level. The ST23 and ST24 were obtained from the same saline area but belong to terrestrial environment due to phyllosphere.

They presented a higher efficiency than other cultures and showed higher solubilizing ability even in the presence of 3% NaCl. This might be attributed to the fact

Table 6. Change in pH of culture filtrate dusring phosphate solubilization by *Streptomyces* in broth culture at different temperature, pH and incubation period.

Organisms	EC		0%			0.20%			3 % NaCl		
	Temperature (ºC)	pH	7 days	15 days	21 Days	7 days	15 days	21 Days	7 days	15 days	21 Days
ST-21	30	7.2	6.15	6.15	6.2	5.25	5.56	5.59	6.13	6.28	6.34
		9	6.6	6.15	6.28	5.42	5.69	5.57	6.53	6.47	6.34
		10.5	7.06	7.15	6.9	6.37	6.09	5.99	6.76	7.24	6.86
	37	7.2	6.25	6.35	6.37	5.41	5.4	5.4	6.09	6.6	6.48
		9	6.55	6.46	6.43	5.54	5.71	5.5	6.56	6.59	6.56
ST-22	30	7.2	6.18	6.15	6.26	5.15	5.37	5.63	6.39	6.55	6.43
		9	6.29	6.3	6.47	5.72	5.42	5.78	6.48	6.55	6.49
	37	7.2	6.3	6.6	6.29	5.08	5.6	5.56	6.41	6.65	6.5
		9	6.44	6.45	6.49	5.5	5.6	5.67	6.47	6.55	6.51
ST-23	30	4.5	5.91	6.24	6.2	5.02	5.56	5.53	6.08	6.63	6.4
		7.2	5.95	6.35	6.26	5.22	5.53	5.46	6.08	6.54	6.46
		9	6.08	6.51	6.18	5.53	5.58	5.58	6.12	6.64	6.43
		10.5	6.47	7.12	7.04	6.19	5.78	6.25	6.62	7.06	7.11
	37	4.5	5.93	6.53	6.26	5.29	5.52	5.47	5.87	6.38	6.45
		7.2	5.86	6.38	6.24	5.45	5.49	5.57	6	6.45	6.37
		9	6.18	6.61	6.08	5.51	5.53	5.67	6.13	6.52	6.4
ST 24	30	7.2	5.98	6.22	6.29	5.29	5.54	5.63	6.04	6.51	6.5
		9	6.17	6.26	6.32	5.49	5.6	5.67	6.17	6.51	6.55
		10.5	7.05	7.09	7.05	6.17	6.11	6.03	6.69	7.08	7.15
	37	7.2	6	6.33	6.37	5.17	5.61	5.53	6.15	6.43	6.47
		9	6.22	6.31	6.45	5.11	5.8	5.6	6.16	6.52	6.51
ST-26	30	4.5	6.18	6.33	5.85	5.09	5.48	5.68	6.48	6.52	5.86
		7.2	6.12	6.34	6.27	5.35	5.6	5.57	6.35	6.66	6.47
		9	6.29	6.49	6.31	6.01	5.42	5.56	6.42	6.5	6.52
	37	4.5	6.24	6.2	5.87	5.03	5.59	5.36	6.41	6.26	5.87
		7.2	6.05	6.38	6.27	5.33	5.37	5.4	6.34	6.51	6.43
		9	6.77	6.23	6.25	5.81	5.56	5.41	6.46	6.61	6.63

that these isolates developed adaptability towards high salinity. Dissolution of TCP may be due to the production of organic acids. Yadav and Dadarwal (1997) reported the role of acidic metabolites in the release of insoluble phosphorus. In the present study, the drop in pH of culture filtrate confirmed the assertion that phosphate solubilising microorganisms solubilize insoluble phosphates mainly by secreting acids into the medium (Dave and Patel, 2003; Chung et. al., 2005). It was also suggested that the production of organic acids by mangrove rhizosphere microorganisms is a possible mechanisms involved in the solubilization of insoluble calcium phosphate (Vazquez et. al., 2000). Since all the isolates showed a shift in pH towards acidic range, it gives a clue that organic acids might be involved. Further investigation may be carried out to analyse the quality and quantity of organic acids produced during solubilization process. It was also evidenced that production of acidic metabolites occurred preferentially upon addition of 0.2% NaCl into the culture medium. This was reflected in the amount of phosphorus released. The salt tolerance potential also varied with the different isolates of ST21, ST24 and ST23 (Narsian and Patel, 1997). Variation among the different isolates of *Streptomyces* of *Heritiera fomes* in terms of solubilization ability may be due to species level morphological, physiological and metabolic differences (Kundu et. al., 2002). Since, phosphorus deficiency in soil is caused by its insoluble form and chemical fixation, phosphate solubilising microbes play an important role in soil fertility and plant growth. Very rare reports are available on phosphate solubilization by *Streptomyces* as well mangrove microbes (Vazquez et. al., 2000). Since, these experiments were carried out under laboratory conditions, the field performance of these isolates may be affected by the presence of other microflora. Therefore, further studies at field level can also be proposed to obtain fruitful beneficial results from these *Streptomyces*. However, the present work adds another significant literature into the field of biofertilisers.

ACKNOWLEDGMENT

Authors are thankful to the Department of Ocean development (DOD), Government of India, for financial assistance.

REFERENCES

Aneja KR (1993). Experiments in microbiology plant pathology and tissue culture Wihwa prakashan Delhi pp. 83-84.

Booth C (1978). Introduction to general methods In Methods in Microbiology Eds C Booth Acad Press NY 4: 57-91.

Bhargava BS, Raghupathi HB (1993). Analysis of plant materials formacro and micronutrients In: Methods of Analysis of soil plants waters and fertilizers edited by HLST and on published by Fertilizer development and consultation organization 204-204 A Bhanot corner 1-2 Pamposhenclave New Delhi-10048 p. 49-82.

Chung H, Park M, Madhaiyan M, Seshadri S, Song J Cho, Hand Sa T (2005). Isolation and characterization of phosphate solubilising bacteria from the rhizosphere of crop plants of Korea. Soil Biol. Biochem. 37: 1970-1974.

Dave A, Patel HH (2003). Impact of different carbon and nitrogen sources on phosphate solubilization by *Pseudomonas fluorescens*. Indian J. Microbiol. 43: 33-36.

Kothamsi D, Shalini Kothamasi, Bhattacharya A, Khuhad RC, Babu CR (2006). Arbuscular mycorrhiza and phosphate solubilising bacteria of the rhizosphere of the mangrove ecosystem of great nicobar islands India. Biol. Fertile. soils 42: 358-361.

Kundu BS, Gera R, Sharma N, Bhatia A, Sharma R (2002). Host Specificity of phosphate solubilising bacteria. Indian J. Microbiol. 42: 19-21.

Narsian V, Patel HH (1997). Salt tolerance and phosphate solubilizing activity of *Aspergillus aculeatus.* Indian J. Microbiol. 37: 43-44.

Narsian V, Patel HH (2000). *Aspergillus aculeatus* as a rock phosphate solubiliser. Soil Biol. Biochem. 32: 559-565

Obojska AB, Leiczak M (1999). Degradation of phosphonates by Streptomycete isolates. Appl. Microbiol. Biotechnol. 51: 872-876.

Seshadri S, Ignacimuthu S, Lakshminarsimhan C (2002). Variation of heterotrophic and phosphate solubilizing bacteria from Chennai Southeast coast of India. Indian J. Marine Sci. 31: 69-72.

Srivastav S, Yadav KS, Kundu BS (2004). Prospects of using phosphate solubilizing Pseudomonas as biofungicide. Indian J. Microbiol. 44: 91-94.

Vazquez PG, Holguin ME, Puente A, Cortes L, Bashan Y (2000). Phosphate-solubilising microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. Biol. Fertil. Soils 30: 460-468.

Yadav KS, Dadarwal KR (1997). Phosphate solubilization and mobilization through soil microorganisms In: Biotechnological approaches in soil microorganisms for sustainable crop production edited by KR Dadarwal Dept. Mirobiol. CCS Hariyana Agricultural University Hisar India Published by Scientific publishers PO Box 91 Jodhpur India pp. 293-305.