

Marine Actinobacteria showing Phosphate solubilizing efficiency in Chorao Island, Goa, India

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Abstract

The occurrence and distribution of an actinobacteria group of bacteria capable of dissolving insoluble phosphates were studied in marine environments, especially in sediments of Chorao Island, Goa Province, India. A total of 200 bacterial isolates of actinobacteria were isolated. All isolates were screened for phosphate solubilizing activity on Pikovskaya's agar. Thirteen different isolates exhibiting maximum formation of halos (zone of solubilization) around the bacterial colonies were selected for quantitative estimations of P-solubilization. Quantitative estimations for P-solubilization were analyzed for up to 10 days at an interval of 24h. Maximum solubilization from 89.3 ± 3.1 to $164.1 \pm 4.1 \mu\text{g mL}^{-1}$ was observed after six days of incubation in all isolates, while isolate NII-1020 showed maximum P-solubilization. The increase in solubilization coincided with the drop in pH. Many of these species showed wide range of tolerance to temperature, pH and salt concentrations. Further, 16S rRNA gene sequence analyses were carried to identify the bacterial groups which are actively solubilized phosphate in vitro. Gene sequencing results reveals that, all isolates were clustered into six different actinobacterial genera like, *Streptomyces*, *Microbacterium*, *Angustibacter*, *Kocuria*, *Isoptericola* and *Agromyces*. The presence of phosphate solubilizing microorganisms and their ability to solubilize phosphate were indicative of the important role of bacteria in the biogeochemical cycle of phosphorus and the plant growth in coastal ecosystems.

Key words: Sediments; Mangroves; P-solubilization; Actinobacteria; Cultured organisms

Introduction

Phosphorus is a limiting nutrient, inspite of being a major component of seawater. Major forms are either bound to or strongly adsorbed to silt and clay. Phosphorus normally occurs as phosphates in both inorganic and organic compounds. [3, 14, 26]. Moreover phosphate uptake has been found to be dominated by bacteria [5]. Microorganisms assimilate inorganic phosphate and mineralize organic phosphorus compounds and microbial activities were involved in the solubilization and mobilization of phosphates. Orthophosphate appears to be the preferred and apparently universal substrate. Mainly phosphorus is solubilized enzymatically or by acid hydrolysis. Though phosphate solubilizing bacteria (PSB) like *Pseudomonas*, *Serratia*, *Bacillus*, *Flavobacterium* and *Corynebacterium* have been reported from various ecosystems, such as terrestrial especially from soil and water ecosystem including coastal, offshore and mangrove [4, 23]. A series of observations indicates that the highest population of PSB (an average of 108 CFU per gram of sample) was found in fertile soils of forests, organic farming and rhizosphere [30-34], as well as mangrove and shorelines samples [8]. Phosphate solubilizing bacteria from sea sediments were reported to be capable of accelerating the dissolution of apatite phosphate within the phosphorus cycle and interacting with the carbon cycle [24]. Phosphate solubilizing bacteria living in both terrestrial and water ecosystem play a vital role in supplying phosphorus to plants [10]. The present work aims to study the occurrence and identification of phosphate solubilizing actinobacteria in the sediments of Chorao Island, Goa, India.

Materials and Methods

Sampling and Collection of sediment samples

Chorao Island: The Chorao Island is located between the Mandovi and the Mapusa Rivers in Goa, India. The western side of the island is taking a peninsular shape and occupied by a thick mangrove forest of about 1.78 km². The geographical location of the station is 15° 32' 34" latitude and 73° 55' 15" longitude (Figure.1). Samples were collected from the middle of the section of the river and from the surface layer of the sediments with a Peterson grab. The central portions of the samples were aseptically transferred to sterile glass bottle and transferred to the laboratory for further analysis.

Phosphate Solubilizing Bacteria

Solubilization of precipitated tricalcium phosphate-TCP ($\text{Ca}_3(\text{PO}_4)_2$) in unbuffered solid agar medium plates has been used widely as the initial criterion for the isolation of phosphate solubilizing microorganisms. The sediment samples were serially diluted, spread plated on Pikovskaya's agar (g l^{-1}) (glucose – 10 g, $\text{Ca}_3(\text{PO}_4)_2$ – 5 g, $(\text{NH}_4)_2\text{SO}_4$ – 0.5 g, KCl – 0.2 g, MgSO_4 – 0.1 g, MnSO_4 – traces, FeSO_4 – traces, Yeast Extract – 0.5 g, Agar – 15 g, pH–7.0) and incubated at $30\pm 2^\circ\text{C}$ for 1-2 weeks. A total of 200 bacteria isolates with p-solubilization halo zone on plates were purified with repeated culturing and maintained in 20% glycerol at -80°C . All the subsequent experiments were conducted after raising fresh culture. Potential isolates which are showing more halo zone of P-solubilization ($>9\text{mm}$) were assessed for further characterization.

Characterization, identification and phylogenetic analysis

Preliminary biochemical characterization was carried out as per standard methodologies [6]. Growth versus incubation period was checked for maximum of 240h, temperature range for growth was checked on nutrient broth from 10, 25, 37, 45 and 55°C by inoculating 1 ml of an exponential culture (10^8 CFU ml^{-1}) in 100 ml of nutrient broth and estimating the cell population at 12h intervals. The effect of salt concentration of the growth organisms were performed from 0, 3, 5, 7, 9 and 12 % (w/v) for up to 240h at 30°C . The identity of the isolate was revealed on the basis of Biolog carbon source utilization, for carbon source utilization bacterial suspensions were inoculated into Biolog GN2 Microplates as described in instruction manuals and incubated at 30°C for 24 h. The results were interpreted with Biolog Micro Log 34.20.04 software (Biolog, Hayward, CA). And further validated by the 16S rRNA gene sequencing performed using an ABI PRISM BigDye Terminator cycle sequencing kit (as recommended by the manufacturer). The universal primer 27F and 1492R was used for the partial sequencing of the 16S rRNA gene. Sequences of the isolates were compared with sequences available by the BLAST search in the NCBI, GenBank database (<http://www.ncbi.nlm.nih.gov>) [1]. Multiple sequence alignments were performed using CLUSTALW [25]. The method of Jukes and Cantor [13] was used to calculate evolutionary distances. Phylogenetic and molecular evolutionary analyses were conducted using mega version 4.0 [16]. The phylogenetic tree were constructed by the neighbor-joining method [22] using the distance matrix from the alignment. Distances were calculated using the Kimura method [15]. All the sequences were submitted to NCBI GenBank.

Phosphate solubilization

After qualitative estimation of the P-solubilizing activity which was carried out on Pikovskaya's agar. Quantitative estimation of P-solubilization was carried out as per standard methodology [17], by inoculating 1 ml of bacterial suspension (3×10^7 cells ml^{-1}) in 50 ml of National Botanical Research Institute Phosphate broth (NBRIP) in Erlenmeyer flasks (150ml), which contained the following ingredients (g.l^{-1}): glucose, 10.0; tricalcium phosphate (TCP), 10.0; $\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$, 5.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.25; KCl, 0.2; $(\text{NH}_4)_2\text{SO}_4$, 0.1 and incubating the flasks for 10 days at 30°C . At the end of the incubation period the cell suspension was centrifuged at $10,000 \text{ rev min}^{-1}$ for 10 min, and the phosphate content in the supernatant was spectrophotometrically estimated by the ascorbic acid method [18]. All the studies were repeated on three independent experiments to confirm the results. Sterile water inoculated media was treated as blank. After incubation for 10-days, pH of the medium was recorded with a pH meter equipped with glass electrode.

Results

Isolation of Phosphate solubilizing bacteria (PSB)

A total of 200 bacterial isolates of actinobacteria were isolated. All isolates were screened for phosphate solubilizing activity on Pikovskaya's agar. Thirteen different isolates exhibiting maximum formation of halos (zone of solubilization) 9-23mm zone of phosphate solubilization after 6 days incubation around the bacterial colonies were selected for quantitative estimations of P-solubilization. (Figure.1). Phenotypic and biochemical characterization were done using Biolog based carbon source utilization and Hi25 Kit (HiMedia, Mumbai) presented in Table.1. Molecular analysis based on 16S rRNA gene sequencing results reveals all isolates grouped together into six different genera of actinobacteria, some of them are being reported for the first time as P-solubilizers. The isolates were showed optimum growth incubation of 144h, 3-7% (w/v) tolerance to salinity and temperature of 37°C (Table.1), which can be used for preparation of biofertilizer from local isolates based on geographical conditions.

Phosphate solubilization activity

The details of the pH and amounts of soluble-P in the medium from 12-240 h of incubation were presented in Table 2. The solubilization of $\text{Ca}_3(\text{PO}_4)_2$ in the liquid medium by different strains was accompanied by a significant drop in pH (3.0 to 4.5) from an initial pH of 7.0 ± 0.2 after 144 h were

recorded. The soluble-P concentration in the medium ranged between 89.3 ± 3.1 to 164.1 ± 4.1 $\mu\text{g ml}^{-1}$ with variations among different isolates. In the blank treatment with addition any bacterial isolate, no soluble-P was detected as well no drop in pH was observed. The maximum P-solubilization was recorded by *Kocuria* sp. NII-1020 and NII-1014 followed by *Microbacterium* sp NII-1011, NII-1021, NII-1012, *Isoptericola* sp NII-1016, 1029, *Streptomyces* sp NII-1008, NII-1005 and *Agromyces* sp NII-1018 and very less P-solubilization were observed in *Angustibacter* sp NII-1013 and two *Streptomyces* sp NII-1007 and NII-1002. Maximum drop in pH was associated with higher levels of P solubilization, *Kocuria* sp. (NII-1020), where pH was decreased to 4.0 from initial pH 7.0, comparatively higher amounts of soluble-P (164.1 ± 4.1 $\mu\text{g ml}^{-1}$) was detected in the medium. Maximum P-solubilization was detected on 144h of incubation. The decrease in pH clearly indicates the production of organic acid and phosphatase, which is considered to be responsible for phosphate solubilization (Table.2).

Phylogenetic analysis

The identification of phosphate solubilizing bacterial strains based on 16S rRNA gene sequence and their phylogeny are presented in Supplementary Fig. 1. All isolates grouped together into six genera, in which four strains grouped to *Streptomyces*, three isolates to *Microbacterium*, two isolates to *Kocuria*, two isolates grouped to *Isoptericola* sp, and one each to *Agromyces* and *Angustibacter*, some species showed less sequence similarities with closest known species in the NCBI GenBank.

Nucleotide Accession Numbers

The 16S rRNA sequences of phosphate-solubilizing actinobacteria were deposited in the NCBI GenBank database and the accession numbers are presented in Table.3.

Discussion

Usually phosphates precipitate because of the abundance of cations in the interstitial water of mangrove sediments making phosphorus largely unavailable to plants. Phosphate-solubilizing bacteria as a potential supplier of soluble forms of phosphorus have a great advantage for mangrove plants. The generally anoxic conditions of the sediments beneath the aerobic zone would tend to favor dissolution of non-soluble phosphate through sulfide production. However, depending on the degree of aeration of the rhizosphere by root oxygen translocation, the bacteria might be of value in solubilizing phosphate near the roots where sediments are not always completely anoxic, similar to the reason that upland rice tends

to require more phosphate than paddy rice [35]. Surprisingly, very limited research has been focused p-solubilizing efficiency of actinobacteria in the marine environment, either in temperate or tropical regions [2, 7, 9, 20, 28]. In an arid mangrove ecosystem in Mexico, nine strains of phosphate-solubilizing bacteria were isolated from black mangrove (*A. germinans*) roots; *Bacillus amyloliquefaciens*, *B. atrophaeus*, *Paenibacillus macerans*, *Xanthobacter agilis*, *Vibrio proteolyticus*, *Enterobacter aerogenes*, *E. taylorae*, *E. asburiae*, and *Kluyvera cryocrescens*, and three strains from white mangrove (*Languncularia racemosa*) roots; *B. licheniformis*, *Chryseomonas luteola*, and *Pseudomonas stutzeri* [27]. Phosphate solubilizing actinobacteria were recorded from all the sampling stations in Choro Island, among them 13 isolated of six different genera showed positive phosphate solubilizing activity. Production of halo zones on solid agar and release of phosphate in the medium could be ascribed to the release of organic acids by the microbes [23]. The ability of PSB to quantitatively dissolve phosphate and pH of the liquid media with various P sources after 6-day incubation was measured using spectrophotometer. It shows that all of the tested isolates were able to dissolve the inorganic phosphate of $\text{Ca}_3(\text{PO}_4)_2$ within the liquid medium of NBRIP. The highest concentration of dissolve P was found in *Kocuria* sp NII-1020 and NII-1014 with $164.1 \pm 4.1 \mu\text{g ml}^{-1}$ and $145.1 \pm 2.7 \mu\text{g ml}^{-1}$ respectively (Table. 2). The lowest concentration was found in *Streptomyces* sp NII-1002 with $89.3 \pm 3.1 \mu\text{g ml}^{-1}$. Jeon et al., [12] showed that *Pseudomonas fluorescens* dissolves $\text{Ca}_3(\text{PO}_4)_2$ after 5-day incubation and pH drops to 4.4. Perez et al. [19] stated the acidity of a liquid culture is the main mechanism of phosphate dissolution. Whitelaw et al., [29] also stated that the concentration of phosphate dissolved in the culture media is in line with the acidity and concentration of amino acid as well as pH shift. Ramachandran et al., [21] stated that since the isolate is able to release inorganic phosphate from $\text{Ca}_3(\text{PO}_4)_2$ in the liquid media, the bacteria have the potentials to dissolve the bonded P and thus make it available for the plants.

De Souza et al., [8] reported the percentage occurrence of phosphate solubilizing bacteria from different niches viz. beaches, Islands, coasts and offshore and it was found that phosphate solubilizing bacterial population is higher in coastal areas than offshore areas. It is probably that the offshore organisms are poor solubilizers of inorganic phosphate because these organisms are hampered by an insufficient supply of easily degradable organic compounds and low uptake of carbon [8,11]. Hence, the lower percentage occurrence of phosphate solubilizing bacteria observed in the present study may be due to the location of the stations in the deeper waters. But, in terms of phosphate solubilization efficiency, the isolates showed a good result. This is the only report of the phosphate-solubilizing

capacity of actinobacteria belonging to the genera *Streptomyces*, *Microbacterium*, *Angustibacter*, *Kocuria*, *Isoptricola* and *Agromyces*, and of their presence in mangrove sediments. The phosphate-solubilizing activity of all of these species was demonstrated first by halo formation around bacterial colonies growing on a solid medium supplemented with insoluble calcium phosphate.

Conclusion

From the above study it is concluded that, actinomycetes/actinobacteria are also an efficient strains for phosphate solubilization activity. The phosphate solubilizing bacteria are widely distributed in different niches with the coastal area having a high density. Sediment was found to be the most efficient carrier. Hence these isolates could serve continuously to fertilize a niche by solubilizing insoluble phosphorus compounds especially in environments where a low concentration of phosphorus causes various limitations. The contributions of phosphate solubilizing bacteria to phosphorous transfer can be analyzed by using these bacteria in the sediments. It is hoped that these potential studies will enhance our awareness of the roles of microorganisms in aquatic systems and the mechanisms of eutrophication.

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References

1. Altschul S.F., Madden T.L., Schaffer A.A., Zhang J., Zhang Z., Miller W., Lipman O.J., 1997, Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acid Res.* 25, 3389–3402.
2. Ayyakkannu K., Chandramohan D., 1971, Occurrence and distribution of phosphate solubilizing bacteria and phosphatase in marine sediments at Porto Novo. *Marine Biology.* 11, 201–205.
3. Biswas D. R., Narayanasamy G., 2006, Rock phosphate enriched compost: An approach to improve low-grade Indian rock phosphate. *Biores. Technol.* 97, 2243–2251.
4. Chen Y. P., Rekha P. D., Arun A. B., Shen F. T., Lai W. A., Young C. C., 2006, Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl. Soil Ecology.* 34, 33–41.

5. Chung H., Park M., Madhaiyan M., Seshadri S., Song J., Cho H., et al. 2005, Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. *Soil Biology & Biochemistry*. 37, 1970–1974.
6. Collins C.H., Lyne P.M., 1980, *Microbiological methods*. London: Butterworth and Co.
7. Craven P.A., Hayasaka S.S., 1982, Inorganic phosphate solubilization by rhizosphere bacteria in a *Zostera marina* community. *Can. J. Microbiol.* 28,605–610.
8. De Souza M.J.B.D., Nair S., Chandramohan D., 2000, Phosphate solubilizing bacteria around Indian Peninsula. *Indian J. Marine Science*. 29, 48-51.
9. Devendran K., Sundararaj V., Chandramohan D., Krishnamurthy K., 1974, Bacteria and primary production. *Indian J. Marine Science*. 3,139–141.
10. Gyaneshwar P., Kumar G.N., Parekh L.J., Poole P.S., 2002, Role of soil microorganism in improving P nutrition of plants. *Plant Soil*. 245, 83-93.
11. Hoppe H.G., Ullrich S., 1999, Profiles of ecto-enzymes in the Indian Ocean: phenomena of phosphatase activity in the mesopelagic zone. *Aqua. Microbial Ecology*. 19, 139-148.
12. Jeon J.S., Lee S.S., Kim H.Y., Ahn T.S., Song H.G., 2003, Plant growth promotion in soil by some inoculated microorganisms. *J. Microbiol.* 41(4), 271-276.
13. Jukes T.H., Cantor C.R., 1969, Evolution of protein molecules. In: Munro HN (Eds) *Mammalian protein metabolism*. Academic Press, New York, pp 21–132.
14. Kanako O., Motohiko K., Ho A., Kenichi K., 2004, Phosphorus application affects root length distribution and water uptake of upland wheat in a column experiment. *Soil Science. Plant Nutrition*. 50, 257–261.
15. Kimura M., 1980, A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mole. Evol.* 16,111–120.
16. Kumar S., Tamura K., Nei M., 2004, MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Briefings in Bioinformatics*. 5, 150–163.
17. Mehata S., Nautiyal C.S., 2001, An efficient method for qualitative screening of phosphate-solubilizing bacteria. *Curr. Microbiol.* 43, 51–56.
18. Murphy J.P., Riley J.P., 1962, A modified single solution method for the determination of the phosphate in natural waters. *Analytica Chimica Acta*. 27, 31–36.
19. Perez E., Sulbaran M., Ball M.M., Yarzabal L.A., 2007, Isolation and characterization of mineral phosphate solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuela region. *Soil. Biol. Biochem.* 39, 2905-2914.
20. Promod K.C., Dhevendaran K., 1987, Studies on phosphobacteria in Cochin Backwater. *J. Mar. Biolog Assoc. India*. 29, 297–305.
21. Ramachandran K., Srinivasan V., Hamza S., Anandaraj M., 2007, Phosphate solubilizing bacteria isolated from the rhizosphere soil and its growth promotion on black pepper (*Piper nigrum* L.) cutting. *Plant. Soil. Sci.* 102, 325 -331.

22. Saitou N., Nei M., 1987, The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mole. Biol.Evol.* 4, 406–425.
23. Seshadri S., Ignacimuthu S., Lakshminarsimhan C., 2002, Variations of heterotrophic and Phosphate solubilizing bacteria from Chennai, southeast coast of India. *Ind.J. Mar.Sci.* 31(1), 69-72.
24. Thingstad T.F., Rassoulzadegan F., 1995, Nutrient limitations, microbial food webs, and ‘biological pumps’: suggested interactions in a Plimited Mediterranean. *Marine. Ecol Progr. Series.* 117, 299-306.
25. Thompson J.D., Higgins D.G., Gibson T.J., 1994, CLUSTAL W: improving sensitivity of progressive multiple sequence alignments through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acid Resear.* 22, 4673–7680.
26. Vassilev N., Medina A., Azcon R., Vassilev M., 2006, Microbial solubilization of rock phosphate on media containing agro-industrial wastes and effect of the resulting products on plant growth and P-uptake. *Plant.Soil.* 287, 77–84.
27. Vazquez P., Holguin G., Puente M.E., Lopez-Cortes A., Bashan Y., 2000, Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biol.Fertil. Soils.* 30, 460–468.
28. Venkateswaran K., Natarajan R., 1983, Seasonal distribution of inorganic phosphate solubilizing bacteria and phosphatase producing bacteria in Porto Novo waters. *Ind. J. Marine Sci.*12, 213–217.
29. Whitelaw M.A., Harden T.J., Heylar K.R., 1999, Phosphate solubilization in solution culture by the soil fungus *Penicillium radicum*. *Soil Biol.Biochem.* 31, 655-665.
30. Widawati S., Suliasih., Latupuapua H.J.D., Sugiharto A., 2005, Biodiversity of soil microbes from rhizospore at Wamena Biological Garden (WBiG), Jayawijaya, Papua. *Biodiversitas.* 6(1), 6-11. (Indonesia)
31. Widawati S., Suliasih., 2006, Augmentation of potential phosphate solubilizing bacteria (PSB) stimulate growth of green mustard (*Brassica caventis* Ocd.) in marginal soil. *Biodiversitas.* 7(1), 10-14. (Indonesia)
32. Widawati S., Rahmansyah M., 2009, The influence of bacterial inoculation to jarak pagar (*Jatropha curcas* L.) growth. *J. Biol. Indonesia.* 6(1), 107-117.[Indonesia]
33. Widawati S., Suliasih., 2009, The role of phosphate solubilizing bacteria and free living nitrogen fixing bacteria on the growth and adaptation of *Gmelina arborea* Roxb. grown on degraded land. *International Seminar to Protect Diversity of Bioresource in the Tropical Area.*Research Center for Biology LIPI, Cibinong, 25-26 November 2009.
34. Widawati S., 2011, Diversity and phosphate solubilization by bacteria isolated from Laki Island coastal ecosystem. *Biodiversitas.* 12(1), 17-21(Indonesia)
35. Zuberer D.A., Silver W.S., 1979, N₂-fixation (acetylene reduction) and the microbial colonization of mangrove roots. *New Phytologist.* 82, 467–471.

Table.1. Biochemical properties of P-solubilizing strains isolated from Chora, Island.

1

Biochemical reactions	PSB isolates													
	NII 1002	NII 1005	NII 1007	NII 1008	NII 1011	NII 1012	NII 1013	NII 1014	NII 1016	NII 1018	NII 1020	NII 1021	NII 1029	
Gram staining	+	+	+	+	+	+	+	+	+	+	+	+	+	
NaCl tolerance (0-12%)	+	+	+	+	+	+	+	+	+	+	+	+	+	
Optimum Growth (3-7%)	+	+	+	+	+	+	+	+	+	+	+	+	+	
Catalase	-	-	-	-	-	+	+	-	-	-	+	+	-	
Oxidase	-	-	-	-	+	+	+	-	-	+	-	+	-	
Optimum temp (°C)	37	37	37	37	37	37	37	37	25	37	37	37	37	
IMViC Test														
Indole production	-	-	-	-	-	-	-	-	+	-	+	+	+	
Methyl Red	-	-	+	+	+	+	+	-	-	+	+	+	+	
Voges-Proskauer	-	-	-	+	-	-	-	+	+	-	-	-	-	
Citrate (Simmons)	+	+	-	+	-	-	+	-	+	+	+	-	+	
Lysine decarboxylase	+	+	+	-	+	-	+	+	+	+	-	-	-	
Ornithine decarboxylase	+	+	+	-	+	-	-	+	+	+	-	+	-	
Phenylalanine deamination	-	-	-	-	-	-	-	-	-	-	-	-	-	
Urease	-	-	-	+	-	+	-	-	-	-	-	-	-	
H ₂ S Production	-	-	-	-	-	+	+	-	-	-	-	+	+	
Nitrate reduction	+	+	-	+	+	-	+	+	-	+	-	+	+	
Carbon source utilization														
Malonate	+	+	-	+	-	-	+	-	+	+	-	-	-	
Esculin	+	+	+	+	+	+	+	+	-	+	-	-	+	
Arabinose	+	+	+	+	+	w	-	+	+	+	+	+	-	
Xylose	+	+	-	-	-	w	-	+	+	+	+	+	+	
Adonitol	+	+	+	-	+	w	+	-	-	-	-	-	-	
Rhamnose	+	+	+	w	+	+	-	-	-	+	+	+	+	
Cellobiose	+	+	+	-	+	-	-	-	-	+	-	-	+	
Melibiose	+	+	+	+	+	-	-	-	-	+	-	-	+	
Saccharose	+	+	+	-	+	-	+	-	-	+	-	-	+	
Raffinose	+	-	+	+	+	-	-	-	+	+	-	-	+	
Trehalose	+	-	+	-	+	-	+	-	-	+	-	-	+	
Glucose	+	+	+	w	+	-	+	+	+	+	+	+	+	
Lactose	+	-	+	-	+	-	-	-	-	+	+	-	+	

Table 2. P-solubilization with respect to zone of solubilization, in-vitro activity and pH decline.

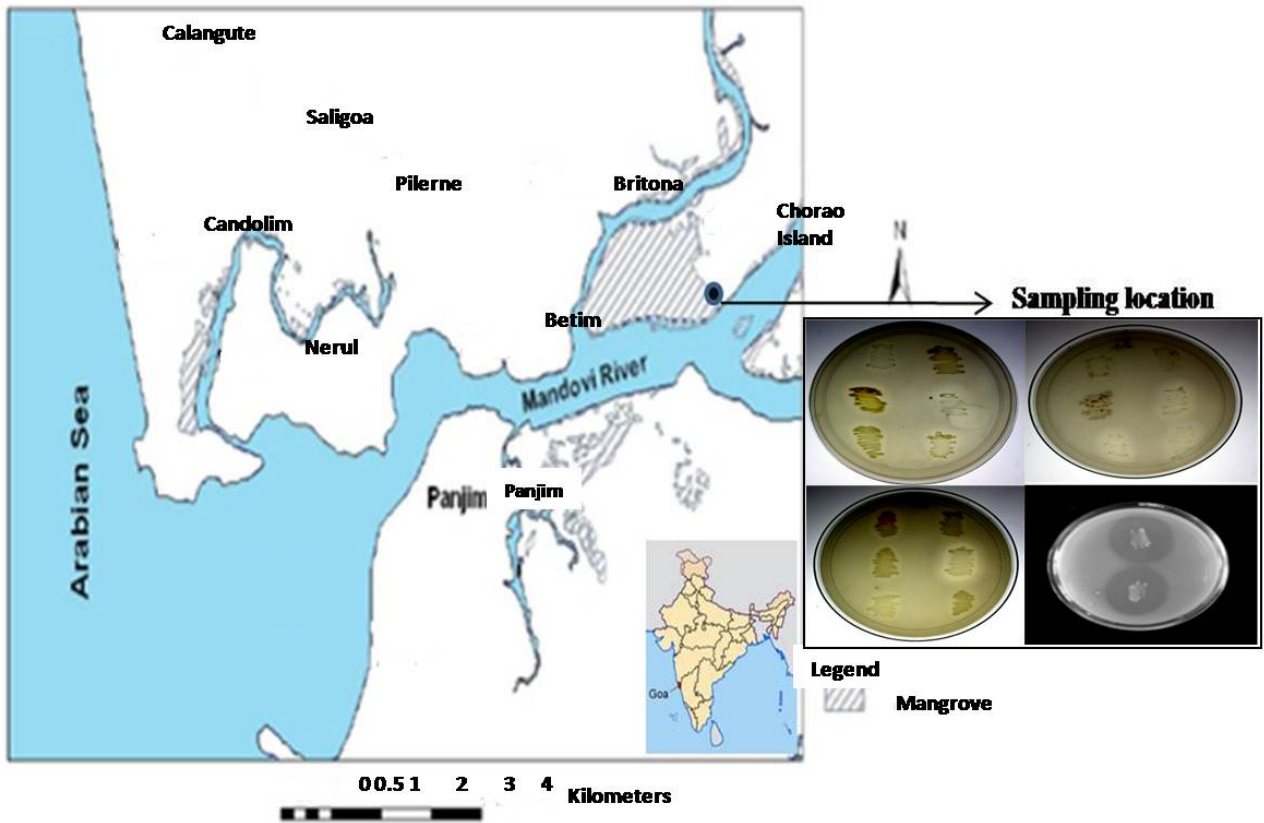
Isolates	Phosphate solubilization ($\mu\text{g P mL}^{-1}$)			
	P-solubilization Zone (mm)	Calcium phosphate $\text{Ca}_3(\text{PO}_4)_2$	pH of the medium	Incubation period (h)
NII-1002	9.0	89.3 \pm 3.1	4.5	144
NII-1005	10.0	101.2 \pm 5.1	4.5	144
NII-1007	9.0	98.2 \pm 5.9	4.5	144
NII-1008	12.0	112.1 \pm 3.1	4.0	144
NII-1011	11.0	113.7 \pm 2.1	4.0	144
NII-1012	10.0	110.2 \pm 3.1	4.0	144
NII-1013	9.0	93.3 \pm 2.6	4.5	144
NII-1014	21.0	145.1 \pm 2.7	3.0	144
NII-1016	12.0	112.2 \pm 2.1	3.6	144
NII-1018	11.0	110.1 \pm 3.2	4.0	144
NII-1020	23.0	164.1 \pm 4.1	3.0	144
NII-1021	14.0	123.4 \pm 2.1	3.5	144
NII-1029	10.0	100.2 \pm 3.4	4.0	144

(Initial pH of liquid medium was 7.0)

Table 3. Genetic characterization of phosphate solubilizing bacterial strain to genomic level.

PSB isolates	Genus/species	Accession no.	Length of nucleotides	16S rRNA gene similarity (%)
NII-1002	<i>Streptomyces violascens</i>	HM036772	988bp	99.7
NII-1005	<i>Streptomyces noboritoensis</i>	HM036675	1140bp	97.9
NII-1007	<i>Streptomyces cinereorectus</i>	HM036677	1474bp	99.6
NII-1008	<i>Streptomyces cinnabarinus</i>	HM036678	1074pb	99.3
NII-1011	<i>Microbacterium aurantiacum</i>	HM036662	1060bp	99.4
NII-1012	<i>Microbacterium kitamiense</i>	HM036663	1458bp	98.0
NII-1013	<i>Angustibacter luteus</i>	HM036657	1427bp	93.9
NII-1014	<i>Kocuria flava</i>	HM036661	995bp	99.4
NII-1016	<i>Isoptericola hypogeus</i>	HM036658	993bp	98.9
NII-1018	<i>Agromyces soli</i>	HM036655	1454bp	98.1
NII-1020	<i>Kocuria palustris</i>	HM036660	993bp	99.0
NII-1021	<i>Microbacterium yannicii</i>	HM036664	995bp	98.1
NII-1029	<i>Isoptericola variabilis</i>	HM036659	919bp	98.9

Fig.1. Sampling site location and Phosphate solubilizing ability of isolates on Pikovskaya's media. .



1

Supplementary Fig.1. Neighbour-joining tree showing the phylogenetic affiliation of isolates to their respective genera. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are shown at branch points. Bar, 0.01 substitutions per nucleotide position.

