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# Phosphate Solubilising Bacteria from Mangrove Soils of Mahanadi River Delta, Odisha, India

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**Abstract** The present study was conducted to isolate phosphate solubilising bacteria from mangrove soils of Mahanadi river delta, Odisha, India and evaluate their phosphate solubilising ability. In total forty-eight phosphate solubilising bacteria were isolated from different soil samples. Based on their size of halo zone formation on NBRIP agar medium and decrease in intensity of colour of the broth medium fourteen isolates were selected as efficient phosphate solubilising strains. Their efficiency on NBRIP agar medium were ranged from 108-175. Their ability to decrease the intensity of bluecolour of the NBRIP-BPB broth medium was ranged from 0.87 and 1.188 (O.D at 600nm). Phosphate solubilising ability test of these fourteen isolates showed that they can solubilise tricalciumphosphate from 8.21 to 48.70µg/ml and most of the isolates could acidified the medium supernatant below 4.0 from the initial pH 7.0. Morphological and biochemical characterisation of the isolates allowed us to identify them as members of the following genera: *Pseudomonas, Bacillus, Alcaligens, Klebsiella, Serratia, Azotobacter*s and *Micrococcus.* All the fourteen PSB isolated from mangrove soil of Mahanadi river delta could efficiently solubilise tricalcium phosphate in the medium which could probably help for future application in biotechnology.

***Keywords:*** *mangrove, tricalcium phosphate, phosphate solubilisation, bacteria*

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## 1. Introduction

Mangrove forests stand as barriers between the land and the sea act as a bridge between terrestrial and marine ecosystems and one of the unique feeding and breeding grounds for diverse groups of organisms [1]. These ecosystems are characterized by periodic tidal flooding which makes the environmental factors such as salinity and nutrient availability highly variable resulting in unique and specific characteristics. Various groups of bacteria like nitrogen fixers, phosphate solubilizers, cellulose decomposers, nitrifiers, denitrifiers, sulphur oxidizers, iron oxidizers and iron reducers are usually present in the mangrove environment [2]. Complex interactions of these microbes maintain the nutritional status and ecological balance of mangrove ecosystem [3]. Muddy mangrove soils have a strong capacity to absorb nitrates and insoluble phosphates carried by the tides [4]. Fungi and inorganic phosphate solubilizing bacteria present in the mangrove rhizosphere participate in releasing soluble phosphate in to mangrove water [4].

Phosphorus (P) is one of the essential elements for the growth and reproduction of bacteria and plays a very significant role in many aspects of cell metabolism. It is the second most important plant nutrient after nitrogen [5]. Phosphorous usually precipitate because of the abundance of cations in the interstitial water of mangrove sediments making phosphorus largely unavailable to plants, thus organisms that solubilise P can have important implications for plant growth, especially in nutrient-limited environments. Bacteria solubilise phosphate in areas where the soil is oxygenated (e.g., near the mangrove roots) and may, therefore, serve an important role in P uptake by the plant. It is generally accepted that the mechanism of mineral phosphate solubilization by phosphate solubilising bacteria (PSB) is associated with the release of low molecular weight organic acids [6], which through their hydroxyl and carboxyl group chelate the cations bound to phosphate, their by converting it into soluble form [7]. Phosphate solubilizing microorganisms have attracted the attention of agriculturalists as soil inoculums to improve the plant growth and yield [8].

A very little information is available about phosphate solubilizing bacterial diversity [9] and their activity in Indian mangroves. Though extensive studies have been carried out for isolation, identification and estimation of P solubilisation by different microorganism inhabiting in estuarine and marine habitats, still no attempts made to understand the diversity and role of P solubilising bacteria in mangrove soil of Mahanadi river delta, Odisha, India.

Keeping these in view this existing scenario inspired the layout of the present study to isolate, characterize and identify some of the predominant phosphate solubilizing bacteria from mangroves of Mahanadi river delta, Odisha, India.

## 2. Materials and Methods

### 2.1. Soil Sample Collection and Isolation of Phosphate Solubilising Bacteria

The soil samples were collected from different location of mangrove forest such as Jumbo, Kharnasi, Triveni, Nuagada, Atharabanki and Mangrove forest at Indian Farmer fertilisers Corporation (IFFCO). After collection of the soil samples the samples were stored at $4^0$C in sterile containers. For each soil sample, several sub-samples were taken, homogenized in sterile MilliQ water containing 0.85% NaCl (w/v) and serially diluted. Aliquots of each dilution were spread on (National Botanical research Institue, Pune) NBRIP-agar medium [10], and incubated at $30^0$C for 24-48 hours. Colonies were selected from the plates on the basis of the appearance of clear halo zone. The ratio between the diameter of the halo zone and diameter of the bacterial colony was calculated and multiplied by 100 [11]. The colonies were further purified on minimal medium based on AT salts [12]. Once purified the isolates were maintained in the laboratory and were used for the further study.

The phosphate Solubilising efficiency of each of the isolates was also determined by following the protocol of Mehta and Nautiyal [13]. In brief, the isolates were grown for 3 days at $30^0$C with continuous agitation in NBRIP-broth medium containing Bromophenol blue (BPB) as pH indicator. At the end of the incubation period the final $OD_{600}$ values were subtracted from the control values.

### 2.2. Quantitative Estimation of Available/Soluble Phosphate

The quantitative estimation of phosphate, solubilized by each isolates was determined according to Murphy and Riely [14]. Quantitative assessment of P solubilization was carried out using NBRIP broth medium. Erlenmeyer flasks containing 100 ml of medium were inoculated with different bacterial culture. Uninoculated medium served as the control. The flasks were incubated at $30^0$ C for 264 h at 100 rpm. The pH of the culture medium was measured every day at specific interval of incubation period. Every day the culture was harvested by centrifugation at 10,000 rpm for 10 minutes. The supernatant was separated from the bacterial cells by successive filtration through Whatman paper 42 # followed by 0.22 µm Millipore membranes and used to estimate the phosphate release in triplicate. The amount of P released was compared with the standard curve taking specific amount of Potassium di-hydrogen phosphate as standard.

### 2.3. Identification of the Isolates

The bacterial isolates were identified by means of morphological examination and some biochemical characterisation. The parameters investigated included colony characteristics, shape, size, spore, motility, Gram's reaction, catalase production, urease production, Voges-Proskauer (V-P) reaction, Indole production, Nitrate reduction, citrate utilization, carbohydrate metabolism (acid-gas production), starch hydrolysis, Tributyrin (or vegetable oil) hydrolysis, Tween-80 hydrolysis, Cholesterol hydrolysis, gelatin hydrolysis, Casein hydrolysis, Growth at different pH and Temperature, Pectin hydrolysis and chitin hydrolysis test were carried out following the standard methods described in Bergey's Manual of Determinative Bacteria [15].

### 2.4. Statistical Analysis

Statistical analysis was performed by SPSS, version 10 for windows (SPSS Inc; Chicago, IL, USA).

## 3. Results and Discussion

**Table 1. Phosphate solubilisation and pH reduction by bacterial isolates**

| Isolates | Soluble P µg/ml | pH reduction | P solubilising | Efficiency Decrease in colour intensity |
|---|---|---|---|---|
| PSB-12 | 38.69 ± 1.23 | 4.2 ± 0.33 | 130.0 ± 2.91 | 0.655 ± 0.003 |
| PSB-15 | 36.69 ± 2.75 | 4.29 ± 0.33 | 123.0 ± 1.35 | 0.662 ± 0.004 |
| PSB-16 | 38.0 ± 2.71 | 3.85 ± 0.27 | 136.36 ± 1.73 | 0.841 ± 0.006 |
| PSB-18 | 39.79 ± 3.59 | 3.56 ± 0.47 | 166.66 ± 0.57 | 0.692 ± 0.003 |
| PSB-21 | 33.0 ± 1.75 | 4.42 ± 0.32 | 120.0 ± 1.62 | 0.712 ± 0.003 |
| PSB-26 | 48.70 ± 3.54 | 3.23 ± 0.26 | 170.0 ± 2.41 | 1.188 ± 0.003 |
| PSB-27 | 33.94 ± 1.33 | 3.9 ± 0.27 | 111.11 ± 1.29 | 0.833 ± 0.003 |
| PSB-28 | 37.35 ± 3.88 | 4.05 ± 0.19 | 112.5 ± 2.91 | 0.935 ± 0.002 |
| PSB-29 | 42.34 ± 1.42 | 3.4 ± 0.47 | 150.0 ± 1.33 | 1.123 ± 0.005 |
| PSB-34 | 41.94 ± 3.44 | 4.01 ± 0.29 | 112.5 ± 1.59 | 0.518 ± 0.006 |
| PSB-37 | 44.84 ± 3.19 | 3.15 ± 0.18 | 175 ± 0.98 | 1.145 ± 0.002 |
| PSB-41 | 36.60 ± 2.35 | 4.21 ± 0.16 | 116.66 ± 2.57 | 0.835 ± 0.001 |
| PSB-44 | 42.73 ± 3.54 | 3.75 ± 0.51 | 112.5 ± 2.77 | 0.893 ± 0.003 |
| PSB-48 | 36.75 ± 3.76 | 4.1 ± 0.22 | 125.0 ± 2.09 | 1.085 ± 0.003 |

P solubilisation efficiency = Zone ratio/Colony ratio x 100. Decrease in color intensity = O.D. of control at 600 nm (1.488) – O.D of the culture medium at 600 nm.

Phosphate solubilising bacteria were isolated from mangrove soils of Mahanadi delta using NBRIP- agar medium. Forty eight morphologically distinct bacterial isolates which were giving halo zones in NBRIP- agar medium were selected as phosphate solubilising bacteria and named as PSB1 – 48. Their phosphate solubilizing efficiency in NBRIP-agar medium in terms of size of halo zone ranged from 108-175. Higher ranges of halo zones were also reported by Joseph and Jisa [16], who reported phosphate solubilising efficiency of bacterial isolates

ranged from 100-575, from rhizosphere soil sample of Kerala. Several workers have reported that many isolates which did not produce any visible halo zone on agar plate could solubilise various type of insoluble inorganic phosphate in liquid medium [17]. This may be because of varying diffusion rates of different organic acids secreted by an organism [18]. Therefore phosphate solubilising bacteria were further screened in NBRIP-BPB broth for quick and easy evaluation of their phosphate solubilising efficiency. The phosphate solubluilizing efficiency of the isolates in NBRIP-BPB broth was studied in terms of decrease in intensity of the colour of bromophenol blue due to the production of acid in the medium which ranged from 0.87 and 1.188 (O.D at 600nm) (Figure 1). The phosphate solubluilizing efficiency of the isolates in NBRIP-BPB broth was also studied earlier in terms of decrease in intensity of the colour of bromophenol blue by the phosphate solubilising bacteria, isolated from different alkaline soil sites in and around Lucknow, India [13]. Finally out of 48 bacteria, only 14 bacterial isolates (PSB-12, PSB-15, PSB-16, PSB-18, PSB-21, PSB-26, PSB-27, PSB-28, PSB-29, PSB-34, PSB-37, PSB-41, PSB-44 and PSB-48) were selected based upon their decolorization of bromophenol blue as well as efficiency of producing prominent halo zone formation on NBRIP-agar plate (Table 1). Further experiments were conducted with this 14 bacterial isolates.

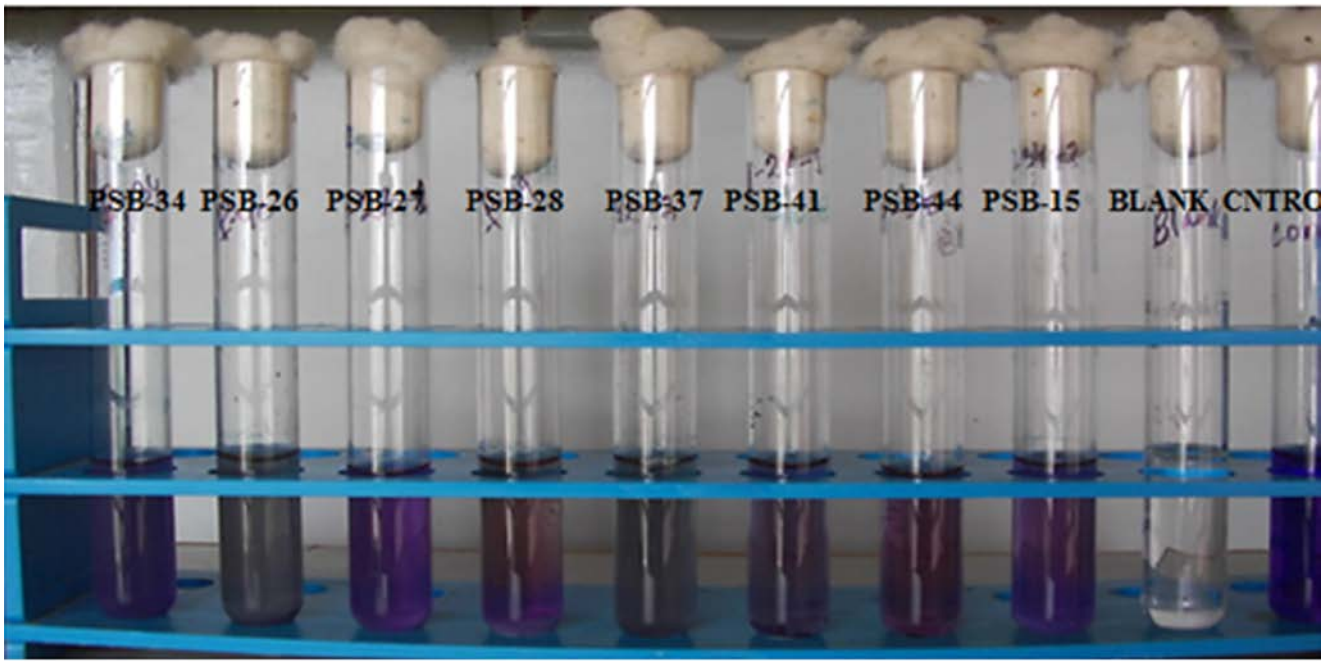


**Figure 1.** Changes of colour intensity of bromphenol blue by P solubilising bacteria in NBRIP-BPB broth medium

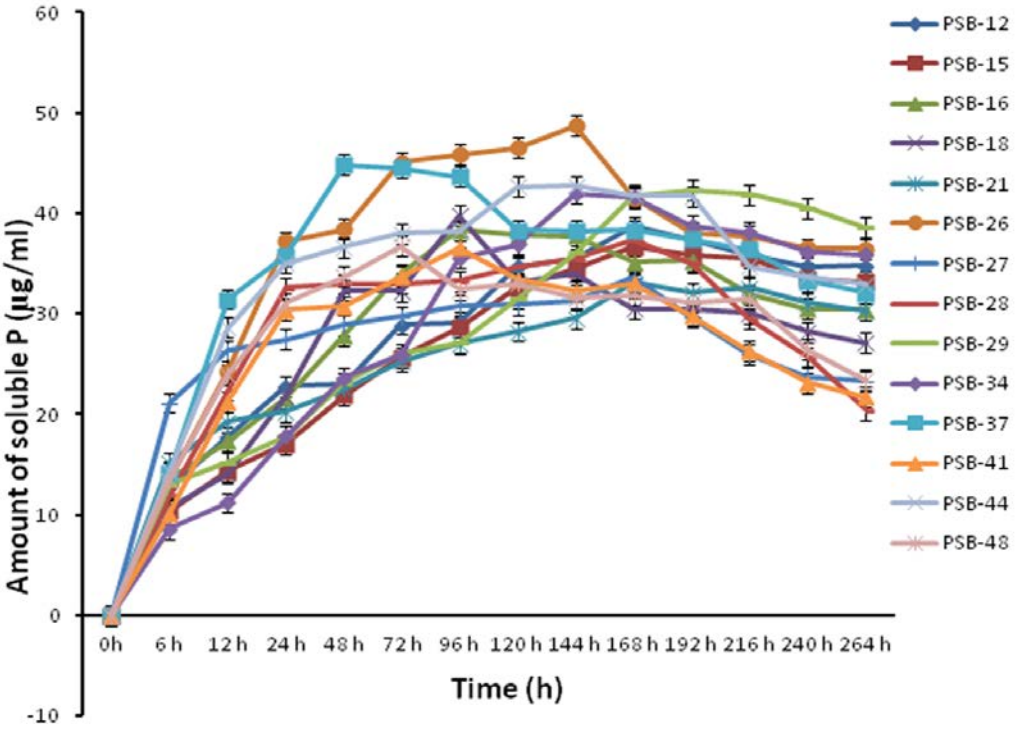


**Figure 2.** Phosphate solubilising ability (µg /ml) of fourteen solubilising bacteria at different incubation periods

The details of the maximum amount of soluble P and reduction of pH in the medium are presented in Table 1. The solubilisation of TCP in the liquid medium by different strain from 24 h to 264 h was accompanied by significant drop in pH from an initial pH of 7.0. The soluble P concentration in the medium ranged from 8.21 to 48.70µg/ml. In the blank treatment no soluble-P was detected as well no drop in pH was observed. The maximum P solubilisation was recorded by the bacterial isolate PSB-26 which showed highest phosphate solubilizing ability (48.70 µg /ml) (Figure 2) followed by PSB-37with phosphate solubilising ability of 44.84 µg/ml with a maximum drop in the pH to 3.15 (pH data not showed). Among the isolates the minimum concentration of soluble P (33.0 µg/ml) was observed in the culture medium of PSB-21(Figure 2) where the pH of the medium was relatively higher (4.5) (pH data not showed). Phosphate solubilisation in the same ranges were also reported from bacteria isolated from other mangrove ecosystems. Pramod and Dhevendran [19] reported phosphate solubilisation by IPSB *Vibrio* sp. and *Pseudomonas* sp. of 0.5-0.55 mg/l from Cochin, India. Kathiresan and Selvam [9] isolated 24 phosphate solubilising bacteria from rhizosphere mangrove soil samples taken from *Rhizophora mucronata* Poir in the Vellar estuary at Parangipettai, southeastern coast of India and showed P solubilisation in the range of 0.012mg/l-0.141 mg/l. Much higher P solubilising activity (400 mg $l^{-1}$) was also reported from the bacterial abundance from an arid mangrove ecosystem in Mexico [4]. Similarly seven bacterial sp such as, two *Bacillus subtilis*, three *Pseudomonas sp.* and two *Azotobacter sp*. reported from mangrove soil of Chollangi, East Godavari exhibited solubilising ability of 80-100 ug/ml of phosphate [20].

**Table 2. Biochemical identification of phosphate solubilising bacteria**

| **Characters** | **PSB12** | **PSB15** | **PSB16** | **PSB18** | **PSB21** | **PSB26** | **PSB27** | **PSB28** | **PSB29** | **PSB34** | **PSB37** | **PSB41** | **PSB44** | **PSB48** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Shape | Rod | Rod | Rod | Rod | Rod | rod | Rod | Rod | Rod | Rod | Rod | Rod | cocci | cocci |
| Cell diameter(µm) | 0.8-1.1 | 0.7-0.8 | 0.8-0.9 | 0.76-0.88 | 1.0-1.05 | 0.55-0.95 | 0.9- 1.0 | 0.75–0.9 | 0.8-1.45 | 0.4- 0.8 | 0.7-2.3 | 1.55-1.98 | 1.5- 2. 0 | 0.9-2.1 |
| Spore | - | - | - | + | + | - | - | - | + | - | - | | - | - |
| Motility test | + | + | + | + | + | + | + | + | + | - | + | - | + | - |
| Aerobic growth | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Urease production test | - | - | - | - | - | - | + | - | - | + | + | + | - | + |
| Gram stain | - | - | - | + | + | - | - | - | + | - | - | - | - | + |
| PHB | + | - | + | - | - | + | - | - | - | - | - | - | - | - |
| Catalase production test | + | + | + | - | + | + | + | + | + | + | + | + | + | + |
| MR | - | - | - | + | + | - | + | - | - | - | - | - | + | - |
| VP | - | - | - | + | + | - | - | - | - | + | + | - | - | - |
| Citrate utilisation test | + | + | - | + | + | + | + | - | + | + | + | + | + | + |
| **Protease:** | | | | | | | | | | - | | | | |
| Gelatin hydrolysis test | + | - | - | + | + | - | - | - | + | - | + | - | - | - |
| Casein hydrolysis test | - | - | - | + | + | - | - | - | + | - | + | - | - | - |
| **Lipase:** | | | | | | | | | | | | | | |
| Tributyrin hydrolysis test | + | + | + | + | - | - | - | - | - | - | + | - | - | |
| Tween 80 utilisation test | + | + | + | + | + | - | - | - | - | + | + | - | - | - |
| Lecithinase egg yolk test | - | - | - | - | - | - | + | - | - | - | - | + | - | - |
| Chitinase production test | - | - | - | + | + | | - | - | - | - | | - | - | |
| Argine dihydrolase test | - | - | + | + | + | - | + | + | + | - | - | - | - | - |
| Strach hydrolysis test | - | + | - | - | + | - | + | - | + | + | - | - | + | - |
| Oxidase production test | + | + | + | + | + | + | - | + | + | - | - | + | + | + |
| Nitrate reduction test | + | + | - | - | + | - | - | - | - | + | + | + | + | + |
| **Acid from:** | | | | | | | | | | | | | | |
| Glucose | + | + | + | + | + | - | + | + | + | + | + | - | + | - |
| Fructose | + | + | + | + | + | - | + | + | - | + | + | - | + | - |
| Mannose | + | + | + | + | + | - | + | + | - | + | + | - | + | - |
| **Gas production** | | | | | | | | | | - | | | | |
| Glucose | + | + | - | - | - | + | - | + | - | + | - | - | + | |
| Indole | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Growth pH 5-7 | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Growth 40°C | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

In the present study all the isolates showed 3.5 to 4 units of pH decrease which is similar to the findings recorded by Prez et al. [21], who also observed 3.2 to 4 unit decrease of Ph by most of the P solubilising bacteria. It is previously reported that the pH drops is due to the production of acid (organic acid) which is the sole reason for the solubilization of phosphate in the medium [22,23,24]. From the results of this study, again it is being reaffirmed that the phosphate solubilization by different PSBs is involved with the production of organic acids [6]. The inverse relationship observed between the pH and soluble-Pconcentration indicates that organic acid production by these PSB strains plays a significant role in the acidification of the medium facilitating the P solubilization. Similar inverse relationship between pH and soluble phosphate was also reported earlier [25].

These fourteen selected bacterial isolates were subjected to various morphological and biochemical characterization (Table 2), with a view to identify them. On the basis of their morphological and various biochemical characterisation these isolates were identified as *Pseudomonas cepacia* (PSB12), *Pseudomonas stutzeri* (PSB15), *Pseudomonas sp.* (PSB16), *Bacillus pumilus*.(PSB18), *Bacillus subtilis* (PSB21), *Bacillus sp.* (PSB 41), *Pseudomonas sp.* (PSB27), *Pseudomonas sp.* (PSB28), *Bacillus megaterium* (PSB29), *Alcaligens Sp.* (PSB-26), *Klebsiella* sp. (PSB 34), *Serratia sp.* (PSB-37), *Azotobacter* sp. *(*PSB44) and *Micrococcus sp.* (PSB-48) (Table 2). All of them were also reported previously as a key phosphate solubiliser in mangrove soil as well as from other environment. Audipudi et al. [21] reported phosphate solubilising activity of two *Bacillus subtilis*, three *Psudomonas spp* and two *Azotobacter spp*, from Chollangi mangrove in south east coast of India. Similarly Ghosh et al. [26] reported some phosphate solubilising bacteria belongs to the genus *Bacillus spp*, *Citrobacter spp*, *Shigella spp*, *Klebsiella spp*, from sea-grass rhizosphere soil. Vazquez et al. *[4]* reported bacterial spp. such as *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Bacillus atrophaeus*, *Paenibacillus macerans*, *Vibrio proteolyticus*, *Xanthobacter agilis*, *Enterobacter aerogenes*, *Enterobacter taylorae*, *Enterobacter asburiae*, *Kluyvera cryocrescens*, *Pseudomonas stutzeri*, and *Chryseomonas luteola* from maxico mangrove soil.

## 4. Conclusion

From the present study it can be concluded that all the fourteen PSB isolated from mangrove soil of Mahanadi river delta could efficiently solubilise tricalcium phosphate in the medium which could probably help taxonomists, agriculturalists and even some industrialists in their own researches. Use of these PSB as bio-inoculants can be incorporated to enhance organic matter decomposition in soil to increase soil fertility which can minimize the fertilizer application and also promotes sustainable agriculture.

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