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Study on P solubilizing efficiencies of native PSB isolates from acid soils of Odisha

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Abstract
Soil acidity contributes 70 per cent of the total cultivated land in Odisha. We have collected two hundred fifty (250) nos. of GPS based rhizospheric soil samples from Cuttack, Khordha, Balasore, Mayurbhanj, Keonjhar districts of Odisha and 106 nos. of samples showed pH below 5.5, which were screened for the enumeration and isolation of P- solubilizing bacteria. Five districts of Odisha were selected on the basis of their extent of soil acidity. Three districts viz; Cuttack, Khordha and Balasore with more than 80 % coverage of acid soils and the two with less than 50 % coverage were taken in to account for collection of rhizospher soil samples. The target soils of the five districts mostly come under the sub groups viz; Typic Ustorthents, Typic Tropaquepts, Vertic Tropaquepts, Typic Haplustalfs, Typic Ustochrepts, Typic Ustropepts, Vertic Ustochrepts, Typic Haplaquepts, Ultic Paleustalfs, Rhodic Paleustalfs, Aeric Ochraqualfs, Aeric Tropaquepts and Typic Rhodustalfs. A total of two hundred eight (208) nos. of PSB isolates were isolated from the villages of five districts. Out of 208, highest no. of PSB i.e. sixty five (65) isolates were isolated from Mayurbhanj district followed by fifty two (52) from Balasore district. Cuttack, Khordha and Keonjhar accounted for 36, 26 and 29 PSB isolates respectively. Forty five (45) PSBs with ≥ 180 % P solubilization efficiency on NBRIP agar medium were further screened for the P-solubilization efficiency on NBRIP Broth with $Ca_3(PO_4)_2$, $FePO_4$, $Fe_3(PO_4)_2$ and $AlPO_4$ as inorganic phosphate sources. Based on the soluble P recovery by the 45 PSB isolates at 48 and 72 h of incubation; five (5) isolates viz; BLS18 (Sarupala, Balasore), CTC12 (Echhapur, Cuttack), KHD08 (Balarampur, Khordha), KJR03 (Rangadihi, Keonjhar) and K1 (Chitrada, Mayurbhanj) from each district were selected for further screening and characterization of their P solubilizing efficiency. These native PSB isolates can be further characterized to screen their P solubilization efficiencies in field conditions.

Keywords: acid soil, P fixation, PSB, Al-P, Fe-P, PSB

Introduction
Soil pH directly or indirectly governs majority of the chemical or biological properties of soil, eventually regulates the biochemical environment of plants. Soil chemical reaction determines the soil types. Acidic soils are mostly categorized of having lower pH (below 6.5), CEC and base saturation percentage while higher amounts of Al, Fe, Mn. P, S, Ca, Mg are poor in concentration due to chemical fixation. B and Mo like micronutrients are also deficient (Jakovljevic *et al*., 2005) [14].

In India, acid soil constitute around 30 per cent of cultivated land whereas in Odisha the area is around 70 per cent (Mitra *et al*., 2002) [18].

Odisha is located on eastern corner of India, between 17^0 47' to 22^0 33' N latitude and 81^0 21' to 87^0 30' longitude covering 1557 million hectares geographical area, of which 8.67 M ha is acidic. According to soil taxonomy, the soils of the state were categorized into four (4) orders. The order Inceptisols (7.49M ha) is the dominant followed by Alfisols (5.62 M ha), Entisols (1.53 M ha) and Vertisols (0.93 M ha) (Panda, 2009) [21].

Again very precisely the state soils can be classified into eight (8) groups viz; Red Soil (Haplustalfs, Rhodustalfs, Ustorthents), Mixed red and Yellow Soil (Haplustalfs, Paleustalfs, Ustochrepts), Black Soil (Chromusterts, Ustorthents), Laterite Soil (Haplustalfs, Plinthustalfs, Ochraqualfs), Deltaic alluvial Soil (Haplaquepts, Fluvaquents, Ustochrepts), Coastal Saline and Alluvial Soil (Halaquepts, Halaquepts), Brown Forest Soil (Haplustalfs, Ustochrepts, Rhodustalfs) and Mixed red and black soil (Association of Alfisols, Vertisols and Vertic Intergrades). Soils belonging to groups red, mixed red and yellow, laterite come under moderately acidic to strongly acidic soils and the groups brown forest and deltaic alluvial impart slightly acidic soil. (Sahu and Mishra, 2005) [27]. The rest three groups contribute mildly acidic to alkaline soil. Problematic acid soils in the state are mostly due to the influence of climate, topography and parent material.

Drastic weathering of parent material under hot humid climate and heavy precipitation (>1500 mm) in the state are responsible for formation of acid soils which are predominately red and lateritic in origin (Panda, 2009) [21].

The acid soils of Odisha are mostly deficient in available-N, low to medium in available-P and medium to high in available-K. Soil acidity triggers the formation of Al-P and Fe-P. These minaerals are hard to solubilize, and therefore can't be taken up by crop, although soils contain high concentration of total P (Merbach *et al*., 2010) [17].

Phosphorous is the major essential macronutrient second only to nitrogen and regulates majority of metabolic processes viz; energy transfer, signal transduction, macro-molecular biosynthesis, photosynthesis, respiration as the key ingredient (Shenoy and Kalagudi, 2005) [28]. But in acid agricultural soils deficiency of this plant nutrient can further initiate variety of symptoms in plants. Hence, to avoid this type of problematic soils, farmers are advised to apply quite higher amounts of inorganic P fertilizer, which again get fixed soon after application, eventually accelerate the process of P fixation (Goldstein, 1986) [8]. However, soil is the natural environment to variety of microorganisms, which provide firm participation in transformation of most macro and micro nutrients. Bacteria from genera such as *Achromobacter, Agrobacterium, Bacillus, Enterobacter, Erwinia, Escherichia, Flavobacterium, Mycobacterium, Pseudomonas* and *Serratia* are highly efficient in solubilizing unavailable complexed phosphate into available inorganic phosphate ion, commonly known as P solubilizing bacteria or PSB (Goldstein, 2001) [9]. The alliance between PSB and plant roots could play the vital role of P nutrition particularly in these problematic acid soils (Jorquera *et al*., 2008) [15].

Considering the fact, that P is the most essential and very often limiting plant nutrient, screening for potential native P-solubilizing bacteria from P deficient acid soils is necessary for plant P nutrition. The present work is designed to explore and screen effective native PSBs from acid soil zones (five districts) of Odisha and to evaluate their P solubilizing ability with Ca-P, Al-P and Fe-P.

Materials and Method

Collection of rhizospheric soil samples

GPS based rhizospheric soil samples (0-15cm depth) were collected from Mayurbhanj, Balasore, Cuttack, Khordha and Keonjhar districts of Odisha. The samples were air dried and passed through 60 mesh sieve for analysis of soil chemical parameters.

Soil chemical parameters

Soil pH

Soil pH was determined in 1: 2.5 soil: water ratio using pH meter (Systronics Digital pH meter 335) as described by Jackson (1975) [13].

Available phosphorous

The available P of the soil samples were determined by Bray's method. One gm of the soil samples were taken in 100 ml conical flasks followed by addition of Bray's -P extracting solution. It was shaken for 1min and was filtered into 50ml conical flasks. 1ml of aliquot was transferred to 25 ml volumetric flask, 2ml of ammonium chloromolybdate was added followed by addition of 6ml distilled water and 2ml Stannous chloride. The volume was made up to 25 ml by addition of distilled water. Absorbance of the prepared samples was noted in Spectrophotometer (Visible Spectrophotometer- CL 320) at wavelength of 660 nm wavelength (Page *et al*., 1982) [20].

Soil biological parameters

Enumeration of soil microbial population

The soil microbial population (heterotrophic and P-solubilizing bacteria, actinomycetes) was determined by serial dilution and spread plate technique. 1 g of the soil samples were added to 10 tubes containing 9ml of distilled water, serially diluted and spreaded over Nutrient Agar, National Botanical Research Institute's phosphate (NBRIP) growth medium and Actinomycetes Isolation Agar for enumeration of total heterotrophic bacteria, P-solubilizing bacteria and actinomycetes respectively. The plates were incubated at 30°C for 24 hours for bacterial isolation and 48 hrs for P-solubilizing bacteria and actinomycetes.

Soil microbial biomass carbon

Microbial biomass carbon was measured employing fumigation and extraction procedure as described by Vance *et al*. (1987) [31]. The process involved collection of filtrate using Whatman filter paper no. 2 by shaking unfumigated soil (5 g) with 20 ml 0.5M K_2SO_4 for 30 minutes. Similarly another set of filtrate was collected using fumigated soil (5 g) exposed to ethanol free chloroform vapour for 24 hrs. Organic carbon in both the extract was analysed using the method of digestion titration. 10ml of filtrate was transferred into a conical flask and 10ml of $K_2Cr_2O_7$ followed by 20ml of conc.H_2SO_4 were added and the entire content undergo digestion for 30 mins at 170^0C. After the content in the flask cool down, 25ml distilled water and 5 ml orthophosphoric acid was added to the digested material and titrated against 0.04M Ferrous ammonium sulphate with Ferroin as the indicator.

Calculation

$$\text{Microbial biomass carbon} = \frac{\text{EC fumigated soil} - \text{EC of Unfumigated soil}}{\text{Kc}}$$

Where, EC = Extractable carbon in fumigated and unfumigated soil samples

Kc = 0.379, Kc is the K_2SO_4 extract efficiency factor, for microbial carbon (Hu and Cao, 2007) [12]

Evaluation of P solubilization efficiency of the isolates

Isolation and screening of PSB isolates

Soils samples were stored at 4^0C for microbial analysis. For isolation of PSB, serial soil dilutions were spread plated on National Botanical Research Institute's phosphate (NBRIP) growth medium (Nautiyal, 1999) [19] with insoluble tricalcium phosphate (TCP) and the colonies were counted (cfu g^{-1} dry wt. of soil). Each PSB isolates were spotted on NBRIP Agar plates for determination of the P solubilization efficiency. The plates were incubated at 30 ± 2^0C for 48 h and the diameter of the colony as well as the halo zone was measured. P-solubilizing index (PSI) and P solubilization efficiency (PE %) were calculated as:

$$PSI = Z / C, \quad PSI (\%) = (Z-C) / C \times 100$$

Where Z = Halo zone diameter, C = colony diameter

Screened PSBs were further examined in NBRIP liquid medium with inorganic phosphates [$Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$ and $Fe_3(PO_4)_2$]. The flasks were incubated at 30 ± 2^0 C for 48 h and the contents were centrifuged at 10,000 rpm for 30 min. Soluble free phosphate in culture supernatant was estimated

from the absorbance values obtained using the calibration curve with KH_2PO_4 at 660 nm for each strain (Jackson, 1975; Page *et al*., 1982; Balamurugan *et al*., 2010) [13, 20]. The potent strains were incubated till 8th day for estimation of soluble P content and P solubilization efficiency by using the above mentioned method.

Result

Collection of soil samples

Soils of Odisha account for more than 70% acidic soil (Figure 1). Out of 30 districts of Odisha, we have selected only five (5) viz; Balasore, Cuttack, Khordha, Keonjhar and Mayurbhanj with around 41.5, 96.5, 82.3, 30.9 and 81.0 per cent acid soil areas respectively. GPS based rhizospheric soil samples (250 nos.) were collected from these five districts and out of these 250 only 106 nos. with pH ≤ 5.50 were selected for enumeration of phosphate solubilizing bacteria. Further these samples were analyzed for chemical parameters viz; reaction (pH) and available phosphorous and microbial parameters viz; total heterotrophic bacteria, actinomycetes, phosphorous solubilizing bacteria and soil microbial biomass carbon.

The study area covered seven (7) blocks in Balasore (Oupada, Bahanga, Nilagiri, Remuna, Balasore Sadar, Basta, Soro); four (4) blocks in Cuttack (Baranga, Athagarh, Tigiria, Narasinghapur); three (3) blocks in Khordha (Begunia, Balianta, Bhubaneswar); five blocks in Keonjhar (Banspal, Sadar, Khireitangiri, Turumunga, Patana) and nine (9) blocks in Mayurbhanj district (Muruda, Rasagovindapur, Shyamakhunta, Baripada Sadar, Betanati, Saraskana, Sukuruli, Karanjia, Jashipur). Soils of Balasore district belonged to the subgroups viz; Typic Ustorthents, Typic Tropaquepts, Vertic Tropaquepts, Typic Tropaquepts, Typic Haplustalfs while that of Cuttack belonged to Typic Ustorthents and Typic Tropaquepts. Three blocks of Khordha covered mainly soil series of Typic Ustorthents, Typic Tropaquepts and Vertic Ustochrepts. Areas of Keonjhar and Mayurbhanj districts mainly covered soil subgroups viz; Typic Ustorthents, Typic Haplaquepts, Ultic Paleustalfs, Rhodic Paleustalfs, Aeric Ochraqualfs and Typic Rhodustalfs (data not shown).

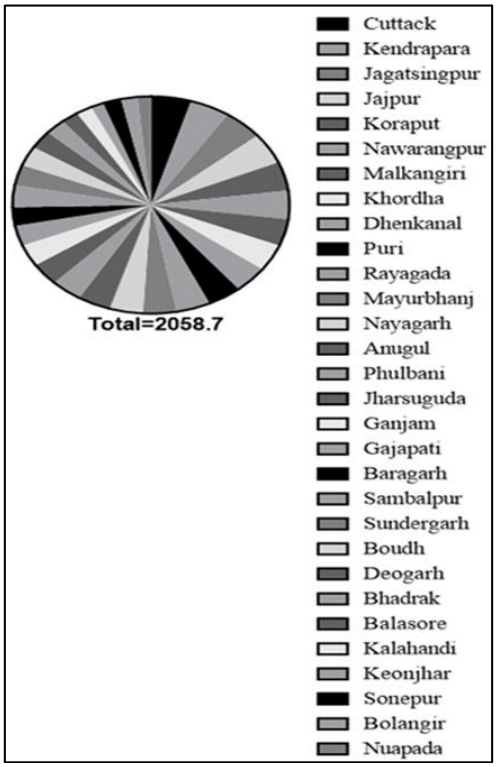


Fig 1: Pie chart showing extent of distribution of acid soils in Odisha

Chemical properties of soils from different villages

GPS based rhizospheric soil samples were collected from five districts of Odisha and their pH and available P were tested (Figure 2; Figure 3). Twenty nine (29) nos. of rhizospheric soil samples collected from Balasore district showed a pH range of 4.41 to 5.50 with village Purusottampur of Remuna block recording lowest pH (4.41) while the villages Gaudadanda and Sanakudi recorded the highest pH (5.50). Available phosphorous ranged between 8.30 to 18.56 kg ha^{-1}. Soil sample collected from the village Purusottampur recorded lowest available phosphorous (8.30 kg ha^{-1}) and Mangasarpur recorded the highest (18.56 kg ha^{-1}).

Out of the twenty (20) soil samples collected from Cuttack district lowest pH (4.67) was recorded from the village Gadisahi. Kankadasahi and Achalakota showed highest pH i.e. 5.50. Available phosphorous of samples from Cuttack district ranged between 9.26 to 18.93 kg ha^{-1}. Village Dhaipur recorded the lowest available phosphorous (9.26 kg ha^{-1}) while Kendupali recorded highest (18.93 kg ha^{-1}).

Fourteen (14) nos. of soil samples were collected from Khordha district. Soil reaction (pH) in Khordha district ranged from 4.50 to 5.50. Soil sample collected from Central Farm, OUAT, Bhubaneswar recorded lowest pH (4.50). Jagiribadi village recorded the highest pH (5.50). Lowest available phosphorous (10.00 kg ha^{-1}) was recorded from the soil sample collected from Central Farm, OUAT, Bhubaneswar but sample from Balarampur village recorded the highest available phosphorous (18.48 kg ha^{-1}).

Sixteen (16) nos. of soil samples were collected from Keonjhar district. Soils of Turumunga district showed lowest soil reaction (4.33) while the highest (5.49) was recorded from Jamudiha village. Available phosphorous ranged from

10.00 kg ha^{-1} (village – Turumunga) to 17.30 kg ha^{-1} in Dhanagadi.

Twenty seven (27) nos. of rhizospheric soil samples were collected from Mayurbhanj district. The samples showed a pH range of 3.66 (village - Manada) to 5.50 (village - Chandua). Soil available phosphorous of Mayurbhanj district ranged between 9.32 kg ha^{-1} (village - Angarpada) to 15.48 kg ha^{-1} (village - Chandua).

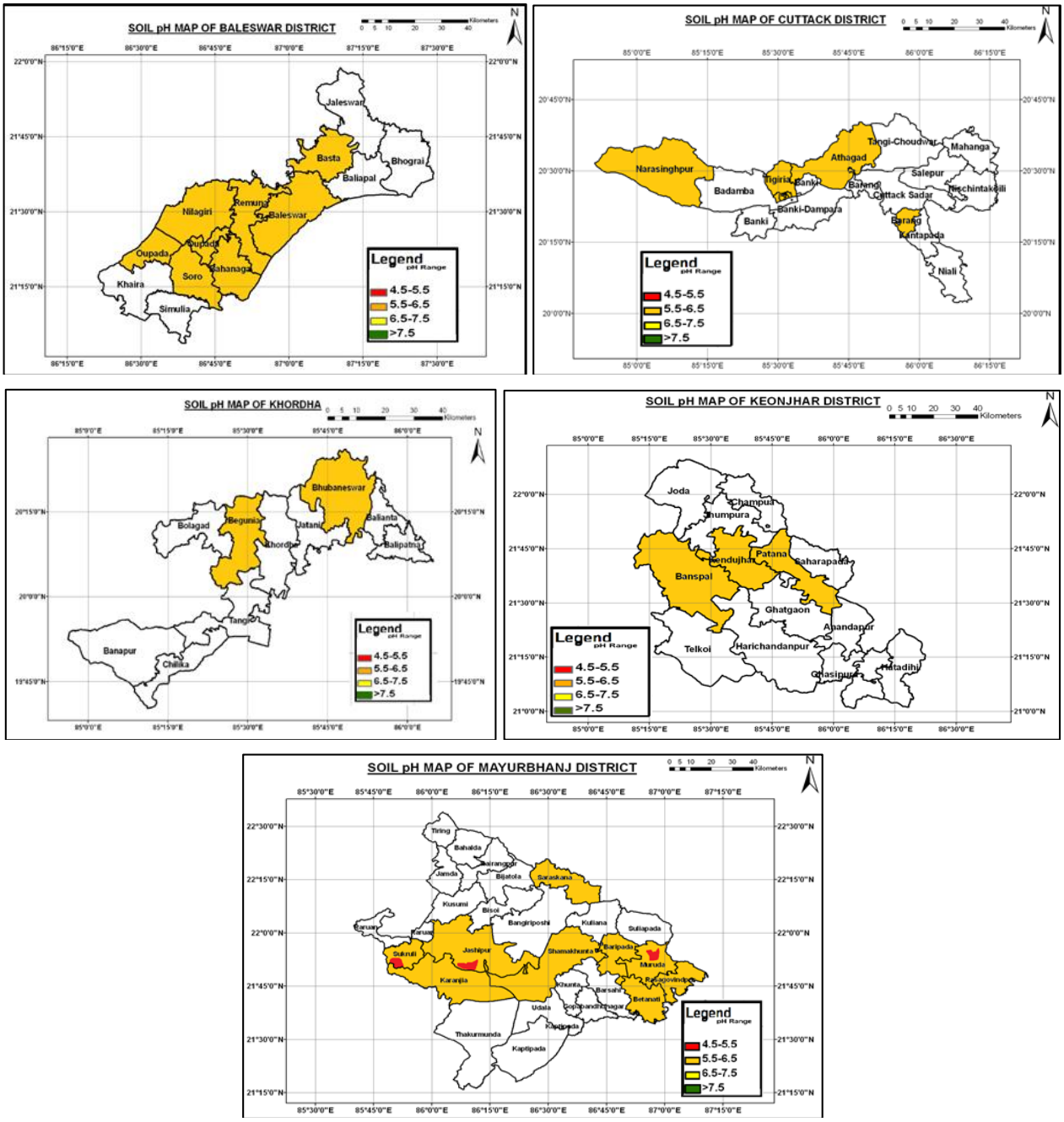
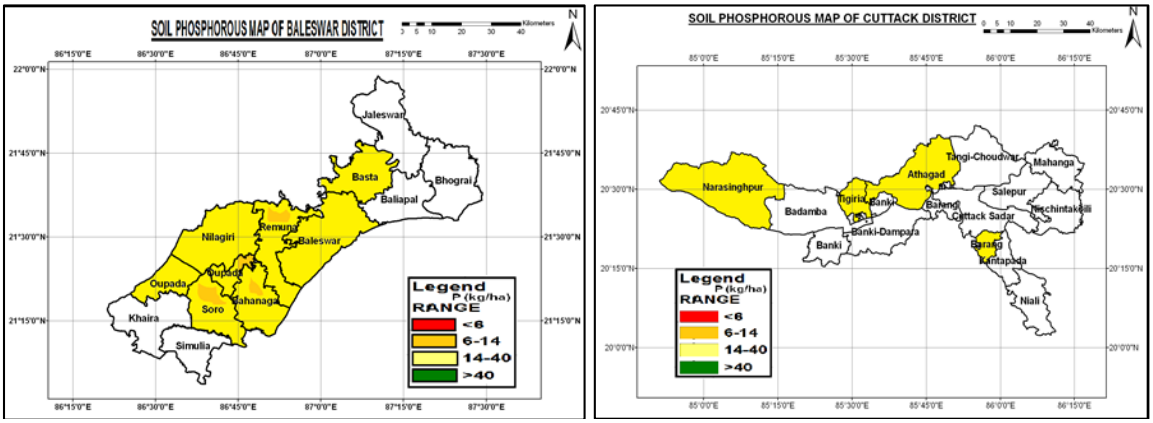


Fig 2: GPS based soil (pH) maps of five districts of Odisha



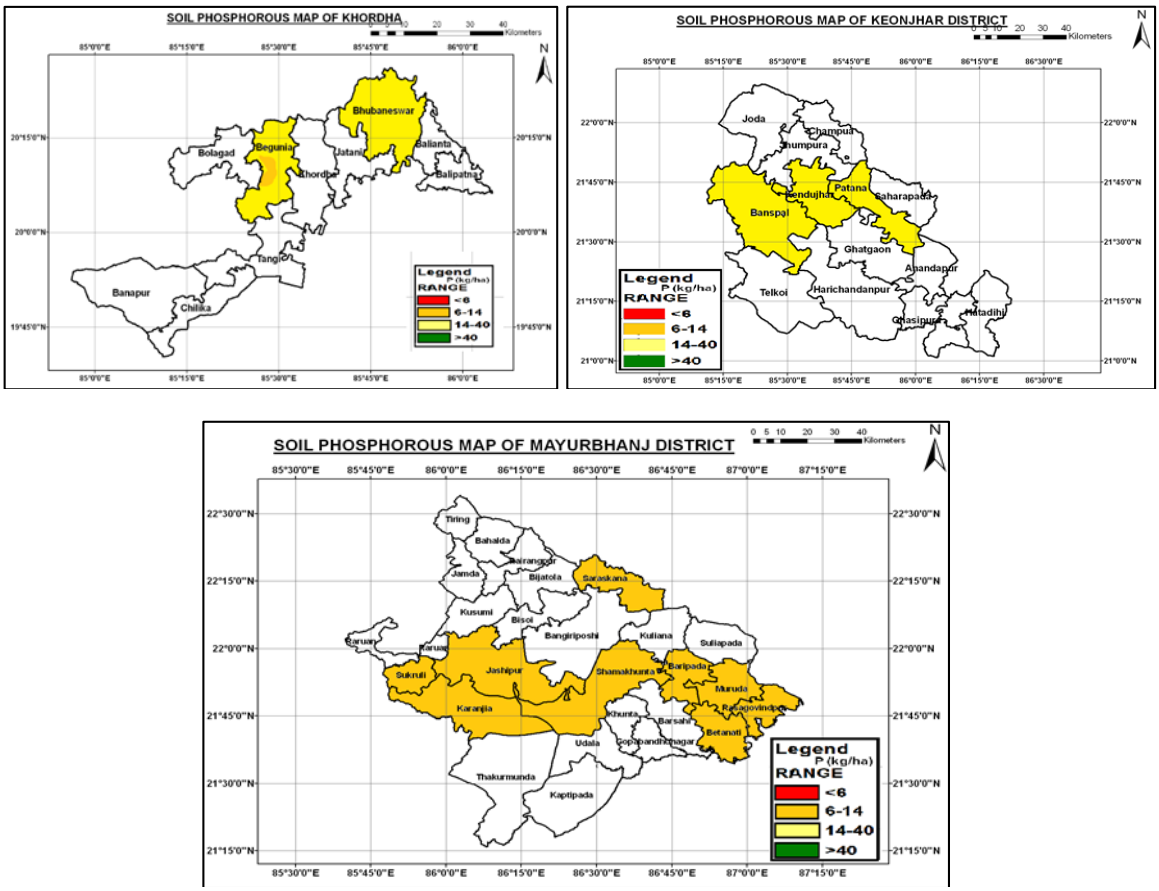


Fig 3: GPS based soil (av. P) maps of five districts of Odisha

Microbial properties of soils from different villages

Soil microbial properties of all the samples (106 nos.) were analyzed for population of heterotrophic bacteria, actinomycetes and phosphorous solubilizing bacteria (PSB) (Figure 4) and microbial biomass carbon. Samples from Balasore district showed highest no. of total heterotrophic bacteria (285 X 10^4 CFU g^{-1} soil) in soil collected from village Sarupala while the lowest (68 X 10^4 CFU g^{-1} soil) was from Gobindapur. Highest population of actinomycetes (278 X 10^4 CFU g^{-1} soil) and PSB (190 X 10^3 CFU g^{-1} soil) were recorded from villages Sampur and Singla respectively. The village Alasuaa recorded lowest population of actinomycetes (95 X 10^4 CFU g^{-1} soil) and PSB (35 X 10^3 CFU g^{-1} soil). Microbial biomass carbon of soil samples from Balasore ranged from 150.46 00 µg C g^{-1} soil (village - Krushnapur) to 386.00 µg C g^{-1} soil (village - Gangapur) (data not shown in table).

Soils collected from Cuttack district recorded highest population of total heterotrophic bacteria (295 X 10^4 CFU g^{-1} soil) in the sample from village Barahmpur while lowest (84 X 10^4 CFU g^{-1} soil) was from village Gadisahi. The soil sample from village Maragotha recorded highest population of actinomycetes (275 X 10^4 CFU g^{-1} soil) as well as PSB (173 X 10^3 CFU g^{-1} soil). Lowest population of actinomycetes (102 X 10^4 CFU g^{-1} soil), PSB (49 X 10^3 CFU g^{-1} soil) as well as microbial biomass carbon (69.00 µg C g^{-1} soil) were obtained with sample from village Achalakota. Highest soil microbial biomass carbon (365.89 µg C g^{-1} soil) was recorded from soils of village Nuabandha.

Population of total heterotrophic bacteria in Khordha district varied from 86 X 10^4 (village - Routpada) to 189 X 10^4 CFU g^{-1} soil (village - Khamangasasan). Soil collected from Routpada village exhibited lowest PSB population (40 X 10^3 CFU g^{-1} soil).

Among the soil samples collected from Keonjhar district, soil from the village Sarasaposi recorded highest population of heterotrophic bacteria (190 X 10^4 CFU g^{-1} soil) as well MBC (278.52 µg C g^{-1} soil). Soil collected from Bauripada accounted for highest population of actinomycetes (280 X 10^4 CFU g^{-1} soil) as well PSB (174 X 10^3 CFU g^{-1} soil).

Rhizospheric soils from Mayurbhanj district showed highest bacteria population (59 X 10^4 CFU g^{-1} soil) in soil from village Musamari and the lowest (216 X 10^4 CFU g^{-1} soil) was from Mohaniganj. Soil from Angarpada recorded lowest (33 X 10^3 CFU g^{-1} soil) population of PSB while soil from Rautara village recorded highest (180 X 10^3 CFU g^{-1} soil) PSB population.

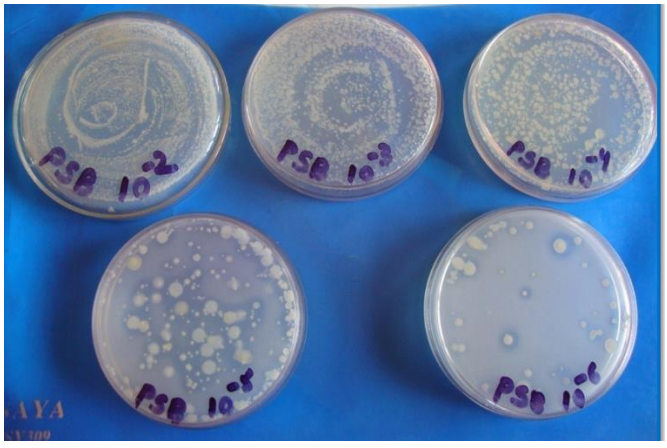


Fig 4: Enumeration of PSB population by serial dilution method

PSB isolates from soils of different villages

A total of two hundred eight (208) nos. of PSB isolates were screened from the selected villages of five districts. Out of

208, highest no. of PSB i.e. sixty five (65) isolates were isolated from Mayurbhanj district followed by fifty two (52) from Balasore district. Cuttack, Khordha and Keonjhar district accounted for 36, 26 and 29 PSB isolates respectively.

PSB isolates with P solubilizing efficiencies

All together we have isolated fifty two (52) isolates producing halo zones on NBRIP Agar Medium with diameter ranging between 10 to 31 mm and phosphorus (P) solubilizing efficiency 66.67 to 416.67 % after 48 h of incubation (data not shown in table). Thirty six (36) PSB isolates from Cuttack district exhibited with halozone diameter ranging 9 to 31 mm at 48 h of incubation. The P solubilization efficiencies of 26 isolates from Khordha ranged between 66.67 to 250.00% while the range varied between 66.67 to 233.33% in case of Keonjhar and 66.67 to 333.33% in case of Mayurbhanj.

Out of 208, only forty five (45) PSB isolates were selected on the basis of higher P solubilization efficiency (PE) i.e. ≥ 180.00% on NBRIP Agar plates after 48 h of incubation. Balasore district accounted for five (5) PSB isolates with more than 180.00 % PE. Among these five BLS18 recorded highest clear zone (31 mm) and PE (416.67 %) followed by BLS09 (18 mm, 200.00 %). The rest three (BLS10, BLS38 and BLS48) showed smaller halo zones (17 mm) and PE (183.33 %). Nine (9) PSB isolates from Cuttack district exhibited P solubilization zones ranging 17 – 31 mm on NBRIP agar medium. Maximum (416.67 %) PE was observed with the isolate CTC12 while the isolates viz; CTC13, CTC30 and CTC33 showed minimum (183.33 %). Eight (8) isolates were selected from Khordha district. Ten (10) isolates from Keonjhar and thirteen (13) from Mayurbhanj districts were selected and among them KJR03 (233.33 %) and K1 (333.33 %) respectively from Keonjhar and Mayurbhanj districts showed highest PE.

Soluble P recovery of 45 PSB isolates with different inorganic P sources

P solubilizing efficiencies and the soluble P recoveries from different inorganic phosphates [$Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$ and $Fe_3(PO_4)_2$] by the 45 PSB isolates at 48 and 72 h of incubation were characterized (Table 1). Isolates from all the five districts exhibited higher PE with $Ca_3(PO_4)_2$ followed by $FePO_4$. Among the isolates of Balasore district BLS18 recovered highest soluble P with $Ca_3(PO_4)_2$ (110.40 mg l^{-1}), $AlPO_4$ (5.29 mg l^{-1}), $FePO_4$ (34.10 mg l^{-1}) and $Fe_3(PO_4)_2$ (5.29 mg l^{-1}) in mediums at 48 h of incubation. The soluble P recovery of BLS18 increased after 72 h of incubation as compared to other isolates from Balasore district. BLS18 was followed by BLS09, which solubilized the 89.00 mg l^{-1} phosphorous in the medium supplemented with $Ca_3(PO_4)_2$ followed by 23.40 mg l^{-1} in the medium with iron (III) phosphate as inorganic phosphate at 48 h. Lowest soluble P was recovered from aluminium phosphate and iron (II) phosphate supplemented medium i.e. 3.25 and 3.95 mg l^{-1} respectively at 48 h. All other isolates recovered low soluble P compared to BLS18 and BLS09. Moreover, all the five isolates from Balasore district showed an increasing trend in soluble P recovery after 72 h of incubation. Noticeably, BLS18 showed highest increase in the soluble P recovery compared to other isolates of Balasore district.

Out of the nine (9) isolates from Cuttack district, CTC12 exhibited highest recovery of soluble P with $Ca_3(PO_4)_2$ (127.25 mg l^{-1}), $AlPO_4$ (5.29 mg l^{-1}), $FePO_4$ (69.25 mg l^{-1}) and $Fe_3(PO_4)_2$ (18.00 mg l^{-1}) as unavailable P sources at 48 h followed by isolates CTC01 and CTC33 for the medium supplemented with $AlPO_4$ (4.35 mg l^{-1}) and CTC13 for the medium with $FePO_4$ (32.50 mg l^{-1}) and $Fe_3(PO_4)_2$ (5.29 mg l^{-1}).

Among the eight (8) isolates from Khordha district KHD08 solubilized highest amount of P with all the given inorganic phosphates in NBRIP medium. KHD08 recorded highest soluble P with $Ca_3(PO_4)_2$ (209.25 mg l^{-1}), $AlPO_4$ (20.05 mg l^{-1}), $FePO_4$ (45.83 mg l^{-1}) and $Fe_3(PO_4)_2$ (7.15 mg l^{-1}) compared to other isolates at 72 h of incubation.

Isolate KJR03 from Keonjhar district recovered highest soluble P with all the inorganic phosphates. KJR03 recovered highest (3.75 mg l^{-1}) soluble P with $AlPO_4$ closely followed by KJR14 (3.60 mg l^{-1}) at 48 h. KJR03 also recovered highest soluble P with $FePO_4$ (25.55mg l^{-1}) and $Fe_3(PO_4)_2$ (7.80 mg l^{-1}) and was closely followed by KJR06.

Among the isolates from Mayurbhanj district, K1 found superior in solubilizing P with $Ca_3(PO_4)_2$ (102.80 mg l^{-1}), $AlPO_4$ (2.35 mg l^{-1}), $FePO_4$ (37.25 mg l^{-1}) and $Fe_3(PO_4)_2$ (7.07 mg l^{-1}) in mediums at both 48 h of incubation. After 72 h all the isolates showed an increasing trend but K1 exhibited highest soluble P.

pH of the cultured supernatant of 45 PSB isolates with different inorganic P sources

The pH of all the cultured NBRIP broths with $Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$ and $Fe_3(PO_4)_2$ at 48 and 72 h of incubation were found below 5.30 (Table 2). Five isolates (BLS18, CTC12, KHD08, KJR03 and K1) from the five districts lowered the pH of the cultured supernatant appreciably compared to rest other isolates at both 48 h and 72 h incubation.

Selection of one efficient P- solubilizing bacteria from each location (district)

Basing on the soluble P recovery by the 45 PSB isolates at 48 and 72 h of incubation (Table 3) five (5) P– solubilizing bacteria one from each district viz; BLS18 (Sarupala, Balasore), CTC12 (Echhapur, Cuttack), KHD08 (Balarampur, Khordha), KJR03 (Rangadihi, Keonjhar) and K1 (Chitrada, Mayurbhanj) were chosen (Table 1) for further screening and characterization of their P solubilizing efficiency, biocontrol and crop growth promotion effects. BLS18 and CTC12 were gram positive rods and the rest three were found to be gram negative rods.

Discussion

Soil acidity is one of the major constraints for sustainable agriculture. It triggers an essential nutritional problem i.e. phosphorus (P) fixation in soil resulting in unavailability of P and ultimately affecting crop uptake (Bolan *et al*., 2003) [4]. Acidic soils are characteristically inefficient in providing optimum P nutrition to plants as the inorganic P most often complexes with Al and Fe and very less with Ca. P being the vital macronutrient second only to nitrogen (N) when becomes unavailable for uptake results in hazardous growth effect of plants. P occurs in all basic forms of life i.e. DNA, RNA and ATP and hence extremely important for all metabolic activities. Thus, P uptake can be directly correlated to plant growth and yield.

In Odisha as the severity of soil acidity is more, thus it seems a bigger problem for the farming community. For maximizing the yields P fertilizers can be added to soil but the negative aspects here are these fertilizers are costly and also if added to soil the P from the fertilizer again goes for fixation.

Thus, the need is to establish the P-solubilizing bacteria which could efficiently solubilize inorganic P particularly from the

complexes of aluminium and iron. These efficient PSB strains could possibly be further exploited as biocontrol agents against soil borne fungal pathogens of groundnut. Though the concept of biofertilizers is very old, but still the present investigation tried to establish the native bacterial strains from acid soils of Odisha as phosphate solubilizing bacteria and studied the efficiency of strains in solubilizing inorganic phosphates.

In the present context, we have targeted five (5) districts i.e. Balasore (extent of soil acidity- 41.5 %), Cuttack (96.5 %), Khordha (82.3 %), Keonjhar (30.9 %) and Mayurbhanj (81.0 %). A total of 250 nos. of GPS based rhizospheric soil samples collected from different villages and blocks of these districts of Odisha were subjected for analysis of soil reaction (pH). Out of which 106 nos. of soil samples with pH ≤ 5.50 were selected for enumeration of mineral or inorganic P solubilizing bacteria. The state receives broad range variations with respect to climate, geology, rainfall, land forms and vegetation resulting in different variety of soil forms (Panda, 2009) [21].

The target soils mostly come under the sub groups viz; Typic Ustorthents, Typic Tropaquepts, Vertic Tropaquepts, Typic Haplustalfs, Typic Ustochrepts, Typic Ustropepts, Vertic Ustochrepts, Typic Haplaquepts, Ultic Paleustalfs, Rhodic Paleustalfs, Aeric Ochraqualfs, Aeric Tropaquepts and Typic Rhodustalfs. Red soils (Haplustalfs, Rhodustalfs and Ustorthents) occupy a sizable area (7.14 M ha) in the state and are mildly acidic in nature. Mixed Red and yellow soils (Haplustalfs, Paleaustalfs, Ustochrepts) constitute 5.5 M ha and are moderately acidic. Laterite soils (Hapustalfs, Plinthustalfs, Orchaqualfs) constitute 0.70 M ha and are moderately to strongly acidic. Coastal saline soils (Haplaquepts and Haloquents) constitute 0.25 M ha and some of these are acidic. Brown forest soils (Haplustalfs, Ustochrepts and Rhodustalfs) constituting 0.17 M ha are acidic (Panda, 2009) [21].

The available phosphorous content of all the collected samples ranged from very low to medium depending directly on soil reaction (pH). Most of the red and laterite soils of Odisha reported to be low in Brays' available P (1.3 – 5.9 mg kg^{-1}) while maintaining a higher total P (Panda and Mishra, 1969) [22]. Bolan *et al*. (1999) [5] reported that lower the pH of soil higher is the anion exchange capacity (AEC), ultimately increasing the fixation of P. In lower pH soils, higher concentrations of Fe and Al are prevalent making easy adsorption or fixation of P (Bolan *et al*., 2003) [4].

Soil acidity causes harmful effects to both plants and soil organisms (Robson and Abbott, 1989; Runge and Rode, 1991) [25, 26]. In excessively acid soils (pH- 4.0) many plants do not grow well while activities of soil micro flora are mostly reduced, resulting in the inhibition of beneficial microbes in root surrounding soils. The concentrations of Al and Mn become toxic to plant growth. Phosphorus (P) becomes insoluble or unavailable by getting fixed to more with $AlPO_4$, $FePO_4$ and $Fe_3(PO_4)_2$ and less with $Ca_3(PO_4)_2$ (Bashan *et al*., 2013) [3]. This is a major drawback for optimum crop productivity and soil health particularly in acid soils.

The management practices for acid soils include liming. Through it the acidity of soils can be neutralized and the hazardous problems associated with soil acidification can be overcome. Liming enhances the physical, chemical and biological properties of soil through its direct effect on the amelioration of soil acidity and its indirect effect on the mobilization of plant nutrients (P, K and S). Most plants grow well at a pH range of 5.5–6.5 and liming can maintain the pH at this range (Bolan *et al*., 2003) [4]. But this practice has to be done during every cropping season and again application of liming material can maintain the pH of the soil up to a certain period, but not throughout the crop growth period (Pradhan *et al*., 2013) [24].

Alternatively, efficient native strains of P- solubilizing bacteria can be characterized for utilization as biofertilizer and their application in crop field could help to manipulate the available phosphorous in crop rhizosphere from sparingly or insoluble sources of inorganic P. According to Zou *et al*. (1992) [33] only 0.1 % of the total P exists in a soluble form, which is available for plant uptake irrespective of the soil type.

Thus in the present context, we have analyzed all the 106 nos. of soil samples for enumeration of total heterotrophic bacteria, actinomycetes, P solubilizing bacteria and microbial biomass carbon. The native P solubilizing bacteria from each village of all the districts were catalogued and their inorganic P solubilization efficiency on NBRIP agar plates were calculated from the halo or clear zones produced by them.

A total of two hundred eight (208) PSB isolates were screened in which Mayurbhanj district ranked the top in the no. of PSB isolates followed by Balasore and Cuttack district. Basing on their P solubilization efficiency (≥180.00%) on NBRIP agar medium with tricalcium phosphate as the P source (Nautiyal, 1999) [19], they are again screened to forty five (45) numbers.

However, NBRIP (National Botanical Research Institute Phosphate) growth medium with tricalcium phosphate as the insoluble P source should not be considered as the sole selector for isolation of efficient P- solubilizers (Bashan *et al*., 2013) [3]. As here is the case of acid soils, which predominantly contains $AlPO_4$, $FePO_4$ and $Fe_3(PO_4)_2$ (Bashan *et al*., 2013; Adhya *et al*., 2015) [3, 1] as the inorganic phosphate complexes. These P compounds are stable minerals with very low solubility which directly hinder the P uptake by plants. P in the forms of orthophosphates ($H_2PO_4^-$ and HPO_4^{-2}) can be taken up by the plants (Tinker, 1980) [30]. Therefore, these isolates were further characterized with different mineral phosphate sources viz; Ca-P, Al-P and Fe-P (II and III) in NBRIP broth mediums.

The soluble P recovery by all the isolates with different P sources followed the order $Ca_3(PO_4)_2$ > $FePO_4$ > $AlPO_4$ > $Fe_3(PO_4)_2$. Further, there was an increasing trend in the soluble P recovery with increase in incubation period. Thus, after 72 h incubation more phosphorus has been recovered compared to 48 h by all the 45 PSB isolates irrespective of the P sources supplied. Chung *et al*. (2005) [7] also reported 13 best isolates based on the solubilization of insoluble phosphates ($Ca_3(PO_4)_2$, $FePO_4$ and $AlPO_4$) in liquid culture. However, the cultured supernatant when subjected to analysis of pH at 48 and 72 h, all the culture mediums showed a decrease in pH i.e. below 5.30, which implied direct linkage of P- solubilization with reaction (pH) of the cultured broth. Higher the soluble P, lower is the pH of the medium. One of the best understood mechanisms of P solubilization is the secretion of various low molecular weight organic acids viz; succinic, oxalic, malic, propionic, gluconic, 2-ketogluconic, citric, acetic, isovaleric, heptanoic, caproic, formic, n-butyric, oxalic, methylmalonic acids (Kpomblekou and Tabatabai, 1994; Chen *et al*., 2006; Panhwar *et al*., 2012) [16, 6, 23]. Decrease in pH of the medium at 48 and 72 h of incubation indicate production of organic acids resulting in mineral P solubilization (Zeng *et al*., 2017) [32].

As stated in the objective of the current investigation, to maintain nativity of the strain, we have selected one isolate

from each district with highest P solubilization potency and optimum P recovery with the given inorganic P sources. The isolates were BLS18 (Sarupala, Balasore), CTC12 (Echhapur, Cuttack), KHD08 (Balarampur, Khordha), KJR03 (Rangadihi, Keonjhar) and K1 (Chitrada, Mayurbhanj). BLS18 and CTC12 were gram positive rods and the rest three were gram negative rods. Earlier workers have stated that, variety of microflora either gram positive or negative could efficiently solubilized the inorganic phosphorus from its unavailable sources (Goldstein, 2007; Jorquera *et al*., 2008; Guiñazu *et al*., 2010) [10, 15, 11].

Table 1: Soluble phosphorous recovery by the bacterial isolates with different inorganic P sources

	Soluble P (mg l^{-1})							
Isolate Codes	**48 h incubation**				**72 h incubation**			
P sources	$Ca_3(PO_4)_2$	$AlPO_4$	$FePO_4$	$Fe_3(PO_4)_2$	$Ca_3(PO_4)_2$	$AlPO_4$	$FePO_4$	$Fe_3(PO_4)_2$
District- Balasore								
BLS09	89.00	3.25	23.40	3.95	105.00	3.85	30.00	6.65
BLS10	67.90	2.75	21.30	2.30	80.00	2.90	30.00	3.85
BLS18	110.40	5.29	34.10	5.29	185.00	6.50	84.00	17.50
BLS38	82.008	2.75	16.05	4.75	102.50	4.00	24.45	6.30
BLS48	57.50	1.50	3.40	2.20	69.40	3.25	4.40	2.85
District- Cuttack								
CTC01	82.50	4.35	20.80	5.00	89.00	5.00	21.10	5.25
CTC02	101.35	3.75	25.40	2.75	115.35	4.40	25.40	4.35
CTC11	82.70	2.90	20.55	2.95	94.50	3.20	20.95	4.20
CTC12	127.25	5.29	69.25	18.00	327.34	18.80	39.20	8.30
CTC13	57.40	2.15	32.50	5.29	71.60	3.25	35.00	6.05
CTC19	71.30	2.50	26.40	3.40	82.80	3.40	26.95	4.75
CTC29	87.50	5.00	15.30	4.45	117.20	5.00	15.00	3.35
CTC30	81.70	2.80	17.90	4.75	88.70	3.50	19.75	5.00
CTC33	58.30	4.35	19.65	3.85	60.00	3.25	19.95	4.25
District- Khordha								
KHD03	60.30	2.90	39.45	3.90	72.70	3.25	40.00	6.00
KHD08	115.50	8.25	45.83	10.35	209.25	20.05	45.83	7.15
KHD13	109.45	4.35	39.50	1.50	120.00	6.10	39.25	3.20
KHD14	71.90	2.95	25.35	3.30	83.50	4.25	29.50	3.90
KHD17	106.30	4.65	17.85	3.90	153.00	3.45	19.00	4.00
KHD18	98.80	5.00	25.30	1.60	119.00	7.35	29.00	2.70
KHD19	105.00	2.80	24.50	4.45	109.50	4.50	26.15	2.40
KHD25	94.45	2.95	19.65	4.00	95.00	3.90	22.80	4.10
District- Keonjhar								
KJR01	84.30	2.75	20.10	3.00	85.00	4.35	30.00	3.70
KJR02	82.80	2.30	21.15	5.75	87.50	5.00	20.00	10.00
KJR03	116.21	3.75	25.55	7.80	156.85	9.50	42.50	21.00
KJR05	69.40	3.08	22.00	2.40	140.00	4.85	29.50	3.40
KJR06	89.30	2.00	24.25	6.00	138.25	8.25	26.60	5.25
KJR07	87.80	2.25	20.00	4.40	120.45	8.50	28.30	7.00
KJR08	54.00	2.20	20.00	5.25	88.50	3.85	35.00	5.30
KJR09	62.35	2.15	17.15	4.25	95.00	7.50	40.00	4.50
KJR14	60.00	3.60	10.00	2.90	70.00	4.75	14.80	3.40
KJR28	60.30	2.50	16.50	2.90	75.30	8.40	34.00	3.25
District- Mayurbhanj								
K1	102.80	2.35	37.25	7.07	201.75	7.15	47.15	13.30
K9	97.80	1.90	31.70	4.45	145.00	2.00	35.00	4.80
K10	82.20	1.30	27.95	4.85	97.00	2.50	30.00	7.75
K16	73.25	0.50	28.95	4.95	84.40	1.00	30.00	6.00
K23	96.80	1.05	24.50	4.85	184.50	2.85	29.50	7.15
K31	100.10	0.55	25.00	1.90	160.55	2.05	26.60	2.60
K33	94.50	1.20	22.75	2.80	138.25	1.50	40.00	6.25
K35	98.10	1.50	20.30	5.15	189.00	5.00	32.25	8.30
K47	74.50	2.20	23.50	4.65	82.70	3.20	24.00	4.95
K48	55.00	0.90	27.00	2.90	62.50	2.35	34.00	4.30
K54	63.00	1.95	24.50	3.10	88.40	3.85	26.15	4.75
K57	89.45	1.75	19.80	4.65	98.25	4.90	23.00	6.10
K64	78.15	2.00	26.60	3.35	87.00	3.90	30.40	3.75

Table 2: pH of the cultured supernatant as influenced by different inorganic P sources

	pH of the cultured supernatant							
Isolate Codes	**48 h incubation**				**72 h incubation**			
P sources	$Ca_3(PO_4)_2$	$AlPO_4$	$FePO_4$	$Fe_3(PO_4)_2$	$Ca_3(PO_4)_2$	$AlPO_4$	$FePO_4$	$Fe_3(PO_4)_2$
District- Balasore								
BLS09	5.00	3.80	4.09	3.76	4.90	3.80	4.00	3.55

BLS10	4.90	3.95	4.40	3.58	4.88	3.50	4.40	3.55
BLS18	4.89	3.78	4.23	3.45	4.73	3.53	3.93	3.41
BLS38	5.21	3.95	4.10	3.94	5.20	3.67	4.00	3.95
BLS48	5.07	3.50	3.90	3.47	5.03	3.40	3.87	3.44
District- Cuttack								
CTC01	4.22	3.90	4.00	3.86	4.12	3.90	3.97	3.84
CTC02	4.50	3.85	3.99	3.90	4.46	3.80	4.12	3.85
CTC11	5.00	3.78	4.08	3.98	4.87	3.66	4.00	3.65
CTC12	4.35	3.70	3.90	3.84	3.53	3.48	3.65	3.40
CTC13	5.18	3.79	3.95	4.05	4.05	3.40	3.55	3.79
CTC19	5.34	3.90	4.00	4.00	4.21	3.53	3.70	3.84
CTC29	5.00	3.91	4.15	3.99	4.95	3.80	3.76	3.40
CTC30	4.56	4.06	4.08	3.93	4.33	3.85	3.57	3.72
CTC33	4.40	3.70	4.03	3.86	4.15	3.66	4.00	3.80
District- Khordha								
KHD03	4.26	3.67	3.80	3.90	4.12	3.48	3.77	3.90
KHD08	4.53	3.68	4.11	3.92	4.46	3.66	3.70	3.86
KHD13	4.85	3.80	4.00	3.88	4.50	3.66	4.00	3.50
KHD14	5.20	3.70	3.50	3.79	3.53	3.48	3.65	3.72
KHD17	4.25	3.79	4.15	4.05	4.05	3.40	3.55	3.40
KHD18	5.14	3.75	3.80	4.00	4.21	3.64	3.57	3.80
KHD19	5.06	3.59	3.95	3.99	4.95	3.80	3.46	3.40
KHD25	4.96	3.90	3.95	3.93	4.33	3.90	3.90	3.68
District- Keonjhar								
KJR01	5.20	3.80	4.10	3.90	4.53	3.67	4.00	3.90
KJR02	4.06	3.79	4.14	3.55	4.05	3.60	4.27	3.64
KJR03	4.04	3.33	3.61	3.33	4.27	3.73	3.99	3.58
KJR05	5.00	3.80	3.80	3.59	4.21	3.44	3.88	3.96
KJR06	4.90	3.36	4.20	3.80	4.50	3.53	3.48	3.34
KJR07	4.96	3.90	3.69	3.39	4.80	3.90	3.80	3.66
KJR08	5.22	3.98	3.37	4.00	5.08	3.95	3.30	3.40
KJR09	4.08	3.74	4.00	3.99	4.10	3.68	3.53	3.70
KJR14	5.16	3.90	3.60	4.07	5.00	3.38	3.62	3.98
KJR28	5.02	3.85	3.55	4.00	4.74	3.51	3.55	3.90
District- Mayurbhanj								
K1	4.43	3.79	3.57	3.57	4.00	3.75	3.35	3.33
K9	4.04	3.57	3.60	3.80	4.00	3.57	3.52	3.93
K10	4.50	3.60	3.40	4.08	4.56	3.64	3.31	4.00
K16	4.11	3.40	3.70	4.00	4.10	3.40	3.62	3.66
K23	4.04	3.98	4.00	3.95	4.00	3.43	4.10	3.98
K31	4.18	3.66	3.96	4.06	4.20	3.60	3.90	4.11
K33	4.07	3.66	4.05	4.16	3.90	3.65	4.00	4.02
K35	5.22	3.90	3.76	3.97	4.62	3.90	3.70	3.95
K47	5.03	3.32	3.68	4.16	4.54	3.33	3.60	4.03
K48	4.98	3.98	3.73	3.96	5.08	3.95	3.70	3.48
K54	4.00	4.00	3.74	3.99	4.10	3.58	3.44	3.70
K57	5.20	3.60	3.60	4.07	5.00	3.33	3.62	4.00
K64	5.00	3.64	3.65	4.00	4.74	3.51	3.60	3.92

Table 3: Location details of the selected PSB strains

Districts	Blocks	Village	pH	Isolate Code	Diameter of clear zone at 48 h (mm)	Gram's reaction	Shape
Balasore	Nilagiri	Sarupala	5.39	BLS18	31.0	+ ve	rod
Cuttack	Athagarh	Echhapur	5.05	CTC12	31.0	+ ve	rod
Khordha	Begunia	Balarampur	5.37	KHD08	21.0	- ve	rod
Keonjhar	Bansapal	Rangadihi	5.38	KJR03	20.0	- ve	rod
Mayurbhanj	Muruda	Chitrada	4.92	K1	26.0	- ve	rod

Conclusion

In acid soils particularly, P forms are mainly fixed by aluminum and iron-free oxides and hydroxides which limit P availability in crops and increase toxicity of Al and Fe. Liming can be practiced to correct soil acidity and create suitable environment (pH 5.5–6.5) for the beneficial microflora to increase the aerobic N fixation, organic matter decomposition and nutrient mineralization, thereby releasing inorganic plant nutrients to soil solution. Chemical amelioration of acidic soil for increasing the phosphorus availability is highly cost effective. Hence, exploring the alternative approaches for retrieving phosphorus from problematic soil by use of efficient native P- solubilizers has become a necessity for sustainable approach of the agricultural system.

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