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ISOLATION OF P- SOLUBILIZING BACTERIA FROM ACID SOILS OF ODISHA WITH ABILITY TO SOLUBILIZE INORGANIC PHOSPHATES

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Abstract- Soils vary in reaction, which directly correlates with availability of one of the major plant nutrients i.e. Phosphorous. Acid soil infertility mainly associated with fixed phosphates of Al and Fe, restrict the soluble P for crop uptake. Besides liming, P- solubilizing bacteria with the capacity to dissociate these Al and Fe bound P is the next viable alternative for sustainable agriculture. GPS based twenty (20) nos. of soil samples (pH ≤ 5.50) from coastal district, Cuttack of Odisha were analyzed and in all 36 native PSB isolates were selected out of which nine (9) isolates with higher P solubilization efficiency were further screened in liquid NBRIP broth supplemented with inorganic phosphates of Calcium, Aluminium, Iron (III) and Iron (II). Identification of PSBs by using TCP usually produces many, but only few are true PSBs. Since soils greatly vary by pH, hence there is a need to identify native and potential isolate with ability to solubilize the phosphates of Al and Fe in acidic soils. Thus the present study aim to establish the P solubilizing efficiency of some PSB strains with inorganic P compounds of Ca, Al, Fe (III) and Fe (II). The native strain CTC12 was observed to have significantly maximum P solubilizing potential with all the inorganic phosphates. Based on the biochemical and molecular characterization (16s rDNA sequencing) isolate CTC12 was identified as *Bacillus amyloliquefaciens* strain CTC12 (NCBI accession no. KT633845).

Keywords- Acid soil, PSB, $AlPO_4$, $FePO_4$.

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Introduction

Soil acidity is a major constraint in quality crop growth and production. In India acid soil covers an area of around 49 million ha, out of which 25 million ha show pH less than 5.5 and 24 million ha between 5.5 and 6.5. These soils are often referred to as problematic soils, mainly due to the production constraints involving the deficiency of some major and micronutrients. Acid soil constitutes around 70 % of the total geographical area of Odisha. Out of 30 districts, 15 districts have soil acidity >70 per cent areas, 8 districts between 50-70 per cent and 7 districts < 50 per cent areas. Drastic weathering of parent material under hot humid climate and heavy precipitation (>1500 mm) during monsoon in the state are responsible for formation of acid soils which are predominately red and lateritic in origin [1].

Major problems associated with acid soils are severe toxicity of Fe, Al and Mn accompanied with unavailability of some vital nutrients (P, Ca and Mg) and low microbial activity leading to poor yield of crops. Mainly, P (second most important macronutrient of crop) forms are aluminum and iron-free oxides and hydroxides [2], limiting P availability to crops. Unlike N, P forms (both available and unavailable) existing in soil, can only be taken up by the plants in its available or soluble form. A major part of fertilized phosphorous incorporated in soil often gets into the immobile pool due to acidic soil pH [3].

Previous workers have reported that, negatively charged soluble phosphates react with positively charged clay, iron, and aluminum compounds in the soil. Through the process of phosphorus fixation, readily soluble forms of phosphates are converted to less available forms. Moreover in acid soils Fe and Al levels are high, which reacts with P forming stable compounds like variscite ($AlPO_4.2H_2O$), strengite ($FePO_4.2H_2O$) [4] and vivianite ($Fe_3(PO_4)_2.8H_2O$). These phosphatic forms are unsuitable for plant uptake. Hence, very often it is suggested to ameliorate these problematic acid soils with suitable liming material. The present investigation therefore focused on an alternative approach to manage not only the problematic acid soils but also simultaneously committed to contribute maximum P uptake. This piece of work also reports the isolation and screening of native strains of P- solubilizing bacteria from acid soil (pH≤ 5.50) of Odisha.

Materials and Methods

Collection of rhizospheric soil samples

GPS based rhizospheric soil (30 cm depth) samples were collected from Cuttack district of Odisha. The samples were air dried and screened through 60 mesh sieve for determination of soil physico-chemical parameters following standard methods [5].

Isolation and screening of PSB isolates

Rhizospheric soil samples were subjected to enumeration of P-solubilizing bacteria using National Botanical Research Institute's phosphate (NBRIP) growth medium [6] with insoluble tricalcium phosphate (TCP). Further each of the PSB isolates were spotted on NBRIP Agar plates and incubated at 30 ± 2°C for 48 h for estimating P solubilization efficiency. The diameter of the colony as well as the halo zone was measured and the P-solubilizing index (PSI) and P solubilization efficiency (PE %) were computed as below.

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

where Z = Halo zone diameter, C = Colony diameter

Screened PSBs were further examined for solubilization efficiency in NBRIP liquid

medium with inorganic phosphates of Calcium, Aluminium, Iron (III) and Iron (II). The broth cultures were incubated at 30 ± 2^0 C for 48 hr as well as 72 hr and then centrifuged at 10,000 rpm for 30 min. Following standard protocols, the soluble p fraction in the broths was determined and only the potent strain exhibiting maximum efficiency was continually incubated for eight (8) days to find out both soluble P content and P solubilization efficiency [5].

Biochemical characterization of the isolates

Morphological characteristics, *viz.* shape, size, motility and Gram's stain of the PSB isolates were examined under phase contrast light microscope (×100 objective). The electron micrograph of the isolates were generated using scanning electron microscope (model EVO MA 15 Carl Zeiss SMT, Germany). Various physiological and biochemical tests such as triple sugar iron , mannitol, motility, methyl red; voges-proskauer (acetoin production), citrate utilization (Simmon's Citrate Agar), anaerobic growth, amino acid decarboxylase, indole production, nitrate reduction, carbohydrate oxidation and fermentation and enzyme activities, i.e. urease, oxidase, catalase, cellulose, amylase, chitinase, lipase (Tributyrin), caseinase, Dnase, nitrate reductase, nitrite reductase, gelatinase were tested following standard methods (Bergey's manual) [7,8]. The isolates were further screened for antibiotic resistance by disc diffusion method on Mueller-Hinton agar plate [9].

Molecular characterization

The DNA was isolated PureLink Genomic DNA kit (Invitrogen). Amplicon was electrophoresed in a 1% Agarose gel and visualized under UV-VIS gel doc system. The 16S rDNA was PCR amplified using the forward primer (5'AGAAAGGAGGTGATCCAGCC3') and reverse primer (5'AGAGTTTGATCMT GGCTCAG3') at 94°C for 4 min, 94°C for 1 min, 58°C for 1 min, 72°C for 1.30 s for 30 cycles, and then 72°C for 8 min. The PCR reaction mixture consisted of template DNA (150 ng), enzyme: *Taq* polymerase (1.5 U/µl), 10 X *Taq* polymerase buffer (100 mM Tris (pH 9), 500 mM KCl ,15 mM $MgCl_2$, 0.1% gelatin), dNTP mix (10 mM), 10 µm each primers. The amplified full length products (1.4 kb) were sequenced using ABI 3130xl analyzer following Sangers dideoxy termination method. The sequences were BLAST at ncbi.nlm.nih.gov and the Phylogenetic tree was constructed after multiple sequence alignment in CLUSTALW software. The Phylogenetic tree was constructed after multiple sequence alignment using cluster algorithm [10]. The phylogenetic tree was built on the matrix of pair distances between sequences. In the boot strap a multiple alignment was resembled 100 times.

Isolation of genomic DNA, plasmid and cellular protein

The bacterial culture incubated overnight in nutrient broth at 150 rpm and 30 ± 0.1 ^{0}C were used for isolation of both genomic DNA and plasmid following the standard techniques [11,12]. Standard protocols were followed for extraction of cellular proteins [13]. The profiles were visualized as fluorescent bands in a UV transilluminator (312 nm) and photographed through a gel photo documentation system.

Statistical Analysis

The software R version 3.2.2 was employed for statistical analysis with Duncan's new multiple range test at 1% critical range using the package "agricolae". Three replicates were used for arriving at mean values.

Results

Collection of rhizospheric soil samples

Out of 35 soil samples collected from Cuttack district, twenty (20) samples (pH ranged between 4.67 to 5.50) were analyzed for chemical parameters *viz;* organic carbon, exchange acidity, exchangeable aluminium, available P and microbial properties *viz*; total bacterial count and microbial biomass carbon [Table-1]. The PSBs isolated from different locations are coded and presented [Table-1].

Isolation and screening of P- solubilizing isolates

Out of 36 P- solubilizing isolates that were enumerated only 9 exhibited phosphate solubilization zones ranging from 17 – 21 mm on NBRIP agar medium with TCP as inorganic P source [Table-2]. Maximum P solubilization index (PI) and efficiency (PE) was observed with the isolate CTC12 [Fig-12] and the minimums were CTC13, CTC30 and CTC33.

The nine (9) isolates were screened in NBRIP broths with inorganic phosphates [$Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$ and $Fe_3(PO_4)_2$] and incubated respectively for 48 and 72 hrs [Table-3]. Isolate CTC12 expressed maximum efficiency with all the inorganic phosphates at 48 and 72 hrs of incubation followed by isolates CTC01 and CTC33 for the medium supplemented with $AlPO_4$ and CTC13 for the medium with $FePO_4$ and $Fe_3(PO_4)_2$. Furthermore, negative correlations were also observed between the soluble P and pH of the cultured supernatant at 48 and 72 hrs of incubation [Fig-1-8]. The pH of the supernatant showed a sharp reduction ranging from 4.35 to 5.18, 3.70 to 4.06, 3.90 to 4.15, 3.84 to 4.05 and 3.53 to 5.02, 3.40 to 3.90, 3.55 to 4.12, 3.40 to 3.85 respectively at 48 and 72 hrs of incubation. The best performer among the nine isolates was screened and identified following biochemical and molecular techniques.

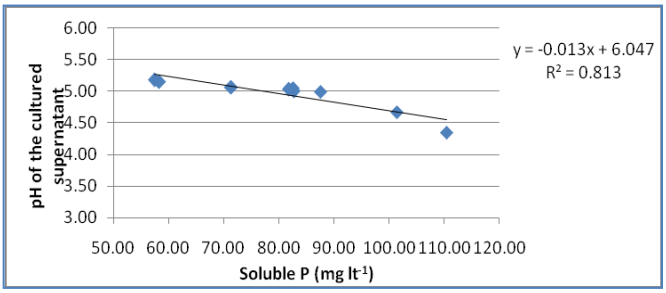

Fig-1 Correlation between available P and pH of NBRIP broth ($Ca_3(PO_4)_2$) over 48 h of incubation

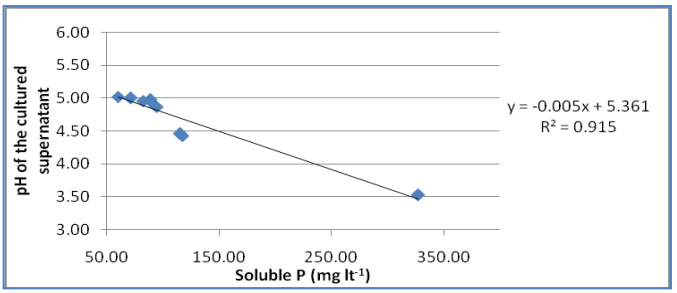

Fig-2 Correlation between available P and pH of NBRIP broth ($Ca_3(PO_4)_2$) over 72 h of incubation

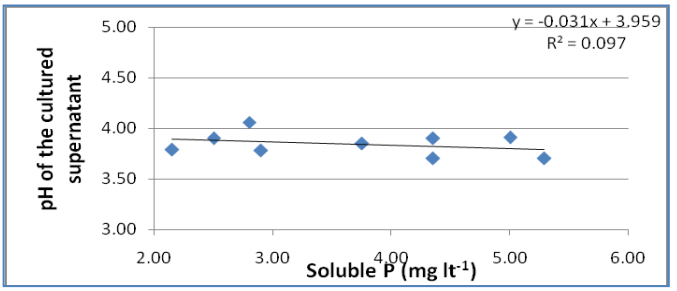

Fig-3 Correlation between available P and pH of NBRIP broth ($AlPO_4$) over 48 h of incubation

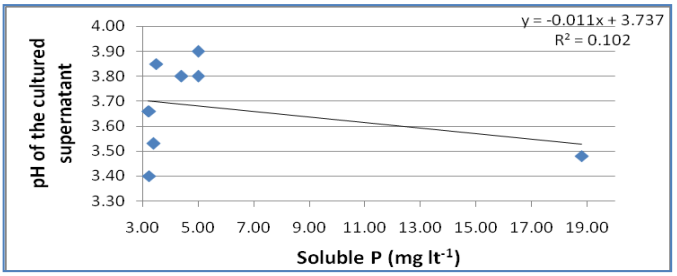

Fig-4 Correlation between available P and pH of NBRIP broth ($AlPO_4$) over 72 h of incubation

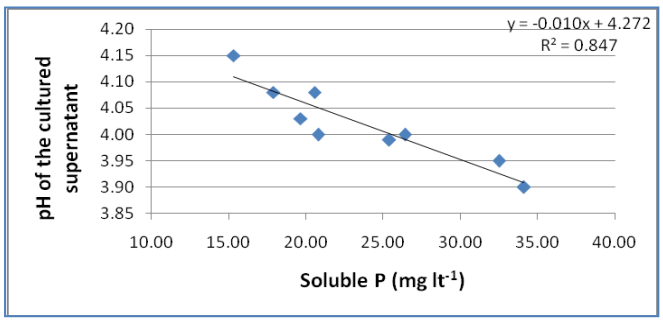

Fig-5 Correlation between available P and pH of NBRIP broth ($FePO_4$) over 48 h of incubation

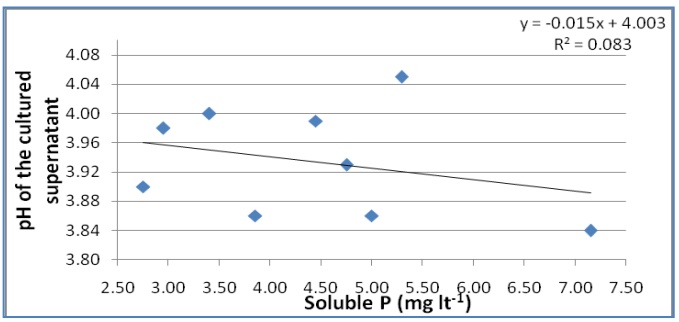

Fig-7 Correlation between available P and pH of NBRIP broth ($Fe_3(PO_4)_2$) over 48 h of incubation

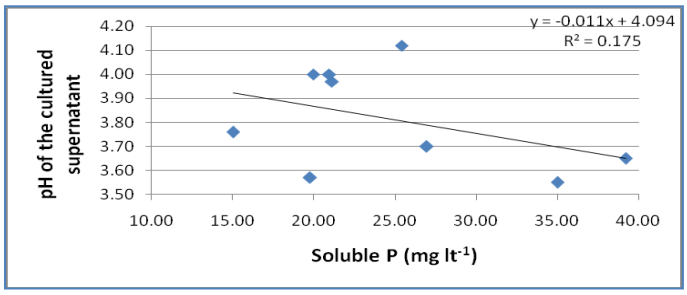

Fig-6 Correlation between available P and pH of NBRIP broth ($FePO_4$) over 72 h of incubation

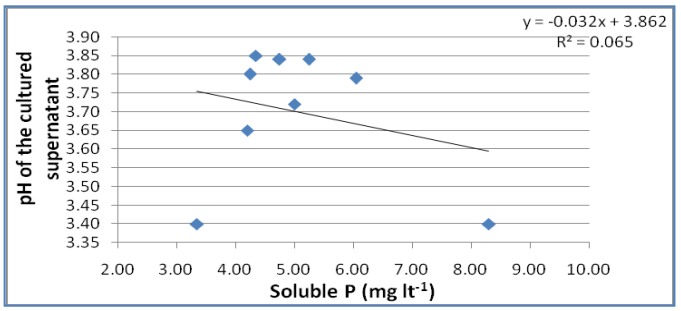

Fig-8 Correlation between available P and pH of NBRIP broth ($Fe_3(PO_4)_2$) over 72 h of incubation

Table-1 *Characteristics of the collected rhizospheric soil samples*

Sl. No.	GPS Location	pH	OC (%)	Ex. Acidity [cmol(p+)kg^{-1}]	Ex. Al^{3+} [cmol(p+)kg^{-1}]	Available P (kg ha^{-1})	Total heterotrophic bacteria (CFU x 10^4 g^{-1}soil)	MBC (µg C g^{-1} soil)	PSB Isolates
1	N20^{0}25.477' E85^{0}49.884'	5.47	0.55	0.61	0.10	16.10	256.00	316.46	CTC01
2	N20^{0}27.135' E85^{0}47.441'	4.67	0.50	0.95	0.54	10.30	84.00	157.75	CTC02 CTC03
3	N20^{0}29.786' E85^{0}40.093'	4.89	0.46	0.92	0.50	9.43	120.00	189.46	CTC04 CTC05 CTC06
4	N20^{0}30.191' E85^{0}38.331'	4.95	0.46	0.90	0.52	9.26	148.00	145.47	CTC07 CTC08
5	N20^{0}30.899' E85^{0}36.700'	5.23	0.51	0.74	0.32	12.82	157.00	179.56	CTC09 CTC10
6	N20^{0}31.105' E85^{0}33.624'	5.05	0.40	0.78	0.46	16.50	95.00	260.53	CTC11 CTC12
7	N20^{0}29.083' E85^{0}34.719'	5.42	0.37	0.64	0.10	17.60	180.00	285.64	CTC13 CTC14
8	N20^{0}28.993' E85^{0}34.020'	5.38	0.38	0.60	0.14	17.52	226.00	320.50	CTC15 CTC16
9	N20^{0}29.035' E85^{0}34.564'	5.40	0.49	0.47	0.03	18.22	289.00	365.89	CTC17 CTC18
10	N20^{0}30.223' E85^{0}36.532'	5.50	050	0.44	0.00	18.36	250.00	321.30	CTC19 CTC20
11	N20^{0}31.910' E85^{0}30.880'	5.50	0.52	0.40	0.00	18.50	85.00	69.00	CTC21 CTC22
12	N20^{0}29.771' E85^{0}39.921'	5.49	0.56	0.40	0.00	18.63	148.00	135.60	CTC23
13	N20^{0}26.359' E85^{0}31.644'	5.17	0.40	0.70	0.26	17.56	159.00	185.75	CTC24 CTC25
14	N20^{0}25.792' E85^{0}29.073'	4.97	0.50	0.96	0.54	11.00	184.00	335.49	CTC26 CTC27
15	N20^{0}46.125' E85^{0}38.921'	5.44	0.53	0.47	0.12	17.30	160.00	237.67	CTC28
16	N20^{0}27.214' E85^{0}06.147'	5.36	0.50	0.52	0.16	18.93	286.00	252.68	CTC29 CTC30 CTC31
17	N20^{0}25.674' E85^{0}06.010'	5.23	0.56	0.64	0.21	17.40	295.00	250.45	CTC32
18	N20^{0}25.674' E85^{0}06.010'	5.06	0.36	0.82	0.43	16.20	284.00	210.50	CTC33
19	N20^{0}25.267' E85^{0}08.172'	4.83	0.40	0.82	0.46	10.50	169.00	135.00	CTC34
20	N20^{0}26.803' E85^{0}06.620'	5.31	0.50	0.46	0.15	15.60	215.00	157.30	CTC35 CTC36

Table-2 *PSI and PE (%) of PSB isolates*

PSB Isolates	Diameter (mm) of clearing zone 'Z'	Diameter (mm) of bacterial colony growth 'C'	P solubilizing Index (SI)	P solubilization efficiency PE (%)
CTC01	19	6	3.17	216.67
CTC02	18	6	3.00	200.00
CTC03	10	6	1.67	66.67
CTC04	10	6	1.67	66.67
CTC05	15	6	2.50	150.00
CTC06	10	6	1.67	66.67
CTC07	13	6	2.17	116.67
CTC08	12	6	2.00	100.00
CTC09	13	6	2.17	116.67
CTC10	15	6	2.50	150.00
CTC11	19	6	3.17	216.67
CTC12	21	6	3.50	250.00
CTC13	17	6	2.83	183.33
CTC14	16	6	2.67	166.67
CTC15	14	6	2.33	133.33
CTC16	11	6	1.83	83.33
CTC17	9	6	1.50	50.00
CTC18	11	6	1.83	83.33
CTC19	19	6	3.17	216.67
CTC20	12	6	2.00	100.00
CTC21	10	6	1.67	66.67
CTC22	10	6	1.67	66.67
CTC23	15	6	2.50	150.00
CTC24	10	6	1.67	66.67
CTC25	13	6	2.17	116.67
CTC26	12	6	2.00	100.00
CTC27	13	6	2.17	116.67
CTC28	15	6	2.50	150.00
CTC29	19	6	3.17	216.67
CTC30	17	6	2.83	183.33
CTC31	16	6	2.67	166.67
CTC32	14	6	2.33	133.33
CTC33	17	6	2.83	183.33
CTC34	16	6	2.67	166.67
CTC35	14	6	2.33	133.33
CTC36	11	6	1.83	83.33

Table-3 *P solubilization efficiency (PE %) in liquid NBRIP medium supplemented with inorganic phosphates*

Isolate codes	48 hrs incubation				72 hrs incubation			
	$Ca_3(PO_4)_2$	$AlPO_4$	$FePO_4$	$Fe_3(PO_4)_2$	$Ca_3(PO_4)_2$	$AlPO_4$	$FePO_4$	$Fe_3(PO_4)_2$
CTC01	8.25 ± 0.361ab	0.34 ± 0.012ab	2.02 ± 0.025abc	0.57 ± 0.038ab	8.90 ± 0.584b	0.39 ± 0.006b	2.05 ± 0.025abc	0.60 ± 0.012ab
CTC02	10.14 ± 0.070a	0.30 ± 0.015ab	2.47 ± 0.044abc	0.32 ± 0.031b	11.54 ± 0.693b	0.35 ± 0.006b	2.47 ± 0.025abc	0.50 ± 0.029b
CTC11	8.27 ± 0.506ab	0.23 ± 0.021ab	2.00 ± ab0.031c	0.34 ± 0.026b	9.45 ± 0.539b	0.25 ± 0.012b	2.03 ± 0.006abc	0.48 ± 0.031b
CTC12	11.04 ± 0.350a	0.42 ± 0.021a	3.31 ± 0.056a	0.82 ± 0.053a	32.73 ± 0.938a	1.48 ± 0.032a	3.81 ± 0.121a	0.95 ± 0.017a
CTC13	5.74 ± 0.444b	0.17 ± 0.015b	3.16 ± 0.021ab	0.61 ± 0.026ab	7.16 ± 0.050b	0.26 ± 0.006b	3.40 ± 0.042ab	0.70 ± 0.015ab
CTC19	7.13 ± 0.194ab	0.20 ± 0.012ab	2.56 ± 0.056abc	0.39 ± 0.021b	8.28 ± 0.130b	0.27 ± 0.010b	2.62 ± 0.066abc	0.55 ± 0.017ab
CTC29	8.75 ± 0.175ab	0.30 ± 0.017ab	1.49 ± 0.010c	0.51 ± 0.015ab	11.72 ± 0.203b	0.39 ± 0.032b	1.46 ± 0.015c	0.39 ± 0.006b
CTC30	8.17 ± 0.112ab	0.22 ± 0.010ab	1.74 ± 0.067bc	0.55 ± 0.012ab	8.83 ± 0.101b	0.28 ± 0.015b	1.92 ± 0.010bc	0.57 ± 0.035ab
CTC33	5.83 ± 0.476b	0.34 ± 0.015ab	1.91 ± 0.015abc	0.44 ± 0.021ab	6.00 ± 0.181b	0.26 ± 0.006b	1.94 ± 0.029bc	0.49 ± 0.010b

Tested by Duncan's Multiple Range Test with 1% critical range. Means represented by the same letter are not significantly different. Data given in above are average values of three replicates ± standard error of mean (SEM).

P solubilization efficiency of the PSB isolate CTC12 with different P sources

The PSB isolate CTC12 observed to solubilize unavailable P as tricalcium phosphate [$Ca_3(PO_4)_2$], aluminium phosphate [$AlPO_4$], ferric phosphate [$FePO_4$], ferrous phosphate [$Fe_3(PO_4)_2$] supplemented in the NBRIP broth medium [Fig-9] & [Table-4]. In the present study, the isolate incubated over 8 days recorded solubilization efficiency in the range of 11.04 to 46.78%, 0.42 to 2.37%, 3.31 to 6.33% and 0.61 to 2.10% when the broth was supplemented respectively with $Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$ and $Fe_3(PO_4)_2$. The pH of the supernatant showed the range 3.53 to 4.35, 3.48 to 3.84, 3.44 to 3.99 and 3.79 to 4.03 respectively with $Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$ and $Fe_3(PO_4)_2$ over 8 days of incubation.

Biochemical and molecular characterization of the efficient isolate

The PSB isolate CTC12 was a Gram positive, aerobic, spore forming, motile, rod shaped (2.185–2.275 µm X 717.2–832.6 nm) bacterium [Fig-11]. The isolate utilized glucose, sucrose and lactose as C source.

Table-4 *Reaction (pH) of the cultured supernatant till 8th days of incubation*

Incubation Period	pH of the cultured supernatant			
	$Ca_3(PO_4)_2$	$AlPO_4$	$FePO_4$	$Fe_3(PO_4)_2$
48 hrs	4.35	3.70	3.99	3.84
72 hrs	3.53	3.48	3.65	3.79
96 hrs	3.80	3.58	3.44	3.80
120 hrs	3.74	3.66	3.52	3.94
144 hrs	3.82	3.84	3.70	4.00
168 hrs	3.90	3.84	3.75	4.03
192 hrs	3.90	3.70	3.82	3.96

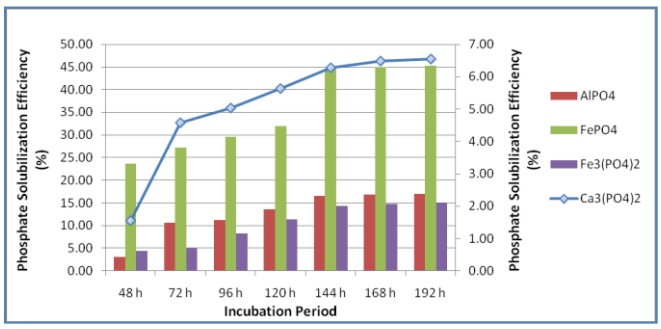

Fig-9 P Solubilization efficiency of the isolate CTC12

The isolate failed to utilize ornithine and lysine but was able to decarboxylate arginine. It showed growth on medium with 10% NaCl, lactose and glucose. The isolate was methyl red negative but voges–proskauer and indole positive. The PSB isolate was observed to be capable of utilizing dextrose, cellobiose, trehalose, fructose, raffinose, sorbitol, arabinose, mannitol and salicin as C source through both oxidative and fermentative pathways. The isolate was positive to urease, catalase, oxidase, amylase, caseinase, Dnase, nitrate reductase, nitrite reductase and gelatinase. Antibiotic screening showed that, CTC12 was sensitive to erythromycin, bacitracin, chloramphenicol, ciprofloxacin, tetracycline, vancomycin, neomycin, amikacin, streptomycin and resistant to amphotericin, penicillin G, polymyxin B. The 16s rDNA analysis and homology pattern suggested it to be *Bacillus amyloliquefaciens* strain CTC12 (NCBI accession no. KT633845). The Phylogram of the isolate was presented in [Fig-10].

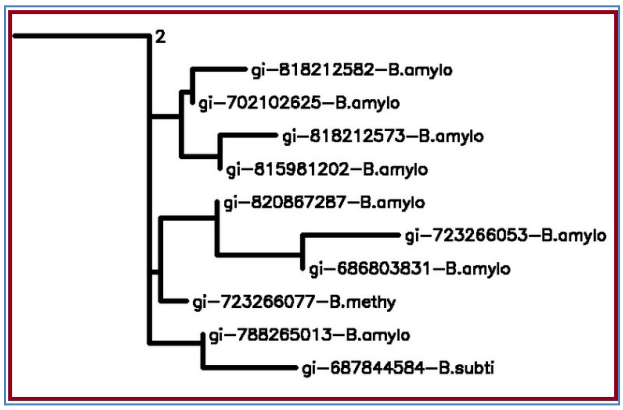

Fig-10 Phylogram based on 16S rDNA sequence of *Bacillus amyloliquefaciens* CTC 12

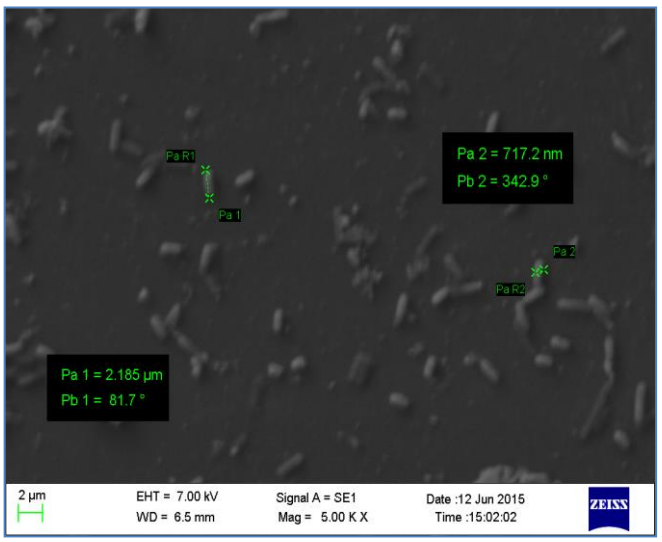

Fig-11 Electron micrograph of *Bacillus amyloliquefaciens* CTC 12

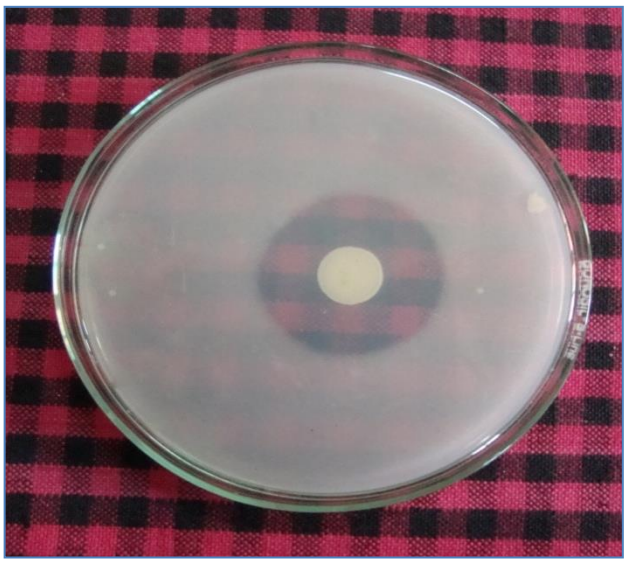

Fig-12 *Bacillus amyloliquefaciens* CTC 12 showing halozone in NBRIP Agar Medium

Molecular characterization of *Bacillus amyloliquefaciens* strain CTC12

The genomic DNA profile of the isolate exhibited 23.947 kbp molecular weight [Table-5] & [Fig-13]. Five (5) plasmids have been isolated ranging from 2.249-10.000 kbp. Protein profile of the isolate showed variations and the organism produced 18 bands ranging size class 12.864 to 756.944 kDa.

Table-5 *Molecular characterization (Genomic DNA, Plasmid and Protein profile) of the isolate of Bacillus amyloliquefaciens CTC 12*

Molecular weight (kbp) of genomic DNA	No. of plasmids	Molecular weight (kbp) of plasmids	Protein profile (kDa)
23.947	5	10.000, 6.771, 5.433, 2.776, 2.249	756.944
			664.109
			441.940
			325.276
			300.441
			162.111
			116.304
			93.812
			76.323
			60.240
			48.516
			43.728
			39.075
			24.106
			21.948
			18.488
			15.136
			12.864

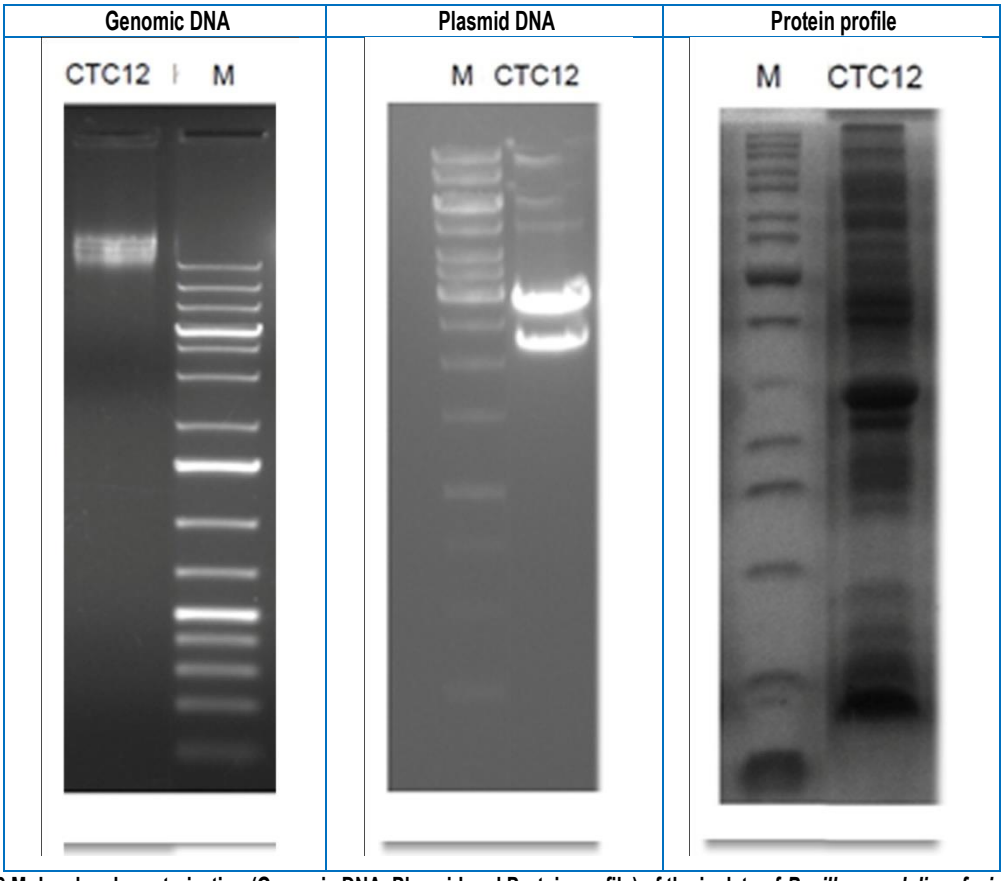

Fig-13 Molecular characterization (Genomic DNA, Plasmid and Protein profile) of the isolate of *Bacillus amyloliquefaciens* CTC 12

DISCUSSION

Odisha accounts for 0.70 mha lateritic soils spread over districts of Puri, Khordha, Nayagarh, Cuttack, Dhenkanal, Keonjhar, Mayurbhanja and Sambalpur. These soils being predominantly composed of hydrated oxides of iron and aluminium with minor amounts of manganese, titanium and quartz show moderately acidic to strongly acidic soil reaction ranging between 4.5 to 5.8. Therefore, one of the coastal districts Cuttack was targeted and altogether, 35 GPS based rhizospheric soil samples were collected and screened for soil reaction (pH). Out of 35 only 20 samples recorded pH ≤ 5.5 were characterized for further chemical analysis *viz*; available P, exch. acidity, exch. Al and organic carbon. The pH of the samples ranged between 4.67 to 5.50, exch. acidity (0.40 to 0.98 cmol(p+)kg^{-1}), exch. Al (0.02 to 0.70 cmol(p+)kg^{-1}), low to medium organic carbon (0.36 to 0.56 %) and low available P (9.32 to 14.23 kg ha^{-1}). Availability of both native and applied water soluble P are low because of high P fixation capacity of acid soils. Most of red and lateritic acid soils of Odisha are low in available P (Bray's 1P: 1.3 to 5.9 ppm) although their total P_2O_5 content is quite adequate ranging from 0.08 to 0.35 % [14]. High P fixation has been observed in lateritic soils of Odisha [15], Sikkim [16] and in Bihar [17].

P fixation is a major problem for quality crop growth. Hence, unless limed the soil cannot be utilized for higher crop yield. However, use of liming materials is not cost effective, since majority of the farmers in Odisha are economically backward. Hence, there has been always an effort to find a cost effective alternative approach, rather eco-friendly. The next best suitable alternative could be use of native strains of phosphorous solubilizing rhizobacteria from acid soils of Odisha with higher P solubilization efficiency. In the present investigation efforts were focused on identifying isolates exhibiting phosphate solubilization ability not only with $Ca_3(PO_4)_2$ but also with $AlPO_4$, $FePO_4$ and $Fe_3(PO_4)_2$. Acidic soils tend to contain higher quantities of acidic cations *viz*; aluminium (Al) and iron (Fe), which destined to react with the phosphatic anions leading to unavailability of the vital macro nutrient as well as toxicity of Al and Fe [4].

In the current study thirty six (36) PSB isolates were cultured, out of which nine (9) were selected on the basis of the bigger halozone on the NBRIP agar medium with TCP as the insoluble source of P [6]. Further, the soluble P produced by the organisms were quantified by inoculating them in the NBRIP liquid broth supplemented with TCP as the insoluble P source at both 48 h and 72 h of incubation. It appeared the solubilization of TCP increased at 72 h of incubation. Out of nine, the isolates CTC12 and CTC02 measured significantly higher soluble P compared to the rest at 48 h. However, at 72 h CTC12 recorded significantly highest P solubilization efficiency compared to rest. Negative correlations were also observed between the soluble P and pH of the cultured supernatant at 48 and 72 h of incubation 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, methymalonic acids [18-21].

TCP though primarily an insoluble P (always associated with alkaline soil) was not very hard to dissolve, compared to other bound P, particularly aluminium phosphates and iron phosphates (most prevalent in acidic soils) [4]. Hence, isolate CTC12 was further inoculated with comparatively harder to dissolve P ($AlPO_4$, $FePO_4$ and $Fe_3(PO_4)_2$) in the NBRIP broth medium for a period of eight (8) days. The solubilization efficiency followed the order $Ca_3(PO_4)_2$ > $FePO_4$ > $AlPO_4$ > $Fe_3(PO_4)_2$. PSBs have the ability to solubilize iron phosphates and aluminum phosphates [22]. Previous researcher also identified an efficient PSB which could solubilize $AlPO_4$ and $FePO_4$ [23]. However, reports suggested that PSBs solubilized different types of P sources [$Ca_3(PO_4)_2$, $AlPO_4$, $Mg_3(PO_4)_2$, $FePO_4$] in the liquid medium with comparatively lower P Solubilization with $AlPO_4$ than other sources [24-27].

Additionally, the pH of the incubated NBRIP Broth cultures showed acidic reaction but medium treated with TCP showed marginally higher pH compared to others [$AlPO_4$, $Fe_3(PO_4)_2$, $FePO_4$], which may be due to the presence of Ca^{2+} ions. Further, it was observed that all the mediums continued to maintain acidic pH till 8^{th} day of incubation irrespective of the P sources, which perhaps owing to release of organic acids in broth cultures. PSB strains produced organic acids *viz*; succinic, oxalic, malic and propionic acids which chelate the cation bound phosphate and convert it to soluble forms through their hydroxyl and carboxyl

groups [21]. Bacterial strain could synergistically enhance P Solubilization in acidic medium [28].

The PSB isolate then, identified as *Bacillus amyloliquefaciens* strain CTC12 (KT633845). Several soil bacteria, particularly from genera *Pseudomonas* and *Bacillus* efficiently solubilize the insoluble phosphate into soluble form [29].

Acid soil infertility status, more often linked to P deficiency and Al, Fe toxicity, seems to be a big barrier in reaching to the sustainable approach in the agriculture system. The present study tried to search an alternative way of P solubilization and availability. The native P- solubilizing bacteria *Bacillus amyloliquefaciens* strain CTC12 from acid soil could solubilize Al-P and Fe-P, but less in comparison to TCP. Hence, further in vitro as well as field level studies are needed to substantiate the P- solubilizing bacteria as both plant growth promoting rhizobacteria and biocontrol agent in soil-plant system.

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Conflicts of Interest

The authors declare no conflict of interest.

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