Phosphate Solubilizing Microorganisms Associated with Chollangi Mangrove Soil in East Coast of India

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Abstract: Twenty three bacterial strains isolated from the soil of Chollangi mangrove environment and demonstrated Phosphate solubilization potential of isolated bacterial strains. Seven isolates showed phosphate solubilization activity. Morpho physiologically and biochemically identified two isolates (CMB1 and CMB2) as Bacillus subtilis, three isolates (CMFP3, CMFP4 and CMFP5) as fluorescent Pseudomonas and two isolates (CMAZ1 and CMAZ2) as Azotobacter sp. Plant growth promoting ability of seven bacterial strains tested for optimization of P^H, carbon source and temperature and estimated phosphate solubilization quantitatively. Isolated strains visualized colonies at P^H 8.6 after 72 hrs, where as pure cultures visualized early within 18 hrs at P^H 6.8-7.2 range. Observations indicated bacterial isolates of arid mangrove environment can easily adapted to normal soil P^H and enhance phosphate content in soil.

Key Words: *Azotobacter*, *Bacillus subtilis*, *Fluorescent Pseudomonas*, Mangrove soil, Phosphate solubilization.

1.INTRODUCTION

Diverse microbial community living in mangrove ecosystems continuously transforms nutrients from dead mangrove vegetation into sources of nitrogen, phosphorus, and other nutrients that can be used by the plants and in turn the plant-root exudates serve as a food source for the microbes. According to Forest Survey of India (FSI), mangrove wetland is 3, 48,710 ha .out of which nearly 56.7 % is present along the East Coast, 23.5 % along the West Coast and the remaining 19.8% in Andaman Nicobar islands.

Microbial biodiversity in mangrove ecosystem is one of the difficult areas of biodiversity research. Study of biogeography, community assembly and ecological processes in mangrove ecosystem require extensive exploration, isolation and identification of potential microorganisms having specificity for recalcitrant compounds. Physical and chemical factors of mangrove ecosystem control the abundance and activities of bacteria in mangrove environment. Mangrove forests in India are productive ecosystems and sensitive to the environmental changes [1]. In the mangrove ecosystem, microorganisms perform various activities such as photosynthesis[2], nitrogen fixation[3], methanogenesis[4],agarolysis[5], production of antibiotics and enzymes etc., result in high productivity[6].

In the terrestrial environment, inoculation of insoluble phosphate solubilizing bacteria (IPSB) isolated from rhizosphere either alone or in combination increased the phosphate

content in soil and benefit crop plants like legume, sorghum and lettuce[6,7,8,9]. Where as in the marine environment IPSB isolated from rhizosphere of mangrove plants were reported to be potential for solubilization of insoluble calcium phosphate[10,11],but so far no report on potential phosphate solubilizing soil bacteria growing at different depths in mangrove environment . Therefore, presently an attempt was made to demonstrate the presence of insoluble phosphate solubilizing bacterial species from soil samples collected at a depth of 3 mts from Chollangi mangrove forest of East Coast to isolate, identify the species and to measure phosphate solubilizing potential invitro.

2.MATERIALS AND METHODS

2.1Collection of soil sample

Soil sample were collected from mangrove environment of Chollangi of east coast at a depth of 3 ft and placed in zip locked plastic bags at 4^0 C .The soil contained 3.8% of organic matter and P^H8.8.

2.2Isolation of Bacterial strains*:*

1 gm of soil was separately suspended in 9 ml of physiological saline soil in a flask and placed on an orbital shaker (at 100 rpm) at room temperature(28± 2^0C) for 1 hr . At the end of shaking the soil samples were serially diluted upto 10^{-6} with physiological saline . 10^4-10^6 dilutions were placed on modified nutrient agar medium containing Flucanazole (antifungal antibiotic)by pour plate technique and incubated at 28^0C The most prominent colonies were isolated maintained on Nam slants at 4^0c for further studies[12](Strickland and Parson 1972).

2.3Flouresent production*:*

The protocol [13] was used for fluorescence production .Bacteria were streaked on King's B agar and incubated at 28± 2^0C for 48 h . at the end of incubation the plates were observed under UV light for production of fluorescence.

2.4 Morphophysiological and biochemical studies: Morphological characters such as shape and color of the colonies were examined. Grams staining and motility were also done. Isolates were biochemically analyzed for the activities of oxidase, catalase, MR-VP test, starch hydrolysis and gelatin hydrolysis, indole production, hydrogen sulphide test, nitrate reduction, , sugar fermentation and citrate utilization. The results were compared with Bergey's Manual of Systematic Bacteriology.

2.5 Phosphate solubilisation:-

Solubilisation of Tri-calcium phosphate was detected in Pikovskaya's agar[13] . Each bacterial isolate was inoculated on the surface of Pikovskaya agar medium and phosphate solubilising activity was estimated after 1 to 5 days of incubation at room temperature phosphate solubilisation activity was determined by the development of the clear zone around bacterial colony.

2.6Quantitative estimation of phosphate:-

Quantitative estimation of inorganic phosphate solubilization was done as per methodology described by Nautiyal and Jackson .Bacterial isolates were grown in national Botanical Research Institute phosphate (NBRIP) broth containing 0.5% Tri calcium phosphate (TCP). The flask containing 50ml medium was inoculated with 500µl bacterial culture in triplicates and incubated at 30±0.1 at 180 rpm for 5 days in incubator shaker. Simultaneously the uninoculated control was kept under similar conditions. The cultures were harvested by centrifugation at 10,000 rpm for 10min. The phosphorus in supernatant

was estimated by vanado-molybdate-yellow color method. To a 0.5 ml aliquot of the supernatant , 2.5 ml Barton's reagent was added and volume was made to 50 ml with de-inonized water. The absorbance of the resultant colour was read after 10 min at 430 nm in UV/Visible spectrophotometer. The total soluble phosphate was calculated from the regression equation for standard curve. The value of soluble phosphate liberated were expressed as µg ml^{-1} over control. The P^H of culture supernantant were also measured using a P^H meter[13,14]

3.RESULTS

Twenty three bacterial strains were isolated from mangrove soil of Chollangi ,East Godavari.The isolates were subjected to Phosphate solubilization and seven isolates were identified as phosphate solubilizers. These isolates were subjected to morphological,biochemical and physiological characterization with a view to identify them(Table1 and 2) .The isolates CMB1 and CMB2 were positive to catalase and amylase where as negative to oxidase.CMFP3, CMFP4 and CMFP5 were positive to catalase ,amylase and oxidase.CMAZ1and CMAZ2 were catalase negative and positive to oxidase and amylase(Table 3)Fermentation of carbon components by the organisms was not identical and most of them were fermentative.(Table4)

Based on morpho physiological and biochemical characters,two isolates, CMB1 and CMB2 were identified as Bacillus sps ,three isolates CMFP3, CMFP4 and CMFP5 belong to the genus Pseudomonas sps and two other isolates namely CMAZ1and CMAZ2 belong to the Azotobacter sps. Optimization of temperature, $P^H$and carbon source were also studied . CMB1 ,CMB2 CMFP5 bacterial isolates showed maximum growth at 40^{o}C and CMFP3, CMFP4 , CMAZ1 and CMAZ2 Bacterial isolates at 45^{o}C .All isolates showed maximum growth at PH 8.8 in Mannitol medium and colonies were visualized after 72 hrs . Pure cultures showed maximum growth atPH6.8 in nutritive agar medium and showed visible colonies with in 18 hrs and may be due to adaptation to osmotic stress. All the isolates were phosphate solubilizers. Quantitative analysis showed that the concentration of soluble phosphate increased abruptly with time and then decreased (Fig 2). All seven bacterial sps were capable of dissolving insoluble phosphate with different extent(Fig 2).The species with highest solubilizing capacity were CMFP3 and CMFP4(Fig 2 C,D),where as CMB1 And CMB2 dissolved moderately (Fig2 A,B) and CMAZ1 and CMAZ2 dissolved less. The increase in solubilization in all seven isolates was corresponding roughly to the logarithmic phase of growth of bacteria.Based on capacity to solubilise phosphate CMFP isolates were best and CMAZ were less potential.

4.DISCUSSION

The average concentration of dissolved orthophosphates in seawater is 73µg/l[14] and concentration found in sediment of study site was 31 µg $ml^{-1}$and it was moderately higher than phosphorus of sea water,composed of insoluble phosphorus .VazQuez et al(2000) reported that fungi and IPSB present in the mangrove soil participate in releasing soluble phosphate into the pore water and may reach mangrove plant rhizosphere . The flow of nutrients like phosphorous between sediment and water is complex phenomenon influenced by bacterial activitybecause of bacterial abundance(91%) of total microbial mass of mangrove soils[15]

Significant occurrence of PSB indicate that phosphotase enzyme from that group of bacteria plays crucial role in phosphorous cycling in the soil sediments of Mangrove forest[15]and also agood indicator of recycling of organic and iinorganic matter in mangrove environment IPSB bacillus strains solubilized 112-157mg/L of phosphate[7] and 0.5- 0.55 mg/l phosphate by marine sediment IPSB Vibrio sp and Pseudomonas sps[12].Our results of phosphate solubilizing activity are comparable to this study

Adaptation to osmotic stress was an exclusive character of moderately osmotic tolerant non halophylic microbes[16] (Rosenberg 1983). In Present investigation we are first time reporting that all seven ISPB isolates showed growth atP^H6.8 in nutritive agar medium and showed visible colonies with in 18 hrs may be an indicator of adaptation of PSB of mangrove soil growing under severely osmotic stress as halophylic microbes exhibiting exclusive character of terrestrial ISPB microorganisms.Extensive exploration identification ,isolation ,screening and use of this type of adaptable ISPB may speed up the development of mangrove plants for reforestration as well as plant growth promotion in terrestrial crops like pulses, cereals etc.

ACKNOWLEDGEMENT

Authors are thankful to UGC-New Delhi for financial assistants from the grant released for research project (F.No. 40-132/2011(SR)) and Central Instrumentation Centre of Acharya Nagatjuna University for laboratory facilities.

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TABLE 1
MORPHOLOGICAL CHARECTERS OF BACTERIAL ISOLATES FROM CHOLLANGI MANGROVE ENVIRONMENT

isolated strain	Morphological characters			
	Gram stain	Spore formation	motile	pigmentation
CMB1	+ve	+ve	+ve	-ve
CMB2	+ve	+ve	+ve	-ve
CMFP3	-ve	-ve	+ve	+ve
CMFP4	-ve	-ve	+ve	+ve
CMFP5	-ve	-ve	+ve	+ve
CMAZ1	+ve	-ve	+ve	+ve
CMAZ2	+ve	-ve	+ve	-ve

TABLE 2
BIOCHEMICAL ANALYSIS OF FOR BACTERIA ISOLATEDFROM CHOLLANGI MANGROVE ENVIRONMENT

Isoolate	BIOCHEMICAL TESTS						
	I	MR	VP	C	G	H_2S	NR
CMB1	-Ve	-Ve	+ve	+ve	+ve	+ve	+ve
CMB2	-Ve	-Ve	+ve	+ve	+ve	+ve	+ve
CMFP3	-Ve	-Ve	-Ve	+ve	-Ve	+ve	+ve
CMFP4	-Ve	-Ve	-Ve	+ve	-Ve	+ve	+ve
CMFP5	-Ve	-Ve	-Ve	+ve	-Ve	+ve	+ve
CMAZ1	-Ve	+ve	+ve	+ve	-Ve	+ve	+ve
CMAZ2	-Ve	+ve	+ve	+ve	-Ve	+ve	+ve

I-Indole, MR-Methyle Red, VP-Voges-Proskaver, C-Citrate, G Gelatin , H_2S , NR-Nitrate reduction.

TABLE3
EXTRA CELLULAR ENZYMATIC ACTIVITIES OF BACTERIAL ISOLATED FROM CHOLLANGI MANGROVE ENVIRONMENT

Isolated srrain	Extra cellular enzyme activity		
	C	O	A
CMB1	+ve	-ve	+ve
CMB2	+ve	-ve	+ve
CMFP3	+ve	+ve	+ve
CMFP4	+ve	+ve	+ve
CMFP5	+ve	+ve	+ve
CMAZ1	-ve	+ve	+ve
CMAZ2	-ve	+ve	+ve

***C=catalase *O=oxidase *A=amylase**

TABLE 4
ANALYSIS OF SUGAR FERMENTATION FOR BACTERIA ISOLATED FROM CHOLLANGI MANGROVE ENVIRONMENT

Isolated strain	Sugar fermentation		
	D	S	M
CMB1	+ve	+ve	+ve
CMB2	+ve	+ve	+ve
CMFP3	+ve	+ve	-ve
CMFP4	+ve	+ve	-ve
CMFP5	+ve	+ve	-ve
CMAZ1	+ve	+ve	+ve
CMAZ2	+ve	+ve	+ve

***D=dextrose *S=Sucrose *M=mannitol**

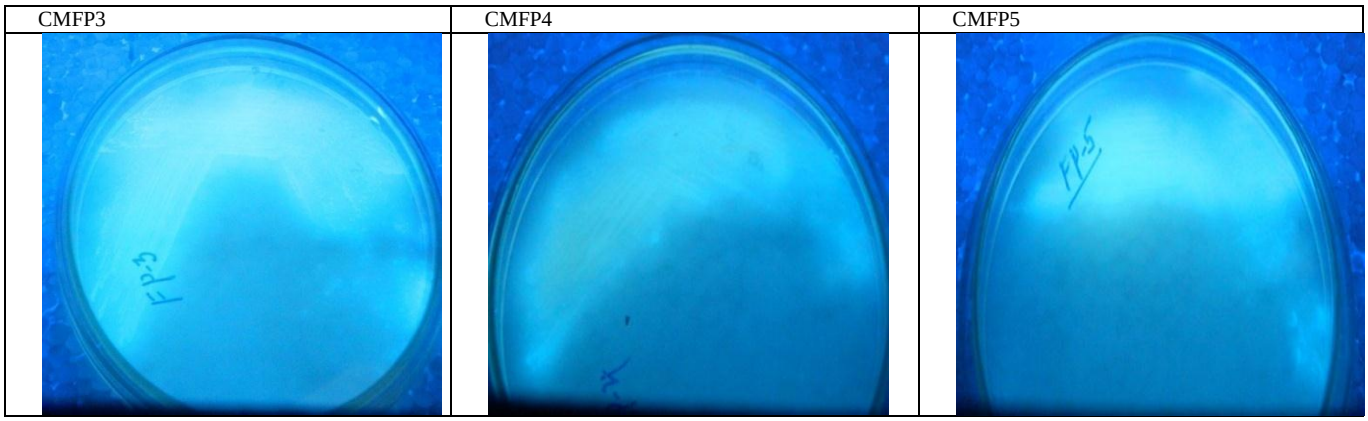
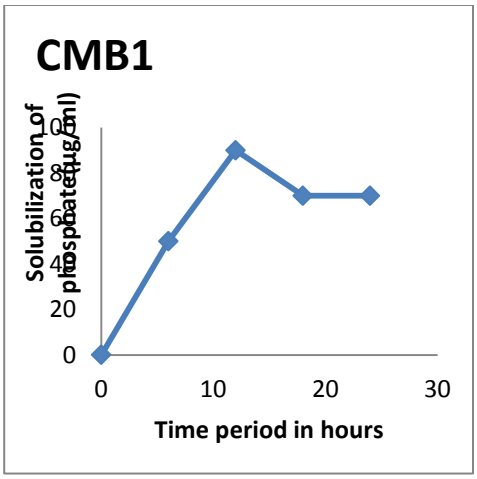
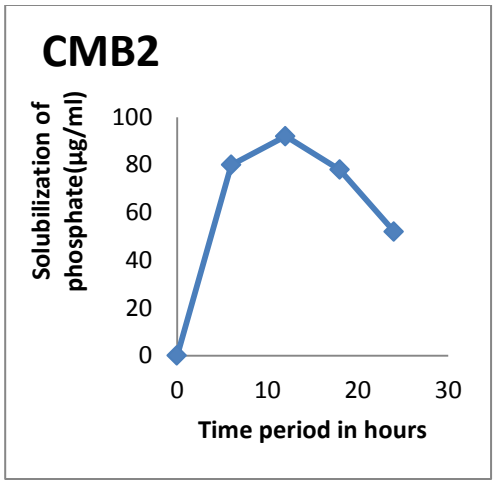
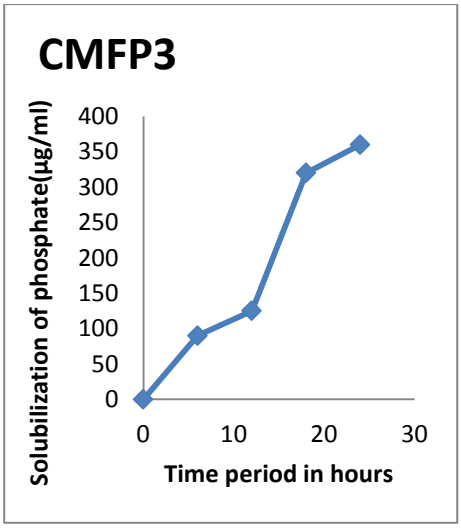
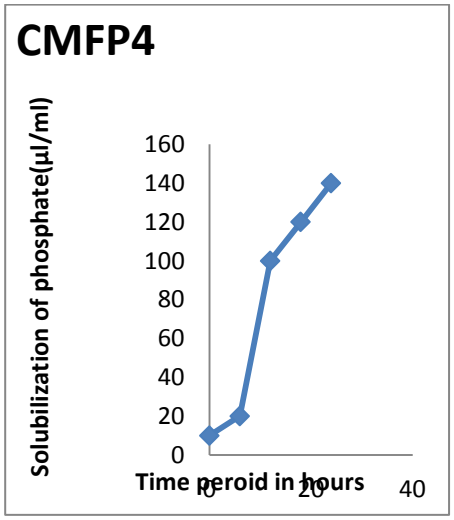


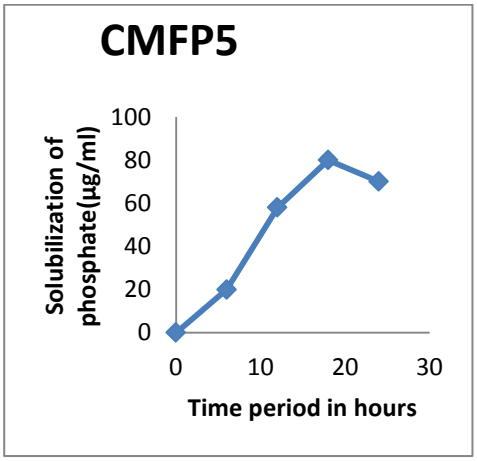
Fig 1:Flouresence Pseudomonas species isolated from Chollangi Mangrove Environment


CMB1
Solubilization of phosphate(µg/ml)
0
20
40
60
80
100
0
10
20
30
Time period in hours


CMB2
Solubilization of phosphate(µg/ml)
0
20
40
60
80
100
0
10
20
30
Time period in hours


CMFP3
Solubilization of phosphate(µg/ml)
0
50
100
150
200
250
300
350
400
0
10
20
30
Time period in hours


CMFP4
Solubilization of phosphate(µl/ml)
0
20
40
60
80
100
120
140
160
0
20
40
Time peroid in hours


CMFP5
Solubilization of phosphate(µg/ml)
0
20
40
60
80
100
0
10
20
30
Time period in hours

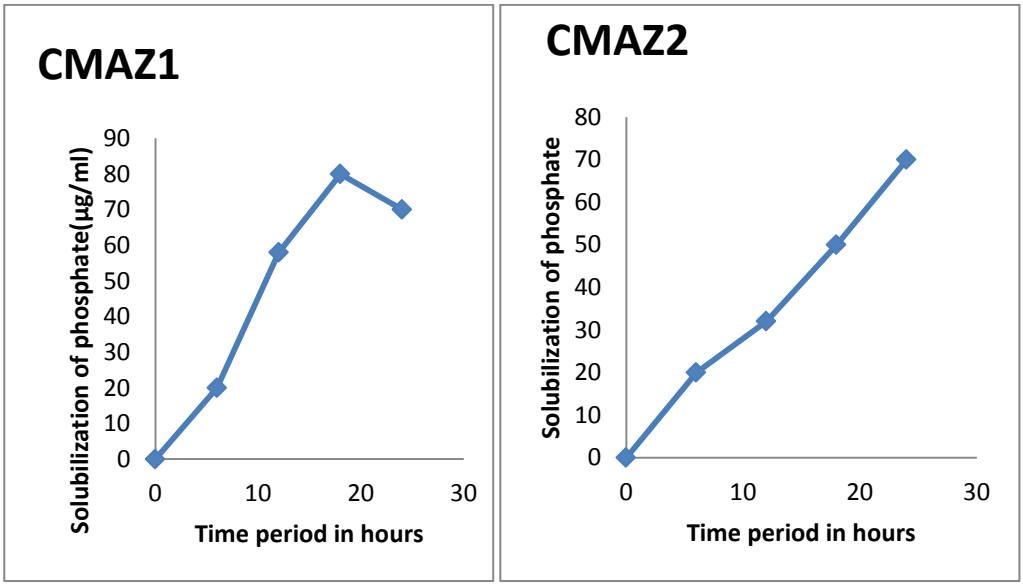


Fig 2: Phosphate solubilization of six phosphate solubilizing bacteria sps in culture medium contain insoluble calcium phosphate

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