



PHOSPHATE SOLUBILIZING MICROORGANISMS ASSOCIATED WITH MANGROOVE SOIL IN EAST COASTAL OF INDIA

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1. INTRODUCTION

Phosphate solubilizing bacteria (PSB) are beneficial bacteria capable of solubilizing inorganic phosphate from insoluble compounds. P-Solubilization ability of Rhizosphere microorganisms is considered to be one of the most important traits associated with plant phosphate nutrition [1]. It is generally accepted that the mechanism of mineral phosphate solubilization by PSB strains is associated with the release of low Molecular weight organic acids, through which their hydroxyl and carboxyl groups chelate the actions bound to phosphate, there by converting it into soluble forms. PSB have been introduced to the Agricultural community as phosphate Biofertilizer [2], [3], [s4], [5].

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 [6]. 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. In the mangrove ecosystem, microorganisms perform various activities such as photosynthesis [7], nitrogen fixation [8], methanogenesis [9], agarolysis, production [10] of antibiotics and enzymes etc., result in high productivity [11], [12].

Special Mangrove soil niches possess valuable microbial resources. Unfortunately, up to the present, so far there is very little knowledge on the mangrove soil microbial communities. It mainly due to the limitations of research methods. Culture independent approaches based on 16S rRNA, 18S rRNA gene analysis open the window to study microbial diversity in mangrove soil. The progress had achieved about studying on mangrove soil microbial species diversity, metabolic diversity and treating environmental pollutions was summarized in the paper. In addition, the bright future of mangrove soil microorganisms was described. Phosphate (p) is one of the major essential Macronutrients for plants and is applied to soil in the same form of phosphate fertilizer [11], [13], [14].

However, a large portion of soluble inorganic phosphate, which is applied to the soil as chemical fertilizer is immobilized rapidly and become unavailable to plants. Currently the main purpose in managing soil phosphate is to optimize crop production and minimize p loss from soils. PSB have attracted the attention agriculturists as soil Inoculants to improve plant growth and yield. When PSB as Inoculants increase p uptake by plants. Simple inoculation of seeds with PSB gives crop yield response equivalent to 30kg p₂O₅ ha or 50 percent of the need for phosphatic fertilizers. Alternatively, PSB can be applied through Fertigation Or in hydroponic operations. Many different strains of then bacteria have been identified as PSB including *Pantoea agglomerans*, *Mycobacterium laevaniformans* and *Pseudomonas putida* [15], [16].

Additionally, phosphate (p) compounds are capable of immobilizing heavy metals, especially pb, in contaminated environments through phosphate – heavy metal precipitation. However, most p compounds are not readily soluble in soils so it is not readily used for metal immobilization [17].

Some soil bacteria, isolated from the root region of plants, are known to enhance growth of the plants. These beneficial free-living soil bacteria are termed as plant growth promoting rhizobacteria (PGPR). These include nitrogen-fixing azotobacters and phosphate solubilizing bacteria etc. The beneficial effect of PGPR is mediated through either direct or indirect mechanisms [18]. The direct effects are most commonly attributed to the production of plant hormones such as auxins, gibberellins and cytokinins; or by supplying biologically fixed nitrogen. The PGPR also improve growth by indirect mechanisms such as, suppression of bacterial, fungal and nematode pathogens, and production of siderophores, HCN, ammonia, antibiotics, volatile metabolites etc. [18]. Some studies are available for the beneficial bacteria associated with the natural mangrove habitats [19]. However, no such studies are available for artificially developed mangrove habitats. Hence, the present study has been undertaken to isolate and enumerate the azotobacters and phosphate solubilizing bacteria associated with the rhizosphere soil of mangrove tree species, and to relate them with the nutrient levels.



FIGURE: 1 MANGROOVE SAMPLE

Higher classification: Bacillus

Scientific name: Bacillus circulans

Phylum: Firmicutes

Class: Bacilli

Order: Bacillales

Family: Bacillaceae

Phosphate (p) is a Macronutrient that plays essential roles in plant growth and participates in many metabolic reactions. It is a vital element for life as it is a vital element for as it is present in biological molecules, including nucleic acids, co enzymes, phosphoproteins and phospholipids, Additionally P is one of the main limiting elements for biomass production in terrestrial ecosystem and the reason for the ongoing eutrophication of contional utilization of p- fertilizer the p cycle exists with in individual ecosystem including soil : stream, forest, and marine, which are closely related to key security issues of surroundinenvironment and human society.

P cycle differs from the N and c biogeochemical cycle since it does it not form any stable gaseous species at earth temperature and atmospheric pressures., only small amounts of phosphoric acid (H_3PO_4) may enter the atmosphere and contribute to acid rain in some cases. Permitted by consumption of fossil fuels and biofuels into the atmosphere, which has been listed as one of the ten critical “ planetary boundaries “ of the earth system will be subsequently transported to a aerosol- bore P and rapidly deposited in terrestrial and aquatic ecosystem[20].

Through inorganic PR denudation and sediment immobilization on medium – term timescales activity, human perturbation, and climate change as important factors impacting global P cycling by increasing P concentrations from both external and internal sources, with consequences for terrestrial and aquatic ecosystems. Human activities, including the development and utilization of organophosphorous chemicals, extraction of geological P reserves to produce p- fertilizer[21]. Diverse microbial community living in mangrove ecosystemscontinuously transforms nutrients from dead mangrove vegetation into sources of nitrogen, phosphate, 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[22].

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[23].

In the mangrove ecosystem, microorganisms perform various activitiesactive such as photosynthesis[7], nitrogen,fixation[8]methanogenesis[11],agarolysis,production of antibiotics and enzymes etc., result in high productivity[19]. 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.

Where as in the marine environment IPSB isolated from rhizosphere of mangrove plants were reported to be potential for solubilization of insoluble calcium phosphate [20], 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 *in vitro* [24], [25].

Phosphate-solubilizing bacteria (PSB) were found in majority of soils [23] though their population was generally low in arid and semi arid regions, possibly due to the low level of organic matter and high temperature regime [26]. The PSB population was higher in soils under mild and moist climates than in dry ones (Subba Rao, 1982). Phosphorus is second only to nitrogen as mineral nutrient required by both plants and microorganisms. Its major physiological role being in certain essential steps in accumulation and release of energy during cellular metabolism [27].

Phosphorus in soils is immobilized or becomes less soluble either by adsorption, chemical precipitation or both. Moreover, Iraqi soils are mainly calcareous with tendency to fix phosphorus [26]. The role of PSB is important through the release of neutral and alkaline phosphatase enzymes that is capable of solubilizing fixed phosphorus [27].

However, no information is available on the abundance of such bacteria in Iraqi soils, and their biological significance in phosphorus cycle. In the present investigation, an attempt has been made to study the influence of some soil properties and the plant cover on the abundance of PSB [28].

2. MATERIALS AND METHODS

COLLECTION OF SOIL SAMPLE:

Soil samples were collected from mangrove environment of east coast at a depth of 3 ft and placed in zip locked plastic bags at 40°C. The soil contained 3.8% of organic matter and PH 8.8.

SERIAL DILUTION:

Prepare the 10 set of test tubes name it as 10 and 10. Add 10 ml of water in 10 test tube .9 ml of water in rest of the test tube mix it well. Add 1g of mangrove soil in 10 test tube mix it well. Take 1ml of water from 10 test tube and pour it in 10 test tube make it as 10ml. Continue the same process until it reaches 10 test tube.

ISOLATION OF BACTERIAL STRAIN:

Prepare the petriplates. Pour the nutrient agar in petriplates. Take 1ml of sample from 10 test tube and inoculate in the petriplate and inoculate in the petriplate by streaking method as directed. Continue the process as take 1ml from 10 and 10 respectively. After inoculation the colonies in the nutrient broth and incubate it for 24 hrs at 37°C. After the incubation period PSB were identified.

ANTIMICROBIAL ACTIVITY:

Prepare the nutrient agar with petriplates respectively. Inoculate the organism in the petriplates by using spread plate method. Place the disc with the standard as amoxalin. Incubate the plates at 37°C for 24 hrs. After 24 hrs, 0.3 ml of alpha-naphthol and 40% potassium hydroxide was added and the colour change was observed.

CITRATE UTILISATION TEST:

Prepare simon citrate agar slant and inoculated with colony and incubated at 37°C for 24-48 hours. After incubation, color change was observed.

BIOCHEMICAL STUDIES:

INDOLE TEST:

The trypan broth was prepared and sterilized at 110°C for 30 minutes. After cooling, the colony was incubated at 37°C for 24 hrs. After 24 hrs, few drops of KOVAC'S reagent was added and the colour change was observed.

METHYL RED TEST:

MR-VP medium was prepared and sterilized at 110°C for 30 minutes. After cooling, the colony was inoculated and incubated at 37°C for 24 hrs. Few drops of Methyl red indicator were added and the colour change was observed.

VOGES PROSKAUER TEST:

MR-VP medium was prepared and sterilized at 110°C for 30 minutes. After cooling, the colony was inoculated and incubated at 37°C for 24 hours. Prepare Urea agar slant and inoculated with colony and incubated at 37°C for 24-48 hours. After incubation, colour change was observed.

TRIPLE SUGAR IRON TEST:

Prepare triple sugar iron agar slant and inoculated with colony and incubated at 37°C for 24-48 hours. After incubation, colour change was observed.

CATALASE TEST:

Test tube contain the 24 hrs culture and mix with 1-2 ml of hydrogen peroxide solution. Observe the result.

OXIDASE TEST:

24 hrs cultures was swab on the filter paper and add a drop of oxidase reagent. Observe the result.

HYDROGEN CYANIDE (HCN) PRODUCTION:

HCN production was checked by streaking the culture on nutrient agar medium supplemented with glycine (4.4 g/l). Whatman filter paper no.1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed on the top of the plate. Plates were sealed with parafilm and incubated at 30°C for 48hrs. Development of orange to color indicated HCN production.

3. RESULT

ANTIMICROBIAL ACTIVITY:

In antimicrobial activity test, the zone of inhibition was observed in plate 2 with amoxalin 500mg capsule was prepared in 1ml solution. In this solution 80µl was used in plate 2, although plate 1 and 3 was using 40µl, 60µl. But the significant zone was observed only on plate 2. These plates were indicated that the phosphate solubilizing bacteria is inhibited by amoxalin.

FIGURE: 2 ZONE OF INHIBITION

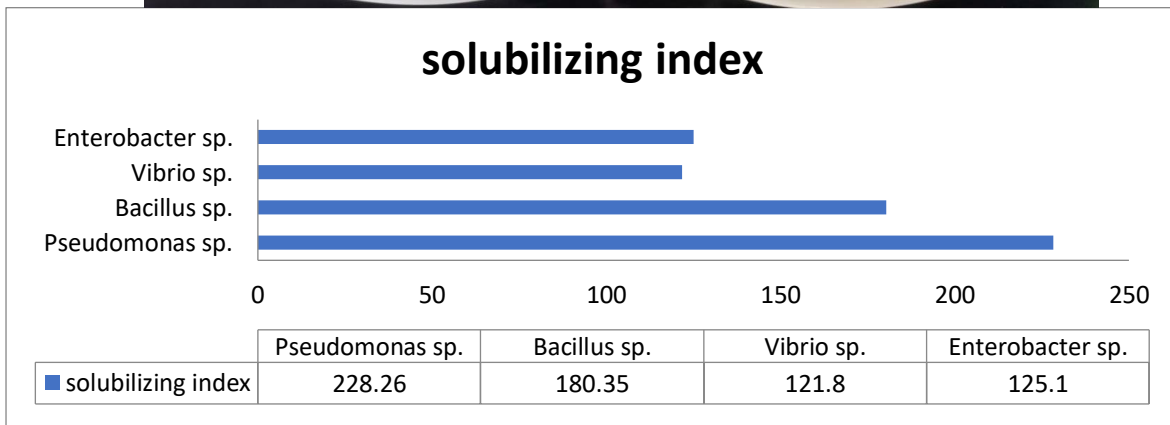
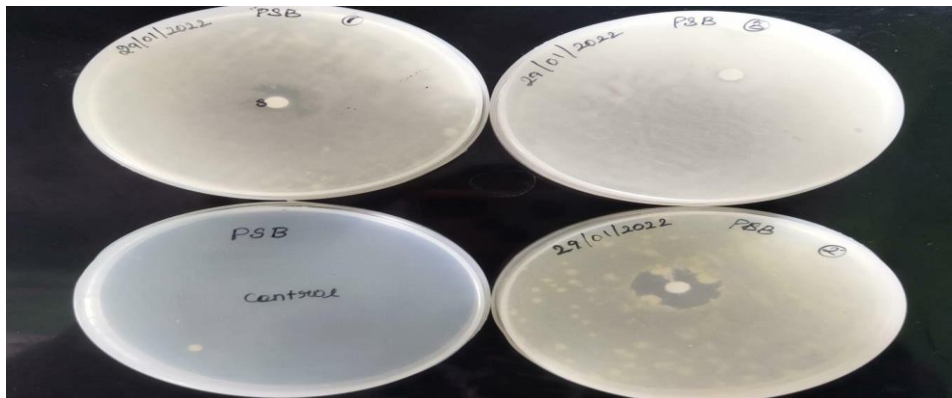
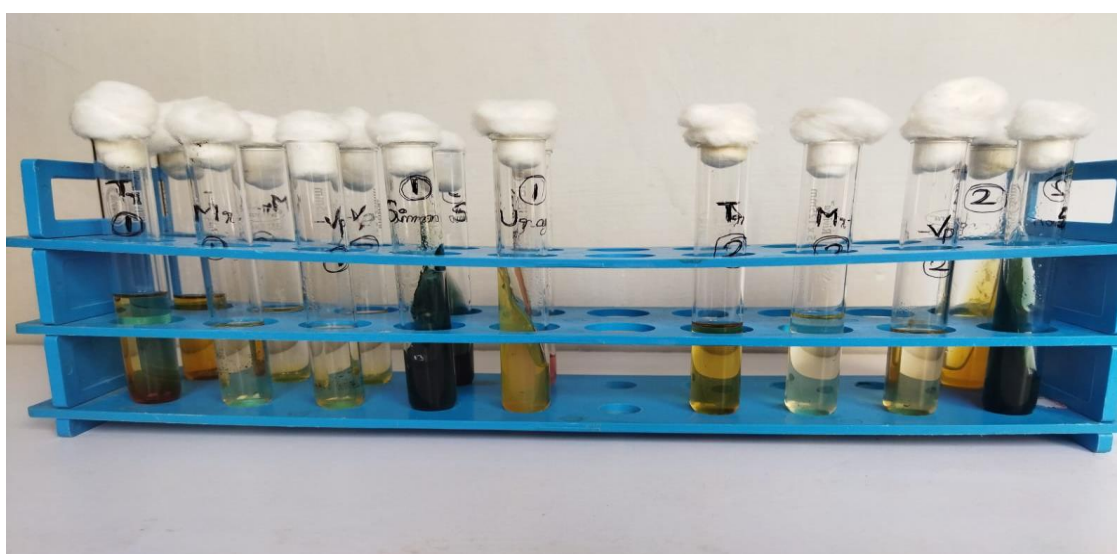


FIGURE: 3 DIFFERENT CONCENTRATION OF SOUBILIZING

BIOCHEMICAL TEST:

Some growth-promoting indicators of primary screening strains were determined, including the capacity of phosphorus-solubilized, nitrogenase activity, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, production of indole-3-acetic acid (IAA), secretion of iron carrier and so on. Finally, the screening multifunctional phosphorus-dissolving bacteria were identified, which were combined with colony characteristics, physiological and biochemical tests and molecular biotechnology. The conventional biochemical identification of phosphate-dissolving bacteria is carried out according to the methods in the "Common bacterial system identification manual", which mainly includes Gram stain, glucose hydrolysis test, lactose hydrolysis test, methyl red test, VP test, hydrogen sulfide production test, gelatin liquefaction test, citrate utilization test, malonate utilization test and denitrification test. Biochemical tests are among the most important methods for microbial identification. Microbial biochemistry tests shorten the time required to identify microbes, reduce costs, and ensure or enhance the accuracy of identification of an unknown sample. It is the fastest developing trend in microbial identification. In recent years, the rapid commercial test kits for anaerobic bacteria have become available. In this test results strain 1, 2 and 3 have mixed positive and negative results. The study found that most of the phosphate solubilizing bacteria screened out was Gram-negative bacteria, which was consistent research on the isolation of endophytes from rhizosphere soil, indicating that Gram-negative bacteria strains have the characteristics of dissolving insoluble phosphate. Therefore, 16SrDNA gene sequence sequencing to identify bacteria has been widely used in the biological field. In this paper, combining the characteristics of the colony, physiological and biochemical tests, 16SrDNA sequence comparison, the strains are identified to species.

NO	INDOLE TEST	METHYL RED TEST	VOGES PROSKAUER TEST	CITRATE TEST	UREASE TEST
STRAIN 1	-VE	+VE	-VE	+VE	-VE
STRAIN 2	-VE	-VE	-VE	+VE	+VE
STRAIN 3	+VE	+VE	-VE	+VE	-VE

TABLE: 1 BIOCHEMICAL TEST**FIGURE: 4 BIOCHEMICAL TEST****HCN TEST:**

Phosphate solubilizing bacteria also protect plants by avoiding phytopathogens, typically owing to the production of antibiotics, hydrogen cyanate (HCN), and antifungal metabolites. Furthermore, Phosphate solubilizing bacteria supports plant growth through production of siderophore and increases efficiency of nitrogen fixation. Besides, Phosphate solubilizing bacteria acts as a biocontrol against plant pathogens via production of antibiotics, hydrogen cyanate (HCN), and antifungal metabolites. Thus, Phosphate solubilizing bacteria potential substitutes for inorganic phosphate fertilizers to meet the Phosphate demands of plants, improving yield in sustainable agriculture. Their application is an ecologically and economically sound approach. Further investigation, therefore, is crucial to explore effective biofertilizers with Phosphate solubilizing bacteria multiple growth-stimulating attributes at the field trial. The test plates shows that the indication of orange colour, is established the presence of phosphate solubilizing bacteria.

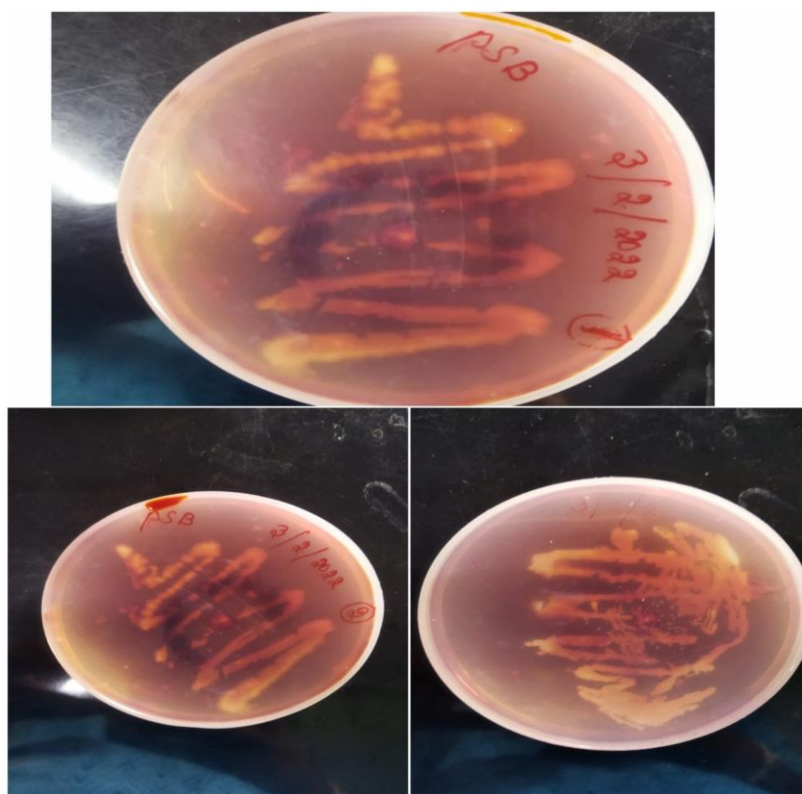


FIGURE: 5 HCN TEST

4. DISCUSSION

Phosphorus is one of the significant developments restricting nutrients expected for legitimate plant development, especially in tropical regions, because of its low accessibility in the dirt. It represents somewhere in the range of 0.2 and 0.8% of the dry load of plants, and it is held inside nucleic acids, compounds, co-proteins, nucleotides, and phospholipids[29]. P is fundamental in each part of plant development and improvement, from the atomic level to numerous physiological and biochemical plant exercises including photosynthesis, advancement of roots, fortifying the stalks and stems, arrangement of blossoms and seeds, crop development and nature of yield, energy creation, stockpiling and move responses, root development, cell division and amplification, N obsession in vegetables, protection from plant illnesses, change of sugar to starch, and moving of the hereditary qualities. Sufficient P accessibility is additionally expected for setting out the primordia of plant regenerative parts during the beginning stages of plant advancement. In the, below chart showed the some bacteria's with their solubilisation index[30].

Phosphate is the second most significant macronutrient expected by the plants, close to nitrogen. However, the accessibility of solvent types of P for plants in the dirt is restricted as a result of its obsession as insoluble phosphates of iron, aluminum, and calcium in the dirt. Most soils have extensive measures of Phosphate; however a huge extent will undoubtedly soil constituents. Soil with low complete P can be enhanced with P manure however can't hold the additional P. Around 75-90% of the additional compound P manure is encouraged by metal-cation buildings and quickly becomes fixed in soils and affects the climate as far as eutrophication, soil richness exhaustion, and carbon impression. Microorganisms are necessary in the normal phosphate cycle[31]. The utilization of phosphate solubilizing microorganisms (PSMs) as biofertilizers for agribusiness upgrade has been a subject of review for a really long time. This audit is planned to give a brief on accessibility of soil P and variety of PSM, instruments of P solubilization, how PSM incite plant development, and their conceivable job as biofertilizer in crop creation[8], [19].

Phosphate (P) is the second generally significant supplement for plant development, representing 0.2% (w/w) of plant dry weight. P assumes an indispensable part in the biological system by partaking in many parts of energy digestion, nucleic corrosive and protein combination, and kinase guideline. The normal P content in soil is almost 0.05% (w/w) with the vitally two structures being inorganic P (Pi) and natural P (Po). By the by, just 0.1% of P can be used by plants, delivering accessible P a prohibitive component for plant development. Phosphate anions in synthetic compost accessible to plants are very receptive and become fixed through collaborations with Ca^{2+} , Fe^{3+} , and Al^{3+} particles in the dirt to frame insoluble phosphate salt edifices; notwithstanding, the plant usage effectiveness for P in substance manures is just 5-25% [1], prompting P advancement in the dirt and the deficiency of soil richness. These real factors make expanding the P use rate for plant development an earnestly requiring circumstance. Phosphate solubilizing microorganisms (PSBs) convert inaccessible P (both Pi and Po) into accessible P to fulfill the necessities of plants through disintegration and ingestion. As per the different P-dissolving designs, PSBs can be separated into two classes:

- (a) Pi-solubilizing microorganisms that emit natural corrosive to break down Pi mixtures and
- (b) Po-mineralizing microorganisms that discharge phosphatase to enzymatically mineralize Po compounds[32].

The utilization of the two classes of PSBs in soil diminishes the pH of the dirt and structures a P-offering microarea around the plant rhizosphere, therefore further developing the P supply accessible to the plant and fortifying the action of other gainful microorganisms, like Rhizobium and Trichoderma. These applications advance the ingestion of nutritive component particles. As of late, following the advancement of energy bases and mines investigated in Shanxi Province, China, a tremendous measure of developed land was defiled with gangue, mineral waste, weighty metal, and rock flotsam and jetsam. Roughly 68 km² of cultivated land lost their harvest efficiency. Consequently, objective and logical measures for soil recovery in the mining region ought to be created and applied immediately. Their P-solubilizing limits under various circumstances were researched. The impact of PSB S32 on P dissolvability in the recovered soil recuperation, plant and root development, and the P take-up of rice were likewise assessed. The current information recommends that the utilization of the separated PSB would be vital in the bioremediation of recovered soil in mining regions[18].

Various microbial local areas living in mangrove biological systems consistently changes supplements from dead mangrove vegetation into wellsprings of nitrogen, phosphate, and different supplements that can be utilized by the plants and thusly the plant-root exudates fill in as a food hotspot for the organisms. As per Forest Survey of India (FSI), mangrove wetland is 3, 48,710 ha .out of which almost 56.7 % is available along the East Coast, 23.5 % along the West Coast and the excess 19.8% in Andaman Nicobar islands. Microbial biodiversity in mangrove environment is one of the troublesome areas of biodiversity research. Investigation of biogeography, local area gathering and natural cycles in mangrove biological system require broad investigation, disengagement and ID of potential microorganisms having particularity for unmanageable mixtures. Physical and synthetic elements of mangrove biological system control the overflow and exercises of microorganisms in mangrove climate. Mangrove backwoods in India are useful biological systems and touchy to the natural changes. In the mangrove biological system, microorganisms perform different exercises, for example, photosynthesis[12], nitrogen fixation[7], methanogenesis[12], agarolysis[20], creation of anti-toxins and chemicals and so on, bring about high productivity. In the earthly climate, vaccination of insoluble phosphate

Solubilizing microscopic organisms (IPSB) disconnected from rhizosphere either alone or in mix expanded the phosphate content in soil and advantage crop plants like vegetable, sorghum and lettuce. Where as in the marine climate IPSB detached from rhizosphere of mangrove plants were accounted for to be potential for solubilization of insoluble calcium phosphate[5],but up until this point no report on potential phosphate solubilizing soil microorganisms developing at various profundities in mangrove climate .

5. CONCLUSION

The normal centralization of broken down orthophosphates in seawater is 73µg/l and fixation found in silt of study site was 31 µg ml⁻¹ and it was reasonably higher than phosphate of ocean water, composed of insoluble phosphate . The progression of supplements like phosphorous among dregs and water is intricate peculiarity affected by bacterial activity because of bacterial abundance (91%) of absolute microbial mass of mangrove soils Significant event of PSB demonstrate that phosphatase catalyst from that gathering of microorganisms assumes pivotal part in phosphorous cycling in the dirt residue of Mangrove forest and additionally a good mark of reusing of natural and inorganic matter in mangrove climate IPSB bacillus strains solubilized 112-157mg/L of phosphate and 0.5-0.55 mg/l phosphate by marine dregs IPSB *Vibrio* sp and *Pseudomonas* sps. Our consequences of phosphate solubilizing action are equivalent to this concentrate on Adaptation to osmotic pressure was a select person of modestly osmotic open minded non halophylic microbes.

In Present examination we are first time detailing that each of the seven IPSB disconnects showed development at pH 6.8 in nutritive agar medium and showed apparent states inside 18 hrs might be a mark of variation of PSB of mangrove soil developing under seriously osmotic pressure as halophylic organisms displaying selective person of earthly IPSB microorganisms. Extensive investigation distinguishing proof ,disengagement, screening and utilization of this sort of versatile IPSB might accelerate the improvement of mangrove plants for reforestation as well as plant development advancement in earthbound yields like heartbeats, grains and so on.

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