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Research Article

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Solubilization of phosphate by *Bacillus Sps*, from groundnut rhizosphere (*Arachishypogaea L*)

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ABSTRACT

The phosphate solubilizing microorganisms were isolated from the groundnut rhizosphere soil. The isolated colony were confirmed as Bacillus subtilis and Bacillus cereus. The effect of different pH, temperature, carbon sources, nitrogen sources, potash sources on the phosphate solubilization was optimized. Maximum solubilization was recorded at temperature range 40^{0}C.Screening of phosphate solubilizing bacteria was done in Bacillus subtilis, Bacillus cereus in both solid & liquid medium..Bacillus subtilis is the most efficient for phosphate solubilizing activity, at pH 7, Carbon sources [0.35$\pm$0.02]Nitrogen sources sources[0.28$\pm$0.03]Potash sources[0.12$\pm$0.09] strains on the basis of their phosphate solubilizing activity. Further research should be continued with efficient phosphate solubilizing isolates and their antimicrobial activity could be exploited as biofertilizer as well as bio pesticide. Chemical fertilizers are predominantly used to enhance crop yield but continuous use causes various draw back like sterility of soil. The use of biofertilisers avoids side effects and retains the fertility of the soil and can be well exploited as phosphatic biofertilizers Screening of phosphate solubilizing bacteria in both solid & liquid medium. Quantitative analysis of organic acid produced by phosphate solubilizing Bacteria.

Keywords: Phosphate solubilization,*Bacillussubtilis,Bacilluscereus*,Groundnut,Rhizosphere.

INTRODUCTION

The term 'rhizosphere' was introduced in 1904 by the German Scientist Hiltnes to denote that region of the soil which is subject to the influence of plant roots. Rhizosphere is characterized by greater microbiological activity depends on the distance to which exudations from the root system can migate. The term 'rhizosphere' effect indicates the overall influence of plant root on soil microorganics. It is now clearly established that greater number of bacteria, fungi and actinomycetes are presents in the rhizosphere soil than in non-rhizosphere soil and there are innumerable reports in literature to substantiate this fact. Several factors such as soil type, its moisture, pH and temperature and the age and condition of plants are known to influence the rhizozphere effect. Phosphorus is one of the essential nutrients required for plant growth but most of it existing in soil is in insoluble metallic complexes with iron aluminium etc., in acidic soil [1] or with calcium carbonate in allcalinesoil ,[2] As a result only a small fraction of phosphate available for plant growth [3].

The concentration of soluble phosphate in soil is usually very low which leads to deficiency of soluble phosphate and make it a limiting factor in plant nutrient [4]. Lower the quantity of phosphate solubilizing microorganisms (PSM) play an important role in supplementing phosphorus to the plant, allowing a sustainable use of phosphate fertilizer [5]. Groundnut *(Arachishypogaea L*) is unique among major crop plants in that the flower is pollinated aerially, and the seed matures understand. The soil immediately surrounding the pod is termed the geocarposphere [6].

The pointed are major anatomical differences between roots and fruits , and that intense physiological activity occurs in soil when peanut pistils develop into fruits. This suggests that the geocarposphere may, like the rhizosphere, consist of an area containing greater amounts of carbohydrates and amino acids that plant free soil and three by contain higher populations of diverse microorganisms. Phosphorus compounds in Indian alluvial soils are predominantly inorganic chiefly locked as tricalcium phosphate.

The group of microorganisms dissolving tricalcium phosphate appears to have implication in Indian agriculture rhizosphere microflora play significant role in mineralization / Solubilization of bound phosphates which are either inform of organic or inorganic phosphatic compounds, and makes available to higher plants. Various bacteria including actinomycetes, cyanobacteria, fungi and yeasts, are known to solubilize phosphates [7] micro organisms play dominant role in soil processes of nutrient cycling and interactions with all other soil inhabiting organisms including plants. The aim of the study is to estimates the solubilization of phosphate by Bacillus sps isolated from groundnut rhizosphere.

EXPERIMENTAL SECTION

Collection of sample
Sample were collected from groundnut rhizosphere soil from orathanadu, Thanjavur district, Tamil nadu.Collected soil sample were stored in polythene bags aseptically and maintained at the laboratory for further study.

Isolation of Bacteria
Rhizosphere soil was diluted 10 times with water , and kept for use 1 ml of diluted sample was taken it was added to 9 ml of sterile 0.85% saline water and then mixed well. This gives 10^{-1} dilution after well mixing 1 ml of sample from the test tube was pipette and transferred to the second test tube containing 9 ml saline and mixed thoroughly which gives 10^{-2}dilution the same procedure was continued upto 10^{-7}dilution .10ml king's B medium plates and incubated at 28^0C for 24 hours. Isolated colonies were subcultured on nutrient slants.

Identification of Bacteria
The morphological and Biochemical tests were done by the methods described in experiments in microbiology, plant pathology and biotechnology [8].

Screening of isolates for phosphate solubilization
Qualitative method
All the suspected colonies were screened for phosphate solubilization on pikovskayas medium. Isolates showing phosphate solubilizing ability were spot inoculated at the centrepikovskaya's plate and incubated at 37^0C.Diameter of clearance zone was measured successively after 24 hours, upto 7 days. The phosphate solubilization efficiency (PSE) is the ratio of total diameter .i.e.clearance zone including bacterial growth(Z)and the colony diameter ,(C), multiplied by 100 PSE=Z/C100 [9].

Quantitative method
100 ml of pikovskaya's broth medium with 250mg of Tricalcium phosphate was prepared and sterilized ,1ml of each isolates was inoculated into the broth medium. Then the inoculated sample were incubated for 14 days on rotatory shaker 37^0C,after incubation culture broth was centrifuged at 10,000 rpm for 30min,pH of all the isolates were measured.

Phosphate estimation
The amount of phosphorus present in the isolates was determined by Subba Rao and Fiske method. 1ml of sample was taken in 2 test tubes and volume was made upto 8.6ml with distilled water.1ml of Ammonium molybdate was added to the sample 0.4ml, of 1,2,4 aminonapthalsulphuric acid was added to the tubes and vortexed. The colour intensity was read out after 10 min at 660nm.Concentration of phosphorus in the sample was calculated [10].

Phosphate solubilization in liquid medium
Conical flask containing 200ml growth medium was inoculated with 1% inoculum coming from pre-culture grown in the same medium. The flask were incubated at 30^0C on a shaker at shaker at medium speed. Samples were taken aseptically at different times and used to determine pH and growth (OD at 600nm).Due to the presence of suspended particles of insoluble.

Tricalcium phosphate in the supernatant ,the sample were first allowed to sediment for 15min at room temperature and were centrifuged at very low speed.The supernatant was removed and diluted 1:1 with 1N in order to dissolve the residual insoluble phosphate.

Optimization of physiological conditions (Temperature and pH)
The phosphate solubilization efficiency (PSE) of the *Bacillus* isolates was studied on pikovskaya agar and (pH 7.0) and incubation temperature 25^0C,30^0C,35^0C,and 40^0C and pikovskaya agar adjusted at different pH Values 5,6,7,8, and 9,with incubation temperature 28^0C.

Effect on different sources on phosphate solubilization
1. Carbon sources
2. Nitrogen sources
3. Potash sources

Quantitative analysis of organic acid produced by phosphate solubilizing Bacteria
The test organism were grown in 50 ml Pikovskayas medium for 10 days. The supernatant was centrifuged at 10,000 rpm for 15 minutes. The supernatant was concentrated at low temperature to $1/40^{th}$ of its original volume. The fraction was spotted on to chromatographic technique using n-Butanol, acetic acid and water solvent in 12:3:5 ratio [11].

RESULT AND DISCUSSION

The soil sample were collected from different rhizosphere soil showed presence of phosphate solubilizing microorganisms. The isolated colonies, *Bacillus subtilis, Bacillus cereus*, exhibiting maximum phosphate solubilization and have been found to be active in solubilization of tricalcium phosphate under in-vitro condition. According to the biochemical tests,the isolated organisms were identified as *Bacillus subtilis* and *Bacillus cereus*.[Table-1].The phosphate solubilizing microorganisms have been reported to produce organic acids in liquid medium for simple carbon Nitrogen and potash source. Studies on different carbon sources like glucose, sucrose, lactose, mannitol and sodium acetate on phosphate solubilization revealed that incorporation of glucose followed by lactose increased solubilization of phosphate and enhanced acid production efficiency

The phosphate solubilizing *Bacillus subtilis* and *Bacillus cereus* were identified with the help of the zone formation in the pikovskaya'smedium. The zone is formed due to the phosphate solubilizing organisms which cleave phosphate molecules present in the pikovskaya's agar medium.

The phosphate solubilizing bacteria appeared on soil extract agar showing zone of solubilization. They were selected for further works. Studies on different carbon soures like glucose, sucrose, lactose, mannitol and sodium acetate on phosphate solubilization revealed that incorporation of glucose followed by lactose increased solubilizationof phosphate and enhanced acid production efficiently.[Table-2].

The phosphate solubilization of *Bacillus cereus*[0.32 ± 0.05] and *Bacillus subtilis*[0.38 ± 0.01] were analysis using various parameters such as pH[7-9], temperature[30^0C - 45^0C],and nutrient supplementation.[Table-3,4].The role of phosphorus in increasing the yield and improving the quality of is well know phosphate, next to nitrogen is a vital nutrient for plant and microorganisms.

Table 1:Morphological and Biochemical Characterization of Isolated Bacteria

Morphological and Biochemical Characters	***Bacillus subtilis***	***Bacillus cereus***
Cultural character	White colour colony	White waxy growth
Gram staining	Positive	Positive
Motility	Motile	Motile
Shape	Rod	Rod
Indole production test	-	-
Methyl Red test	-	-
Voges –proskauer test	+	±
Citrate Utilization test	+	-
Catalase test	+	-
Oxidase test	+	+
Urease test	-	-
Carbohydrate fermentation	±	-

\+ - *Positive*
\- - *Negative*

Table 2: Estimation of Phosphate solubilization

Microorganisms	**Phosphate solubilization(mg/l)**
Bacillus subtilis	0.649
Bacillus cereus	0.864

Table 3: Optimization of pH on phosphate solubilization

pH Range	**Phosphate solubilization(mg/l)**	
	Bacillus subtilis	***Bacillus cereus***
5	0.20±0.03	0.19±0.01
6	0.25± 0.07	0.20±0.09
7	0.38± 0.06	0.30 ±0.05
8	0.34± 0.04	0.25±0.02
9	0.28 ±0.08	0.23±0.06

Values are expressed as mean ± Standard deviation

Table 4: Optimization of Temperture on phosphate solubilization

Temperture(C^0)	**Phosphate solubilization(mg/l)**	
	Bacillus subtilis	***Bacillus cereus***
25	0.25 ± 0.06	0.22 ± 0.06
30	0.26 ± 0.04	0.23 ± 0.09
35	0.33 ± 0.09	0.26 ± 0.10
40	0.36 ± 0.08	0.28 ± 0.02
45	0.31 ± 0.07	0.24 ± 0.01

Values are expressed as mean ± Standard deviation

CONCLUSION

Finally, concluded that *Bacillus subtilis* is the most efficient strains on the basis on their phosphate solubilizing activity. Further research should be continued with efficient phosphate solubilizing isolates, from rhizosphere these may be used for inoculum production and the screening of selected phosphate solubilizing isolates was evaluated.

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REFERENCES

[1]White law,M.A(**2002)***Adv.Agcon***.,**69:99-104.
[2] P.Gyaneshwar,G.Nareshkumar,L.J.Pareskh,andPoole,P.S.(**1998)***Plants soil***,**245:83-89.
[3]Goldstenin,A.H(**1994)** Silver ASM Press,Washington.195-198.
[4]Forster.J.C(**1995)** Metods in Applied soil Microbiology and Biochemistry, National *Acadamic press London*.63-69.

[5]Gaur,A.C(**1990)**Phosphate solubilizing microorganisms as biofertilizer,Omega scientific publishers ,New Delhi,India.149-157.
[6]Bagyaraj,D.J.Krishnaraj,P.L.andKhanuja,S.P.F(**2000)***Proceedings of IndianNationalSciencAcadamic*.66:66-82.
[7] Hasan,H.A.H(**1999)***Soil Folia microbial***,44**:77-84.
[8]Aneja,K.R**(2005**) Experiments in Microbiology,plant pathology,Tissueculture and Mushroomproduction technology, 4thed new age international(P) Ltd,New Delhi,161-162.
[9]R.Gothwal,K.Nigam,V.K.Mohan,M.K..Sasmal and DandGosh,P(**2006**) *Plant and soil*,1:51-58.
[10]SubboRao,N.S.S(**1982)** Advances in Agricultural Microbiology**,**Oxford and IBH Publishing co.New Delhi,296-298.
[11]Narman,J and Norman,R**(1990**) Organic acids in chromatographic and electrophoretic techniques,Interscience Publishers inc. New york,272-289.