International Journal of TechnoChem Research
ISSN:2395-4248 www.technochemsai.com
Vol.06, No.01, pp 35-39, 2020

Screening of phosphate solubilizing fungi from Rhizosphere soil and study on its alkaline phosphatase activity

Rathi Devi P[1] and Packialakshmi N[2]

[1]Department of Microbiology & CLT, Dr.Umayal Ramanathan college for women, Karaikudi-630003, Tamilnadu,India
[2]PG & Research Department of Microbiology and Biotechnology, Jamal Mohammed college (Autonomous) Tiruchirappalli – 620 020, India.

Abstract : Soil is a mixture of organic nutrients, which provides nutrients for plant growth. Plants require N, P & K for their growth and vegetative propagation. Among vital nutrients, Phosphorus is less available to plants and microorganisms aid in phosphate solubilization. The present study is an ecofriendly method to induce phosphate solubilization in Rhizosphere soil. Totally 25 isolates were isolated from rhizosphere using Pikovskaya mediumand screened for phosphate solubilization using Tricalcium phosphate supplemented minimal medium.Of these, Aspergillus sp and *Penicillium* sp were found to produce remarkable solubilization index. Hence these two isolates were used for further assays. Enzyme assay was carried out to determine the enzyme activity using cell free extractsand the activity was analyzed by spectrophotometry.In addition, HPLC also performed to determine the end products of phosphate solubilization.The results of HPLC studies revealed that tricalcium phosphate was solubilized into organic acids like citric acid, gluconic acid and malic acid. The results of present study are an evidence for the production of organic acids by *Aspergillus* sp and phosphate solubilization which induce crop improvement as well as sustainable agriculture.
Keywords : Fungi, Phosphate solubilization, Enzyme assay, HPLC, Organic acids

Introduction

Phosphate is an important nutrient for plant growth and development. All plants require phosphate for metabolic activities like nucleic acid synthesis, respiration, energy production, energy storage and transfer, cell division and cell growth. Phosphorus fertilizers, when applied during the early stages of plant growth promotes early root formation, and is important for development of primordia for reproductive parts of plants. Seed formation requires phosphorus and its content is higher in seeds than in any other part of the plant. It improves survival of plants in winter climates. Phosphorus is available in rocks primarily as minerals like oxypatite, hydroxypatitite and apatite. These are highly insoluble and constitute about 40% of total phosphorus in Indian soils. Indigenous microorganisms convert insoluble phosphates into soluble phosphates and aid in the bioavailability of phosphorus. Phosphate solubilizing microorganisms assimilate phosphorus and release them to plants. Phosphate solubilization is a complex phenomenon which highly depends on many factors such as nutritional, physiological and growth conditions1.*Bacteria such as Pseudomonas, Alcaligens, Rhizobium, Seratia, Erwinia* are capable of phosphate solubilization 2.

Mechanism of mineral phosphate solubilization by PSB strains is associated with the release of low molecular weight organic acids3, which through their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms. There is experimental evidence to support the role of organic acids in mineral phosphate solubilization4.

Phosphate solubilizers possess enzymes that convert insoluble phosphate into soluble phosphate. Phosphate solubilizers like soil fungi are capable to produce extracellular enzyme, i.e. group of phosphatase enzyme which are able to mineralize organic P into inorganic P so that high P is available for plant. There are several soil phosphatases and the most commonly determined are: phosphomonoesterases, phosphodiesterases and phytases. Phosphomonoesterases act on phosphate monoesters and according to their optimum pH are divided in acid and alkaline phosphomonoesterases[5]. Both are adaptive enzymes: acid phosphomonoesterase predominates in acid soils while alkaline phosphomonoesterase predominates in neutral and basic soils 5.

An ecofriendly approach towards converting insoluble phosphates by potential phosphate solubilizing fungi from the soil is inevitable. The present study was designed to determine alkaline phosphatase activity of indigenous fungi from the natural rhizosphere soil.

Experimental methods

Collection of soil sample.

Rhizospheric soil samples near the roots of rice *Oryzae sativum aestivum* (rice var.) plants (ten plants each from ten fields) grown in the Sarugani region of Sivagangai, India were collected under aseptic condition in sterile polyvinyl bags separately by the composite sampling method and was transported under normal conditions.The roots were selected and the loosely adhered soil of about 10 g each was collected. Then isolated rhizosphere soil samples were sieved through 2 mm mesh.

The physico-chemical characteristics, namely pH, conductivity and salinity, were determined using a multi-parameter electrode tester. The carbon and nitrogen content of the soil was estimated with a C-H-N analyzer. The iron, phosphorus and magnesium contents of soil were determined by inductively coupled plasma atomic emission spectroscopy

Isolation of Phosphate solubilizing Fungi.

Phosphate solubilizing fungi were isolated from rhizosphere soil of crop fields using the standard dilution plate method. Soil sample was serially diluted by using air dried soil (10 g) mixed with 90 ml sterile distilled water.Soil suspension was vigorously shaken and allowed to stand for 5 min.Dilutions from 10^{-4}, $10^{-5}$and 10^{-6}were inoculated on Rose Bengal Chloramphenical Agar(RBCA) and incubated at 28 ± 2°C for 4-8 days. Isolated fungal strains were sub-cultured on RBCA slants for further studies. Phosphate solubilizing ability of the fungal colonies was confirmed by inoculating on Pikosvikyos medium(1) at 28 ± 2°C for 7 days. Diameter of clearance zone was measured after every 24 h, up to 7 days. The Solubilization Index (SI) is the ratio of total diameter i.e. clearance zone including fungal growth and the colony diameter. All the results were recorded in triplicate.

Screening for Phosphate solubilizing fungi

Phosphate Solubilization in broth culture

Microorganisms were inoculated in to liquid media to determine their capability of releasing P from insoluble sources. Modified PVK broth (100 ml with 0.5% Tricalcium phosphate) was poured into 500 ml Erlenmeyer flasks and the pH was adjusted to 7.0. After sterilization, 1mlof fungal suspensions is inoculated into the flasks in triplicate. Non-inoculated flasks supplemented with Tricalcium phosphate were marked as controls. Then the flasks were incubated at 28 ± 2°C, 150 rpm for 30 days with intermittent sample withdrawal (centrifuged at 17250 g for 10 min) for P solubilization. Measurements of pH and organic acid analysis on 7th, 14th and 21st day were carried out. The quantitative estimation of solubilized P was done by the Vanadomolybdophosphoric yellow color method6**.**

Analysis of Organic acid content by HPLC

Organic acid production by fungi was estimated by High Performance Liquid Chromatography (HPLC) analysis. HPLC operating parameters for the analysis of organic acids werestandard method7.

Determination of Phosphatase enzyme activity

The isolated fungal species showing maximum phosphate solubilization were selected for phosphatase enzyme studies using Pikovskaya's broth medium enriched with three different treatment of bound phosphate (TCP) and available phosphates (KH2PO4): viz

Treatment(A)TCP[10g/l]+KH2PO4[0.5g/l];

Treatment(B)KH2PO4[0.5g/l] onlyand

Treatment(C)TCP[10g/l] only.

The supernatant was used as crude enzyme extract for determining extracellular phosphatase enzyme activity at 405nm spectrophotometrically8,9.

Results and discussion

Fungalisolates wereidentified by observing colony characteristics on RBCA plates. Growth pattern of the isolates were identified as *Aspergillus* spand *Penicillium* sp(Fig 1,2). Further it was confirmed by microscopic analysis of colony using lacto phenol cotton blue staining method.

Fig 1.Isolation of fungi on RBCA plates **Fig 2.Blackcoloured colonies**

Solubilization index for the 24 isolates was in the range from 0.5cm-0.10cm. solubilization index for *Pseudomonas* sp was observed as 0.9cm10,11and present study also revealed that fungal isolates was 0.6cm for *Aspergillus* sp and 0.5cm for *Penicillium* sp.

In liquid media, fungal isolates produced acid in high amount which is evident from decolourization of broth. Phosphate solubilization by these isolates was observed for 28 days. Quantitative estimation revealed that solubilization initiated after 3 days and was highest on the 7th day. Decrease in pH was observed in all the isolates. Phosphate solubilization was observed in the range of 100µg ml^{-1}to250µg ml^{-1}.Maximum phosphate was solubilizedby *Aspergillus* sp(190µg ml^{-1}), and also it produced significant amount of citric acid, gluconic acid and moderate amount of oxalic acid. Organic acids were also produced including succinic, glycolic and malic acidin small amounts. *Penicillium* sp solublized phosphate(150µg ml^{-1})and also produced low levels of glycolic, citric, succinic,gluconic and oxalic acids.

Phosphatase activity

Phosphatase activity of fungi (*Aspergilus* spand *Penicillium* sp.) were higher than bacteria10.Phosphatase activity of fungi (*Aspergillus* sp and *Penicillium* sp.) were higher than bacteria (*Pseudomonas mallei, Bacillus subtilis*) as reported earlier11**.***Aspergillus* sp solubilised phosphste into organic acid and accumulated organic acids having retention times of 5.5 (approximating the retention time ofcitric acid), and 7.1 min. *Aspergillus* sp isolate also accumulated a small amount of organic acids having retention time of 7.8 min (approximating the retention time of malic acid), and 8.3 min. *Penicillium* sp accumulated malic acid. The results of the present study revealed that *Aspergillus* sp proved to be an efficient strain for phosphate solubilization and *Penicillium* sp needs small genetic manipulation. In addition, no significant amounts of organic acid production was observed from a phosphate solubilizer fungus, *Penicillium*

sp12'.Tricalcium phosphate induced extracellular phosphatase production(119U/ml) compared to other phosphate sources14,15,similar result was observed when tricalcium phosphate was added (10g/l) in the medium. Phosphatase activity by fungal isolates was maximum in the pH4.0-6. 5 , which was similar to the results obtained during optimization studies13for *Trichoderma* sp

The physico chemical characteristics of soil such as pH was neutral, the nitrogen content of the rhizosphere soil was found to be low ,while the amount of Potassium in soil was quite high and phosphorus content was also low14,16,17. The results of the present study on soil properties were in agreement to the results obtained by15which reported that the fertility of soil was moderate.

Conclusion

Two phosphate solubilizing fungi were isolated and identified as *Aspergillus* sp and *Penicillium* sp. Phosphorus solubilization activity of PSM is associated with the release of organic acid and a drop in the pH of the medium.In the present study, there is a decrease in the pH values of the Pikosvikyos media because of the release of organic acids in the cultural medium. Furthermore, the results of HPLC studies revealed that fungi solubilizes Phosphate into organic acid viz., Citric acid, Gluconic acid, Glycolic acid, Malic acid, Succinic acid and oxalic acid, which is evident from their retention time. Consequently it was proved that the isolated *Aspergillus* sp and *Penicillium* sp have the ability to solubilize phosphate which was confirmed by its phosphatase activity. Further molecular characterization of the isolates will provide their genetic relationship with other phosphate solubilizers.

Acknowledgements

Authors are thankful to Dr. Eswarapriya.B, Head, Department of Microbiology & CLT and Management of Dr. Umayal Ramanathan college for women,Karaikudi, Tamilnadu for Technical support, encouragement and guidance during research work.

References

1. Pikovskaya RI (1948) Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Microbiologia .,1948,17: 362-370.
2. Rodriguez H, Fraga R .Phosphate solubilizing bacteria and their role in plant growt promotion.Biotechnol Adv.,1999,17:319-339 .PubMed Abstract.
3. Goldstein, A.H. Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by Gram negative bacteria. Biol. Agric. Hort.,1995,12: 185–193.
4. Rodriguez H, Fraga R, Gonzalez T, Bashan Y. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil.,2006, 287:15-21.
5. Cunningham JE, Kuiack C. Production of citric and oxalic acids and solubilization of calcium phosphate by *Penicillium billai*. Appl Environz Microbiol. 1992;52:1451–1458. [PMC free article] [PubMed]
6. SubbaRao NS .Advances in agricultural microbiology. India: Oxford and IBH Publications Company.,1982 : 229-305
7. Watanabe, F.S., Olsen, S.R. Test of an ascorbic acid method fordetermining phosphorous in water and NaHCO3 extracts from soil.Soil Sci. Soc. Am. Proc.,1965,29: 677–678.
8. Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J. Protein measurement with the folin phenol reagent. *J. Biol. Chem.*,1951.193:265-75
9. Bradford, M.M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*,1976,72:248-254
10. Chen,Y. P., Rekha, P. D., Arun, A. B., Shen, F. T., Lai,W.-A. &Young C.C.Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities.Appl Soil Ecol ., 2016,34: 33–41.
11. Pemila Edith ,Chitraselvi R, Kalidass S, Rajiv Kant. Efficiency of Rhizosphere Bacteria in Production of Indole Acetic Acid, Siderophore and Phosphate Solubilization International Journal of ChemTech Res.2014-2015,7(6),pp 2557-2564

12. Hilda, R., Fraga, R., 1999.Phosphate solubilizing bacteria and their rolein plant growth promotion. *BiotechnoL Adv.*,1999,17: 319–339.
13. Rasha. Daoud, Mohammad.Kher. Tahla., Mohammad Fawaz. Azmeh. Optimization of polygalacturonase production by Trichoderma harzianum on orange peels in submerged fermentation. International.Journal of ChemTech Res., 2016,9(1):359-365.
14. Priya D, D.J.Mukesh kumar, P.T.Kalaichelvan.Optimization and production of Extracellular alkaline phosphatase from Bacillus megaterium .Int.J. ChemTech Res.,2014,6(9): 4251-4258.
15. Manal A.H. El-Gamal, Hanaa A. Abo-Kora and O.N. Massoud.Impact of formulated Azospirillum lipoferum, Bacillus polymyxa and Nostoc muscorum on Wheat productivity .International Journal of ChemTech Res. 2015, 8 (9),: 100-113.
16. S.Anbuselvi L,Jeyanthi Rebecca , Jitendra kumar.Isolation and Characterization of Phosphate solubilizingbacteria from Corn stalk and its activity on soil.Int.J. ChemTech Res., 2015, 8(8):194-196.
17. Hanan S. Siam, Samia H. Ashmaye, Mona G. Abd El-Kader and Awatef A. Mahmoud.Partial Replacement of Integrated Mineral Fertilizer through Biofertilization to Maximize Economic Yield of Faba Bean under Saline Soil Conditions.International Journal of ChemTech Research,2016,9(6): 153-164.

*****