Study of phosphatase, phytase and plant growth promoting activity of phosphate solubilizer strains *Aspergillus awamori* and *Burkholderia latens*

Solanki Vikram[1*], Parmar Himanshu[2] and Bhatt Shreyas[1]
1. Department of Life Sciences, Hemchandracharya North Gujarat University, Patan, Gujarat, INDIA
2. Vrundavan Science College, Ganeshpura, Mehsana, Gujarat, INDIA
*vcsolankicr@gmail.com

Abstract
Enzymes are biocatalysts and they accelerate and mediate the rate of biochemical reactions in the biological system. Sidereophores are small, high-affinity iron chelating compounds secreted by microorganisms such as bacteria and fungi. Indole-3-acetic acid (IAA, 3-IAA) is the most common naturally occurring plant hormone of the auxin class. In the present study, two phosphate solubilizing potent strains, Aspergillus awamori and Burkholderia latens were tested for phosphatases (acid and alkaline) activity, phytase activity, indole acetic acid production and siderophore production.

It was proven that both isolates were able to produce acid phosphatases, alkaline phosphatases and phytase. Both isolates have ability to produce significant amount of IAA. No siderophore production was found for both isolates.

Keywords: Bacteria, fungi, enzymes, IAA and sidephores.

Introduction

Enzymes are biocatalysts and they accelerate and mediate the rate of biochemical reactions in the biological system. Phosphatases are enzymes which hydrolyze the complex organic phosphates into simpler inorganic phosphates by dephosphorylation process which will be further used by the cell to construct the nucleic acid, phospholipids and ATP molecule[19].

There are two types of phosphatases enzyme: alkaline phosphatases and acid phosphatases. The species of soil bacteria like *Bacillus spp., Pseudomonas spp., Enterobacter spp., Azotobacter spp., Burkholderia spp.* etc. and species of soil fungi like *Aspergillus spp., Penicillium spp., Mucor spp., Fusarium spp., Absidia spp.* etc. produce extracellular phosphatases[17-19].

Phytate is a phosphorylated derivative of myo-inositol which is important to store and retrieve phosphorus, inositol and ions in plant development and germination[20]. Phytase effectively catalyzes the release of phosphate from phytate. Phytase hydrolyzes phytic acid to myo-inositol and phosphoric acid[10]. The fungal isolates from genera *Aspergillus, Penicillium, Mucor* and *Rhizopus* are the most active phytase producing microorganisms producing it through phosphates fermentation[12].

Biochemical characteristics of *Burkholderia latens* and *Aspergillus awamori*: Plant growth promoting rhizobacteria (PGPR) and plant growth promoting fungi (PGPF) increase the plant growth through nitrogen fixation, siderophore production, indole acetic acid production, gibberellic acid production, antibiotic production, enzymes production, solubilization of phosphate and antagonism to phytopathogens[11].

Indole acetic acid: The production of indole-3-acetic acid is widespread among fungi and bacteria [5]. IAA is the major and most abundant auxin in plants; it regulates plant growth and development[14]. Amino acid L-tryptophan serves as a precursor for biosynthesis of auxin in higher plants and microbes[15]. More specifically, IAA is a phytohormone which is known to be involved in root initiation, cell division and cell enlargement in plant[1]. Some soil bacteria such as *Pseudomonas spp., Azotobacter spp., Bacillus spp.* and *Burkholderia spp.* as well as some soil fungi like *Aspergillus spp., Trichoderma spp.* and *Penicillium spp.* were reported as potent IAA producers *in vivo* and *in vitro* condition[7].

Siderophore: Siderophores are low-molecular-weight ligands (20–2000 Dalton) produced by bacteria, fungi and plants to solubilize and to take up iron[3,6]. More than 500 different siderophore from bacteria have been described. The iron ligation groups have been tentatively classified into three main chemical types: hydroxamate, catecholate and hydroxycarboxylates.

However, other varieties of siderophore structures have been resolved which include oxazoline, thiazoline, hydroxypyridinone, alpha and beta-hydroxy acids and alpha-keto acid components. The most important property of siderophore is their denticity (number of iron coordinating atoms per molecule) which ranges from bidentate to hexadentate[21].

Material and Methods

Microorganisms: *Burkholderia latens* and *Aspergillus awamori* were isolated from Bt-cotton rhizosphere. Both isolates were studied for their enzymatic activities such as alkaline phosphatases, acid phosphatases and phytase and some biochemical characteristics.

Inoculum preparation: Inoculum was prepared from 3 to 4 days grown *Aspergillus awamori* on potato dextrose agar slant and 2 days grown *Burkholderia latens* on nutrient agar slant. Sterile 0.01 % (v/v) tween-80 solution was added. Both bacterial and fungal cultures were scrapped using an inoculation needle under aseptic conditions. The spore count in the suspension was 10^7cell ml^{-1}, 2% of the both fungal and bacterial suspension were used as inoculum.

Fermentation condition for acid and alkaline phosphatases: Both isolates were grown in 50 ml of PVK broth and incubated at room temperature (35±2 ºC) for 2 to 3 days for bacteria and 5 to 7 days for fungi with different cultural condition. After incubation periods, fermented broth was withdrawn and centrifuged at 10,000 rpm for 15 min. Supernatant was used for acid phosphatasesand alkaline phosphatases activity.

Fermentation condition for phytase: Each of the isolates was grown in 50ml of liquid phytase screening medium containing (w/v) 0.1 % sodium phytate, 1% peptone, 0.2% $(NH_4)_2SO_4$, 0.05% KCl, 0.05% $MgSO_4.7H_2O$, 0.03% $MnSO_4$, 0.03% $FeSO_4.7H_2O$, pH 7.5 in a 250 ml flask and incubated at 40°C for 3 days on a rotary shaker at 200rpm. Crude enzyme was harvested by centrifugation at 10,000 rpm for 10min at 4°C and the clear supernatant was used as the source of phytase.

Assay of acid and alkaline phosphatases activity: The activity of phosphatases (acid and alkaline) produced by the *Burkholderia latens* and *Aspergillus awamori* was estimated by the methods given by Juma and Tabatabai[9].

Culture supernatant was incubated at 37ºC with p-nitro phenyl phosphate and modified universal buffer (pH adjusted to 6.5 and 11 respectively for acid and alkaline phosphatases assay). Swirl the flask and incubate at 37ºC for 1 hr. After incubation, the flask was removed, 0.5 M $CaCl_2$ and 0.5 M NaOH solutions were added to stop the reaction. The flask was swirled again for few seconds. The assay mixture was filtered through Whatmann no. 42 filter paper and O.D was taken at 420 nm in spectrophotometer.

Assay of phytase activity: Phytase activity of *Burkholderia latens* and *Aspergillus awamori* was estimated by Fiske and Subbarow's[4] colorimetric method. Sodium phytate (0.2%) was used as substrate. Sodium phytate was prepared in 0.1M acetate buffer (pH 5.0). The reaction mixture was prepared by the addition of sodium phytate with crude enzyme and incubated at 35ºC for 20 minutes. After incubation, the enzymatic reaction was stopped by adding 5 % (v/v) trichloroacetic acid solution and colour developed by adding colour reagent and O.D was taken at 700 nm by using Bau et al[2].

Indole acetic acid: For indole acetic acid production, *Aspergillus awamori* was inoculated in 50 mL czapek dox broth (pH 6.5) containing 0.1% DL-tryptophan and incubated in shaker at 30ºC for 7 days whereas *Burkholderia latens* was grown in 50 mL nutrient broth containing 0.1% DL-tryptophan and incubated in shaker at 30ºC for 2 days. After incubation, estimation of IAA in the supernatants was done using colorimetric assay[13]. Culture supernatant was mixed with orthophosphoric acid and Salkowsky's reagent. O.D was taken after 30 min at 535 nm in UV/Visible spectrophotometer. The IAA production was determined using IAA standard curve and the result was expressed as µg ml^{-1}.

Siderophore production: Phosphate solubilizing microorganisms like *Burkholderia latens* and *Aspergillus awamori* were checked for siderophore production by using plate method[23]. Pure cultures of *Burkholderia latens* and *Aspergillus awamori* were streaked on center of chrome azurol sulphonate (CAS) agar plate and incubated at 30°C for 4 to 7 days. The colony showing orange zones was considered as siderophore producing strains. The control plate was also incubated under the same conditions and no colour change was observed.

Results and Discussion

In the present study, two phosphate solubilizing potent strains were tested for phosphatases (acid and alkaline) activity, phytase activity, indole acetic acid production, siderophore production and organic acid production.

Activities of all enzymes were measured after optimum incubation time for both isolates. Acid phosphatases, alkaline phosphatases and phytase activity of *Burkholderia latens* was measured after 3 days and for *Aspergillus awamori*, activity was measured after 8 days. According to obtained result, it was proven that both isolates were able to produce acid phosphatases, alkaline phosphatases and phytase (Figure 1). *Burkholderia latens* produced maximum alkaline phosphatases activity followed by acid phosphatases and phytase. *Aspergillus awamori* produced maximum acid phosphatases followed by phytase and alkaline phosphatases. All enzymes activity was expressed in U mL^{-1} min^{-1}.

Aspergillus awamori produced acid phosphatases, alkaline phosphatases and phytase activities as 0.097 ± 0.09, 0.059 ± 005 and 0.07 ± 0.004 U mL^{-1} min^{-1} respectively. *Burkholderia latens* produced alkaline phosphatases, phytase and acid phosphatases activities as 0.065 ± 0.01, 0.034 ± 0.007 and 0.046 ± 0.003 U mL^{-1} min^{-1} respectively.

Saiyad et al[22] reported that phosphate solubilizing bacteria produced low acid and alkaline phosphatases and thus there was less hydrolysis of the substrate and showed lower activity. Jamshidi et al[8] reported that phosphate solubilizing fungi produced significant amount of acid phosphatases and investigated that phosphatases does not act directly on inorganic phosphate solubilization, but phosphatases activity may participate in lowering the pH of the culture medium by dephosphorylation and the production of acids.

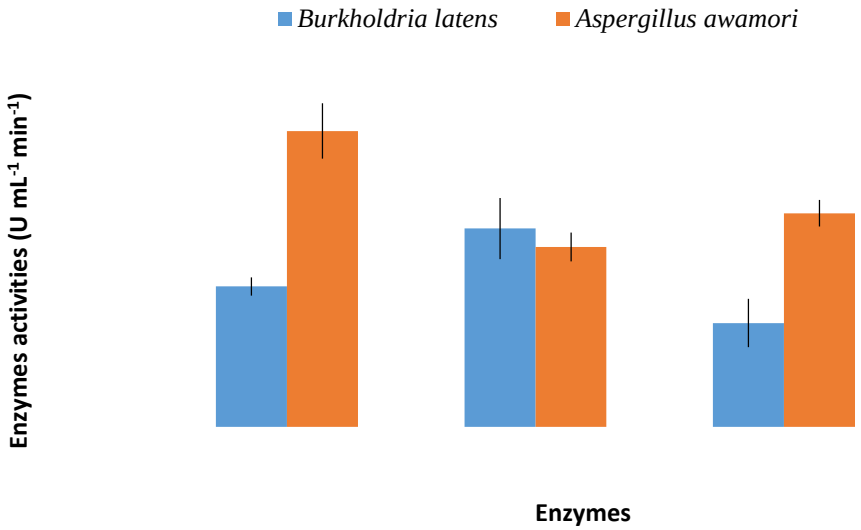

Figure 1: Enzymes activities of *Burkholderia latens* and *Aspergillus awamori*

Indole acetic acid: Both isolates have ability to produce significant amount of IAA. *Burkholderia latens* produced 32.3 ± 2.52 µg mL^{-1} of IAA and *Aspergillus awamori* produced 24 ± 3.51 µg mL^{-1} of IAA. Nailwal et al[16] reported IAA production ranging from 16.4 to 20.8 µg mL^{-1} with tryptophan and 7.8 µg mL^{-1} without tryptophan. Inui-Kishi et al[7] investigated that *Burkholderia spp*. produced 58.34 µg mL^{-1} of IAA.

Siderophore production: PSB and PSF strains were also tested for siderophore production efficiency by CAS plate assay method. In case of both isolates selected for study, there was no siderophore production.

Conclusion

Burkholderia latens and *Aspergillus awamori* produced acid phosphatases, alkaline phosphatases, phytase and indole acetic acid. Both isolates did not show the siderophore production, so these strains can be used as biofertilizers and to check their efficiency at laboratory level to field level.

Acknowledgement

We are thankful to the Principal of Biogas Research and Extension Centre, Gujarat Vidyapeeth, Sadra, Gandhinagar, Gujarat for providing research facility to carry out present work.

References

1. Arshad M. and Frankenberger W.T., Microbial production of plant growth regulators, In: Soil Microbial Ecology: Application in Agricultural and Environmental Management, Metting, Jr. F.B. Eds., Marcel Dekker Inc., New York, USA, 307-347 **(1993)**

2. Bae H.D., Yanke L.J., Cheng K.J. and Selinger L.B., A novel staining method for detecting phytase activity, *Journal of Microbiological Methods*, **39,** 17-22 **(1999)**

3. Chu B.C., Garcia-Herrero A., Johanson T.H., Krewulak K.D., Lau C.K., Peacock R.S., Slavinskaya Z. and Vogel H.J., Siderophore uptake in bacteria and the battle for iron with the host; A bird's eye view, *Biometals*, **23(4),** 601-611 **(2010)**

4. Fiske C.H. and Subbarow Y., The colorimetric determination of phosphorus, *Journal of Biological Chemistry*, **66**, 376-400 **(1925)**

5. Gruen H.E., Auxins and fungi, *Annual Review of Plant Physiology*, **10,** 405-440 **(1959)**

6. Hider R.C. and Kong X., Chemistry and biology of siderophores, *Natural Product Reports*, **27(5),** 637-657 **(2010)**

7. Inui-Kishi R.N., Kishi L.T., Picchi S.C., Barbosa J.C., Lemos M.T.O., Marcondes J. and Lemos E.G.D.M., Phosphorus solubilizing and IAA production activities in plant growth promoting rhizobacteria from Brazilian soils under sugarcane cultivation, *ARPN Journal of Engineering and Applied Sciences*, **7(11),** 1446-1454 **(2012)**

8. Jamshidi R., Jalili B., Bahmanyar M.A. and Salek-Gilani S., Isolation and identification of a phosphate solubilizing fungus from soil of a phosphate mine in Chaluse, Iran, *Mycology*, **7(3),** 134-142 **(2016)**

9. Juma N.G. and Tabatabai M.A., Phosphatase activity in corn and soybean roots: Conditions for assay and effects of metals, *Plant and Soil*, **107(1),** 39-47 **(1988)**

10. Kumar D., Rajesh S., Balashanmugam P., Rebecca L.J. and Kalaichelvan P.T., Screening, optimization and application of extracellular phytase from *Bacillus megaterium* isolated from poultry waste, *Journal of Modern Biotechnology*, **2(2),** 46-52 **(2013)**

11. Lenin G. and Jayanthi M., Indole acetic acid, gibberellic acid and siderophore production by PGPR isolates from rhizospheric soils of *Catharanthus roseus*, *International Journal of Pharmaceutical and Biological Archives*, **3(4),** 933-938 **(2012)**

12. Liu B., Rafiq A., Tzeng Y. and Rob A., The induction and characterization of phytase and beyond, *Enzyme and Microbial Technology*, **22(5),** 415-424 **(1998)**

13. Loper J.E. and Schroth M.N., Influence of bacterial sources of indole-3-acetic acid on root elongation of sugar beet, *Phytopathology*, **76(4),** 386-389 **(1986)**

14. Moore T.S., Biochemistry and physiology of plant, 2nd edition, New York: Springer-Verlag Inc., 285 **(1989)**

15. Muleta D., Microbial inputs in coffee (*Coffea Arabica L.*) production system, Southwestern Ethiopia, 21-23, Doctoral thesis, Uppsala, Sweden **(2007)**

16. Nailwal S., Anwar M.S., Budhani K.K., Verma A. and Nailwal T.K., *Burkholderia sp.* from rhizosphere of *Rhododendron arboretum*: Isolation, identification and plant growth promotory (PGP) activities, *Journal of Applied and Natural Science*, **6(2),** 473-479 **(2014)**

17. Nenwani V., Doshi P., Saha T. and Rajkumar S., Isolation and characterization of a fungal isolate for phosphate solubilization and plant growth promoting activity, *Journal of Yeast and Fungal Research*, **1(1),** 009-014 **(2010)**

18. Oliveira C.A., Alves V.M.C., Marriel I.E., Gomes E.A., Scotti M.R., Carneiro N.P., Guimaraes C.T., Schaffert R.E. and Sa N.M.H., Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome, *Soil Biology and Biochemistry*, **41(9),** 1782-1787 **(2009)**

19. Priya D., Kumar D.J.M. and Kalaichelvan P.T., Optimization and production of extracellular alkaline phosphatase from *Bacillus megaterium*, *International Journal of ChemTech Research*, **6(9),** 4251-4258 **(2014)**

20. Raboy V., Myo-inositol-1,2,3,4,5,6-hexakisphosphate, *Phytochemistry*, **64(6),** 1033-1043 **(2003)**

21. Raymond K.N. and Dertz E., *Biochemical and physical properties of siderophores*, In Crosa J.H., Mey A.R. and Payne S.M., Eds., Iron transport in bacteria, 1st edition, ASM press, Washington, DC, 3-17 **(2004)**

22. Saiyad S.A., Jhala Y.K. and Vyas R.V., Comparative efficiency of five potash and phosphate solubilizing bacteria and their key enzymes useful for enhancing and improvement of soil fertility, *International Journal of Scientific and Research Publications*, **5(2),** 1-6 **(2015)**

23. Schwyn B. and Neilands J.B., Universal chemical assay for the detection and determination of siderophores, *Analyi1cal Biochemistry*, **160(1),** 47-56 **(1987)**.

(Received 06th March 2021, accepted 21st May 2021)