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Short Communication

A Comparative Study of Plant Growth Promoting Potential of Phytase Enzyme from Bacterial and Fungal Source

Seema Dwivedi

Dean, School of Biotechnology, Gautam Buddha University, Gr. Noida, India.

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**Corresponding Author:*
E-Mail: *seemaadwivedi@gmail.com*

Abstract

In this investigation, we compare plant growth promotion of phytase isolated from bacteria and fungus. In the plate assay, fungal isolates (genera Aspergillus sps.,Penicillium sps. And Trichoderma sps.) and bacterial isolates (genera Streptomyces sps., Cohella sps. and Microbispora sps.) were selected to evaluate phytase activity. Penicilliumsps.showed the highest degradation ability and Cohella sps. exhibited comparatively high degradation ability. Anphytase enzyme was identify and usable for industrial application. The enzyme was purified by different method. This protein exhibited a gel filtration and SDS-PAGE. In plant growth promoting activity the isolated PPB14 produced the highest shoot and root lengths of 16.22 and 20.44 cm, corresponding to increases of 61.23 and 50.23 % . The amount of all fungal isolates was showed to be significantly ($p < 0.04$) higher over uninoculated control.

Keywords: Phytase, purification, enzyme assay, Aspergillus spp., microbial sources

Introduction

Phytase, extracellular enzymeswhich is use in non-ruminant nutrition. These enzyme effect on nutrient retention, availability of many nutrients (Trace minerals and macroelements) and performance **[20, 27, 34, 35, 47, 48].**

Phytateenzyme used by plants as phosphorous, inositol and a range of minerals **[3, 37].** Phytases assemble the decompose of phytate enzyme into phosphate (inorganic) and lower phosphorylated myo-inositols. While phytases have been isolated from a microorganism such as plants and animal tissues **[26, 28, 32]**

Plant-microbe interaction has specially regarding beneficial bacteria. Plants are inhabited by endophytes, which control the host (plant) resolving responses as a result of interactions**[13]** without harm the host (plant) **[38]** (shown in **Fig 1**). The endophytesare likely to have different specialization, functions, adaptations and competence**[2, 30]**. Furthermore, it is likely that only specialized endophytic strains are capable of colonizing and surviving in the reproductive plant organs **[2, 53]** and that only bacteria with competitive and adaptive

colonization characteristics could inhabit the seeds **[30]**.

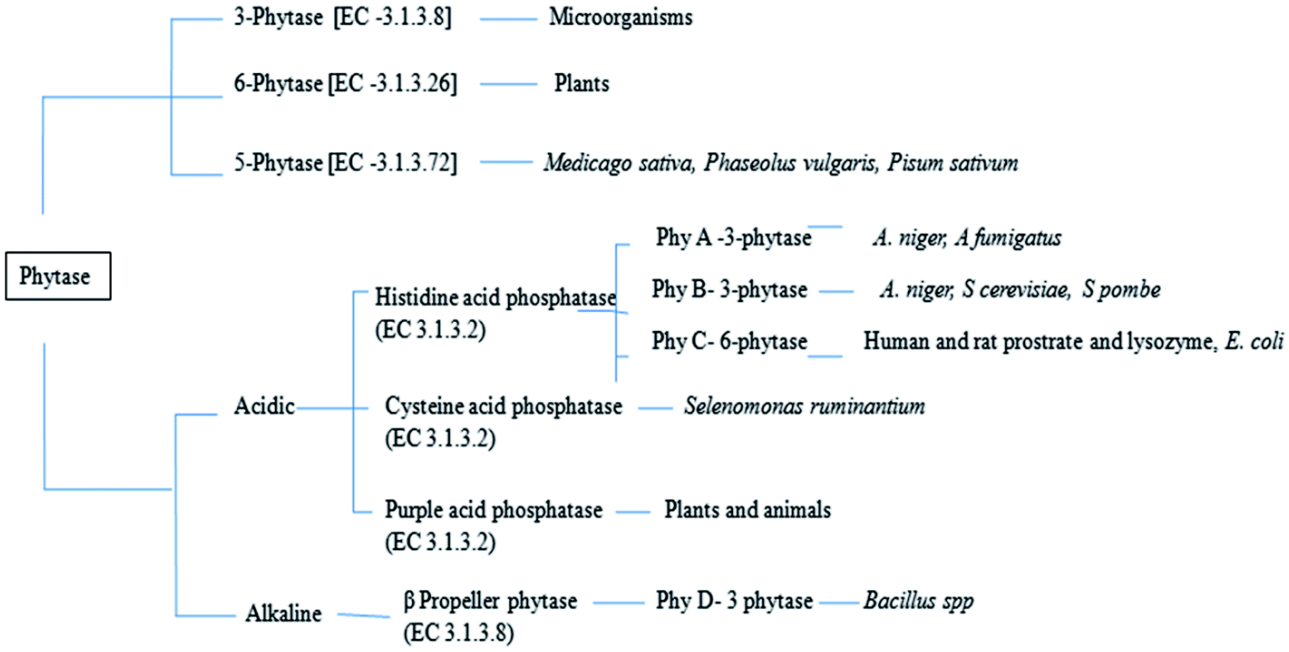

Figure 1: Classification of Phytase

Phosphorus is the most important element in the nutrition of plants. Phosphorous plays an important role in virtually all major metabolic processes in plant including photosynthesis, signal transduction, macromolecular biosynthesis, energy transfer, and respiration **[22]**and nitrogen fixation in legumes **[46].** Although Phosphorous is abundant in soils (inorganic and organic forms) it is a major limiting factor for plant growth as it is in an unavailable form for root uptake. Inorganic Phosphorous occurs in soil, insoluble mineral complexes, some of them appearing after frequent application of chemical fertilizers. These insoluble, precipitated forms cannot be absorbed by plants **[39]**.

The degradation of phytate is catalyzed by enzymes called phytases, produced by various organisms (plants, animals, and micro- organisms) **[23]** as shown in **Table 1.** A large number of microorganisms have been identified as phytase producers **[18, 19, 31, 36, 57]**. Phytate-hydrolyzing microorganisms inhabit wide and diverse environments, indicating that the biodegradation of phytate might be accomplished in a variety of ecosystems **[16]**. It has been shown that phytate-utilizing bacteria may improve the acquisition of Phosphorous by plants **[41]**; however, few studies have focused on the degradation of phytate in organic wastes **[8]**. Composting may improve the bioavailability of Phosphorous through the degradation of organic Phosphorous by microorganisms **[9]**.

Table 1. Microorganisms and enzymes responsible for plant growth promotion

Enzyme type	Microbial strains	Reference
Acid phosphatase	*Pseudomonas sp.*	Richardson *et al.* 2001a, b
	Burkholderiacepacia	Unno*et al.* 2005
	P. fluorescens	Ryu*et al.* 2005
	Citrobacterfreundi	Thaller*et al.* 1995
	Proteus mirabali	Thaller*et al.* 1995
Phytase	*B. subtilis*	Ryu*et al.* 2005
	Pseudomonas putida	Richardson and Hadobas 1997
	B. circulans	Hameeda*et al.* 2006
	Aspergillusniger	Hayes *et al.* 2000

Material and Methods

Isolation of Bacterial isolates and Fungal isolates-

Sabouraud"s dextrose agar medium used for the growth of fungus. Nutrient agar medium (NAM) for the growth of bacterial cultures **[33]**.

Production and determination of phytase enzyme Activity-

The isolated organisms weredetermined for their ability to produce phytaseenzyme on the phytase-screening medium (PSM) **[21].**Phytaseenzyme activity in liquid Phytase screening medium was determined by following method of **[7]** as used in **[24].**

Plant growth promoting activities of isolated Bacterial *sps*.-

Biological Nitrogen Fixation

Nitrogenase activity of isolates was resolved by the ethylene production assay/acetylene reduction assay **[14]**. Isolated culture were inoculated to an airtight 30 ml vial containing 10 ml nitrogen-free basal semi-solid medium and grown for 48 h at 28 ± 2°C. In pellicle formation, the bottles were injected with 10% (v/v) acetylene gas and incubated at 28 ± 2°C for 24 h. Ethylene production was measured using a G-300 Gas Chromatograph fitted with a Flame Ionization Detector and a Porapak-N column. Carrier gas were used such as hydrogen and oxygen, with a flow rate of 4 kg/cm2, and the column temperature was maintained at 165°C. The rate of N2 fixation expressed as the quantity of ethylene accumulated based on the standard curve and peak-area percentage.

Indole-3-Acetic Acid Production

For detection and quantification of indole-3-acetic acid (IAA) production by bacterial isolates, isolated colonies were inoculated into Jensen's broth **[1]**. The culture was incubated at 28 ± 2°C with continuous shaking at 125 rpm for 48 h**[41].** Approximately 2 mL of culture solution was centrifuged at 15000 rpm for 1 min, and a 1 mL aliquot of the supernatant was mixed with 2 mL of Salkowski's reagent and incubated 20 min in darkness at room temperature **[11]**. IAA production was observed as the development of a pink-red color, and the absorbance was measured at 530 nm using a spectrophotometer.

Analysis of Phosphate Solubilization efficiency-

Phosphate solubilization by bacterial isolates was done by the method of **[33]**. Plates were made in duplicate for each bacterial isolate using Pikovskaya agar medium. Bacterial culture was point inoculated at the centre of Pikovskaya agar plate and incubated in incubator at 28°C for 7 d. **[27]**.

Production and purification of phytase enzyme from isolates

Crude extracellular phytase was concentrated using an Amicon ultrafiltration cell with a 30 kDa molecular cut off PM 30 Amicon membrane at 4 °C. The resulting retentate was acidified with 0.1 M HCl until it reached pH 2.0, it was maintained under slow agitation for 30 min at 4 °C and centrifuged (10,000 × g , 10 min at 4 °C). A 10 mL aliquot of the supernatant was loaded onto a 20 mL DEAE-Sepharose CL-6B column that had been pre-equilibrated with 50 mM sodium acetate buffer, pH 5.0 containing 0.5% (w/v) sucrose**[42]**. Unbound protein was removed by washing with four bed volumes of equilibration buffer. Bound protein was then eluted using a linear salt gradient at 1 mL/min; 4 mL fractions were collected. Pooled activity (3 mL) was loaded onto a Sephacryl S-300 HR column (2.6 × 60 cm) that had been pre-equilibrated with a 25 mM sodium acetate buffer, pH 5.0, containing 0.5% (w/v) sucrose at 0.8 mL/min using a AKTA Purifier fast protein liquid chromatography, from which 2.5 mL fractions were collected. Protein concentration was determined by the Bradford method using bovine serum albumin as the standard at 0–20 µg/mL **[14]**.

Effect of Phytase enzyme on Plant growth

The microbial population in the rhizosphere utilizes the organic substances exuded from plant roots for their growth and reproduction **[17, 56].** Therefore these microbes have to compete with plant roots for other elements such as phosphorus. Major part of soil organic phosphorus is phytates, mainly consisting of inositol penta- and hexaphosphates **[4]**. Microbial population utilizes this Phosphorous by secreting phosphatase and phytase in the rhizosphere **[10, 43, 49, 50, 51, 55, 58, 59].**

Result and Discussion

Isolation and Characterization of Bacterial Isolates and Fungal isolates

The fungal isolates used in this study were obtained on Sabouraud"s dextrose agar (SDA) are ***Aspergillus niger*** **(PPF5)**, ***Penicillium sps.*** **(PPF10)**, ***Trichoderma sps.*** **(PPF19)**. The cellulolytic bacterial isolates examined in this study, which were obtained from Nutrient Agar Media (NAM) were ***Streptomyces sps.*** **(PPB7)**, ***Cohnella*** **(PPB14)**, ***Microbispora*** **(PPB22)** as shown in Fig 2.

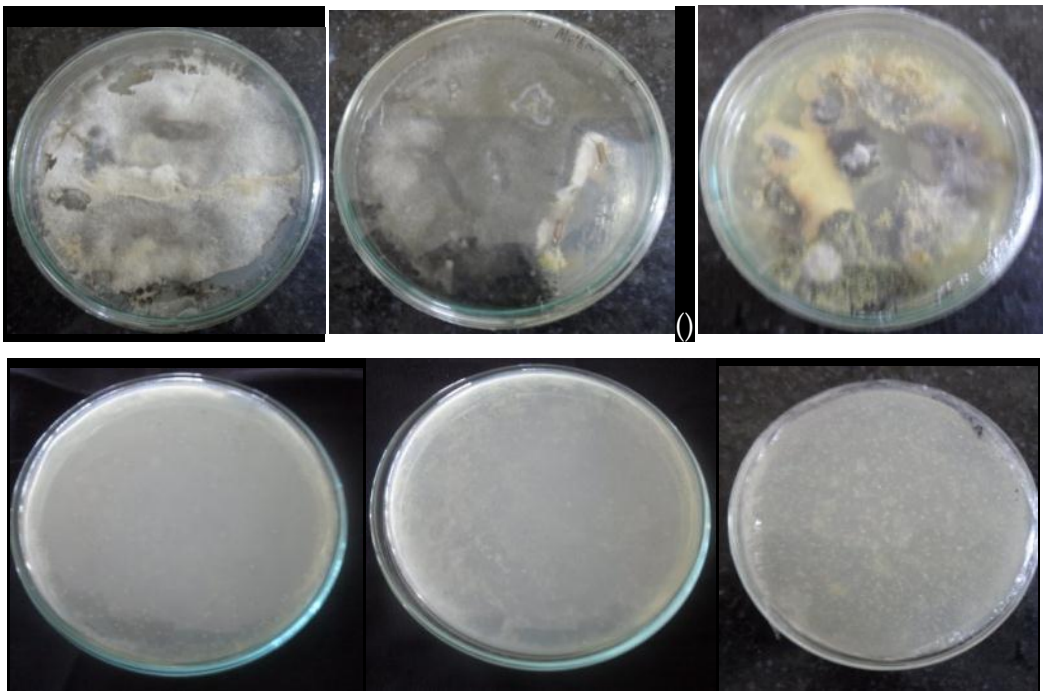

Figure2: PenicilliumSps., Aspergillusniger, Trichodermasps. ,Actinomycetessps.,Cohellasps., Microbisporasps.

****PPF-Phosphate Producing Fungus, PPB-Phosphate Producing Bacteria***

Production and determination of phytase Activity

The liberated inorganic Phosphorous in the solution was resolved by colorimetrically method using reagent (malachite green), and inorganic Phosphorous in the culture was also determined using the reagent such as malachite green.

Plant Growth Promotion Activity of Isolated bacterial sps.

All isolated bacterial *sps.*extremely increased the growth of Plant related to non-inoculated controls. Treatment with isolate PPB14 produced the highest shoot and root lengths of 16.22 and 20.44 cm, corresponding to increases of 61.23 and 50.23 % above control treatments. However, the maximum shoot and root weight enhancement was observed in PPB7 -treated plants. Treatment with isolate PPB7 produced shoot fresh and dryweights of 4.89 and 0.55g plant−1, which were 69.34 and 99% higher than those of control plants. Similarly, treatment with

isolate PPB7 produced root fresh and dry weights of 2.89 and 0.24 plant, corresponding to increase of 90.89 and 125.67% above control treatments.

Analysis of phosphate solubilization efficiency

The isolatedfungus (PSF)ranged from 1.04 to 3.08 at 7 days of incubation at 25–28°C. Among the screened PSF isolates, PPF5 (*Aspergillus sp*.) was the most efficient phosphate solubilizer on PV plates with SI = 2.89 and PPF10 (*Penicillium sp*.) with SI =1.99, whereas the smallest SI of 1.08 was detected from the isolatePPF19(as shown in **Table 2**). The amount of all fungal isolates was showed to be significantly ($p < 0.04$) higher over un-inoculated control. The minimum Phosphate-solubilized from TCP broth on day 5, afterwards the solubilized Phosphorous increased up to 15 days of incubation. Accordingly, the mobilized phosphate values in the medium ranged between (92.07–778.77 µg mL−1) among different fungal isolates during 21 days of incubation. The maximum amount of solubilized phosphate (724.67 µg mL−1) was recorded from PPF5 (*Aspergillus sp*.) inoculated culture filtrates followed by (*Aspergillus sp*.) PPF5 (607.30 µg mL−1), (*Aspergillus sp.)* PPF5 (489.05 µg mL−1), and (*Penicillium sp*.) PPF10 (389.10 µg mL−1). The minimum concentration of soluble-P (230.20 µg mL−1) was recorded in the cultures of (*Trichoderma sp*.) PPF19 during 15 days of incubation time. In further incubation (at day 20), decline in the mobilized phosphate was recorded in all cases of the test fungal isolates that reached up to 165.56 µg mL−1 (minimum value) in case of PPF5 and 489.52 µg mL−1 (maximum value) in case of the isolate PPF10 inoculated culture filtrates.

Production and purification of phytase enzyme from isolates

Purification of enzyme was attained by ultrafiltration followed by ion exchange, acid precipitation and chromatography (Gel filteration). After these steps, the fractions adsorbed on DEAE-Sepharose demonstrated phytase activity. Subsequently, peak with phytase activity was achieve when this fraction was submitted to gel filtration chromatography. A purification factor of 62.33 was reported with 13% yield. Purification was approved by SDS PAGE analysis, and a band was detected on the non-denaturing electrophoresis gels. Previous studies on fungal phytase purifications present comparable purification factors. The PPF5 phytase enzyme was purified 42 times with a 18.4% yield and Thermomycesphytase purification demonstrated a purification factor of 37.88 fold and 2.89% yield.

Table 2: Solubilization index range of the isolates on solid Pikovskaya's agar plate

Isolates	SI range
PPF5	1.20- 2.42
PPF10	1.42-2.48
PPF19	1.08-1.99
PPB7	1.2-2.12
PPB14	0.95-1.12
PPB22	1.1-1.67

Effect of Phytase enzyme on plant growth

Growth-promoting microorganisms (PGPM) show growth promoting effects on plants. The microbes' population in the rhizosphere uses the organic substances exuded from roots for their growth and reproduction. Therefore Plant growth promoting microorganisms have to clash with roots for other elements such as

phosphorus. Many bacterial isolates were able to utilize insoluble phytates. Some PGPM showed positive effect of plant growth promotion. A fungus, *Aspergillussps.* Isolated from rhizosphere exhibit plant growth promoting effects due to the solubilization of inorganic phosphates, production of phytase enzyme. The fungal inoculum significantly increases the length of root and shoots and dry matter in Plants over the control.

Conclusion

Phytase enzyme is essential source of organic phosphorous. It cannot be instantly promote by plant and strongly adsorbed by soil. Rhizosphere supports the evolution and activity of microbial community, including microorganism able to support plant growth. They colonize root and increase plant growth (indirect and direct mechanism). Because of the excellent features displayed, this phytase appears to be a promising enzyme for use in animal feed. Its high thermostability suggests that the enzyme is suitable for industrial use because high temperatures are usually encountered in industrial animal food processing. Its high ability to hydrolyze phytate in acidic conditions as well as its high resistance to proteolytic enzymes also suggest that it may effectively release phytic phosphorus in the animal digestive tract.

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