Original Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ISOLATION, SCREENING OF PHYTATE DEGRADING FUNGI (*ASPERGILLUS NIGER*) AND OPTIMIZATION OF SUBMERGED FERMENTATIVE PRODUCTION OF PHYTASE

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ABSTRACT

Microbial phytases are having several applications and one of the important application is it can be used as an animal feed supplement, not only to improve phytate digestibility and nutritive value of plant based foods but also reduces the environmental phosphorus pollution. In the present study, 12 fungal strains were isolated from different poultry soil wastes (Tirupati & surrounding areas) and screened for phytase activity by using phytase screening medium (PSM). Among the isolates one strain was found to possess potent phytase producing activity and identified as *Aspergillus niger* which has been selected for further studies. Phytase production by *A.niger* was evaluated in submerged fermentation. Effect of different culture media on phytase production was assessed and fermentation parameters were optimized. Physical parameters (temperature, pH), chemical parameters (different carbon and nitrogen sources and mineral salts) Optimization studies were studied. Maximum phytase activity of 465 U/ml was found at 30°C in submerged cultivation. The activity was found to decline above 30°C. The fungus failed to grow above 50°C. Phytase was most active at pH 5 (456 U/ml) with an increase in the pH of the medium enzyme activity declined. Optimization studies indicated that glucose was more suitable carbon source for maximum (478 U/ml) phytase production. Various nitrogen sources were used in optimization process where peptone has exhibited maximum activity of 482U/ml. It was found in our results that all the metal salts (sodium chloride, magnesium sulphate, manganese sulphate and copper sulphate) used in the study had a negative impact on the enzyme production.

KEYWORDS: **phytate-degrading enzyme, feed supplement, *Aspergillus niger*, optimization, submerged fermentation, nitrogen source, carbon source.**

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INTRODUCTION

Phytic acid, Myo-inositol hexaphosphate is the storage form of phytic acid (Phytate) in plant seeds. Phytases catalyze the hydrolysis of phosphomonoester bonds in phytic acid, thereby releasing lower forms of Myo-inositol phosphates and inorganic phosphate. The monogastric animals (pig, poultry, fish) are unable to utilize phosphorous from phytic acids, because their intestine lacks phytase activities hence feed must be supplemented with inorganic phosphate. Phytase enzyme preparations have a wide range of applications in animal and human nutrition. Most foods of plant origin contain 50 to 80% of their total phosphorus as phytate. [1]Phytase-bound phosphorus is poorly utilized by monogastric animals, due to low phytate-degrading capability in the gut. [2, 3]Therefore the manure of the animals contains high amount of phosphorous that may cause environmental pollution, eutrophication of water bodies, particularly in areas of intensive livestock production. To combat environmental pollution there is necessity of phytase production for all commercial and environmental applications. Microbial phytases vary in their enzymatic action depending on the source of phytate may be differences in some properties such as substrate specificity, resistance to proteolysis and catalytic efficiency. [3]

MATERIALS AND METHODS

A phytase-producing strain of *Aspergillus niger* was isolated in our studies (Figure 1). This fungal culture was preserved and maintained by means of monthly subculture on PDA agar and storage at room temperature. Inoculation was done by cutting solid fungal medium (4 x 4 mm) from the growing edge of the culture and transferred to the centre of fresh medium.

Figure 1
Zone of hydrolysis produced by phytic acid degrading organism (A.niger)

Submerged Fermentation (SmF)
Medium with carbon and nitrogen source were used for submerged fermentation studies. [5] In the present study *Aspergillus niger* was cultivated in different nutrient media such as potato dextrose broth (PDB), Czapekdox broth (CDB), Complete medium (CM) and synthetic medium (SM) to monitor the efficiency of phytase enzyme production and growth of fungi. Complete medium (CM) has been used for further optimization studies because of potential enzyme activity.

Phytase Assay
Phytase enzyme assay was carried out by the method of Heinonen and Lahti [4]1 ml diluted crude enzyme sample was mixed with 0.5 ml of 0.2 M acetate buffer and 0.5 ml of 15 mM Sodium phytate. The mixture was incubated for 40 minutes at 45°C. Reaction was terminated by adding 2 ml of 15% Trichloroacetic acid. Aliquot of 0.5 ml was taken from the above reaction mixture and mixed with 4 ml of AAM solution (2: 1: 1, Acetone; Ammonium molybdate; Sulphuric acid). To the same mixture 0.4 ml of 1 M Citric acid was added, after mixing properly O.D was measured at 355 nm. Phytase activity was calculated and expressed in terms of U/ml.

Optimization of physical parameters
Effect of temperature on phytase production
To determine the optimum temperature for maximum phytase production by *Aspergillus niger* the fungus was cultivated at different temperatures from lower to higher range. The temperature ranged from 10- 50°C.

Effect of pH on phytase production
The organism was cultivated at different pH levels ranging from 3.0- 8.0.

Optimization of chemical parameters
Effect of different carbon sources, nitrogen sources and metal salts on the phytase enzyme yield were optimized according to the method of Heinonen and Lahti [4]

Effect of carbon source
The effect of different sugars on the growth and enzyme production by *Aspergillus niger* was studied by supplementing the sugars at 1% in the CM instead of glucose. Complete medium with glucose served as control. The medium with glucose served as control. The sugars used were fructose, lactose, maltose, xylose and sorbitol. Broth was quantitatively checked for phytase enzyme.

Effect of nitrogen source
Different nitrogen sources such as yeast extract, peptone, ammonium sulphate and potassium nitrate were added to complete medium at 0.5% level. Medium with yeast extract served as control. The flasks were inoculated and incubated at 30°C for phytase enzyme activity up to 5 days.'

Effect of metal salts

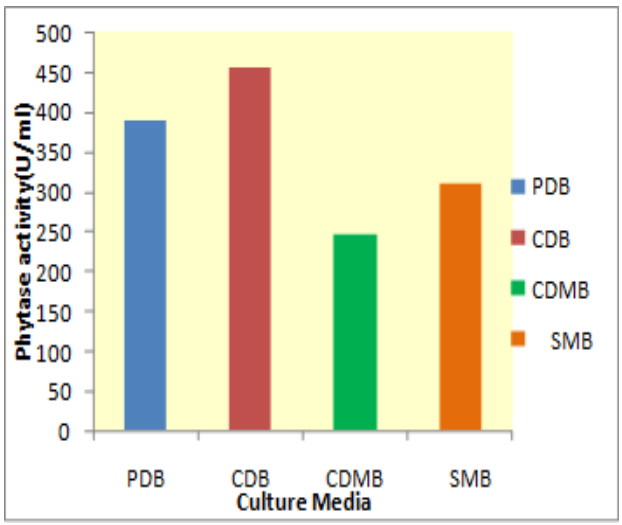
Effect of some of the metal ions on phytase enzyme production by *Aspergillus niger* was determined by incorporating the complete medium with different metal salts such as sodium chloride, magnesium sulphate, manganese sulphate and copper sulphate at a concentration of 0.1%. After incubation broth was assayed for enzyme activity.

RESULTS

Aspergillus niger was cultivated in different broth media to compare the effect of media components on the production of phytase enzyme activities (Figure 2). Complete medium has shown an activity of 455U/ml. This was followed by Potato dextrose medium, synthetic medium (388U/ml, 311U/ml) and. Czapekdox medium (247 U/ml) was found to be less suitable for phytase production in liquid culture medium. The results are shown in Figure.3.

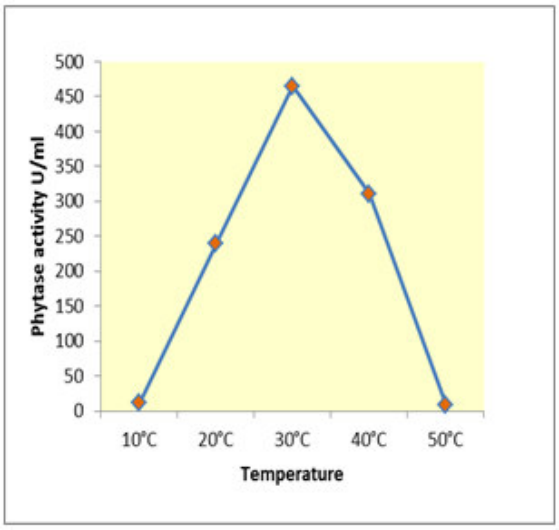
Figure 2
Growth of Aspergillus niger in CDB

Figure 3
Effect of different culture media on phytase production



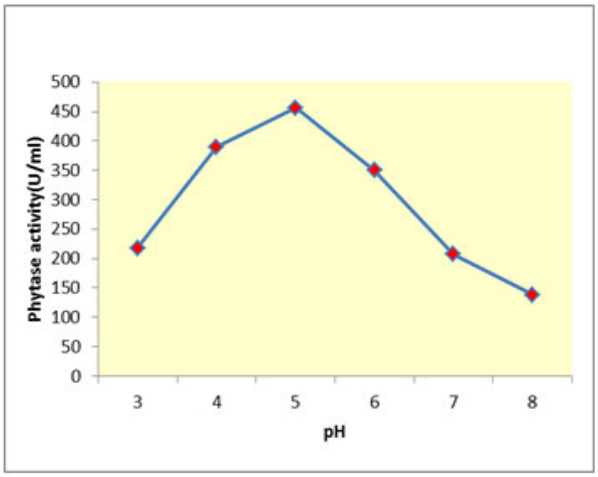
Complete medium was selected for further studies. *A. niger* was cultivated at various temperature ranging from 10- 50°Cunder submerged fermentative conditions. Maximum activity of 465 U/ml was found at 30°C in submerged ultivation. The activity was found to decline above 30°C. The fungus failed to grow at 10°C and above 50°C. Optimum temperature for maximum phytase activity was 30°C (Figure 4).

Figure 4
Effect of temperature on phytase production by Aspergillus niger inSmF



The optimum pH for the phytase enzyme production was carried out at various pH levels ranging from 3.0 to 8.0. Maximum enzyme activity of 456 U/ml was found at pH 5. Enzyme activity declined with increase in the pH of the medium (Figure 5).

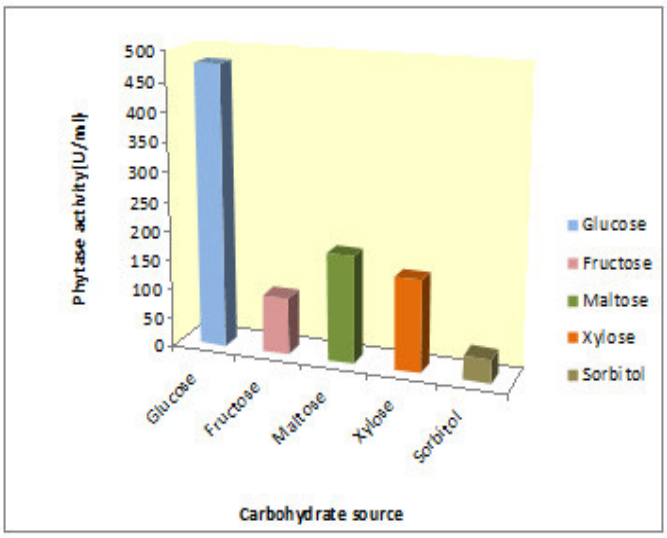
Figure 5
Effect of pH on phytase production by Aspergillus niger in SmF



Optimization of chemical parameters

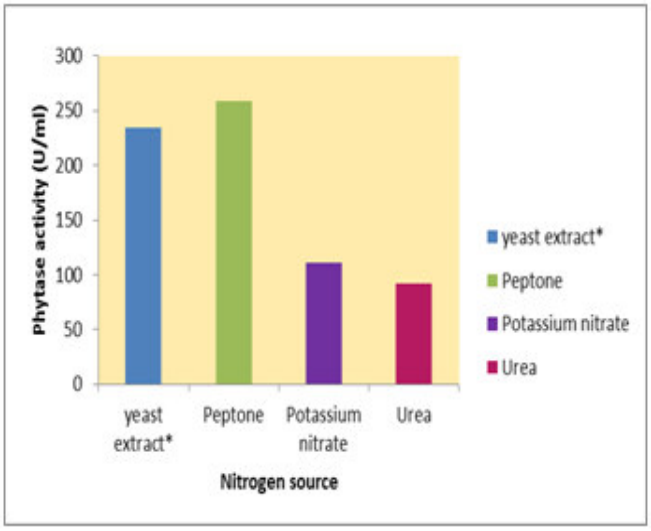
Effect of different carbon, nitrogen and metal salts on the phytase enzyme yield was studied. Fungus was cultivated in a fermentation medium containing specific sugars at 1% level. The medium with glucose served as control and had an activity of 478U/ml activity. The results of SmF indicated that glucose was more suitable for maximum phytase production whereas for other sugars like Fructose, Maltose, Xylose and Sorbitol phytase activity ranged from 40-182U/ml (Figure 6).

Figure 6
Effect of sugars on phytase production by Aspergillus niger



Effect of different inorganic nitrogen sources on the production of phytase enzyme was also studied in medium supplemented with 0.5% of four different nitrogenous compounds such as peptone, potassium nitrate, urea and yeast extract served as control. Among the nitrogen compounds used, the medium with peptone showed maximum activity of 475 U/ml. Potassium nitrate and urea with lowest activities (111 U/ml, 92 U/ml), control activity is 234 U/ml. (Figure 7).

Figure 7
Effect of Nitrogen sources on phytase production by Aspergillus niger



Different metal salts were added to the complete medium and their effect on the phytase enzyme activity was evaluated. It was found in our results that all the metal salts used in the study had a negative impact on the enzyme production except control (466 U/ml) without metal salt addition.

DISCUSSION

The present study was focused on isolation and screening of fungal strains for phytase enzyme from poultry waste soil collected from Tirupati and surrounding areas. Certain physical parameters such as the effect of temperature and pH on phytase production by *A. niger* were studied. Phytase production is influenced by media components, especially carbon and nitrogen sources and metal ions. [5] In the present investigation, the trend in enzyme production increased up to 30°C was observed after that phytase activity gradually decreased. This is in support of earlier reports where they have shown that optimum pH between 4.0 to 6.0 and temperature 25°C-30°C is optimum for filamentous fungi. [7-9]The effect of supplementation of the carbon sources achieved the maximum phytase productivity and Glucose was considered as a simple and easy carbon source consumed by microorganisms.[10]Pinky et al [11] have studied the impact of supplementation of organic and inorganic nitrogen sources to the fermentation medium. Peptone has shown enhancement of enzyme production and represented that there was 1.75-fold increase when compared to the control (without addition of peptone). The study of different organic and inorganic nitrogen sources appeared that peptone was the most favourable

nitrogen source for enzyme production. Metal salts had negative impact on phytase production. The use of microbial phytase as a feed additive has been examined several times over the last20 years, resulting in improved phosphorus availability from poultry and swine feed. Phytase has the potential to reduce the amount of phosphate in poultry and swine excretes by enhancing phosphorus retention by the animal. With the increased concern for the environment and the nutritional bioavailability for the monogastric animals, there is an increased demand for phytase enzyme in feed processing.

ACKNOWLEDGEMENT

The authors thank Department of Applied Microbiology, DST-CURIE central laboratory, Sri Padmavati Mahila Visvavidyalayam for the facilities provided to carry out this research.

REFERENCES

1. Harland BF Morris ER Phytate: a good or a bad food component. Nutrition Research, 1995;(15):733-754.
2. Ravindran V, Bryden LW Kornegay ET. Phytates: occurrence, bioavailability and implications in poultry nutrition. Poult. Avian Biol. 1995;(6):125–143
3. Farrell, DJ. Martin E., duPreez JJ., Bongarts M. Betts M. Sudaman A. Thomson E. et al. The beneficial effects of a microbial phytase in diets of broiler chickens and ducklings. J. Anim. Physiol. Anim. Nutr. 1993;(69):278– 283.
4. Heinonen, JK Lahti RJ. A new and convenient colorimetric determination of inorganic orthophosphate and its application to the assay of inorganic pyrophosphatase. Anal. Biochem. 1981;(113):313-317
5. Sreeraamulu.G Srinivasa, DS. NandK Joseph Lactobacillus amylovorus as a phytase producer in submerged cultureLetters in AppliedMicrobiology1996;(23)385- 388.
6. Ashima V Satyanarayana T. Phytases: Microbial Sources, Production, Purification, and Potential Biotechnological Applications Biotechnol. Lett. 2001;(23)7: 551-554.
7. Casey A, Walsh G. Purification and characterization of extracellular phytase from Aspergillus nigerATCC 9142. Bioresource Technology. 2003;(86) 2:183–188
8. Oh BC, Choi WC, Park S, Kim YO, Oh TK. Biochemical properties and substrate specificities of alkaline and histidine acid phytases. Applied Microbiology and Biotechnology. 2004;(63) 4:362–372.
9. Papagianni M, Nokes SE, Filer K. Production of phytase by Aspergillus niger in submerged and solid-state fermentation. Process Biochemistry. 1999;(35):397–402.
10. Hassouni M, Ismail I, Alaoui PI, Gaime C, Augur S, Rousses. Effect of culture media and fermentation parameters on phytase production by the thermophilic fungus mycelophothora thermophila in solid state fermentation, Mycologi aplicada Int., 2006;(18): 29–36
11. Pinky Gautam, Sabu A, Ashok Pandey, George Szakacs , Carlos R Soccol. Microbial production of extra-cellular phytase using polystyrene as inert solid support. Bioresource Technology 2002;(83) 3: 229- 233.