

Optimization of Production Media of Novel Phytase from *Aspergillus Niger* Using Wheat Bran Waste

Manish Kumar¹, Sushma²

¹Ph.D. Research Scholar, Department of Biochemistry & Biochemical Engineering
Sam Higginbottom Institute of Agriculture, Technology & Sciences
(Deemed-to-be-University) Allahabad-211007 (U.P.) India

²Assistant Professor, Department of Biochemistry & Biochemical Engineering
Sam Higginbottom Institute of Agriculture, Technology & Sciences
(Deemed-to-be-University) Allahabad-211007 (U.P.) India

Abstract: *Phytase, an acid phosphatase enzyme, efficiently cleaves phosphate moieties from phytic acid thereby generating myo-inositol derivatives and inorganic phosphate, was indentified during screening of endophytic fungi Aspergillus niger. Phytase was produced in selective media at room temperature for 3 days. Production media was also optimized varying the concentration of ingredients from 5 to 25g by using solid-state fermentation for 7 days to check the activity of phytase. The zones of inhibition of crude and pure phytase were 2.1 and 2.4cm recorded using well diffusion method.*

Keywords: phytase, wheat bran waste, inorganic phosphate, *Aspergillus niger*, solid state fermentation

1. Introduction

Phytase (myo-inositol hexakisphosphate phosphohydrolase, EC 3.1.3.8) catalyze the hydrolysis of phytate to the inorganic phosphate and less-phosphorylated myo-inositol derivatives (Konietzny and Greiner, 2002) and have a big share in enzyme business due to its widespread application as a feed supplement. One way to enhance phosphate utilization from phytate is by using the phytase. To obtain a good source of phytase, a variety of microorganisms, animal's tissue and plant have been screened. Several plant phytases from wheat, barley, bean, corn, soybean, rice and cotton have been studied extensively by Greiner and Konietzny (2006).

Phytase activity in microorganisms has been found most frequently in fungi (Mullaney *et al.*, 2000). A large number of microbes including bacteria, yeast and filamentous fungi have been used for phytase production. Selection of particular microbe depends on the nature of substrate, environmental conditions and desired final product. Thermophilic fungi have complex or unusual nutritional requirements and well known microbes to produce phytase (Singh & Satyanarayana, 2006). This enzyme produces available phosphate and a non-metal chelator compound. Therefore, phytases are considered to be enzymes of great value in enhancing the nutritional quality of phytate-rich foods and feeds (Oboh and Elusiyan, 2007).

Phytic acid (myo-inositol hexakisphosphate) is a organic phosphorous compound which its component makes approximately upto 75-80% of phosphorus in plant seeds and 1-5% of total weight in most of edible seeds such as legumes, cereal grains and oilseed meals. Phytate contains 14-28% of phosphorus, 12-20% Ca²⁺, 1-2% Zn²⁺ and Fe²⁺ (Vohra and Satyanarayana, 2003). Phytate is not metabolized by monogastric animals, which have low levels of phytate-hydrolyzing enzymes in their digestive

tracts. These un-metabolized phytates pass through the intestinal tract, therefore are excreted leading to environmental problems by eutrophication of surface water resources (Raboy, 2001). Considerable amounts of phytate can be found in plant-based food products such as rice bran, oat flour, barley flour, wheat bran, beans, sesame bran, sunflower meal, soybean, cowpea, and sorghum (Dost and Tokul, 2006).

Solid-state fermentation system has generated much interest in recent years because it offers several economical and practical advantages including high product concentration, improved product recovery, simple cultivation equipment and lower plant operational cost. The use of filamentous fungi for the phytase production through solid-state fermentation has gained much interest for research in the last years (Pandey *et al.*, 2001).

For industrial application, a phytase with a pH activity profile ideally suited for maximal activity in the digestive tract of monogastric animals is desirable. Because of its great practical importance, there is an ongoing interest in isolating new and safe microbial strains producing novel and efficient phytases (Jin *et al.*, 2008). The enzymatic degradation of phytic acid will not produce toxic by-products, so it is environment friendly (Ciofalo *et al.*, 2003). At the end of 20th century, annual sales of phytase as feed additive were estimated at US\$ 500 million and are continuing to rise (Vats and Banerjee, 2004). Nevertheless, majority of them are unable to satisfy requirements necessary for industrial production such as high enzyme activity and thermo-stability. Many attempts have been made in the last 20 years to obtain heat-resistant phytase. Phytase produced by *Aspergillus fumigatus* was found to be thermostable according to Guo *et al.*, 2007. The objective of this study is to optimize phytase production during solid-state fermentation from *Aspergillus niger* endophytic fungi isolated from soil.

2. Materials and Methods

2.1 Isolation of strains from soil

Soil samples were collected from Jacob School of Biotechnology and Bioengineering field, SHIATS, Allahabad. Those soil samples were dissolved in saline solution (0.8%) afterward plated onto a selected media for phytase which contains; glucose-1.5%, ammonium sulphate-0.5%, potassium chloride-0.05%, magnesium sulphate-0.01%, sodium phytate-0.5%, sodium chloride-0.01%, calcium chloride-0.01%, ferrous sulphate-0.001%, magenous sulphate-0.001%, and agar-2% then kept at room temperature for 3 days. The strains that had a clear zone around culture were selected (Xiong *et al.* 2004). Mother culture of *Aspergillus niger* was prepared on potato dextrose agar media for further study.

2.2 Optimization of solid-state medium

Glucose, calcium carbonate and wheat bran concentration were optimized as 5, 10, 15, 20 and 25g/50ml distilled water, respectively. The selected strain was inoculated into Erlenmeyer flasks containing a growth medium for fermentation. The flasks were maintained at room temperature.

2.3 Extraction and activity assessment of phytase (EC 3.1.3.8)

After 7days, fermented production media was taken for extraction and was blended it slowly with 50ml sodium acetate buffer (100mM; pH 4.8). Kept it on water bath for 20 min at 70°C followed by cooling at room temperature, filtration (twice by muslin cloth), and centrifugation at 8000 rpm for 20 min. Supernatant was collected and pellet was discarded. From this, 20µl of crude sample was taken to check the phytase activity by the method of Heeft (1995). Further, crude sample was purified by ammonium sulphate salt precipitation, 20ml of crude sample was precipitated by adding pinch-by-pinch ammonium sulphate salt to isolate the phytase enzyme and centrifuged it again at 8000 rpm for 20 min then pellet was collected slowly and supernatant was discarded. Then pellet was mixed with sodium acetate buffer (100mM; pH 4.8) to check pure phytase activity of sample again as zone of sodium phytate degradation after 18hrs by well diffusion method

3. Results and Discussion

Wheat bran used as nutrient source as and glucose for the growth of *Aspergillus niger* It is clear from the table that maximum zone of phytate degradation of crude sample was 2.1cm and pure sample 1.6 cm, when wheat bran was used as 25g/50ml of distilled water as well as when calcium carbonate concentration was optimized then crude and pure sample had maximum zone of phytate degradation 2.1 cm at 15g/50ml of distilled water and more than this concentration of calcium carbonate, zone of phytate degradation decreases continuously. All the concentration studied, the maximum zone of phytate degradation was seen in samples, where

glucose was used as growth of *Aspergillus niger*. In case of crude sample, maximum zone of phytate degradation was 2.6 cm and pure sample 2.4cm, when glucose was used as 25g and 20g/50ml of distilled water respectively that means, glucose concentration as increases than zone of phytate degradation also increases continuously.

Table 1: Phytase activity screening by zone of sodium phytate degradation after 18hrs

Conc. (g/50ml of d. w.)	Wheat bran		Calcium carbonate		Glucose	
	Crude	Pure	Crude	Pure	Crude	Pure
5	0.0	0.8	1.2	1.4	1.2	1.4
10	0.1	0.9	2.1	2.0	1.0	1.2
15	1.6	1.2	2.1	2.1	1.4	1.4
20	1.8	1.5	1.9	1.8	1.8	2.4
25	2.1	1.6	1.7	1.8	2.6	2.1

4. Conclusion

It was concluded that wheat bran waste can be used efficiently as nutrient source (such as carbohydrate or minerals) for phytase production from *Aspergillus niger* except chemical composition, which will help to reduce the cost of phytase production in manufacture industry.

5. Acknowledgement

All the biochemical analysis carried out in this work was conducted in research lab of Department of Biochemistry and Biochemical Engineering, Jacob School of Biotechnology & Bioengineering, Sam Higginbottom Institute of Agriculture Technology and Sciences, (Deemed-to-be-University) Allahabad.

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Author Profile

Manish Kumar, Ph.D. research scholar in the Department of Biochemistry & Biochemical Engineering, JSBB, SHIATS, Allahabad and received M.Phil degree from Singhania University, Rajasthan, India

Dr. (Mrs.) Sushma, presently working as faculty in the Department of Biochemistry & Biochemical Engineering, JSBB, SHIATS, Allahabad, received her M.Sc. degree from GBPUA&T Udham Singh Nagar and Ph.D. (Biochemistry) from Indian Veterinary Research Institute (IVRI), Bareilly in 1987 and 1992 respectively.