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Original Article

Influence of carbon source on phytase production by *Aspergillus niger*

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Abstract

The present study was undertaken to evaluated the influence of various refined carbohydrate sources such as Fructose, Maltose, Lactose and Sucrose along with standard medium on phytase production and activity by *Aspergillus niger*. Among the different refined carbohydrate sources maximum enzyme activity was recorded in Fructose and Sucrose (33.33 units/ml). Maximum enzyme activity was recorded at pH 6.5 (45.33 units/ml). Among the different temperatures tested maximum activity was recorded at the temperature 60°C (46.33 units/ml) followed by 50°C (42.33 units/ml)
***Key words*:** Phytase, Semi synthetic media, refined carbohydrate source, characterization

Introduction

Phytic acid (*myo*-inositol hexakis phosphate; phytate) is the predominant storage form of phosphorus in cereals, oilseed meals, and legumes (Reddy NR et al, 1982). In terms of animal nutrition, monogastric animals such as swine and poultry are not capable of metabolizing phytate phosphorus owing to the lack of digestive enzymes hydrolyzing the substrate, and therefore inorganic phosphate is added to their diets to meet the phosphorus requirement, while undigested phytate phosphorus is excreted in manure and poses a serious phosphorus pollution problem, contributing to the eutrophication of surface waters in areas of intensive livestock production (Wodzinski et al, 1996). In addition, phytic acid also acts as an anti-nutritional agent by forming complexes with proteins and various metal ions, thereby decreasing the dietary bioavailability of these nutrients.

Phytase (*myo*-inositol hexakisphosphate phosphohydrolase; EC 3.1.3.8) hydrolyzes phytic acid to *myo*inositol and phosphoric acid (Xin gen lei et al, 2003). The supplementation of animal feedstuff with phytase increases the utilization of phosphate and diminishes the anti-nutritional effects of high-phytate diets, ultimately improving the nutritional quality of the diets (Kannan et al, 2008). Recently, phytases have been of interest for biotechnological applications, as environment-friendly feed additives in feed manufacturing industry. In the present study, we describe the influence of various refined carbohydrate sources such as Fructose, Maltose, Lactose and Sucrose on phytase production and activity by *Aspergillus niger* was studied under shake flask culture technique.

Materials and Methods

Microorganism

Phytase producing strain of *Aspergillus niger* was obtained from MTCC,Chandigarh and the pure culture was maintained on czapek yeast extract agar slant as monosporic culture.

Inocula

The source of inocula was obtained from the 7 days old czapek yeast extract agar slant and the spore suspension was obtained by adding sterile distilled water with 0.1% Tween 80 over the slant surface and scrapping the slant surface with glass rod, and the slurry was filtered through cheese cloth to remove mycelia debris and the resulting filtrate was used as source of inocula and the spore count was made using hemocytometer.

Preparation of carbon source

Refined carbon source such as Fructose, Maltose, Lactose and Sucrose was obtained from reagent company Himedia. 0.5g of respective sugars was added along with standard medium individually and used.

Submerged Fermentation

The standard semi synthetic fermentation medium used in this work, contained in g/l: corn starch,28; glucose,5; peptone,18; potassium chloride,0.5; magnesium sulphate,1.5; potassium dihydrogen phosphate,1; calcium chloride,2. Media, 100ml in 250ml

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Erlenmeyer flask, were sterilized at 120^{o}C for 20 minutes. Four different sterile refined carbon sources was added to the sterile medium individually prior to inoculation. The pH after sterilization was adjusted to 5.0 (M.Papagianni et al, 2001). 0.1ml of spore suspension (10^{8} spores/ml) was inoculated from czapek yeast extract agar slant into the flasks were kept at the shaker 150rpm for 2 days at 30^{o} C. After incubation the media was filtered through whatman No1 filter paper. The collected filtrate was centrifuged at 10,000rpm for 10 minutes. Supernatant obtained was used as crude enzyme source.

Phytase Assay

Phytase activity was measured in an assay mixture containing 44.1mM phytic acid and 200mM glycine buffer (pH 2.8) and suitably diluted enzyme. Reaction mixture is incubated at 37^{o}C for 30 minutes, colour reagent was added and the developed colour was read colorimetrically at 400nm. A standard graph is drawn from the obtained values. From the graph the amount of phosphates liberated by phytase is calculated (Heinonen *et al*.,1981).

Effect of pH and Temperature optima of crude phytase

To determine the pH, the enzyme was incubated with phytic acid prepared in 200mM glycine buffer, pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and released phosphate ions were assayed. The temperature optimum was determined by incubating the enzyme with substrate, prepared in 200mM glycine buffer pH (2.8) at different temperature for 30 minutes and the phytase activity was assayed (Chadha *et al*., 2004)

Results and Discussion

Among the various (Fructose, Maltose, Lactose and Sucrose) carbon sources used for phytase production, two carbon sources Fructose and Sucrose, were found to show more phytase activity (Table-1) and the enzyme activity was found to be 33.33units/ml when cultivated by submerged fermentation at 30^{o}C for 2 days (Maria papagianni *et al*., 2001). Of the various carbon sources used, Fructose and Sucrose along with standard medium supported maximal phytase activity. Fructose and Sucrose, besides being a good enhancer source when compared with the other carbon sources (Fig.1).

Table-1: Phytase activity in different carbon source

S.No	Media	Enzyme activity units/ml
1	Standard media	22.66
2	Standard media + Fructose	33.33
3	Standard media + Maltose	22.66
4	Standard media + Lactose	12
5	Standard media + Sucrose	33.33

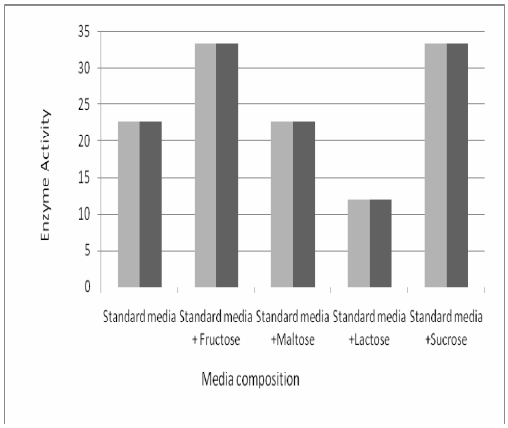

Fig.1: Effect of carbon source on phytase activity

Table.2: Phytase activity at different pH range

S.No	pH Range	Enzyme activity units/ml
1.	5	12
2.	5.5	22.13
3.	6	33.33
4.	6.5	45.33
5.	7	33.33
6.	7.5	22.3

The crude phytase was optimally active at pH 6.5, though the enzyme activity checked between 5.0 and 7.5 (Figure- 2). A broad pH range of activity has also been shown

previously for thermophilic fungi, *Aspergillus fumigates* and *Myceliophthora thermophile* (Wyss *et al.*,1999). Phytase activity was optimally active at 60^{o}C and declined sharply (Figure 3). This observation is consistent with the view that therrnophilic fungi produce phytases that are catalytically more active at higher temperature (Chadha *et al*., 2004).

Table-3. Phytase activity at different temperature range

S.No	Temperature (^{0}C)	Enzyme activity units/ml
1.	30	29.3
2.	40	33.33
3.	50	42.33
4.	60	46.33
5.	70	28.3

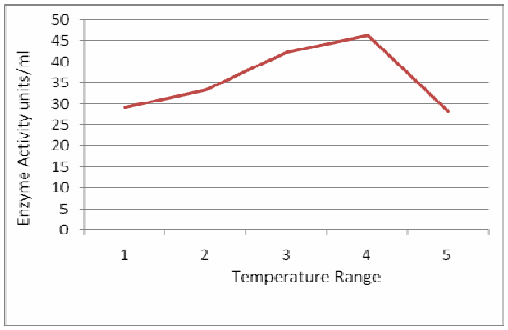

Fig.2: Effect of pH on phytase activity

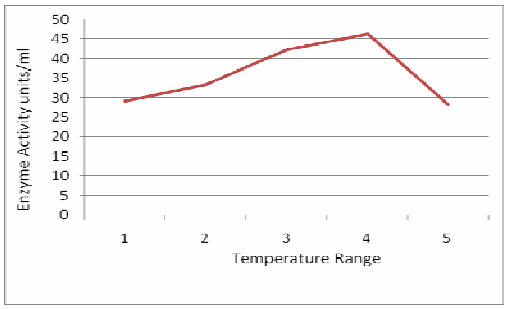

Fig.3: Effect of Temperature on phytase activity

The crude phytase was optimally active at pH 6.5, though the enzyme activity checked between 5.0 and 7.5 (Fig.2). A broad pH range of activity has also been shown previously for thermophilic fungi, *Aspergillus fumigates* and *Myceliophthora thermophile* (Wyss *et al*., 1999). Phytase activity was optimally active at 60^{o}C and declined sharply (Fig.3). This observation is consistent with the view that therrnophilic fungi produce phytases that are catalytically more active at higher temperature (Chadha *et al*., 2004).

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