

Phytase production by *Rhizopus oligosporus* MTCC556 under submerged fermentation conditions

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Phytases catalyse the hydrolytic degradation of phytic acid and its salts and are added to monogastric animal feed to ameliorate the negative environmental and nutritional consequences of dietary phytate. Improvement of phytase production by submerged fermentation from *Rhizopus oligosporus* 556 strain using 1% wheat bran was attempted by optimizing the culture medium. The phytase activity appeared to be more after 8 days of fermentation. Maximum phytase production was 39U/ml after under optimal conditions *i.e.* 5% glucose and 0.5% of peptone at pH 6. Phytase production was affected by inorganic phosphate content and high levels of inorganic phosphorus repress the biosynthesis of phytase.

Key words : Phytase, *Rhizopus oligosporus*556, Submerged fermentation, Phytate degradation

INTRODUCTION

Phytate (myo-inositol hexakisphosphate) is the common storage form of phosphorus in plant seeds and cereal grains (Reddy *et al.*, 1982). Phytate is considered to be an anti-nutritional factor for humans and animals because of its high chelating ability with cations and complex formation with the basic amino acid group of proteins, thus decreasing the dietary bioavailability of these nutrients (Wodzinski and Ullah, 1996; Martinez *et al.*, 1996). Phytate is not metabolized by monogastric animals, which have low levels of phytate-hydrolyzing enzymes in their digestive tracts. These unmetabolized phytates pass through the intestinal tract and are excreted outside and caused environmental problems by eutrophication of surface water resources (Raboy, 2001). In order to increase the bioavailability of essential dietary minerals and decrease environmental pollution, the degradation of phytate in foods and feeds is of nutritional and environmental importance. Phytases (myo-inositol hexakisphosphate phosphohydrolase, EC 3.1.3.8) catalyze the hydrolysis of phytate to the inorganic phosphate and less-phosphorylated myoinositol derivatives (Konietzny and Greiner, 2002). 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 (Martinez *et al.*, 1996; Oboh and Elusiyan, 2007). Phytases are present in plants, certain animal tissues, and microorganisms. They have been studied most intensively in the seeds of plants (Gibson and Ullah, 1988; Greiner 2002). Phytase activity in microorganisms has been found

most frequently in fungi (Ullah and Gibson, 1987; Mullaney *et al.*, 2000), bacteria (Kim *et al.*, 1998; In *et al.*, 2004; Oh and Lee, 2007) and yeast (Quan *et al.*, 2002; Veide and Andlid, 2006; In *et al.*, 2007; Kaur *et al.*, 2007). Among the bacterial phytases, the pH optimum for extracellular and intracellular phytases are 6.0-7.0 and 4.5-6.0, respectively. 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.

MATERIALS AND METHODS

Microorganism:

The *Rhizopus oligosporus* 556 strain which was used for the microbial phytase source in this work was obtained from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India. The stock culture was maintained on PDA slant and subcultured monthly. All other chemicals were of analytical grade.

Fermentation:

Fermentation medium (100 ml in 250 ml Erlenmeyer flask) containing 1% peptone, 4% dextrose and 1% wheat bran was prepared. Prior to sterilization, the initial pH of the medium was adjusted to 5.5 with 1N HCl. The sterilized media inoculated with 1% (v/v) of spore suspension (5 x 10⁷ spores per ml) prepared by suspending the spores from 7 day old sporulated slant of *Rhizopus oligosporus*556 grown on PDA in 10 ml of

sterile distilled water and incubated at 30°C at 200 rpm. Samples were removed after every 24 h and the fermented slurry was filtered through muslin cloth and centrifuged at $11,000 \times g$ for 10 min. The supernatant was used for assay of phytase activity. All experiments were carried out at least twice until the optimal culture conditions were found; as such, the data shown in this paper are representative.

Phytase assay:

Phytase assays were carried out using the Shimizu method (Shimizu, 1992) with some modification. The collected cells were resuspended in 0.2 M acetate buffer (pH 3.6) for the determination of phytase activity. The enzymatic reactions were initiated by incubating 0.1 ml of the cell suspension with 0.9 ml of 2 mM sodium phytate in 0.2 M sodium acetate buffer (pH 3.6). After incubation at 37°C for 10 min, the reaction was stopped by adding 1.0 ml of 10% trichloroacetic acid. The liberated inorganic phosphate was measured according to the ammonium molybdate method (Heinonen and Lahti, 1981). One unit (U) of phytase activity was defined as the amount of enzyme that liberated one micromole of inorganic phosphate per minute under the assay conditions,

Phytase production at different glucose and nitrogen sources concentration using 1% rice bran:

The effect of concentration of carbon and nitrogen sources on the growth and phytase production by *Rhizopus oligosporus*556 strain was investigated. The concentration of carbohydrate and nitrogen source varied from 1-9% and 0.5%-3% was supplemented in the basal medium.

Effect of phosphate and mineral sources:

In order to investigate the effect of the phosphorus content on the phytase production K_2HPO_4 was added supplying a concentration of phosphorus from 2 to 10 mg/dl

Effect of pH on phytase production:

In order to examine the effect of culture pH on phytase production, the experiments were conducted at a range of pH *i.e.* from 2.0 to 10.0 using 0.2 M sodium acetate buffer.

RESULTS AND DISCUSSION

In order to know the optimum fermentation time for the production of phytase the fermentation was performed for various time intervals. The phytase activity appeared

to be maximum after 8 days of fermentation as indicated in Fig. 1 and there was no further activity increase up to 12 days of fermentation. That's why the fermentation experiments were fixed to 8 days.

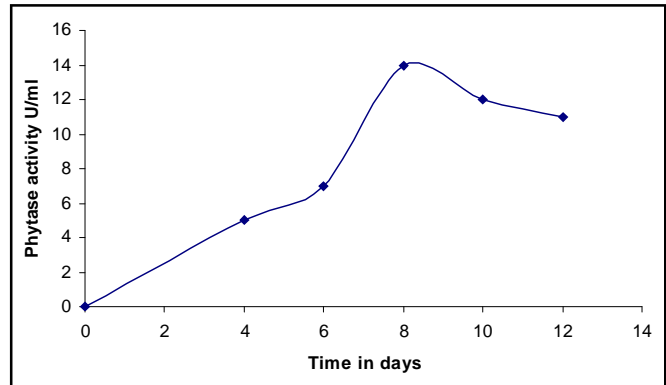


Fig. 1 : Time course of phytase production by *R. oligosporus*

Effect of carbon and nitrogen sources on phytase production:

The effects of carbon and nitrogen sources on the growth and phytase production by *Rhizopus oligosporus*556 strain were investigated. The results were shown in Fig. 2. Among the various tested concentrations of carbon source maximum phytase production (20U/ml of supernatant) was obtained in the medium of 5% glucose. Any increase or decrease in glucose concentration from 5% was not found suitable for phytase titres. Higher concentrations of glucose, such as 9% resulted in reduced phytase titers. However, in case of nitrogen source as shown in Fig. 3, reduced concentration *i.e.* 0.5% significantly enhanced the enzyme production resulting in 25 U/ml of phytase. With the

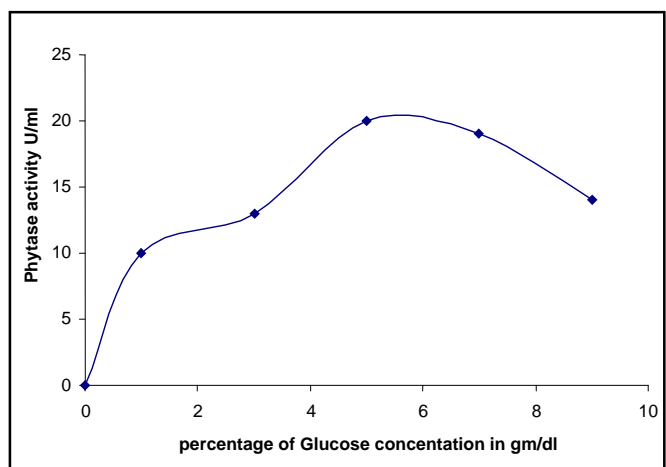


Fig. 2 : Phytase production at different glucose concentrations using 1% wheat bam

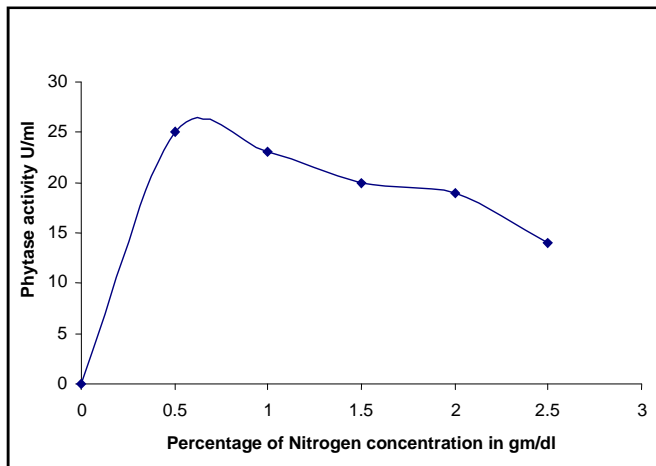


Fig. 3 : Phytase production at different nitrogen concentrations using 1% wheat bam

increase in the concentration, there was gradual decrease in the production of phytase. Since 0.5% of peptone (Lowest experimental concentration) was found most suitable, it was thought desirable to further reduce its concentration and study enzyme production. However, at 0.25% concentration, there was reduction in phytase titres in comparison to 0.5% concentration. (Data not shown).

Effect of phosphate concentration and pH on enzyme production:

The addition of phosphate to the medium influenced the phytase production by *Rhizopus oligosporus* 556. Although the presence of inorganic phosphorus is an essential ingredient of phytase production medium (Soni and Khire, 2007), increasing the concentration of inorganic phosphorus above 0.005% shows adverse effect on phytase production (Kim *et al.*, 1982; Bhavsar *et al.*, 2008). High levels of inorganic phosphorus repress the biosynthesis of phytase (Ogawa *et al.*, 2000; Andlid *et al.*, 2004). In the present studies phytase was produced in greater amounts with phosphate concentration below 6mg/dl. However, phytase production was reduced in significant levels at phosphate concentration greater than 10 mg/dl (Fig. 4). Production of phytase was determined after 8 days of incubation at different initial pH values of the medium. As shown in Fig. 5 maximum enzyme production *i.e.* 39U/ml was observed at pH of 6.

Conclusion:

Present studies on phytase production under submerged fermentation conditions by *Rhizopus oligosporus* 556 indicates that Maximum phytase activity of 39U/ml was produced on the 8th day of fermentation

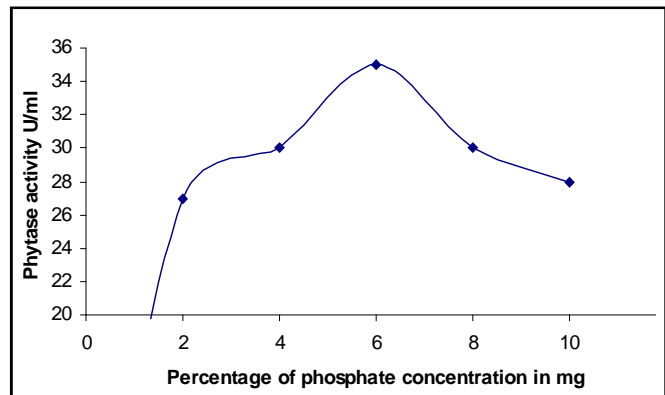


Fig. 4 : Effect of phosphate concentration on phytase production

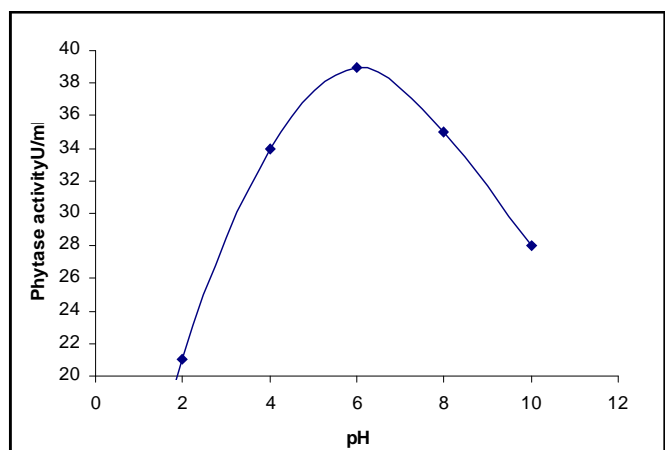


Fig. 5 : Phytase production at different pH

using 1% wheat barn 5% glucose and 0.5% peptone at pH 6. Further experiments in up scaling the process at 10 L fermenter scale and application of phytase in poultry feed are in progress.

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