

## 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 ( $5 \times 10^7$  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