Isolation and Chracterisation of phytase by Aureobasidium pullulans

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Abstract— *Phytate, the major source of phosphorus in seeds, exists as a complex with different metal ions. The phytase production was stimulated by phytate in the medium used and Aureobasidium pullulans showing phytase activity was isolated. the phytase exhibited high stability at temperatures up to 50°C. The maximum production of phytase (108 U/ml) occurred in a medium containing sodium phytaste. The phytase has an optimum pH of 5.5, and an optimum temperature of 50°C.*

Keywords Aureobasidium pullulans, phytase, pH, Tempreture.

I. INTRODUCTION

Phytase (myo-inositol hexakisphosphate phosphohydrolase) effectively catalyzes the release of phosphate from phytate and phosphorylated compounds and is considered to be a unique type of phosphatase (Reddy et al, 1982). In agriculture, phytase is considered to be one of the most important monogastric animals' sources of nutrition because of its significantly improving phosphorus utilization efficiency and its ability to lower anti-nutrients (Gref et al., 1983 and Lei et al., 1993). In addition, phytase is used to improve the bioavailability of iron and zinc for vulnerable human population groups brought about by iron and zinc deficiencies in foods (Thomas et al., 2004). Owing to its ability to reduce the inorganic phosphate content of animal manures, phytase, derived from different sources such as bacteria (Greiner et al., 1993 and Rodriguez et al., 1999), yeasts (Mayer et al.,1999 and Nakamura 2004), fungi (Berka et al.,1998 and Pasamontas et al., 1997), and plants (Gibson et al., 1988 and Hegeman et al.,2004), had been produced. Nevertheless, majority of them are unable to satisfy requirements necessary for industrial production such as high enzyme activity or/and thermostability. Many attempts have been made in the last 20 years to obtain heat-resistant phytase. It suggested that phytase produced by Aspergillus fumigatus or Bacillus sp. DS11, respectively, was thermostable. Then, the A. fumigatus phytase gene was sequenced and expressed in methylotropic yeast P. pastoris(Rodriguez et al., 2000), Hansenula polymorpha (Wyss et al., 1999), respectively. Also, it was reported that Aspergillus awamori (Martin et al.,2006) and Yersinia intermedia (Huang et al.,2006) could produce phytase with high performance. In comparison, it suggested that A. pullulans phytase might have a promising industrial application.

II. T MATERIALS AND METHODS

Microorganism

Aureobasidium pullulans, isolated from the sea mud in the south of Iran. The organism was cultivated on potato dextrose agar slants .Cultures were incubated for 7 days at 28°C. The microorganism was maintained at 4 on potato dextrose agar (PDA) and subcultured every 2 weeks.

Phytase assay

Phytase was assayed by measuring the amount of phosphate released (Fiske & Subbarow 1925) using sodium phytate as the substrate (Vohra & Satyanarayana 2001). One unit of phytase is defined as the amount of enzyme that liberates 1 lmol inorganic phosphate/ min at 60°C under the assay conditions.

Effect of pH and temperature on phytase activity and stability

For determining optimum temperature for the activity of phytase, the enzyme assays were carried out in the temperature range between 30 and 80 °C (pH 5.0). Screening of isolates for phytase production Nine strains were screened for phytase production on solidified PSM screening medium

with sodium phytate as carbon source (Howson & Davis 1983)

To evaluate the effect of pH optimum on enzyme activity, buffers of the varied pH values (0.1 M, glycine–HCl (2.0–3.0), Na-acetate (4.0–6.0), Tris–HCl (7.0–8.0)) were used.

III. Results and Discussion

3.1. Screening of phytase producing organism

Fruit, leaf and seed of the plants samples were extensively screened for the isolation of phytase producing strains. A number of positive isolates were obtained from various sea mud samples on PSM-agar plate and their production pattern was checked in liquid medium (fig. 1). Though a number of positive bacterial, actinomycete, yeast and fungal isolates were obtained, only two yeast isolate showed reproducible phytase activity with pH optima between 5.5 and 6 and temperature optima between 50 and 30 . The selected isolate was found to be A. pullulans on the basis of morphological characteristics, physicochemichal and sequencing data. Under unoptimized conditions, the isolate produced 126 units/ml phytase activity after 8 days of incubation at 28 . This activity is comparable with known phytase producing strains.

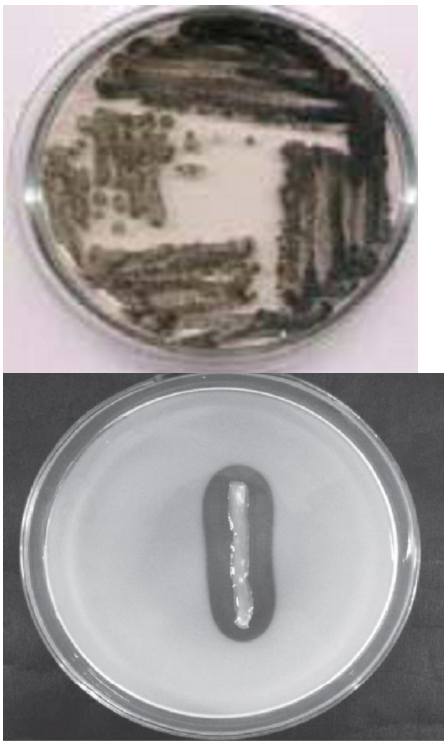

Fig. 1 Growth and clear zone of A. pullulans on phytase screening medium

3.2. Production of phytase in shake flask

When the course of phytase production was checked in starch medium (pH 6.5) at 28 , the isolate produced The temperature and pH optima of phytase activity were 55 and pH 3.5, respectively. After 10 days, there was a decline in phytase production despite continued culture growth. Although there are reports regarding the production of phytase from yeasts (Hellstr?m et al., 2012).

3.3. Effect of environmental factors on the growth and production of phytase

The effect of environmental factors on the growth and production of phytase by A. pullulans was studied in shake flasks. When growth was carried out at different temperatures (28, 30, 37, 45, 50, 60, 70, 80 and 90), the isolate was found to grow best at 28 . Maximum enzyme production was also obtained when the organism was incubated at 50 (Fig. 2).

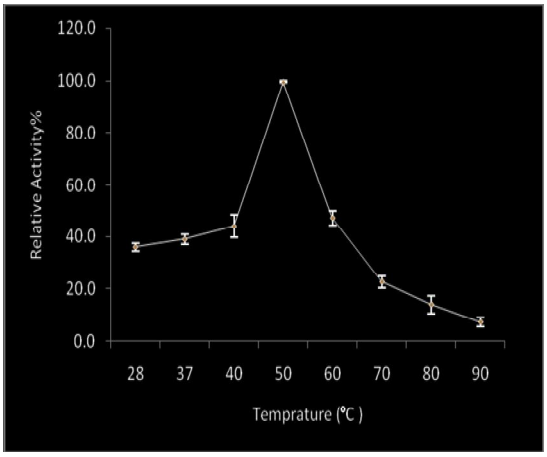

Fig. 2

When phytase production was checked at various pHs in the range 2-9, maximum growth and phytase activity were observed when the initial pH of the medium was adjusted to 7 (Fig. 3). Enzyme activity, extracellular protein content and cell mass concentration increased as the initial pH of the medium moved from acidic towards neutral range, but above pH 7.0, a decline was observed. As the culture started growing, the pH of the medium began to decrease and after 3 days, there was a sharp decline in pH, lowering the pH to 1.9-2.8 where it stabilized.

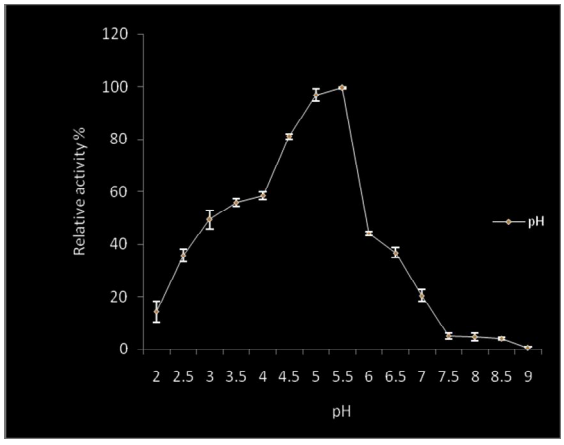

Fig. 3

IV. CONCLUSION

The strain was isolated from the fruit, leaf and seed of the plants showed positive phytase activity (0.2 units/ml) after 4 days of incubation at 28°C in PSM medium containing 1% sodium phytate. This organism was identified as A. pullulans after morphological and sequencing studies. the extracellular phytase had a specific activity of 126 U/mg activity in minimal medium, thus making further purification of enzyme very easy and economical. A. pullulans was found to be an excellent organism for the production of phytase enzyme. Detailed studies on the fermentative production of phytase in a laboratory scale fermentor are required in order to scale up phytase production.

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