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Thermostable Phytase from *Bacillus*

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Phytase producing bacteria were isolated in media (phytase specific medium) with phytin as the only sources of phosphate, from soil and poultry faeces. Among the isolated strains, one identified as *Bacillus* sp. has high phytase activitity and is found to be a thermophilic bacteria. The enzyme produced by this bacteria was thermo stable.

Key words: Phytase, Citric Pulp, Thermostable enzyme.

Phytases (myo-inositol hexakisphosphate phosphohydrolase; EC 3.1.3.8 and EC 3.1.3.26) belong to the family of histidine acid phosphatases (HAP), a subclass of phosphatases, which catalyze the hydrolysis of phytic acid, the principal storage form of phosphorus in cereals, legumes, oil seeds, nuts and others (Mitchell *et al*., 1997).

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 cause 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.

Monogastric animal feed supplemented with microbial phytase effectively improves phytate phosphorus utilization, reduces the excretion of phosphorus in animal manure (Lei et al., 1993) and improves the feed value by removing the anti-nutrient aspect of phytate in intensive livestock production (Mitchell et al.,1997). Although a number of phytase genes have been isolated from various sources Golovan et al.,2000), only a few phytases have been widely used in industry because of the relative instability and cost of the enzyme (Vohra et al.,2003,Simons et al.,1990) The industrial demand for phytase with high specific activity and stability under high temperature conditions for feed pellet production and under acidic conditions in the stomach of monogastric animals continues to stimulate the search for new enzyme sources.

In the present study we have isolated phytase producing Thermophillic bacteria and checked the stability of enzyme.

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MATERIAL AND METHODS

Screening techniques for isolating thermophillic phlytase producing organisms

Enrichment culture media containing calcium phytate as the phosphorus and carbon source were used for the primary screening of phytase producers. The method takes advantage of the insolubility of calcium phytate in aqueous media which gives a white turbidity in an agar plate. The bacterial isolates to be screened for phytase production were cultivated individually in TSB medium (15 gL^{-1} tryptone, 5.0gL^{-1} soyptone, 5.0 gL^{-1} NaCl, 1. 0 L distilled water) at 45° C overnight. And seeded into the phytase screen medium (PSM) (15 gL^{-1} Glucose, 5.0 gL^{-1} NH_4NO_3, 0.5 gL^{-1} KCl, 0.5 gL^{-1} $MgSO_4$. $7H_2O$, 0.01 gL^{-1} $FeSO_4.7H_2O$, 0.01 gL^{-1} $MnSO_4.7H_2O$, 0.5 % Ca-phytate, 20.0 gL-1 Agar; pH adjusted to 5.5), any developing colony which produced a clear zone was considered a potential phytase producer. All reagents used in the present study were obtained from Hi Media, Mumbai

Phytase Production

The media was prepared by replacing the carbon source and nitrogen source of above media by adding extracts of,Citric Pulp and 0.5% of Ammonium nitrate respectively. The fermentations were carried out in 250 mL Erlenmeyer flasks at 30 °C.

Extraction of Phytase

Culture broth was centrifuged by 12000 rpm at 4°C for 5 minutes and the supernatant was determined for phytase activity using calcium phytate as a substrate (Kim *et al.*, 1998). One unit of enzyme activity was defined as the amount of enzyme that catalyses the liberation of 1 ìmole of inorganic orthophosphate from substrate per minute under the standard assay conditions

Inorganic phosphate determination

Spectrophotometric quantification of inorganic phosphate was performed using the Taussky-Shorr reagent. The determinations were performed in triplicate.

Effect of temperature on Enzyme Activity

To determine thermal stability, the crude enzymes were incubated at different temperatures ranging from 20^{0}C to 90^{0}C for 1 hr, cooled to 4^{0}C and assayed.

RESULT AND DISCUSSION

Effect of temperature on Enzyme activity

Because commercial feeds are often pelleted, a process which uses high temperature (60-80^{0}C) and steam. Enzyme thermal stability is very relevant in animal feed application. It is therefore imperative to examine the optimum temperature for reaction and thermal stability of any given phytase inorder to determine its suitability for feed incorporation. The *Bacillus* phytase in the present study maintained phytate degrading activities even at high reaction temperatures.

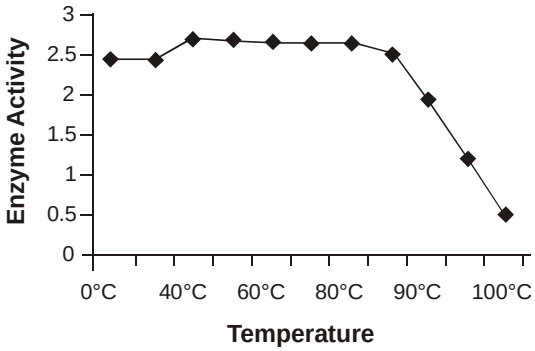

Fig. 1. Effect of temperature on Enzyme activity

CONCLUSION

The ability of soil microorganism to solubilize various forms of precipitated phosphorus is well documented (Rodrigues and Fraga, 1999). Despite the quantitative importance of organic phosphorus compounds (such as phytin) in the soil, knowledge on the extent and mechanisms of the use by plants is still limited (Chunshan et al., 2001; Lan et al., 2002; Rodrigues and Fraga, 1999). Several types of phosphatase, such as phytase, are able to increase the rate of the dephosphorylation (hydrolysis) of organic compound. These enzymes are normally present in soils, where they originate from microorganisms. In this study, we isolated strains with phytate degrading ability. The enzyme produced is highly thermostable Therefore, the organisms isolated and identified could be applied for diet of poultry and pig (Casey andWalsh, 2004).

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