FUNGAL PHYTASE: ISOLATION, GENE CLONING, EXPRESSION AND CHARACTERIZATION

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Ph.D. (BIOTECHNOLOGY)

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ABSTRACT

Phytase is used as an animal feed additive to improve phosphate utilization and mineral absorption in monogastric animals. Soil and bark samples from various places in Thailand were used for fungal phytase isolation. Extracellular phytase was screened by using Phytase Screening Medium, PSM (pH 7.0) and acidic medium (pH 3.5). One hundred and twenty six fungal isolates were obtained and used for DNA extraction and phytase gene amplification. The phytase gene from a selected fungus named Neosartorya spinosa BCC 41923 was cloned and expressed in Pichia pastoris KM71 expression system. The phytase gene (phyN) was fused in-frame with the α-mating factor signal peptide in pPIC9K plasmid and was under the control of the alcohol oxidase I (AOXI) promoter. After 72 h induction with 0.5% methanol, the expressed PhyN was secreted into Buffered Methanol-complex Medium (BMMY) containing 1% sorbitol. The purified enzyme displayed specific activity of 30.95 U/mg at 37°C and 38.62 U/mg at 42°C. Molecular weight of the deglycosylated recombinant phytase as determined by SDS-PAGE was approximately 52 kDa. The optimum pH and temperature for phytase activity were pH 5.5 and 50°C, respectively. The residual phytase activity remained over 80% and 60% of initial activity after the enzyme was stored in pH 3.0 to 7.0 for 1 h and after heating at 90°C for 20 min, respectively. The enzyme exhibited broad substrate specificity with phytic acid as the most preferred substrate. Its apparent $K_m$ and $V_{max}$ for sodium phytate at 37°C for 10 min were 1.39 mM and 434.78 U/mg, respectively. The enzyme was highly resistant to most metal ions tested, including Fe$^{3+}$ and Al$^{3+}$. When incubated with pepsin at the pepsin/phytase ratio of 0.02 (U/U) at 37°C for 2 h, 92% of its initial activity was retained. However, the enzyme was very sensitive to trypsin as 5% of its initial activity remained after treating with trypsin at the trypsin/phytase ratio of 0.01 U/U. Therefore, the enzyme possesses some favorable characteristics useful for the animal feed industry and other industrial applications.

KEY WORDS: NEOSARTORYA SPINOSA / PHYTASE / PHYTIC ACID / PICHIA PASTORIS

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