ABSTRACT

PRODUCTION, PURIFICATION AND CHARACTERIZATION OF PHYTASE FROM PHYTASE PRODUCING FUNGUS

Gamze Sevri EKREN

M.Sc. Thesis, Department of Biology

Supervisor: Assoc. Prof. Dr. Kubilay METİN

2013, 175 Pages

Fungal strains that were isolated from air and soil and registered in Adnan Menderes University Department of Biology culture stocks were screened for phytase activity of 165 fungal strains. *Aspergillus niger* UA-D. strain showed the highest phytase activity. Extracellular phytase produced by *Aspergillus niger* in PSM was purified 73-fold with a recovery of % 2.6 referred to the phytase activity in the crude extract using, ammonium sulphate precipitation, DEAE Sepharose CL-6B and Phenyl Sepharose CL-4B. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the purified enzyme gave a single stained band at a molecular mass of approximately 65 kDa. The temperature and pH for maximum activity of the enzyme were 70 ^{o}C and 3.0, respectively. K_m and V_{max} values for phytic acid of the enzyme were calculated to be, 180 µM and 54.35 U/mL, respectively. The phytase exhibited broad substrate specificity. The enzyme was stimulated by Ba^{2+} and Li^{+} and strongly inhibited by Fe^{2+}, Al^{3+} and Pb^{2+} The enzyme activity was markedly inhibited in the presence of NBS, PMSF, DTNB, 2,3-butanedione suggesting that tryptophan, cysteine, arginine and serine residues play an important role in the catalytic process. Glyserol and sorbitol enhance thermostability of phytase. The enzyme was detemined to be highly stable against organic solvents.

Key Words: Phytase, *Aspergillus niger*, purification, characterization