Abstract

The fungi *Aspergillus niger* RBP7 (KX100578.1) was newly isolated from municipal dumping area of Midnapore town, West Bengal, India for the production of α-amylase. The isolate was identified through phenotypic and microscopic observation. Then the optimization of enzyme production was studied under solid state fermentation (SSF) (using potato peel as substrate) and submerged fermentation (SmF) through one-variable-at-a-time (OVAT) and followed by response surface methodology (RSM) which enhanced enzyme titer. After fermentation acidophilic α-amylase from *Aspergillus niger* RBP7 was purified from the fermented mass. The purified α– amylase (37.5kDa) exhibited the *Km* and *Vmax* of 1.4 mg/ml and 0.992 µ/ mol/ min respectively. The enzyme was found stable in range of pH (2.0-6.0), high NaCl concentration (3M) and at (40-70 °C) temperature. The enzyme showed its optimum activity at pH 3.0 and 45 °C temperature. The stability of enzyme was also tested in presence of different surfactant, inhibitory agent etc and found to be inhibited by Hg^{2+}. After fermentation the hydrolysis of raw starchy food stuff (taro, yam, malanga and sweet potato) by crude enzyme, obtained from SSF was studied. The crude enzyme produced mono-meric and dimeric sugars like glucose and maltose, determined through paper chromatography. These characteristics make the crude enzyme suitable for use as digestive dysfunction and for the improvement of digestibility of animal feed ingredients. The purified acidophilic amylase enzyme can also digest different heterogeneous food materials and its activity almost similar to the commercially available diastase. The enzyme was also applicable in
different waste management processes. There was no cytotoxic effect shown by purified acidophilic amylase in cell viability test. The *In silico* approach has been taken to understand the molecular adaptation mechanisms of α-amylase in low pH medium. Initially 36 α-amylase including 7 acid α-amylase sequences were retrieved from four different biological databases. Protein sequence based comparative study and evolutionary analysis indicated that, though all the selected sequences were functionally similar, they have remarkable sequence diversity. Although having sequence diversity, all acid α-amylase were found in a separate cluster of phylogenetic tree. The secondary and

topology comparison among acid α-amylase and neutral α-amylase showed conserved beta-sheet regions containing the catalytic amino acid residues of 117-D, 204-R, 206-D, 230-E, 296-H and 297-D within all. But changes observed in Ca^{2+}binding site. In all the acid α-amylase, acidic amino acid Glu 210 was observed instead of a basic His210. As Ca^{2+}binding directly proportionate the α-amylase stability at different pH. Change in Ca^{2+}binding amino acid directly indicated their adaptation in changed pH. In future, directed mutation study to alter His to Glu at 210 position may produce more potent genetically engineered acid α-amylase to solve different industrial purpose.

Key words: α- amylase; *Aspergillus niger* RBP7; OVAT; RSM; purification; molecular weight determination, characterization; hydrolysis of raw starchy food stuff; digestion of food material; cell viability test, *In silico* study of amylase