Abstract

Tannin acyl hydrolase (E.C.3.1.1.20) generally referred to as tannase, is an industrially significant enzyme that is mostly used in the beverage, food, chemical, and pharmaceutical industries. In this study, tannase production was studied using *Aspergillus niger* attained from microbial bank of IIB GCU, Lahore and *Saccharomyces cerevisiae* isolated from sachet of Baker's Yeast. Optimization of culture conditions to produce maximum tannase included studying the effects of media, incubation period, incubation temperature, pH, carbon and nitrogen sources, on *A. niger* and *Saccharomyces cerevisiae* and on enzyme activity. The optimum culture conditions determined were Czapeck Dox media and 96hrs of incubation period for both isolates, whereas 30°C for *Aspergillus niger* and 25°C for *Saccharomyces cerevisiae* incubation temperature was optimized, pH 6.0, 0.6%(w/v) of glucose as carbon source and 0.1%(w/v) of sodium nitrate as nitrogen source for maximum tannase activity. Tannase was partially purified by the method of Ammonium salt precipitation. The saturation level of ammonium sulphate precipitataion used was 30-80%w/v where the best enzyme activity was obtained at the saturation level of 60%. Tannase was purified 2.64-fold with a specific activity of 8.56 U/mL protein in case of *Aspergillus niger* and 3.63-fold with the specific activity of 9.44 U/mL in case of Saccharomyces cerevisiae. Characterization results of partially purified showed that optimum activity was at 30°C and pH 6. Tannase was activated by K^+ ions and Na^+ions at concentration of 0.01 and 0.05mM respectively. Addition of metal ions like Zn2+, Cu2+, Ca2+, Mn2+ and Fe2+ inhibited the enzyme activity in both isolates. Kinetics analysis of substrate tested showed that the Km value of methyl gallate was 3.89×10^{-3} M^{-1} respectively in both isolates. Vmax value obtained was 2×10^{3}.