Abstract

Two hundred and sixteen fungal strains were isolated from two hundred fifty soil samples collected from different localities of Punjab and subjected to screening for their pectinolytic ability using submerged fermentation. One hundred and fifty one pectinolytic strains were subjected to secondary screening and isolate IIB-13 gave the maximum pectinase yield (10.78±0.45U/ml/min) and identified as *Aspergillus niger* using morphological characteristics. Random mutagenesis (physical and chemical) was used to improve the pectinolytic activity of strain IIB-13. Nitrous acid mutant H-97 resulted in three fold increase (32.16 ± 0.05U/ml/min) in pectinase production. This H-97strain was further subjected to optimization of media, carbon and nitrogen sources, temperature and pH. Maximum pectinase (37.01±0.11 U/ml/min) was produced using medium containing K_2HPO_4 (4.0 g), yeast extract (0.6 g), KH_2PO_4 (1.28 g), $(NH_4)_2SO_4$ (2.0 g), $MgSO_4$ (1.1 g) and pectin (10 g) per 1.0 L of distilled water at pH and temperature (7.0 and 30°C) respectively after 60 hours of fermentation. Fermenter studies (7.5 L) resulted in increased pectinase activity (40.31±0.07 U/ml/min) with decreased incubation time of 48 h using 1% inoculums with optimized reaction conditions i.e. temperature (30°C), pH (07), agitation (200 rpm) and aeration (1vvm). Fermentation kinetics was applied to validate the results. The crude enzyme was subjected to purification by applying ammonium sulfate precipitation and Ion exchange chromatography which resulted in 44.20 % yield with increased activity i.e. 75.18±0.04 U/mg. Km value of 2.30 mg/ml using pectin as substrate showed its maximum specificity for the enzyme. Activation energy (Ea) i.e. -28.95 KJ/mol and enthalpy of activation (ΔH) i.e. -26.73 KJ/mol were calculated employing thermodynamic studies. Characterization of enzyme revealed catalytic activity optimum temperature and pH as 25°C and pH 7. The enzymes catalytic activity was observed to be reduced by some heavy metals such as Hg^{2+}, Pb^{2+}, Cu^{+} and Cd^{2+}.