Abstract

The main objective of this study was to isolate mannanase producing fungi. Therefore ninty eight soil samples from the surroundings of agricultural and food industries were collected. These samples were subjected to primary screening and forty eight mannanase producing isolates were screened. These isolates were subjected to submerged fermentation to estimate the activity of enzyme. Highest mannanase activity (19.29 ±0.04 U/mL) was obtained by isolate IIB 037. This strain was further identified by 18S rRNA sequencing and was determined as *Aspergillus awamori*. Different cultural conditions i.e. incubation time, temperature, media pH, carbon sources, nitrogen sources and spore inoculum size were optimized. Maximum production was achieved at: 72 h of incubation, 30°C temperature, medium pH 5.0, 4% spore inoculum with yeast extract and ammonium sulfate as nitrogen source and having LBG as carbon source. Ammonium sulphate precipitation followed by dialysis and anion exchange chromatography using Q Sepharose column was done to achieve the purity of enzyme, which resulted in 86.80% yield and 1.22 fold purification with increase in specific activity of (48.83 ±0.03 U/mg). The molecular size of purified mannanase was 66 kDa. Kinetic characterization of an enzyme revealed LBG as specific substrate for mannanase with Km and Vmax value of 11.07 mM and 19.08 U/mL, respectively. Thermodynamic studies of an enzyme revealed enthalpy of activation (ΔH) and activation energy (Ea) as 30.53KJ/mol, 27.76KJ/mol, respectively. The effect of different metal ions, surfactants and organic solvents were checked on mannanase. In the presence of ß-mercaptoethanol activity of mannanase increased notably. While methyl alcohol, ethanol, Hg2+ and Cu2+ inhibited enzyme activity. In addition, considering the biotechnological aspect, clarification of fruit juice was examined and by using 4% of purified enzyme for 1 h, promising results was obtained.