

## Abstract

The present study was carried out to clone a glycoside hydrolase GH 13 family enzyme from *Thermotoga petrophila* into *Escherichia coli* and characterization of the recombinant enzyme. After amplification of the GH 13 family gene of *Thermotoga petrophila*, it was cloned in *E. coli* DH5 $\alpha$  by using pTZ57R/T as a cloning vector. Screening of positive clones was performed by colony PCR, double digestion of recombinant pTZ57R/T containing GH 13 family gene with *Nde*I and *Hind*III as well as by sequencing of cloned gene. Expression of GH 13 family gene was checked in *E. coli* BL21 (DE3) by using pET 21a (+) as an expression vector. The growth conditions i.e temperature, pH, effect of IPTG and time of induction and optical density of culture at the time of induction were optimized for maximum expression of GH 13 family gene. Various other fermentation parameters like size of inoculum, agitation rate, effect of different media, aeration rate and dissolved oxygen were also studied for maximum expression of cloned gene. Purification of the recombinant GH 13 family enzyme was carried out by heat treatment followed by ion exchange chromatography with 34.6-fold purification having specific activity of 126.31 U mg<sup>-1</sup> and a recovery of 56.25 %. Molecular weight of the purified GH 13 family enzyme, 70 kDa, was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The enzyme was stable at 100 °C temperature and at pH of 7.0. The enzyme activity was increased in the presence of metal ions especially Ca<sup>+2</sup> and decreased in the presence of EDTA indicating that the  $\alpha$ -amylase was a metalloenzyme. However, the addition of 1 % Tween 20, Tween 80 and  $\beta$ -mercaptoethanol constrained the enzyme activity to 87, 96 and 89 %, respectively. No considerable effect of the organic solvents (ethanol, methanol, isopropanol, acetone and n-butanol) was observed on enzyme activity. Line-weaver Burk plot showed  $K_m$  and  $V_{max}$  values of 12.35 mM and 25.839 U/ml/min, respectively. Thermodynamic parameters for hydrolysis of soluble starch were found to be  $E_a$ =28.445 KJ/mol,  $\Delta H$ = 34.12 KJ/mol,  $\Delta S$ = -6.7 KJ/mol and  $Q_{10}$ =0.47. Conserved domain analysis of GH13 family protein showed that it comprises of three conserved domain: AmyAc\_MTase (maltosyltransferase), domain of unknown function and AmyA (glycosidase). Homology modelling of GH13 family gene was carried out using

templates 1gjuA and 4gkl.1. Enzyme-substrate docking of GH 13 family enzyme was carried out by using maltotriose and dextrin as substrates. 76% desizing of cotton cloth with purified recombinant GH13 family enzyme was achieved at optimized conditions (with 150U/ml enzyme in a buffer of pH 7.0 at 80°C after 60 min of incubation). In the light of all results obtained in this study it is concluded that this recombinant GH13 family enzyme could be used as beneficial candidate for textile industry.