ABSTRACT

In the present study research was undertaken to clone an endo-1, 4-β-glucanase gene (*bglC*) of glycoside hydrolase family 5 from moderately thermophilic bacterial strain *Bacillus licheniformis* ATCC 14580. After genomic DNA isolation and PCR amplification, *bglC* was cloned into *Escherichia coli* DH5α cells by using pTZ57R/T vector. Screening of positive clones was done through colony PCR and restriction digestion analysis. Endo-1, 4-β-glucanase gene *bglC* (1.5 kb) was further expressed in *E. coli* BL21 (DE 3) strain by ligating it into pET-22b (+) expression vector. Purification of recombinant enzyme was done using ammonium sulphate precipitation followed by immobilized metal affinity chromatography (IMAC) and gel filtration. The enzyme was purified to 5.75 fold having enzyme activity of 7.9 U/ml/min and specific activity of 52.66 U/mg and the molecular weight was found to be 56 kDa using sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis. Characterization of recombinant endo-1, 4-β-glucanase enzyme showed that it was stable over a pH range of 4-7 and retained 100% of its activity at 60°C. Substrate specificity of purified endo-1, 4-β-glucanase showed that enzyme was most active towards CMC. Line-weaver Burk plot revealed K_m and V_{max} values of 3.8 % and 8.38 U/ml/min respectively. pk_{a1} and pk_{a2} of active site ionizable groups were determined to be 4.2 and 7.1 respectively using Dixon plot. Thermodynamic parameters for hydrolysis of CMC were found to be E_a=36.32 kJ/mol, ΔH= 34.12 kJ/mol, ΔS=-6.4 kJ/mol and Q_{10}=0.47. The activity of endo-1, 4-β-glucanase was increased in prescence of Co^{2+} and Mg^{2+} whereas Cu^{2+} and Hg^{2+} greatly reduced the enzyme activity. Bioinformatic analysis showed that *B. licheniformis* endo-1, 4-β-glucanase possess 72% identity with endoglucanase from *Geobacillus stearothermophilus*. The purified endo-1, 4-β-glucanase enzyme was further used for biostoning of denim. The biochemical properties of endo-1, 4-β-glucanase proved it a valuable candidate for use in laundry and textile industries and for utilizing cellulose in industrial bioethanol production.