ABSTRACT

The gene encoding a thermostable β-D-xilosidase from *Geobacillus thermoleovorans* IT-08 (GbtXyl43B) was subcloned from pTP510 into pET30a and expressed in *Escherichia coli*; additionally the characterization and kinetic analysis of GbtXyl43B were carried out. The recombinant gene product was purified to apparent homogeneity showing M, of 72 by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The enzyme exhibited an optimum temperature and pH of 60 °C and 6.0, respectively. In terms of stability, GbtXyl43B was stable at 60 °C at pH 6.0 for 1 h as well as at pH 6–8 at 4 °C for 24 h. The enzyme had a $k_{cat}$ of $3.2 \times 10^{-4}$ s$^{-1}$ and $K_M$ of $6.6 \times 10^{-2}$ mM, or catalytic efficiency ($k_{cat}/K_M$) of 0.0048 s$^{-1}$ mM$^{-1}$ on p-nitrophenyl-β-D-xylopyranoside substrate. Thin layer chromatography product analysis indicated that GbtXyl43B was exoglycosidase cleaving single xylose units from the nonreducing end of xylan. The activity of GbtXyl43B on insoluble xylan was eightfold higher than on soluble xylan. Bioinformatics analysis showed that GbtXyl43B belonging to glycoside hydrolase (GH) family 43 contained carbohydrate binding module (CBM; residues 15 to 149 forming eight antiparallel β-strands) and catalytic module (residues 157 to 604 forming five-bladed β-propeller fold with predicted catalytic residues to be Asp287 and Glu476). CBM of GbtXyl43B dominated by the Phe residues which grip the carbohydrate is proposed as a novel CBM36 subfamily.

**Keywords:** CBM36, *Geobacillus thermoleovorans* IT-08, GH43, Xylanase, β-D-xilosidase