Abstract

Thermostable cellulolytic enzymes having the ability to tolerate high temperature conditions are of substantial importance in biotechnological and industrial processes for which a number of thermophilic microorganisms have been searched for their efficient thermostable cellulases. In this study, a putative peptidase M42 family protein gene (Tpcel42) of 1020 bp encoding for 339 amino acid protein having cellulolytic activity was cloned from thermophilic bacteria *Thermotoga petrophila* RKU-1 into mesophilic expression host for the production of thermostable cellulolytic enzyme. Tpcel42 was cloned initially in pTZ57R/T for propagation in *E. coli* DH5α, and subcloned in pET-21a(+) expression vector for heterologous expression in *E. coli* BL21 CodonPlus (DE3)-RIPL. The multiple sequence alignment of cellulase with other cellulases displayed glutamic acid (E) as conserved catalytic residue. The expression of Tpcel42 gene was induced with 0.5 mM IPTG. The cell fractions were assessed for protein concentration and enzyme activity showing intracellular expression of TpCel42. SDS-PAGE analysis of the intracellularly expressed heterologous protein TpCel42 confirmed it to be of 37 kDa molecular weight. The cellulolytic activity of TpCel42 was assessed using pNPC and CMC at 80 °C and pH 6 resulting in enzyme activity of 96 U/mL/min and 1.67 U/mL/min, respectively. Thus, expressed thermostable cellulase TpCel42 showed exo- and endo-activity like a processive cellulase and can be utilized for various industrial processes and for biofuel production.