ABSTRACT

The pullulanase gene was amplified from the genomic DNA of *Thermotoga petrophila* RKU-I. It was cloned and expressed in *E.coli* BL21 Codon plus. The pullulanase gene was consisted of 2.5 kb encoded a protein of 843 amino acids. The crude enzyme showed a band of 93 kDa on SDS-PAGE. The enzyme showed 106.96 U/ml/min in crude cell lysate with 6.164 mg/ ml of protein concentration against (1%) pullulan as substrate. The crude enzyme was characterized to observe the effect of temperature, pH, and metal ions. The crude enzyme showed maximum activity at 70°C. The optimum pH for pullulanase activity was 6 with McIlvaine buffer (50mM). The effect of different metal ions on enzyme activity was also examined. The recombinant pullulanase activity was not effected by Pb^{+2} whereas other metal ions i.e. Ca^{+2}, Ni^{+2}, Cd^{+2}, Zn^{+2}, Cu^{+2}, Hg^{+2}, Mg^{+2}, Fe^{+3}, Co^{+2} and SDS were used in final conc. of 1mM caused variation in the activity.