ABSTRACT

The present study deals with gene cloning, expression and purification of a thermostable pullulanase from *Thermus aquaticus* (DSM 625). The pullulanase gene was isolated and then purified from the thermophile and cloned successfully using the cloning vector pTZ57R/T. The 670 bp gene was expressed in *E. coli* DH5α host cells. Double digestion was conducted with enzymes *Nde*I and *Hin*dIII to analyse the recombinant plasmid obtained in order to confirm the pullulanase gene cloning in pTZ57R/T. The gene was expressed in the expression vector pET-21a (+) and expression host cells BL21 Codon Plus. Sequencing analysis confirmed nucleotide sequence of the cloned gene and phylogenetic analysis showed homology among many organisms. The ammonium sulfate precipitation technique was used for the purification of pullulanase enzyme from cell lysate. No activity was observed in 20-40% saturation level. The recombinant enzyme had highest specific activity of 66.0 U/mg at 70-80% fraction. The purified 25 kDa enzyme was further characterized through various parameters. Enzyme assays were carried out to determine physical characteristics. Various metal ions were used to determine the effect on pullulanase activity. The Pb^{+2} ions had no effect but Ca^{+2} and Zn^{+2} somewhat reduced the activity. On the other hand, Hg^{+2} decreased it to 89% which is significant. Mg^{+2} and Cd^{+24} both reduced the enzyme activity to more than half and SDS halted it up to 73%. The thermostability of the enzyme was analysed on a wide temperature range (20-100 °C). It was determined that peak activity of the enzyme was at 80 °C with pH 6.0. Further optimization and investigation is required to benefit from the thermostable properties of this enzyme to fulfil potential biotechnological prospects.