ABSTRACT

Xylanases are considered as one of the principal enzymes used in industry for the hydrolysis of a heteropolysaccharide, xylan. The present study refers to the isolation of xylanase encoding gene from *Anoxybacillus rupienesis* and its cloning in *E. coli.* The strain to be used was first sequenced using 16S rRNA sequencing technique. To extract xylanase gene from *Anoxybacillus rupienesis*, a set of specific primers were used in the process of PCR. The amplified product was then purified and pTZ57R/T cloning vector was used to clone and transformed in *E. coli* DH5α. For the purpose of expression, cells of *E. coli* BL21 CodonPlus were used for transformation while pET-22a(+) vector was used for sub cloning . The expression was then confirmed by running on 12% SDS-PAGE. The crude enzyme obtained was then purified by precipitating it with ammonium sulphate salt and further purification was done using gel filtration chromatography technique. Ammonium sulphate precipitation resulted in 70.0 U/mg of specific activity in cell lysate while supernatant showed 72.5 U/mg of specific activity. Gel filtration chromatography resulted in specific activity of 94.44 U/mg and 86.36 U/mg in cell lysate and supernatant respectively. The purified enzyme was then characterized for optimization of temperature and pH. Thermostablility and stability of pH was also calculated. The optimum temperature at which xylanase showed highest activity was 70°C but thermostablility was obtained at 65°C. The optimum pH at which xylanase gave highest activity was 6.0 and was noticed to be stable at same pH. Effect of different ions was also examined on xylanase activity. Some ions such as Ca^{+2} and Mg^{+2} resulted in 2.5 and 2.3 folds increased activity, respectively. While the activity of xylanase enzyme decreased in the presence of Triton-X100, EDTA and cadmium.