ABSTRACT

This is the first research to conduct the gene cloning, expression and characterization of an alkaline phosphatase (AP) gene from a thermophilic bacterial strain *Anoxybacillus rupiensis*. AP has widely applications in the field of biotechnology i.e. blotting, sequencing, immunoassays, non-isotopic probing and biosensor etc. Current study reveals the alkaline phosphatase gene encoding from *Anoxybacillus rupienesis* that leads to cloning in pTG19-T vector and pET-22a (+) use as an expression vector. While *E. coli* BL21 Codon Plus strain use for transformation purpose. 12% SDS-PAGE was used to confirm the AP expression. Ammonium sulphate salt was use for purification of crude enzyme by precipitating process. Gel filtration chromatography was used for further purification of enzyme. In cell lysate, ammonium sulphate $NH_4(SO_4)_2$ precipitation process showed 70.0 U/mg of specific activity. Whereas 72.5 U/mg specific activity shown by supernatant. In supernatant and cell lysate the specific activity of 86.36 U/mg and 94.44 U/mg was given by gel filtration chromatography respectively. Purified alkaline phosphatase was then subjected to characterization such as pH and temperature optimization. Stability of pH and thermostability were also calculated. Enzyme show its optimal catalytic activity at pH 9.0 and also shown stability at this pH. Optimum temperature was 70 °C but thermostability was shown at 65 °C. Effects of various metal ions were also determined on AP activity. Two different ions i.e. Mg^{+2} and Ca^{+2} shown increased in catalytic activity about 2.3 and 2.5 folds respectively. Whereas, alkaline phosphatase activity was decreased by the presence of cadmium, Triton-X100 and EDTA.