ABSTRACT

In current research, study was done to clone esterase gene from newly isolated strain of *Anoxybacillus* sp. Which was recently classified as moderately thermophile. The given strain was biochemically characterized on the basis of different properties. The strain was positive for casein hydrolysis test and negative on the basis of gelatine hydrolysis test. Catalase test also showed positive result. Primers were designed from genome of *Anoxybacillus flavithermus* and esterase gene was amplified by PCR. After amplification, esterase gene cloned into pTZ57R/T vector and Positive clones were confirmed by colony PCR. The esterase gene was then expressed in *E.coli* BL21 by using expression vector pET-22b (+) to enhance expression of gene. Ethyl acetate was used as a substrate to find the enzyme activity. The recombinant enzyme gave optimum activity at 60°C and pH 8.0. The activity of esterase was slightly enhanced in presence of Ca^{2+} whereas other metals Mg^{2+}, Mn^{2+} slightly reduced activity. Hg^{2+} showed drastic effect on activity of recombinant enzyme. The docking results described that ethyl acetate fitted in binding cleft of esterase model. Blast results showed that *Anoxybacillus* esterase have almost 80 % identity to other species of same genus. The recombinant enzyme is potential candidate for industrial application due to distinct properties of thermostability.