ABSTRACT

The present work is focused on the cloning and expression of thermostable alpha amylase in *E. coli* from *Thermotoga* sp. In this context, 1.4 kb gene was selected and primers were designed. This gene sequence was amplified by PCR, followed by ligation of PCR product into pTZ57R/T cloning vector. This recombinant vector was transformed to competent cell of *E. coli* DH5α that were prepared by $CaCl_2$ treatment. Positive clones were selected by colony PCR and from them plasmids were isolated. These plasmids were restricted with *Nde*I and *Sal*I and restriction mixture was run on agarose gel and 1.4 kb band was cut and purified. pET21a(+) and pET28a(+) were also isolated and restricted with the same enzymes and followed by purification. Both purified gene and expression vectors were ligated using T4 DNA ligase. After overnight incubation at 16°C, the ligation mixture was used to transform in competent cells of E. coli DH5α. Colonies obtained after transformation were screened for gene of interest by colony PCR. From positive clones plasmids were isolated and cloning was confirmed with single restriction with *Sal*I enzyme. These recombinant plasmid were transformed to *E. coli* BL21 Codon Plus. The recombinant protein was expressed using IPTG and expression was analyzed by SDS-PAGE and a protein band of 55 kD was observed.