# ABSTRACT

*Thermotoga petrophila*, is hyperthermophile and anaerobic bacterium that is able to hydrolyze a variety of substrates including starch and is a potential source of thermostable amylases. Thus, the present work is focused on cloning and expression of thermostable α-amylase gene from *T. petrophila*. Putative amylase gene sequence (2.4 kb) was retrieved from NCBI genomic database (NCBI Reference Sequence: CP000702.1) and analyzed by BLAST and Conserved Domain Database Tools. The target gene sequence showed the 70-92% homology with amylases to cyclodextrin glucanotransferases from different bacterial species. The conserved domain database search revealed that the target query encodes for a multidomain protein, including AmyAc_AmyMalt_CG (cd11320) domain, DOMON like super family (cl14783) domain and α-amylase (pfam00128) domain. The forward and reverse primers (with *the NdeI restriction site* at the 5′ end of forward primer) were used to amplify 2.4 kb gene by PCR. The PCR product was ligated in TA cloning vector pTZ57R/T followed by transformation of the ligation mixture into the competent cells of *E. coli* DH5α. The recombinant plasmid containing insert+pTZ57R/T was isolated from the PCR positive colonies of *E. coli* DH5α and double digested with *Nde*I and *Sal*I restriction enzymes. The double digested and purified insert (2.4 kb) was subcloned into pET21a(+) expression vector and transformed into *E. coli* BL21 Codon Plus competent cells. The colony PCR was used to confirm the successful transformation and positive colon of *E. coli* BL21 was used for the expression of α-amylase in LB medium. The expression of recombinant protein was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and 94 kDa protein band was obtained.