ABSTRACT

Encoding a wide range of proteolytic enzymes, hyper-thermophilic organisms are able to thrive at or above 80 °C. As they can withstand extreme physiological and culture conditions, these hyper-thermophilic proteases present a compelling framework to be used for their economic value. The protease gene from *Thermotoga petrophila* was cloned and expressed using the bacterial vector pET-21a (+) for its thermophilic capacity. The target gene of interest was amplified using polymerase chain reaction and subsequently cloned. *Escherichia coli* BL 21(DE3) served as the host for recombinant gene expression. The recombinant plasmid was successfully transformed once the plasmid and the desired gene were ligated. By employing the plasmid-colony PCR, the cloning process was substantiated. Purification stages consisted of heat-treatment and Immobilized Metal Ion Affinity Chromatography (IMAC). Consistent with the recorded findings, two-dimensional SDS-PAGE revealed the molecular size of protein to be of 71 kDa aligning with the size of sequenced DNA of the gene of interest. The external growth factors were discovered to have a significant impact on the protease's properties and yield. Elevated temperature shifts from 60 to 80 °C and of pH level 5.0 to 7.0 resulted in a two-fold up-regulation of protease yield. Furthermore, culture medium optimization and enzyme profiling validated the thermophilic potential and stability of the respective protease enzyme. Thus, the proteases derived from thermophilic origin that exhibit distinct biophysical characteristics along with potential optimized function suggest that this system might offer a practical platform to be utilized for several biotechnological purposes. In this study, molecular cloning of protease from thermophile *Thermotoga petrophila* was studied and enzyme's optimized potential was assessed.