ABSTRACT

The quest for thermophilic microorganism for producing functional lipolytic enzymes with the ability to withstand high temperature and maintain their native state under extreme conditions open up new opportunities for biotechnology industry. A peptidase i.e., S9 prolyl oligopeptidase catalytic domain protein (Tnlip) from *Thermotoga naphthophila* RKU-10^{T} having 921 bp and encodes 306 amino acids was cloned in mesophilic host to produce thermotolerant recombinant lipases. Tnlip was initially cloned in pTZ57R/T cloning vector, subcloned in an expression vector pET-21a (+) and overexpressed in *E. coli* BL21 CodonPlus (DE3)-RIPL. Multiple sequence alignment showed 100% homology of lipase (TnLip) with S9 peptidase of *T. naphthophila* and 3D structure of protein confirmed Ser^{160} , His^{246} , and Asp^{196} in its catalytic triad. IPTG was used as an inducer and heterologous expression of protein by SDS-PAGE analysis confirmed the molecular weight of TnLip to be 35 kDa. Moreover, cell fractionations of recombinant TnLip and preliminary characterization showed the maximum activity (540 U/mL/min) with pNPP as substrate, at pH 7 and temperature 80°C by cell lysate (intracellular) fraction of protein. Therefore, this recombinant lipase (TnLip) can be utilized in biodiesel production and will be prove helpful in various other biotechnology processes procuring high temperature for its processing.