ABSTRACT

The present study was carried out to clone thermostable alkaline serine protease gene from a bacterial strain *Geobacillus steraothermophilus* B-1172. Genomic DNA was isolated and gene was amplified by PCR and thermostable alkaline serine protease gene from *G. stearothermophilus* was cloned into *E. coli* DH5α cells using pTZ57R/T as a cloning vector. Positive clones were screened by colony PCR and double digestion of the recombinant pTZ57R/T was performed with *EcoR*I and *Nde*I in order to separate the cloned protease gene. Ligation of protease gene in pET-22b(+) was carried out by using T4 DNA ligase at 22°C overnight and gene was further expressed in *E. coli* BL21 (DE3) strain. The growth conditions i.e temperature, pH, effect of IPTG and time of induction were optimized for the optimal production of the protease. Varying fermentation parameters i.e. size of inoculm, agitation rate, effect of different media, aeration rate and dissolved oxygen were also studied. Protease, thus produced, was purified by ammonium sulfate precipitation followed by Affinity chromatography and Gel filtration chromatography with 16.9 fold purification, specific activity of 120 U/mg, and a recovery of 54.68 %. Molecular weight of the purified protease, 39kDa was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The enzyme was stable at 90°C at pH 9. The enzyme activity was steady in the presence of EDTA indicated that the protease was not a metaloprotease. However, an addition of 1 % Triton X-100 or SDS surfactants constrained the enzyme specific activity to 34 % and 19 % respectively. Among organic solvents, an addition of 1-butanol (20 %) augmented the enzyme activity to 29 %. With casein as a substrate, the enzyme activity under optimized conditions was found to be 73 U/mg. The effect of protease expression on the host cells growth was also studied and observed negative affect on *E. coli* cells to certain extent.

Line-weaver Burk plot showed K_m and V_{max} values of 5.6 mg/mL and 2057.6 U/mL respectively. Thermodynamic parameters for hydrolysis of casein were found to be E_a=14.81 KJ/mol, ΔH= 12.14 KJ/mol, ΔS=-4.3 KJ/mol and Q_{10}=1. Catalatic domains of serine proteases from 08 important thermostable organisms were analyzed through WebLogo and found to be conserved in all serine sequences suggested that protease of *G. stearothermophilus* could be beneficially used as a biocontrol agent and in detergent

industry. The purified alkaline serine protease was examined for its compatibility with detergents in order to determine commercial utilization of protease in detergent industry. Shelf life of protease enzyme was 25 days at which 88 % residual activity was observed. Due to its high stability, alkaline serine protease proved as a valuable candidate for use in detergent industry.