Abstract

In the present study, protease producing thermophilic bacteria were isolated from water samples of Raikot, Gilgit Pakistan and thermostable protease was purified and characterized. The isolated strains were initially screened by using skim milk agar plates and the isolates that formed large clear zones were selected for protease production. Further screening of selected isolates was carried out by culturing the bacteria in shake flasks. After 48 h of incubation, maximum thermostable protease (106 U/mL) was produced by strain IIB-9. It was identified by morphological, microscopic, biochemical and molecular characteristics. Gene sequencing of 16s RNA of IIB-9 revealed that strain was *Geobacillus stearothermophilus*. Studies on the effect of different carbon and nitrogen sources revealed that glucose and peptone when supplemented in the fermentation medium enhanced the enzyme production. Upon optimization of parameters along with selected carbon-nitrogen sources, *G. stearothermophilus* IIB-9 produced maximum protease of 129.57 U/mL at 60°C, at pH 8.0 after 48 h of incubation with 2% inoculum size. Enzyme was partially purified with 1.31-fold and 71% yield by ammonium sulphate precipitation. The molecular weight of enzyme was found to be 37 kDa by SDS-PAGE. Studies on the protease characterization revealed that the optimum temperature and pH of this enzyme was 70°C and 8.0, respectively. The enzyme was stable for 30 min at 80°C, pH 9.0 with 90% and 63% residual activity, respectively. Enzyme showed highest specificity for casein as substrate. Activity was stimulated by Mg^{2+}, Mn^{2+} and Ca^{+2}, indicating that these ions had a functional role in the molecular structure and thermostability of the protease.