



Abstract

The present research work includes the heterologous expression and characterization of a thermostable protease of *Anoxybacillus* sp. *Anoxybacillus* sp. was morphologically, biochemically and physiologically characterized. For characterization light microscopy, gram staining, Catalase test, Casein hydrolysis test and gelatin test were performed. The conditions of growth including time of incubation, temperature and pH showed that *Anoxybacillus* sp. gave maximum growth at pH 7.0 and temperature 55°C after incubation of 24 hours. Genomic DNA was isolated and the thermostable protease gene of the *Anoxybacillus* sp. after its amplification using specific primers was cloned and expressed into *E. coli* BL21 by using the pET-22 b (+) as an expression vector. Positive clones were screened and confirmed by doing colony PCR, restriction digestion analysis as well as characterization. Recombinant protease was characterized to check the effect of temperature, pH, metal ions and thermostability. The enzyme was found to have the pH optimum of 7.5, temperature optimum of 65°C and was thermostable at this temperature even after the incubation of 1 hour. Enzyme gave the maximum activity by giving 5.2 Units/ml/min under the optimized conditions with casein as a substrate. This enzyme was found to be stable over a wide range of temperature and pH. Study of the effects of metal ions showed that activity of this recombinant protease was increased in the presence of Zn^{2+} and Cu^{2+} but it was reduced drastically when enzyme was incubated with Mg^{2+} , Mn^{2+} , Ca^{2+} and Hg^{2+} . Bioinformatics and phylogenetic analysis showed the resemblance of amino acids sequence of this protease with some other genera such as *Bacillus*, *Geobacillus*, and *Parageobacillus*. 3D structure modeling was performed and the interaction of substrate with enzyme in its binding cleft was analyzed through docking. However the biochemical properties of this recombinant protease proved that it could be a valuable and potential candidate for its use as a catalyst in a wide range of biotechnological applications.