1.0 ABSTRACT

The study was aimed at selecting a bacterial strain which can produce highest amount of alkaline protease and to improve the strain for protease production. Commercial strains *Bacillus licheniformis* ATCC 6346 (London) and *Bacillus licheniformis* M27 (CFTRI, Mysore) and isolated strains; BRE isolated from boiled rice extract and CD isolated from cow dung were used in this studies. The highest protease activity of $1.59x10^4$ UmL^{-1} was produced by *B. licheniformis* M27. The optimum temperature for the activities of proteases obtained from all four strains was 70°C. Among the four strains, the performance of *B. licheniformis* M27 was best; and it was selected for further studies. The optimum pH for the activity of protease obtained from *B. licheniformis* M27 was 9.5. The protease showed highest activity of $1.82x10^5$ UmL^{-1} at pH 9.5 and 70°C. The protease showed the K_m value of $11.11gL^{-1}$ with casein at pH 9.5 and 70°C. The stability of the protease was high at and above pH 6.0 and at room temperature. The stability was increased by 89.2% in presence of $25gL^{-1}$ casein at 70°C at 0.5h. Nutritional and physical parameters were optimized for protease production. Optimized fermentation medium contained (gL^{-1}) $(NH_4)_2SO_4$, 2.5; glucose, 9.0; Na_2HPO_4, 8.0; KH_2PO_4, 4.0; $MgSO_4.7H_2O$, 0.5; and $CaCl_2.2H_2O$, 0.025 and Tween-80, 0.5% (v/v). The maximum protease activity of $5.56x10^5$ UmL^{-1} was produced in the optimized fermentation medium at 84h. Optimized culture conditions were 42°C, pH 6.7, agitation rate of 125rpm and inoculum size of 20% (v/v) and the highest protease production was $5.78x10^5$ UmL^{-1}. The strain was improved by three repeated cycles of UV-mutation and chemical mutation to further increase the protease production. By optimizing the medium and culture conditions and by strain improvement the strain was able to produce $7.79x10^5$ UmL^{-1} protease activity which is 49 fold increase in the enzyme activity.