## Section E2

**601/E2**

### Purification and characterization of an extracellular heat stable serine protease from *Bacillus lichniformis NMS – 1*

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Alkaline proteases are used in the food industry, leather tanning and processing industry, and preparation of pharmaceuticals. Proteases produced from thermophilic bacteria isolated from hot water springs are used in a range of commercial applications. Enzymes isolated from these organisms are not only thermostable but are also often resistant to and active in the presence of organic solvents and detergents. In this study we report the isolation of an extracellular protease producing bacteria from a hot water spring and purification and characterization of the protease enzyme. The Nelum wewa hot water spring, situated in Sewanapitiya, in the Polonnaruwa District has a water temperature of 61 $^0$C and an out flow temperature close to the well of 56 $^0$C. This is the highest water temperature reported in Sri Lanka. The pH of the water was 4.8. Water and soil samples were collected under sterile conditions and were inoculated into culture medium containing 0.5% (w/v) yeast extract, 1% (w/v) peptone, and 0.5 g/l glucose, 0.4 g/l $Na_2HPO_4$, 0.085 g/l $Na_2CO_2$, 0.02 g/l $ZnSO_4$, 0.02 g/l, $MgSO_4$, 0.02 g/l $CaCl_2$. Casein was added to promote the production of protease and the bacteria was allowed to grow at 50 $^0$C, in an orbital shaker at 150 rpm. Bacteria were isolated by streak plate and dilution plate methods. Protease producing bacteria were identified by observing a clear zone in skimmed milk media. Out of the three bacterial strains isolated only one showed a clear zone in skimmed milk media. The bacteria was identified as *Bacillus licheniformis* strain NMS 1 by morphological, biochemical tests and 16s r RNA analysis. The maximum supernatant protease activity of 200 mU/ml was found at 50 $^0$C after growing for 28 hours. The extracellular protease enzyme was purified by ammonium sulphate fractionation and DEAE ion exchange chromatography. Two protease activity peaks were observed in ion exchange chromatography indicating the presence of two isoenzymes. One enzyme peak was pooled and the specific activity was reported as 3049 mU/mg with a 56-fold purification and a retained activity of 16%. Polyacrylamide gel electrophoresis showed the presence of two proteins bands indicating that the protein was partially purified. The partially purified enzyme had a maximum activity at 60 $^0$C. Studies on extracellular protease showed a broad pH optimum from 4 – 12 with maximum activity at 9. The enzyme was stable within the pH values of 8 – 12. The $K_m$ and $V_{max}$ values calculated from Lineweaver–Burk plot were $2.7x10^{-3}$ mg/ml and 263 mU/mg. Inhibition studies indicated that the isolated enzyme is a serine protease. The major protease types used commercially are heat stable alkaline proteases. Therefore this isolated protease will be a good candidate for industrial applications.

Keywords:Alkaline protease, extracellular, *Bacillus licheniformis NMS1,* heat stable

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