

## **CHARACTERIZATION OF PROTEASES FROM BACILLUS SUBTILIS.**

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Proteases are one of the most important industrial enzymes accounting for nearly 60% of total world wide enzymes sales. Gram-positive, spore-forming bacterium *Bacillus subtilis* produces several proteases at the end of the exponential phase of growth. The aim of this work was the characterization of extracellular enzymes from *Bacillus subtilis*. The bacteria were grown at 37°C in Soy medium. After incubating for 36h, the extracellular enzymes were obtained by centrifugation (8000 rpm; 4°C; 10 min). Proteolytic activity was determined using azocasein 1% (w/v) as substrate. The protein concentration of the preparation was 0.118 mg.mL<sup>-1</sup>. The enzyme was active in temperature range 45°C-65°C, and optimum pH was 9.0 (0.1M Tris-HCl buffer). The enzyme retained about 70% of initial activity after 120 min at 25-45°C. In the pH range 6-9 the enzymes were stable for 120 min, retaining about 80% of initial activity. None of the metal ions tested showed a significant inhibition of protease. Without ions NO<sub>3</sub><sup>+</sup> was observed an increase in the proteolytic activity (11-64%). The use of specific substrates protease inhibitors identified the presence of serine-protease and chymotrypsin-like enzymes. The Suc-Phe-p-Nan proteolysis was inhibited 96.9% by PMSF and 60.9% by TPCK. It shows that *Bacillus subtilis* is a potential source of alkaline and thermostable serine proteases, which can has several biotechnological applications.

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