

Purification of a subtilisin-like serine proteinase from recombinant *Bacillus subtilis* during different phases of growth

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Abstract

Subtilisin-like proteinase *Bacillus intermedius* which is secreted at different stages of bacterial growth (at 28 h and 48 h) were purified from the culture media of recombinant strain *Bacillus subtilis* JB 20-36(pCS9) by chromatography on CM-cellulose and MonoS columns. MALDI-TOF mass spectroscopy of purified enzymes demonstrated that they were identical in regard to amino acid sequence. The molecular weights of both proteins were 27 kDa. Biochemical analysis revealed differences in K_m values for proteinase isolated at different growth stages (1.85 and 0.86 mM for first and second fractions respectively), and in substrate specificity and sensitiveness to Ca^{2+} ions. Gel-filtration experiments demonstrated that subtilisin-like proteinase *B. intermedius* was produced as an active monomer (27 kDa) during early stationary phase (28 h of growth) and as a dimer (54 kDa) during the late stationary phase (48 h).

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Keywords

Bacillus intermedius, *Bacillus subtilis*, Protein dimer, Subtilisin-like proteinase