

ISOLATION AND CHARACTERIZATION OF A NOVEL EXTRACELLULAR AMYLASE FROM *Photobacterium profundum* SS9

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Microbial alpha-amylases are used in food, textile, and detergent industries. We isolated an amylase from cultures of *Photobacterium profundum* SS9. This bacterium thrives in seawater at more than 3000m depth. Two putative alpha-amylases, both with 474 amino acids, are predicted, but biochemical data are scarce. *P. profundum* was grown in aerobic cultures in synthetic or natural seawater enriched with tryptone and yeast extract, at 15°C, with passages at 96h. Cells were harvested by centrifugation (10000gx10minx4°C). Protein in the supernatants was concentrated 25-fold using an Amicon concentrator (10kDa cutoff). Amylase activity was assayed in seawater, with 50mM buffer (pH 5.0-8.0) 1mM CaCl₂ or 1mM EGTA, 0.1% soluble starch, and variable temperature, using the DNS method. We identified a major band with approximately 52kDa by SDS-PAGE of the concentrates, as expected from analysis of the genome for the *P. profundum* alpha-amylase. Activity was measured directly from the supernatants and on the concentrates, and was maximal at pH 8.0, decreasing at lower or higher pH's. Enzyme presented good activity and stability for more than 3h at 40°C, but was rapidly inactivated at 45°C or above. Although for some industrial applications thermostability is desirable, cold-active amylases might find application in food processing by high hydrostatic pressure at low temperatures.

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