# Heterologous expression and biochemical characterization of a novel cold-active ?-amylase from the Antarctic bacteria Pseudoalteromonas sp. 2-3

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?-Amylase is an endo-acting enzyme which catalyzes random hydrolysis of starch. These enzymes are used in various biotechnological processes including the textile, paper, food, biofuels, detergents and pharmaceutical industries. The use of active enzymes at low temperatures has a high potential because these enzymes would avoid the demand for heating during the process thereby reducing costs. In this work, the gene of ?-amylase from Pseudoalteromonas sp. 2?3 (Antarctic bacteria) has been sequenced and expressed in Escherichia coli BL21(DE3). The ORF of the ?-amylase gene cloned into pET22b(+) is 1824 bp long and codes for a protein of 607 amino acid residues including a His6-tag. The mature protein has a calculated molecular mass of 68.8 kDa. Recombinant ?-amylase was purified with Ni-NTA affinity chromatography. The purified enzyme is active on potato starch with a Km of 6.94 mg/ml and Vmax of 0.27 mg/ml*min. The pH optimum is 8.0 and the optimal temperature is 20