EXTRACELLULAR ALPHA-AMYLASE FROM BACILLUS sp.: TEMPERATURE, SALINITY, PRESSURE AND SUBSTRATE DEPENDENCE.

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Microbial alpha-amylases are used in food, textile, and detergent industries. We previously described a mildly acidophilic, thermotolerant extracellular amylase from Bacillus sp (Soares et cols., SBBq 2006). We now report effects of different media, substrates, and hydrostatic pressure on this activity. Cultures grown in LB medium at 30°C in 250ml Erlenmeyer flasks (125rpm), with passages at 72h, were centrifuged (10000gx10min). The supernatants, stored at 4°C, were precipitated overnight with 62.5% ammonium sulfate at 4°C, centrifuged (18000gx90min), and the pellets re-suspended in 10mM Tris-Cl (pH7.0). Otherwise, an Amicon concentrator (10kDa cutoff) was used. Enzyme was concentrated 50-200-fold and frozen. Activity was assayed by the (DNS) method in freshwater with 0.4M NaCl or synthetic seawater, 20mM buffer (pH 5.5), 0.1% soluble starch, 1mM $CaCl_2$. The synthetic substrate nitrophenyl-maltotrioside and modified starches were also used. Activity at 55°C-75°C in presence of 0.4M NaCl was activated by further salt addition. In seawater, activity was comparatively higher, and activity increased and lasted longer (>120min) when 4M NaCl were added. Modified starch was also a good substrate. CNPG3 was a substrate at all the temperatures, and hydrostatic pressure activated hydrolysis by 3.5-fold at 2.0kbar, with stability for more than 120 min at 55°C. This enzyme might be suitable for industry due to its good thermotolerance, acidophilic preference, substrate broad-specificity, and pressure activation.

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