

1. Title An alkaline active maltooligosaccharide forming alpha-amylase from *Bacillus halodurans*
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Abstract English The East African Rift Valley contains a number of soda lakes, which represent some of the most stable, naturally occurring alkaline environments on earth. Alkaliphilic microorganisms found in these habitats have attracted a great interest due to their ability to produce extracellular enzymes that are active and stable at high pH values for industrial applications. Starch hydrolysing alkaliphilic bacteria were isolated from samples collected from hot springs around Lake Bogoria, Kenya and identified as *Bacillus halodurans*. These isolates were found to produce extracellular amylolytic enzymes active at pH 10.0 and 55 °C. The gene encoding Amy 34, a maltohexaose forming α-amylase from *Bacillus halodurans* isolate LBK 34, was sequenced, cloned and expressed in *E. coli*. From sequence analysis, the four conserved regions within the α-amylase family of enzymes and a carbohydrate binding module (CBM family 25) at the C- terminal were identified, while a structural similarity search revealed that the enzyme is related to cyclodextrin glycosyltransferases (CGTases). Recombinant Amy 34 is a monomer of 119 kDa, exhibiting optimum activity at pH 10.5 - 11.5 and 60 °C. The enzyme hydrolyses starch to form a mixture of maltooligosaccharides, the main product being maltohexaose. The enzyme could also hydrolyse α-cyclodextrin but not β- and γ-cyclodextrins. Thermal unfolding of Amy 34 is irreversible with four transitions, as determined by differential scanning calorimetry. A transition (denaturation) temperature, T_m of 70.8 °C is obtained at pH 9.0, which increases by 5 °C and decreases by 10.4 °C when Amy 34 is heated in presence of 100-fold molar excess of CaCl₂ and metal chelator, EDTA, respectively. These observations, together with the inhibitory effect of calcium ions observed at high concentrations on enzyme activity suggests that calcium plays a role in stabilisation rather than having a direct role in the catalytic activity of Amy 34. Amy 34 amylase was also used to generate starch hydrolysate for subsequent use as a carbon source for *Halomonas boliviensis* LCI, a moderate halophile, to produce a bioplastic, poly(β-hydroxybutyrate). Finally, maltohexaose yield from soluble starch using Amy 34 was improved by *in situ* product recovery as compared to the process run under conventional batch conditions. Subject Chemistry

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