Characterization of α-galactosidase belonging to family-4 glycoside hidrolases from *Bacillus halodurans*

Bacillus halodurans 由来のファミリー4に属するαーガラクトシダーゼ酵素特性

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The α-galactosidase MelA (BH2228) gene of *Bacillus halodurans* was cloned and expressed in *Escherichia coli*. The *melA* gene consists of 1305 nucleotides encoding a protein of 434 amino acids with a predicted molecular weight of 49,761. It was assigned to family 4 of glycoside hidrolases. Almost all of the enzyme was produced as inclusion bodies at 37°C. In order to reduce the expression level, induction temperature was decreased to 20°C so that the enzyme could be produced in soluble fraction. By using His-binding metal affinity chromatography, recombinant α-galactosidase was purified to homogeneity in a single step. The purified enzyme required Mn^{2+} and NAD^{+} for the activity and showed optimum activity at 37°C and pH 7.4. The enzyme hydrolyzed *p*-nitrophenyl-α-D-galactopyranoside, melibiose, raffinose, and stachyose but not guar gum, indicating that this enzyme preferred small saccharides to highly polymerized galactomannans.