P7

Cloning and Characterization of the *Penicillium* sp. α-Galactosidase cDNA

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The gene encoding *Penicillium* sp. α-Galactosidase was cloned by PCR with designed primers based on partial amino acid sequences of the purified protein. The nucleotide sequence and deduced amino acid sequence of the 5′ and 3′ RACE products are shown. Examination of the sequence revealed the presence of one open reading frame of 1,371 bp. The nucleotide sequences of the overlap region of these fragments were identical, and the amino acid sequences of the purified enzyme identified by Edman degradation were found in the sequence. The coding sequence consisted of 19 amino acids of signal sequence and 420 amino acids of mature α-Galactosidase with a molecular mass of 46.3 kDa. Nine putative N-glycosylation sites were found in the sequence, and this is coincident with the reactivity with concanavalin.

P8

Purication of the Recombinant α-Galactosidase Expressed in *Escherichia coli*

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E. coli M15 cells were grown in 500ml of LB broth supplemented with ampicillin at 37℃ for 16 h with shaking. After the A_{600} reached 0.6, 2.5mM isopropyl-β-D-thiogalactopyranoside(IPTG) was added to the culture and the culture continued to grow at 37℃ for 5hr. The cells were harvested; suspended in 5ml of 50mM sodium phosphate, pH 7.8, containing 300mM NaCl; and sonicated on ice. The majority of the heat-labile proteins were precipitated by the heat treatment at 70℃ for 10min and removed by centrifugation. The supernatant was applied to the Chelating Sepharose FF column, which was equilibrated with 10 ml of 20 mM sodium phosphate, pH 7.4, containing 10mM imidazole and 0.5M NaCl. After a washing with the buffer containing 150mM imidazole, The enzyme solution was desalted by dialysis against 20mM sodium phosphate, pH 7.0, and stored at 4℃.