P49

Site-directed mutagenesis of the conserved amino acid residues in levan fructotransferase from *Microbacterium* sp.

Hee-Kyung Sung, Min-Jeong Kim and Jaeho Cha

Department of Microbiology, Pusan National University, Busan 609-735

Asp-63, Asp-195, and Glu-245 of a levan fructotransferase (LFTase) gene of *Microbacterium* sp. AL-210 are highly conserved residues and each was expected to play an important role in catalysis of this enzyme. PCR-based mutagenesis was performed to make point mutation from these three residues by changing with Ala, Asn, or Gln. Eight LFTase mutants were eventually constructed, and then subcloned into expression vector pET-29b(+). Each mutant was expressed in soluble form in *E. coli* BL21(DE3) by 1 mM IPTG induction and purified by using Hi-Trap chelating affinity chromatography and FPLC. The enzyme activities of th mutant enzymes were at least 100 times lower than that of the wild-type enzyme. D63A, D195N, E245A, and E245D showed 1%, 1.04%, 0.62%, and 1.26% activity of the wild-type enzyme, respectively. This result indicates that three residues Asp-63, Asp-195, and Glu-245, are critical in catalysis of LFTase. The kinetic study of each mutant is under progress to examine the detailed role of each residue.