P103

Conversion of Levan Fructotransferase into a Levanase by Directed Evolution

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Levan is a highly branched fructose homopolysaccharide consisting of β(2→6) linked fructosyl units for main chain and β(2→1)-linked fructosyl units for the branched chain. Levan fructotransferase (LFTase) preferentially catalyzes transfructosylation reaction, whereas other levan-degrading enzymes hydrolyze levan into a levan-oligosaccharide and fructose. Although LFTase and other levan-hydrolyzing enzymes show different reaction specificities, both enzymes share six highly conserved regions for fructosyl hydrolase family enzymes which is critical for catalysis. Based on the domain comparison, the major difference between both enzymes is the extra 51 amino acid residues at N-terminal region of LFTase which is not found in other levanase. However, the N-terminal truncated mutant of LFTase did not change the reaction specificity of the enzyme indicating that this domain is not critical for reaction specificity. To identify the amino acid residues that are critical for transglycosylation reaction specificity, we carried out error-prone PCR mutagenesis and screened for *Microbacterium* sp. AL-210 LFTase mutants with increased hydrolytic activity. Approximately ten mutants showed increased hydrolytic activity compared to wild-type LFTase by modified DNS method. The identification of the hydrolyzed reaction products as well as the sequencing of each mutant is under progress.