Cloning, sequencing analysis and expression in *Escherichia coli* of a *Leoconostoc mesenteroides* B-512 FMCM levansucrase encoding gene

Mi Young Seo[1], Hee Kyoung Kang[2], Eun Seong Seo[3], Gwang Ok Lee[3], Doman Kim[1,2]
Faculty of Chemical Engineering[1], Engineering Research Institute[2], Faculty of Applied Chemical Engineering and Institute for Catalysis[3], Chonnam National University, Gwang-ju, 500-757, Korea.
TEL: +82-62-530-0874, FAX: +82-62-530-1849

Abstract

An extracellular levansucrase gene, *m1ft* from *Leuconostoc mesenteroides* B-512FMCM, was isolated from a genomic library and the nucleotide sequence of the *m1ft* structural gene was determined. *m1ft*is composed of 1272 bp and code 424 amino acid residues with calculated molecular mass of 47.1 kDa. It showed an activity band of 103 kDa on a non-denaturing SDS-PAGE. This fact conformed the dimeric form of the M1FT. The amino acid sequence of *m1ft* gene showed good conservation with sequences of reported levansucrase and of the conserved regions through to be implicated in the enzyme activity, comparison of the deduce amino acid sequence certified the dissimilarity of the proteins from various bacteria. Polymers synthezied cell supernatant from *E. coli* transformant show levan, based on acid hydrolysis, NMR anlaysis, and dot blot using anti-levan-ab, which is specific for the ß-2,6 linked fructan composing the majority of *Acetobactor levanicum* polymer. The enzyme converted sucrose to fructooligosaccharides (FOS), small amount of 1-kestose and nystose, liberating glucose. The majority of free fructose and levan were also formed as a consequence of sucrose hydrolysis and fructan polymerase reactions, respectively. We studied the acceptor reaction of levansucrase using sucrose as a glucose or fructose donor. In case of maltose, it produced alose (maltose-fructose). The enzyme can synthesize melibiose, the major products of reaction. The optimum temperature and pH of this enzyme for levan formation was 30℃ and pH 6, respectively.

References

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