Purification and characterization of glucosyltransferase and fructosyltransferase from *Leuconostoc mesenteroides* NRRL B-1149

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Abstract

A glucan and a fructan producing enzymes from *Leuconostoc mesenteroides* NRRL B-1149 were prepared and concentrated from the culture of 1.5% sucrose using polysulfone ultrafiltration hollow fiber in the presence of 0.1% (w/v) Tween 80, 1 mM $CaCl_2$, and 0.02% NaN_3. The molecular masses of the enzymes were estimated to be about 213.6 kDa and 180 kDa, respectively, based on the PAS staining for the glucosyltransferase and Mukasa method for fructosyltransferase. Polymers produced by the enzymes showed different solubility; an insoluble glucan and a soluble fructan. The linkages of polymers were determined by methylation using Hakomori reagent and following acid hydrolysis. The glucan was composed of α-1,6 and 1,3 linkages and the fructan showed similar linkage data of levan.

Introduction

Glucosyltransferases(GTF, EC 2.4.1.5) such as dextransucrase and glucansucrase polymerize the glucose moiety of sucrose to form glucans. Many strains of *Leuconostoc mesenteroides* are known to produce different kinds of glucansucrases or dextransucrase.[1)] Dextransucrase of *L. mesenteroides* NRRL B-512F has been utilized industrially for the production of clinical dextrans and for the synthesis of Sephadexes. Other strains such as *L. mesenteroides* NRRL B-742, B-1299, and B-1355 are known to produce dextrans that have unique branching patterns. Many *Leuconostocs* can produce more than one type of polymer (alternans, dextrans and levans).[1,2,3)] All of these enzymes produced by the wild type *L. mesenteroides* strains require sucrose in the culture medium as an inducer.[2)] Fructosyltransferse(FTF, EC 2.4.1.9) synthesizes extracellular

fructans from sucrose. *S. sobrinus* and *S. mutans* can synthesize both extracellular glucans and fructans from sucrose.[3] The enzyme of *L. mesenteroides* NRRL B-1149 is known to produce an insoluble glucan with continuous α-1,3 glucosidic linkages.[3] In the present study, we found *L. mesenteroides* NRRL B-1149 produced both a glucosyltransferase and a fructosyltransferase. Thus, we tried to purify these two enzymes using a series of column chromatography methods and to characterize their properties.

Materials and methods

Organism and growth conditions

L. mesenteroides NRRL B-1149 were cultured with medium consisted of 1.5% (w/v) sucrose, 0.3% (w/v) yeast extract and peptone, 0.3% (w/v) K_2HPO_4, and 1% mineral solution (2% $MgSO_4 \cdot 7H_2O$, 0.1% NaCl, 0.1% $FeSO_4 \cdot 7H_2O$, 0.1% $MnSO_4 \cdot H_2O$, 0.13% $CaCl_2 \cdot 2H_2O$) at 28℃

Gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoreses (SDS-PAGE) was done, essentially as described by Laemmli, on 7% cross-linked polyacrylamide gels in tris-glycine buffer, pH 8.8. Protein fractions were denatured by heat treatment for 4 min at 100℃ in the presence of SDS (0.05%) and 2-mercaptoethanol (1%). Coomassie Brilliant Blue R-250 was used for staining. Non-denaturing gel electrophoresis was done by the method of Laemmli, excluding heating the sample.

Sugar-releasing activity stains by Mukasa method

After SDS-PAGE, the gel was washed for 60 min with 250 ml of 20 mM sodium phosphate buffer, pH 5.2, with 0.1% Tween 80 and 1 mM $CaCl_2$ at 4°C. The washed gel was partially dried for about 20 min with warm air from a hair drier until the edge of the layer gel dried up slightly, and the staining solutions as reported by Mukasa[5] were smoothly spread on the gel surface by inclining the gel slightly.

Methylation analysis

To determine the linkage of polymers which showed glucan and fructan in acid hydroysis they were methylated using the Hakomori reagent, followed by acid hydrolysis with 2 M trifluoroacetic acid, and analyzed for the methylated products using TLC.[4] The methylated products were separated by two ascents on Whatman K6 plates using 3: 9: 1 (v/v/v) of acetonitrile: chloroform:

methanol, followed by development of the plate using 0.3% N-(1-naphthyl)-ethylnenediamine and 5% sulfuric acid in methanol, and heating at 120°C for 10 min.

Results

Molecular size in SDS-PAGE

PAS staning of SDS-PAGE loaded with supernatants and incubated with sucrose (Fig. 1) indicated that the two bands of proteins showed different mobility; the first one was equivalent to a molecular weight around 213.6 kDa and the second one showed on the 180 kDa. Glucosyltransferase of *S. mutans, L. mesenteroides, S. sobrinus,* and *S. salivarius* show molecular masses between 130 and 180 kDa. Fructosyltransferase enzymes studied in various bacteria have molecular masses between 50 and 100 kDa, whereas a 140 kDa enzyme was reported in *S. salivarius.*

Confirmation of protein properties using monosugars- releasing activity staining

The culture concentrate from *L. mesenteroides* B-1149 contains both the glucosyltransferase and the fructosyltransferase. In figure 2, lane 2 shows glucose-releasing activity of culture concentrate on sucrose. The lane 3 shows glucose and fructose-releasing activity. Lane 2 shows a darker spot at bigger size band and a weak spot at smaller size. It means bigger size band produced much glucose compared with smaller one because it converts sucrose to fructan and glucose. Thus, the bigger band indicates fructosyltransferase. Lane 3 shows glucose and fructose releasing activity. The smaller band has a darker spot unlikely lane 2. It indicates that fructose was produced at that band. So, it could be considered as the glucosyltransferase.

Methylation analysis

Figure 3 shows the methylation analysis of glucan (lane 4) and fructan (lane 7) synthesized by a culture concentrate. Lane 4 shows the glucan has the linkage of α-1,6 and 1,3 like alternan. Lane 7 shows the fructan that shows similar composition data of levan.

Reference

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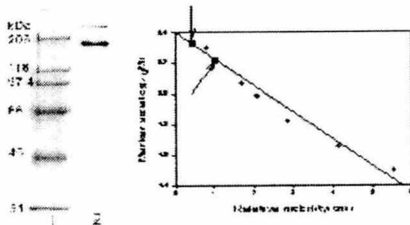

Fig. 1. Activity staining of culture concentrate produced from *Leuconostoc mesenteroides* B 1149. (Lane 1, marker; lane 2. PAS stainning of *L.mesenteroides* B 1149 culture concentrate.)

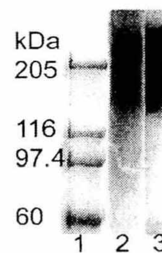

Fig 2. Glucose-releasing or Glucose fructose releasing activity staining of culture concentrate produced from *Leuconostoc mesenteroides* B-1149 on sucrose. (Lane 1. marker, lane 2. glucose releasing activities of *L.mesenteroides* B-1149 culture concentrate on sucrose, lane 3. glucose and fructose releasing activities of *L.mesenteroides* B-1149 culture concentrate on sucrose)

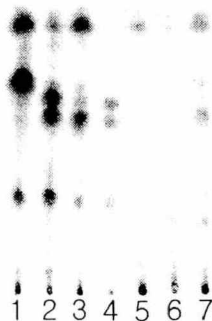

Fig. 3. TLC sepatation of O methylated D glucoses obtained from the methylation analysis analysis of soluble glucan and insoluble fructan (Lane 1. The methylation analysis of maltotriose; lane 2. the methylaion analysis of alternan; lane 3. the methylation)