Characterization of a thermostable fructosyltransferase from *Leuconostoc mesenteroides* TL1

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Abstract

A fructosyltransferase from *Leuconostoc mesenteroides* TL1 was purified. The molecular mass of the enzyme was estimated to be 38 kDa based on the activity staining. The pH and temperature optima of the enzyme were 6.3 and 40℃, respectivly. Structural analysis of the polymer prepared from sucrose by the enzyme was determined by NMR Spectroscopy: It shows the heterogeneous linkages of levan-like fructan and dextran-like glucan.

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 such as 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. Since *S. sobrinus* and *S. mutans* have both enzyme, they can synthesize both extracellular glucans and fructans from sucrose.[3)] In the present study, we isolated *L. mesenteroides* TL1 that produces a heterogeneous polymer of glucose and fructose and characterized the enzyme.

Materials and methods

Organism and growth conditions

L. mesenteroides TL1 was cultured with LWS medium consisted of 2% (w/v) sucrose, 0.3% (w/v) yeast extract and peptone, 0.3% (w/v) K_2HPO_4, and 1% (v/v) 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 40℃.

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. Non-denaturing gel electrophoresis was done by the method of Laemmli, excluding heating the sample. Coomassie Brilliant Blue R-250 was used for protein staining. The molecular weight of active protein was determined by Periodic acid-Schiff method.[4)]

Sugar component analysis of polymer

The sugars constituting the polymer produced by using TL1 was analyzed by TLC after acid hydrolysis. Complete acid hydrolysis of the polymer was performed with 1M hydrochloric acid (HCl) at 80℃ for 30 min. The determination of linkage type of the polymer was conducted with NMR analysis. Analysis for anomeric proton in polymer produced by TL1, 10 mg/ml of D_2O, was done by NMR : ^{1}H-NMR, Bruker AMX-500 (500MHz); ^{13}C-NMR, Bruker AMX-500 (125MHz).

Results

Molecular size in SDS-PAGE

Non-denaturing SDS-PAGE was conducted with the purified enzyme and the gel was incubated with sucrose (Figure 1). There was only an active band that was equivalent to 38 kDa.

Analysis of sugar components

Glucose and fructose were detected as sugar components of the polymer, indicating that the polymer is a heterogeneous polymer of glucose and fructose.

Structural analysis of the polymer by NMR Spectroscopy

There showed several resonances at 104.95, 104.23, 81.76, 81.05, 76.98, 75.92, 64.17, 62.94, 61.13, 60.60 p.p.m. These chemical shifts are similar with those assigned to C-1, C-2, C-3, C-5 of levan and C-3, C-6 of dextran (Figure 2).

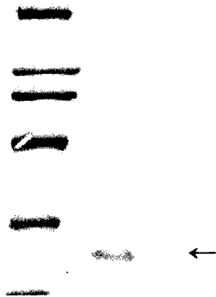
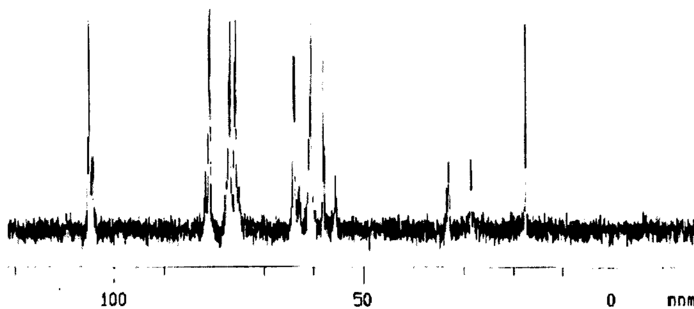


Fig. 1. Activity staining of the purified enzyme prepared *Leuconostoc mesenteroides* TL1. Lane 1, marker(200, 116, 97.4, 66, 45, 31 kDa); Lane 2, PAS staining of *L.mesenteroides* TL1



1 2

Fig. 2. ^{13}C NMR of polymer produced by the enzyme from *L. mesenteroides* TL1

Reference

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