J. Microbiol. Biotechnol. (1999), **9**(2), 219–222

JOURNAL OF
MICROBIOLOGY
AND
BIOTECHNOLOGY

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Facile Purification and Characterization of Dextransucrase from *Leuconostoc mesenteroides* B-512FMCM

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Received: September 7, 1998

Abstract A simple sequence of membrane concentration and DEAE-Cellulose chromatography has been optimized to give a purified dextransucrase from *Leuconostoc mesenteroides* B-512FMCM with the highest specific activity (248.8 IU/mg protein) ever reported in high yield (overall 88.7%) for dextransucrase. When there was no sucrose in the dextransucrase and the dextran reaction digest, the dextransucrase hydrolyzed glucose from dextran. The glucose was transferred to the other glucoses from dextran and formed isomaltose and isomaltodextrin. The transglycosylation efficiency of glucose from dextran was much higher with acceptors. The dextransucrase can be used for the production of various kinds (or structures) of oligosaccharides using dextran and various acceptors with almost 100% theoretical yield.

Key words: Dextransucrase, *Leuconostoc mesenteroides*, transglycosylation, oligosaccharides, purification

Dextransucrase (EC: 2.4.1.5) is a glucosyltransferase that catalyzes the synthesis of dextran using sucrose [3, 12]. Dextran polymerized by dextransucrases from *L. mesenteroides* B-512FMCM is an α-1,6-linked D-glucan having α-1,3-linked branches [7]. The enzyme can also catalyze the transfer of glucose from sucrose to other carbohydrates which were present or were added to the reaction digest, and make new oligosaccharides [1]. Studies for purifying dextransucrase from *L. mesenteroides* B-512F(M) were performed and either had low yields or failed to remove important impurities, especially polysaccharides [8, 9, 14]. This could be overcome by using the dextransucrase constitutive mutant, B-512FMC, developed by Kim and Robyt [4, 5]. It was a partial dextransucrase constitutive mutant with increasing activity. Recently, we have developed a dextransucrase hyper-producing mutant, *L. mesenteroides* B-512FMCM, from *L. mesenteroides* B-512FMC [7]. It was selected after irradiation of photons and produced 13 times higher activity than that of the parental strain. It also showed complete constitutivity for dextransucrase production.

The goals for this study were production and characterization of gram scale purified dextransucrase without dextran contamination, and use for the production of oligosaccharides from dextran and various carbohydrate acceptors with or without sucrose. The enzyme could transglycosylate glucose from dextran and formed a series of isomaltodextrins connected to acceptors with a high yield.

L. mesenteroides B-512FMCM dextransucrase was produced in a 5-liter Fermentor (Bok-Sung Co., Seoul, Korea) in 3 l of LM medium containing 2% (w/v) glucose as carbon sources [4, 5]. The pH and temperature were maintained at 5.5 and 28°C, respectively. The stirring rate was 100 rev/min and there was no aeration. Tween 80, $CaCl_2$, and NaN_3 were added at concentrations of 1 mg/ml, 2 mM, and 0.2 mg/ml, respectively.

For methylation and acid hydrolysis of carbohydrates, 10 mg of the carbohydrate was dissolved in dry DMSO (0.8 ml) by stirring until a clear solution was obtained. Hakomoris reagent (0.2 ml) and methyl iodide (0.2 ml) were added [11]. The reaction was allowed to proceed 2 h to give a darker yellow to brown color; 4 ml of water was then added to the yellow/brown DMSO solution. This mixture was extracted three times with 2 ml of $CHCl_3$. The $CHCl_3$ layer was rotary-evaporated in vacuo at 30°C or lower; 1 ml of 4 M CF_3CO_2H was added and the solution was transferred to a screw-cap tube that was autoclaved for 2 h at 121°C. The sample was made to a syrup by rotary evaporation in vacuo at 30°C or lower, and the syrup was diluted with 1 ml of methanol. Aliquots (1 μl) were placed on Whatman K6 TLC plates; the TLC plate was irrigated

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at room temperature with two ascents of acetonitrile-$CHCl_3$-methanol, 3:9:1 (v/v/v) at room temperature. The plate was thoroughly dried between each ascent. The compounds were developed on the plate by rapidly dipping the plate into a solution containing 3 g of *N*-(1-naphthyl) ethylenediamine and 50 ml of concentrated H_2SO_4 in 1 l of methanol [13]. The plate was dried and then placed in an oven for 10 min at 120°C; blue-black spots appeared on a white background.

The procedure for dextransucrase purification given here is for a one-liter culture. All procedures were conducted at 21°C. Buffers contained 1 mg/ml of Tween 80, 2 mM $CaCl_2$, and 0.2 mg/ml of sodium azide. After removing cells by centrifugation, the culture supernatant was concentrated using membrane filtration (MW 30 kDa cutoff). The concentrated enzyme solution was dialyzed overnight against 8 l of 20 mM sodium acetate buffer (pH 5.2) containing 50 mM NaCl. The dialysate (about 100 ml) was loaded onto a 1.0×15 cm DEAE-Cellulose column that was equilibrated with the same buffer. The column was washed with 200 ml of this buffer and then with 500 ml of 20 mM sodium acetate (pH 5.2) containing 200 mM NaCl. This was followed by 200 ml of 20 mM imidazole-HCl (pH 6.5) containing 200 mM NaCl, and by a linear NaCl gradient (800 ml; 0.2- 1.0 M) run over a period of 6 h.

Dextran size was determined using DAWN-Photometer (Wyatt Technology, U.S.A.) as described previously [6]. The transglycosylation reaction was performed by incubating 50 ml of B-512FMCM dextransucrase (10 IU/ml) and dextran (2%-w/v) with or without 100 mM maltose or isomaltose. Reaction digests were analyzed by TLC [13].

Unlike other *L. mesenteroides* strains, the B-512FMCM produced 81.7% of total protein as dextransucrase in glucose culture [7]. The enzyme solution was concentrated on Amicon PM30 membrane. 92% of the original activity was recovered with the activity of 470 IU/ml (Table 1). The most purification occurred after DEAE-Cellulose with a yield of 96.4% from the previous step and 88.7% of total activity to 1025 IU/ml. The final specific activity, 248.8 IU/mg proteins, is the highest value reported for *L. mesenteroides* B-512(F) dextransucrase. The purified enzyme showed a single band on SDS-PAGE with a size of 180 kDa (±7 kDa); that was the same size as the parental strain (B-512FMC) dextransucrase (Fig. 1) [4]. Several hundred-milligram amounts of dextransucrase had been purified from 3 l fermentation in 88.7% yield on a single DEAE-Cellulose column.

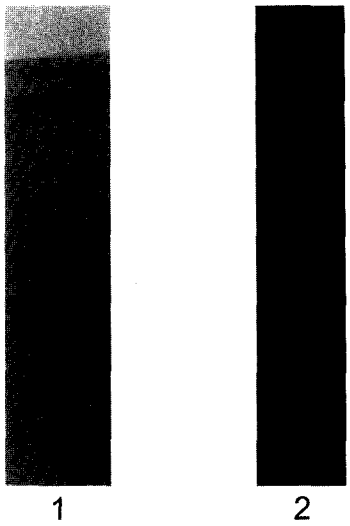

Fig. 1. SDS-PAGE analysis (1) and activity staining for dextran synthesis following SDS-PAGE (2) of the purified dextransucrase of *L. mesenteroides* B-512FMCM.
Dextransucrase activity was detected by incubating the gels in 150 mM sucrose overnight, followed by staining for polysaccharides by a periodic acid-Schiff procedure [10].

Figure 2 shows the TLC of the methylation and acid hydrolysis products of 1 mg of B-512FMCM dextran. The major methylated product was 2,3,4-tri-O-methyl-D-glucose with minor amounts of 2,3,4,6-tetra-O-methyl-D-glucose and 2,4-di-O-methyl-D-glucose. 2,3,4,6-tetra-O-methyl-D-glucose indicates the reducing ends of branched chains and 2,4-di-O-methyl-D-glucose occurred from the α-1,3-branched glucose residues. Based on the results of methylation and the endo-dextranase hydrolysis [7], it is certain that the B-512FMCM dextransucrase synthesized the same structured dextran of B-512FM(C) that have 95% α-1,6-linkages in the main chains with 5% α-1,3-branch linkages.

There were, however, significant differences between the mutant (B-512FMCM) and parental strain (B-512FMC) enzymes in respect to pH and temperature effects on the constitutively produced dextransucrase activities (Table 2). The B-512FMC dextransucrase activity was decreased above pH 7.0, and at pH 9.0 only had 40% of the maximum activity. The B-512FMCM dextransucrase, however, had

Table 1. Summary of the purification of B-512FMCM dextransucrase.

Step[a]	Vol (ml)[b]	Dextransucrase				Protein		Specific Activity
		IU/ml	Total Units	Step Yield (%)	Overall Yield (%)	mg/ml	Total mg	IU/mg Protein
Cell Removal	990	49	48510	100	100	0.24	242	200.5
Concentration	95	470	44650	92.0	92.0	2.35	224	199.3
DEAE-Cellulose	42	1025	43050	96.4	88.7	4.12	173	248.8

[a]Purification steps;
[b]Volume after each step; One IU of dextransucrase indicates the release of 1 μmole of fructose from 100 mM sucrose per min at pH 5.2 and 28°C. The dextransucrase was produced in 3 l of LM medium containing 2% (w/v) glucose as carbon source [4, 5].

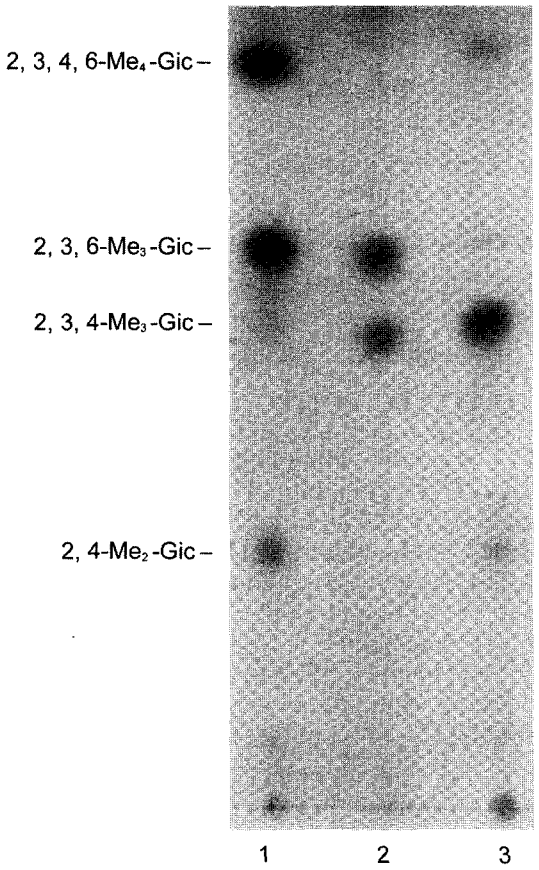

Fig. 2. TLC of O-methylated-D-glucoses obtained from the methylation and acid hydrolysis of B-512FMCM dextran.
Lane 1, standards from top to bottom of 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-glucose, 2,3,4,-tri-O-methyl-D-glucose, 2,4-di-O-methyl-D-glucose; Lane 2, O-methyl-D-glucoses from pullulan; Lane 3, O-methyl-D-glucoses from B-512FMCM dextran. Aliquots (1 μl) were placed on Whatman K6 TLC plates; the TLC plate was irrigated at room temperature with two ascents of acetonitrile-$CHCl_3$-methanol, 3:9:1 (v/v/v) at room temperature. The compounds were developed on the plate by rapidly dipping the plate into a solution containing 3 g of *N*-(1-naphthyl) ethylenediamine and 50 ml of concentrated H_2SO_4 in 1 l of methanol.

88% of its activity at pH 9.0. The temperature optimum of B-512FMCM was 28°C, just as the parental strain [4, 5]. Unlike B-512FMC dextransucrase, the activity was sharply decreased at 37°C and had only 2% activity at 45°C.

Depending on the pH and temperature for the synthesis of dextran, the size of dextran was slightly different. At 4°C the dextran size of 2.6×10^6 was the smallest under the reaction conditions described in Table 2. As the temperature increased, the size of dextran increased; at 10, 28, and 37°C (at pH 5.2), the size of each dextran was 3.7×10^6, 4.7×10^7, and 5.6×10^7, respectively. Above and below pH 5.2 (at 28°C) the dextran size decreased and at pH 8 or pH 4.0, the size was 6.5×10^6 or 3.0×10^7, respectively.

The B-512FMCM dextransucrase hydrolyzed glucose from dextran and transglycosylated into other glucoses (released from dextran by dextransucrase), maltose, and isomaltose. These transglycosylation reaction products were

Table 2. Effects of pH and temperature on dextransucrase activities of *L. mesenteroides* B-512FMCM and B-512FMC.

pH	Relative Activity[a] (%)		Temperature (°C)	Relative Activity[a] (%)	
	B-FMCM	B-FMC		B-FMCM	B-FMC
3.0	61	60	4	88	42
5.2	100	100	28	100	100
7.0	100	82	37	43	84
9.0	88	40	45	2	45

Enzymes were held at various pHs for 12 h or temperatures for 1 h, and then brought back to 21°C for assay.
[a]s.d. of the relative activity: less than ± 6%.

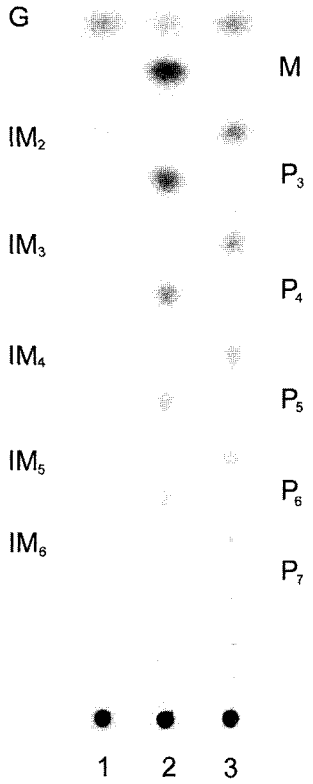

Fig. 3. TLC of the transglycosylation reaction of dextransucrase and dextran with or without maltose or isomaltose.
Lane 1, dextransucrase and dextran; Lane 2, dextransucrase, maltose and dextran; Lane 3, dextransucrase, dextran and isomaltose. G: Glucose; M: Maltose; IM_2: Isomaltose; P_3: Panose, Pn: (IM_{n-1}) connected to maltose; IMn: Isomaltooligosaccharides. The transglycosylation reactions were performed by incubating 100 μl of dextransucrase (10 IU/ml) and 2% dextran with or without 100 mM maltose or isomaltose (100 μl) at pH 5.2 and 28°C.

glucose, series of isomaltooligosaccharides (IMn) (Fig. 3, Lane 1); glucose, panose, IMn, and isomaltooligosaccharides connected to maltose (Fig. 3, Lane 2); glucose and IMn (Fig. 3, Lane 3). By the addition of maltose and isomaltose into the dextran and enzyme reaction digest the

transglycosylation efficiencies were increased up to 24% and 18%, respectively. Around 20 h reaction of the dextran (2%-w/v) with dextransucrase (10 IU/ml) with or without acceptor (50 mM in reaction digest) produced a series of oligosaccharides without leaving dextran. This is a potential way to produce various kinds of new oligosaccharides with almost 100% of theoretical yield.

Using dextransucrase hyper-producing strain *L. mesenteroides* B-512FMCM, we could produce purified dextransucrase that had a specific activity of 248.8 IU/mg with 88.7% yield using a simple ion-exchange chromatographic procedure. The purified enzyme highly transglycosylated glucose from dextran to various kinds of carbohydrate acceptors. These results suggested that the dextransucrase obtained from *L. mesenteroides* B-512FMCM can be used for the production of various kinds (or structures) of new oligosaccharides with almost 100% yield. Production of highly branched oligosaccharides from different structure polysaccharides (such as alternan, B-1299CB dextran) by transglycosylation using different glucansucrases is in progress.

Acknowledgments

This work was supported by the Korea Science and Engineering Foundation through the Basic Research Program (grant No. 971-0604-030-2) and the Research Center for New Bio-Materials in Agriculture at Seoul National University.

REFERENCES

1. Baek, J. S., D. Kim, J. H. Lee, P. S. Chang, N. S. Han, and J. F. Robyt. 1998. Enzymatic synthesis of new oligosaccharides using glucansucrases. *Kor. J. Appl. Microbiol. Biotechnol.* **26:** 179-186.
2. Fu, D. and J. F. Robyt. 1990. A facile purification of *Leuconostoc mesenteroides* B-512FM dextransucrase. *Prep. Biochem.* **20:** 93-106.
3. Jeans, A., W. C. Haynes, C. A. Wilham, J. C. Rankin, E. H. Melvin, M. J. Austin, J. E. Cluskey, B. E. Fisher, H. M. Tsuchiya, and C. E. Rist. 1954. Charcterization and classification of dextrans from ninety-six strains of bacteria. *J. Am. Chem. Soc.* **76:** 5041-5052.
4. Kim, D. and J. F. Robyt. 1994. Production and selection of mutants of *Leuconostoc mesenteroides* constitutive for glucansucrases. *Enzyme Microbial Technol.* **16:** 659-664.
5. Kim, D. and J. F. Robyt. 1994. Properties of *Leuconostoc mesenteroides* B-512FMC constitutive dextransucrase. *Enzyme Microbial Technol.* **16:** 1010-1015.
6. Kim, D., H. C. Seo, and D. F. Day. 1996. Dextran production by *Leuconostoc mesenteroids* in the presence of a dextranase producing yeast, *Lipomyces starkeyi. Biotechnol. Techniq.* **10:** 227-232.
7. Kim, D., D. W. Kim, J. H. Lee, K. H. Park, L. M. Day, and D. F. Day. 1997. Development of constitutive dextransucrase hyper-producing mutants of *Leuconostoc mesenteroides* using the synchrotron radiation in the 70~1000 eV region. *Biotechnol. Techniq.* **11:** 319-321.
8. Kobayashi, M. and K. Matsuda. 1980. Characterization of the multiple forms and main component of dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F. *Biochim. Biophys. Acta* **614:** 46-62.
9. Miller, A. W., S. H. Eklund, and J. F. Robyt. 1986. Milligram to gram scale purification and characterization of dextransucrase from *Leuconostoc mesenteroides* B-512F. *Carbohydr. Res.* **147:** 119-133.
10. Miller, A. W. and J. F. Robyt. 1986. Detection of dextransucrase and levansucrase on polyacrylamide gels by the periodic acid-Schiff stain: Staining artifacts and their prevention. *Anal. Biochem.* **156:** 357-363.
11. Mukerjea, R., D. Kim, and J. F. Robyt. 1996. Simplified and improved methylation analysis of saccharides, using a modified procedure and thin-layer chromatography. *Carbohydr. Res.* **292:** 11-20.
12. Robyt, J. F. 1986. Dextran. pp. 752-767. *In* Mark, H.F., N.M. Bikales,, C.G. Overberger, and G. Menges (eds.), *Encyclopedia of Polymer Science and Engineering*, vol. **4**. John Wiley & Sons, New York, U.S.A.
13. Robyt, J. F. and R. Mukerjea. 1994. Separation and quantitative determination of nanogram quantities of maltodextrins and isomaltodextrins by thin-layer chromatography. *Carbohydr. Res.* **251:** 187-202.
14. Robyt, J. F. and T. F. Walseth. 1979. Production, purification, and properties of dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F. *Carbohydr. Res.* **68:** 95-111.