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Production and Use of Glucosyltransferases from *Leuconostoc mesenteroides* NRRL B-1299 for the Synthesis of Oligosaccharides Containing α-(1→2) Linkages

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ABSTRACT

Glucosyltransferase activities, produced by batch culture of *Leuconostoc mesenteroides* NRRL B-1299, were recovered both in the culture supernatant (SGT) and associated with the insoluble part of the culture (IGT). A total glucosyltransferase activity of 3.5 U/mL was measured in batch culture. The enzymes from the supernatant were purified 313 times using aqueous two-phase partition between dextran and PEG phases, yielding a preparation with 18.8 U/mg protein. It was shown that both SGT and IGT preparations catalyze acceptor reactions and transfer the glucose unit from sucrose onto maltose to produce glucooligosaccharides. Some of the glucooligosaccharides synthesized (L_n series) contain α-(1→6) osidic linkages and a maltose residue at the reducing end. They were completely hydrolyzed by glucoamylase and dextranase. The other glucooligosaccharides synthesized (Bn series) resisted the action of these enzymes. The tetrasaccharide of this series has been characterized by ^{13}C NMR. Its structure was determined as 2-*O*-α-D-glucosylpanose. The oligosaccharides synthesized

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by the maltose acceptor reaction with the SGT and IGT preparations only differed in the relative amounts in which they were produced. The difference may arise from diffusional limitations appearing when the insoluble catalyst is used. Under the assay conditions, the glucanase resistant oligosaccharide yield was 35% with both glucosyltransferase preparations.

Index Entries: Glycotechnology; glucosyltransferase; *Leuconostoc mesenteroides;* acceptor reaction; oligosaccharide; α-(1→2) osidic linkages.

INTRODUCTION

Oligosaccharides have attracted more and more attention in the last few years. Besides their traditional use as a source of energy or sweeteners, they have found new applications in the food and feed industries as stabilizers, bulking agents, or dietary compounds stimulating the growth of beneficial bacteria of the intestinal microflora (*1–3*). The improvement of knowledge concerning the role of the cell-surface carbohydrates and the development of glycotherapeutics also increased the interest in oligosaccharide molecules and has opened new potential applications in immunology, human, and animal health (*1,4,5*). Although such a wide field of applications for oligosaccharides greatly stimulates glycotechnology, oligosaccharide production processes remain limited because of the high structural complexity of these molecules. The main industrial processes consist of plant extraction and acid or enzymatic hydrolysis of plant or microbial polysaccharides. Recent progress in enzymology have demonstrated the potential use of hydrolases in the catalysis of reverse reactions for the synthesis of glucooligosaccharides (*6*) and galactooligosaccharides (*7*). However, the reaction yields and the limited specificity of most hydrolases in reverse reactions limit the application of this technology.

Another enzymatic approach for oligosaccharide production consists of the use of transfer reactions catalyzed by transferases. Industrial enzymatic processes are used to synthesize cyclodextrins (*8*) and fructooligosaccharides (*3*). Glucooligosaccharides can be synthesized using glucosyltransferases (EC 2.4.1.5). These enzymes catalyze the transfer of glucose units from sucrose onto acceptor molecules, mainly sugars, resulting in the synthesis of glucooligosaccharides (*9–11*). In the absence of acceptors the main product of the reaction is dextran, a polymer composed of α-(1→6) linkages in the linear chain and α-(1→2), α-(1→3), and/or α-(1→4) branched linkages (*12,13*). The chemical structure of dextran was shown to be highly dependent on the glucosyltransferase producing strain (*12*). The most studied dextran, which is produced by the strain *L. mesenteroides* NRRL B-512F, contains 95% α-(1→6) linkages and 5% α-(1→3) branched linkages (*14*).

Our research work has been directed toward the design of processes for the production of glucooligosaccharides via acceptor reactions using the glucosyltransferases from *L. mesenteroides* strains. The synthesis of controlled mol-wt dextran by the acceptor reaction using dextransucrase from *L. mesenteroides* NRRL B-512F has been described and the enzymatically synthesized dextrans were shown to have less branched linkages than the dextran fractions produced by acid hydrolysis (*15,16*). More recently, we focused our attention on the production of oligoalternans and α-(1→3) branched oligosaccharides using the glucosyltransferases produced by *L. mesenteroides* NRRL B-1355 (*17,18*) and NRRL B-742 (*19*), respectively. These glucosyltransferases were shown to display a specificity in the acceptor reaction close to that observed in high-mol-wt glucan synthesis. As part of this research dealing with the enzymatic synthesis of oligosaccharides having dietary properties, we also studied the synthesis of oligosaccharides having α-(1→2) glucosidic bonds (*20*). In the present paper, we report oligosaccharide production using the acceptor reaction in the presence of maltose catalyzed by the glucosyltransferases from *L. mesenteroides* NRRL B-1299.

In 1954, *L. mesenteroides* NRRL B-1299 was first reported as producing a highly α-(1→2) branched dextran (*12*). Two fractions were isolated by ethanol precipitation, the L fraction (less soluble) and the S fraction (the more soluble), which contain 27 and 35%, respectively, of α-(1→2) branched linkages (*21–23*). Five fractions of *L. mesenteroides* NRRL B-1299 dextran differing in their solubility and mol wt were analyzed by Kobayashi et al. (*24,25*). They were shown to contain high percentages of α-(1→2) linkages, as well as a small amount of α-(1→3) linkages. Few authors have studied the glucosyltransferases from this strain. Results from Kobayashi and Matsuda (*26*) showed that 40% of the glucosyltransferase activity was recovered in the supernatant and that 60% of the activity was linked to the cells. A lysozyme treatment allowed the solubilization of almost 40% of the insoluble activity. They reported an initial extracellular activity in the supernatant of 0.028 U/mL. The extracellular glucosyltransferases of the supernatant were separated either as a monomer or as highly aggregated forms having different pH and temperature optima (*27*). Two other forms were isolated from the insoluble part of the culture (*28*). These insoluble glucosyltransferases were shown to be closely involved in insoluble dextran synthesis (*29*). Smith reported that 95% of the activity produced by the strain was associated with the cells but he failed to solubilize this activity (*30*).

There are, surprisingly, no reports dealing with glucooligosaccharide synthesis using the acceptor reaction catalyzed by the glucosyltransferases from *L. mesenteroides* NRRL B-1299. It has, however, been shown that these glucosyltransferases were more efficient in catalyzing the transfer of a glucosyl residue coming from sucrose onto lactulose than the dextransucrase from *L. mesenteroides* NRRL B-512F. The structure of the acceptor reaction product was shown to be the same (*31*).

In this paper, we describe the production and purification of two enzyme preparations with glucosyltransferase activity from cultures of *L. mesenteroides* NRRL B-1299. We report the study of the acceptor reaction in the presence of maltose catalyzed by the two enzyme forms and also the structural characterization of the maltose acceptor reaction products.

MATERIALS AND METHODS

Glucosyltransferase Production

Glucosyltransferases were produced by culture of *L. mesenteroides* NRRL B-1299. The culture medium composition was: sucrose (40 g/L), yeast extract (20 g/L), K_2HPO_4 (20 g/L), $MgSO_4 \cdot 7\ H_2O$ (0.2 g/L), $MnSO_4 \cdot H_2O$ (0.01 g/L), $CaCl_2$ (0.02 g/L), NaCl (0.01 g/L) and $FeSO_4 \cdot H_2O$ (0.01 g/L). The phosphate buffer solution was prepared and sterilized separately after the pH had been adjusted to 6.9 using orthophosphoric acid. The batch culture medium (1.5 L) was inoculated with 100 mL of a 15 h preculture. Temperature was maintained at 27°C. The culture was stopped at the end of the exponential growth phase when the pH was 4.5.

Glucosyltransferase Purification

The pH of the culture medium was adjusted at 5.2 at the end of culture. Cells and insoluble glucosyltransferases (IGT) were recovered by centrifugation, washed with 50 m*M* sodium acetate buffer, pH 5.2, and freeze-dried.

Soluble extracellular glucosyltransferases (SGT) from *L. mesenteroides* NRRL B-1299 were recovered in the culture supernatant and purified by aqueous two-phase partition between the dextran produced during the culture and polyethyleneglycol (PEG, mol wt 1500), using a process described by Paul et al. (32). PEG (50% w/v in H_2O) was added dropwise up to a final concentration of 18% (w/v). The mixture was then centrifuged at 16,000*g* for 15 min.

One unit (U) of glucosyltransferase is defined as the amount of enzyme that catalyzes the formation of 1 μmol fructose/min at 30°C in 20 m*M* sodium acetate buffer, pH 5.2, with 100 g/L sucrose. The initial rate of fructose production was measured by the dinitrosalicylic acid method (33). Proteins were assayed using the method of Lowry et al. (34).

Oligosaccharide Synthesis

Reactions were carried out at 30°C in 20 m*M* sodium acetate buffer, pH 5.2, sucrose (100 g/L), maltose (50 g/L or 33.3 g/L), B-1299 SGT (0.3 U/mL), or IGT (1 U/mL). Enzyme action was stopped after the total consumption of sucrose by heating to 75°C for 15 min. No modification of

the composition of the medium appeared after the heat treatment. When IGT was used, 200 ppm of sodium azide, which had no effect on glucosyltransferase activity, were added to the reaction medium to prevent cell growth.

The amount of oligosaccharides synthesized was determined from the area of the peak obtained by HPLC analysis on a C18 column (as described below) using isomaltooligosaccharides as standards. High-mol-wt dextran yield was measured by gel permeation chromatography on a Si 100 column (Merck-Clevenot). After reaction, the insoluble catalyst was removed by centrifugation, washed twice, and dried to weigh the amount of insoluble dextran synthesized during the reaction.

Oligosaccharide Purification

The acceptor reaction medium was first ultrafiltrated using an Amicon hollow fiber system (membrane cutoff = 100,000) to eliminate the endogenous or newly synthesized high-mol-wt dextrans. Afterward, fructose was removed by chromatography on a strongly acidic resin loaded with calcium (Duolite C-204F). Then, the oligosaccharides were hydrolyzed using the conditions described below. Finally, the separation of the different oligosaccharides was achieved by preparative HPLC (as described below).

Oligosaccharide Hydrolysis

Two glucanases were used to ensure the hydrolysis of the linear acceptor products synthesized: The endodextranase from *Penicillium* sp. (EC 3.2.1.11, Sigma Grade I, St. Louis, MO), which hydrolyzes only the α-(1→6)-D-glucosidic bonds (*35,36*) and the glucoamylase from *A. niger* (EC 3.2.1.3, Novo Nordisk, Danbury, CT), which was shown to hydrolyze the α-(1→4), α-(1→3) and α-(1→6) linkages at respectively decreasing rates to produce glucose from the nonreducing end of linear glucooligosaccharides (*37,38*). The acceptor-reaction mixture was first diluted to a total concentration of sugars of 40 g/L and was then incubated for 15 h at 37°C with the glucoamylase (3 U/mL) and/or the dextranase (20 U/mL). The amount of glucanases used was previously shown to catalyze the complete hydrolysis of a 30 g/L oligodextran solution in 2 h. One glucoamylase unit is defined as the amount of enzyme that hydrolyzes 1 μmole of maltose/min at 25°C. One endodextranase unit is defined as the amount of enzyme that catalyzes the hydrolysis of 1 μmole of isomaltose/min at 37°C and pH 6.0.

HPLC Analysis

Gel permeation chromatography (Si 100 column) as well as analytical reverse phase chromatography (C18 column) were carried out using a

Millipore-Waters system (Millipore Waters, Milford, MA) consisting of an M 6000 A pump, a U6K injector, and an R-410 differential refractometer. The ultrapure water eluent had a flowrate of 0.5 mL/min.

Preparative chromatography was carried out using a C18 column Partisil 10 ODS3 M40/50 (Whatman, Maidstone, UK). The system consisted of a Gilson 303 pump (Gilson Medical Electronics, Middleton, WI), a Gilson 131 differential refractometer, a Rheodyne injection valve (Cotati, CA), and a Pharmacia F300 fraction collector (Uppsala, Sweden). 4 mL of the oligosaccharide solution (150 g/L) were injected and eluted with ultrapure water at a flowrate of 15 mL/min.

^{13}C NMR

The ^{13}C NMR spectra of the oligosaccharides (75 to 100 g/L in deuterated water) were recorded at 303°K in a Brucker AC 80 spectrometer (20.1 MHz). The spectral width was 5 KHz, the acquisition time was 3 s, the number of transients was between 3000 and 10,000. The chemical shifts were expressed in ppm relative to acetone in water.

RESULTS AND DISCUSSION

Glucosyltransferase Production

Batch culture of *L. mesenteroides* NRRL B-1299 was carried out under standard conditions. The behavior of the culture parameters is presented in Fig. 1. At the end of the culture, glucosyltransferase activities were recovered both in the supernatant (SGT) and with the cells (IGT) and a total glucosyltransferase activity of 3.5 U/mL was harvested. The fraction associated with the cells called, in the literature, "intracellular activity" (*28*) is designated, in this report, as "insoluble glucosyltransferase activity" (IGT). Addition of maltose (20 g/L) and Tween 80® (10 g/L) to the standard medium reduced the amount of insoluble material formed during the culture and increased the amount of SGT by 60% (Table 1) but had no effect on the total glucosyltransferase activity produced.

Glucosyltransferase Purification

IGT activity reached a maximum of 0.5 U/mg for the batch culture in the presence of maltose. A combined treatment using lysozyme digestion and milling with glass beads resulted in 18% solubilization and in 54% loss of the insoluble activity. This high loss of activity led us to use the crude insoluble material to study the glucosyltransferases. In this case, a preservative was added to the reaction mixture to prevent cell growth.

SGT were purified using aqueous two-phase partition between dextran and PEG. After two successive extractions, 79% of the activity was recovered in the dextran-rich phase with the elimination of 99% of the

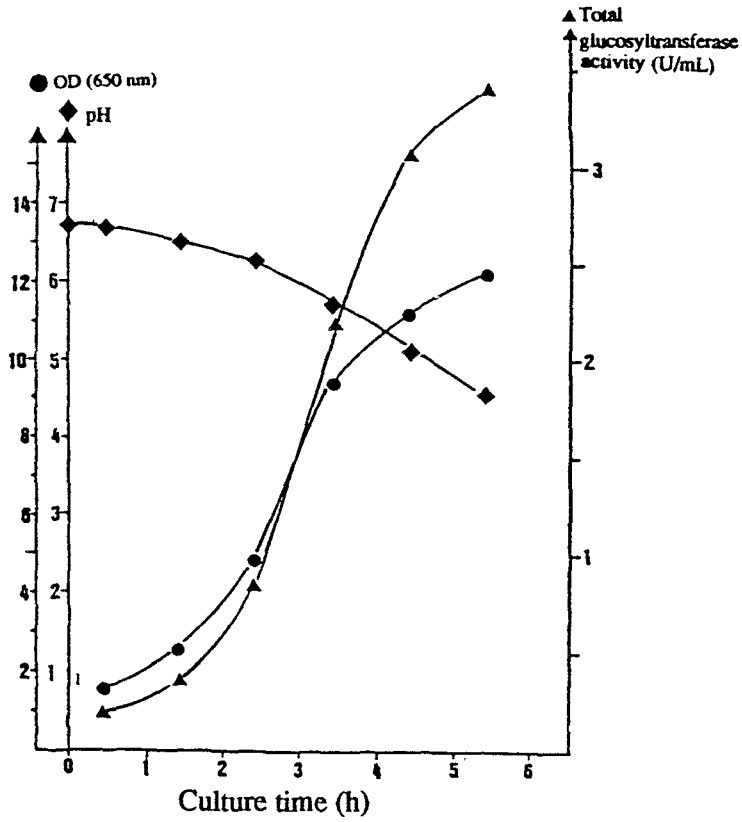

Fig. 1. Production of glucosyltransferases from *L. mesenteroides* NRRL B-1299 by batch culture under standard conditions.

Table 1
Production of Glucosyltransferase Activity
by *L. mesenteroides* NRRL B-1299 Using Different Culture Conditions

Batch culture conditions	Standard	Standard with maltose (20 g/L) and Tween 80 (10 g/L) addition
Culture time, h	5.5	5.5
Soluble glucosyltransferase activity, U/mL	0.37	0.58
Insoluble glucosyltransferase activity, U/mg	0.4	0.5
Insoluble material, g/L	7.9	5.2
Total activity, U/mL	3.5	3.2
Soluble activity, percent of total activity	10	18

Table 2
Purification of the Soluble Glucosyltransferase Activity from *L. mesenteroides* NRRL B-1299

Purification step	Volume, mL	Activity, U/mL	Protein, mg/mL	Total activity, U	Specific activity, U/mg	Yield, percent
Crude supernatant	1,200	0.38	6.1	456	0.06	100
Dextran phase after two extractions[a]	120	3.0	0.16	360	18.8	79

[a]Extractions were carried out using 18% PEG (w/v).

contaminating proteins (Table 2). The complex formed between SGT and the dextran allowed the use of this method, already described for use with the B-512F dextransucrase (32), for the purification of the NRRL B-1299 SGT. The freeze-dried preparation of SGT had a final activity of 0.12 U/mg dry material. The specific activity obtained was 18.8 U/mg, which favorably compares with 12 U/mg and 9.5 U/mg for the soluble and the aggregated forms of the NRRL B-1299 glucosyltransferases purified by Kobayashi and Matsuda with a 2% yield in a four-step purification method (27).

Oligosaccharide Synthesis Using IGT

We carried out the acceptor reaction in the presence of maltose acceptor (33.3 g/L) and sucrose (100 g/L). As shown in Fig. 2A, the NRRL B-1299 IGT catalyzes the synthesis of oligosaccharides. One series of oligosaccharides was readily identified as linear glucooligosaccharides exclusively composed of α-(1→6) linkages and a maltose residue at the reducing end. They are similar to those synthesized by dextransucrase from *L. mesenteroides* NRRL B-512F (*15,19*). They were noted L_n, the subscript n corresponding to the degree of polymerization (d.p.) of the oligosaccharide. When the acceptor reaction mixture was incubated with glucoamylase (Fig. 2B), the L_n oligosaccharides disappeared and hydrolysis resistant oligosaccharides were isolated. A plot (shown in Fig. 3) of the log of retention time of the glucoamylase resistant oligosaccharides vs the degree of polymerization suggests that all these oligosaccharides belong to the same series: They were noted B_n.

The concentration of oligosaccharides B_4 and B_5, which were directly synthesized by the acceptor reaction, increased after glucoamylase hydrolysis. Incubation with glucoamylase and dextranase gave almost the same results except that oligosaccharide B_7 was hydrolyzed and a peak corresponding to another oligosaccharide, noted B_4' appeared (Fig. 2C and 2D), probably resulting from the endohydrolysis of B_7.

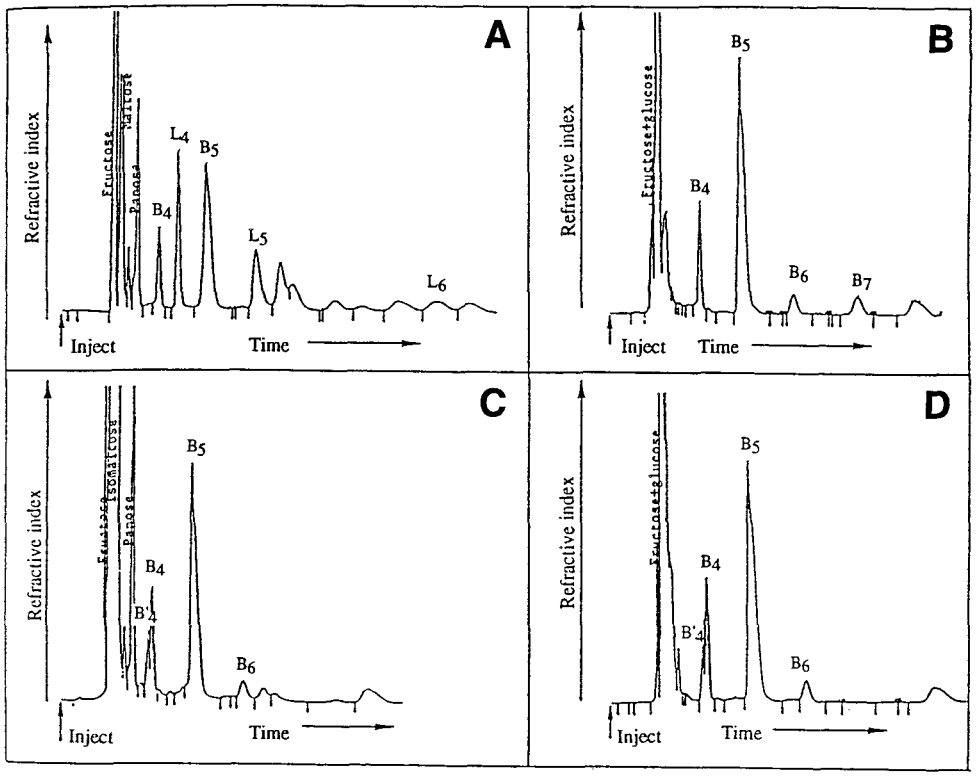

Fig. 2. HPLC analysis of the oligosaccharides synthesized with IGT from *L. mesenteroides* NRRL B-1299. Acceptor reaction was carried out with 100 g/L sucrose, 33.3 g/L maltose, and 1 U/mL IGT. HPLC analysis was carried out on C 18 column using ultrapure water as eluent at 0.5 mL/min. Products noted Li represent linear glucooligosaccharides of d.p.i composed of α-(1→6) linkages and a maltose residue at the reducing end. Products noted Bi represent oligosaccharides containing α-(1→2) linkages. (A) acceptor reaction products; (B) acceptor reaction products after glucoamylase hydrolysis; (C) acceptor reaction products after endodextranase hydrolysis; (D) acceptor reaction products after glucoamylase and endodextranase hydrolysis.

Structure of Oligosaccharide B_4

Oligosaccharide B_4 was purified using preparative HPLC (Fig. 4) to reach 98% purity as calculated from the area of the peaks observed on the chromatogram of the purified oligosaccharide. The preparation was then examined by ^{13}C NMR (Fig. 5). The chemical shifts reported in Table 3 were assigned to a given carbon by comparison with the spectra of maltose, panose and B-1299 dextran.

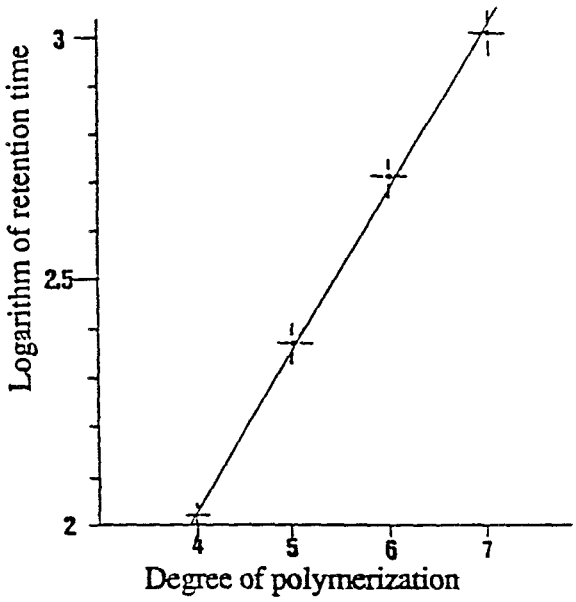

Fig. 3. Plot of the log retention time versus degree of polymerization of the oligosaccharides of the B_n series resistant to glucoamylase hydrolysis.

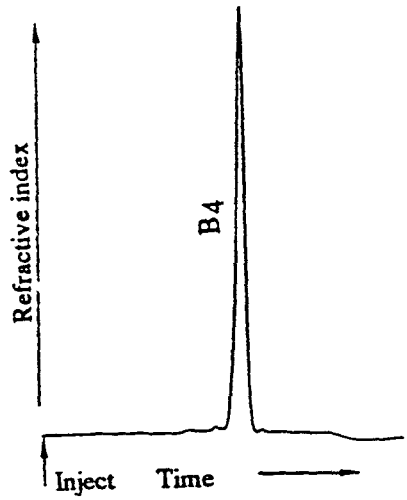

Fig. 4. HPLC chromatograms of oligosaccharide B_4 after preparative chromatography purification. The preparative separation was carried out using a C 18 Partisil 10 ODS3 M40/50 column (Whatman).

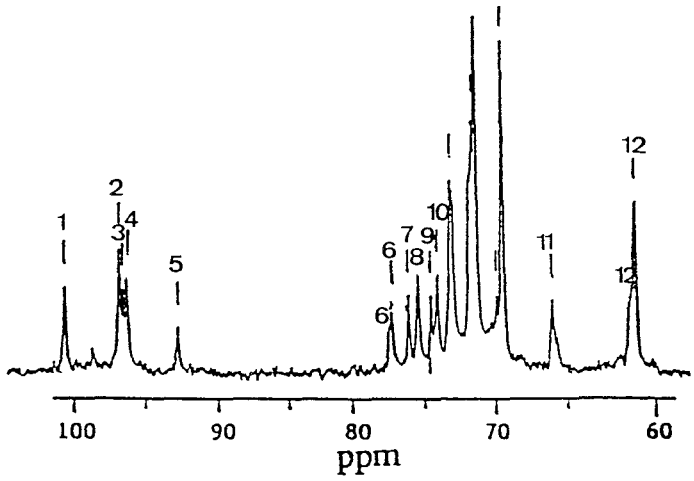

Fig. 5. ^{13}C NMR spectrum of oligosaccharide B_4 produced by acceptor reaction using the glucosyltransferases from *L. mesenteroides* NRRL B-1299.

Table 3
Chemical Shifts of Oligosaccharide B_4

Peak number	Assigment	Oligosaccharide B_4	Maltose[a]	Panose	Dextran B-1299[b]
1	C-1→4	100.7	100.7	100.7	
	C-1→6			99.1	98.7
2	C-1→2	97.1			97.2
3	C-1 β	96.8	96.8	96.8	
4	C-1→6	96.5			96.4
5	C-1 α	92.9	92.8	92.9	
6′	C-4→1	78.5	78.5	78.6	
6	C-4→1	78.3	78.2	78.3	
7	C-3[c]	77.1	77.1	77.2	
8	C-2→1	76.5			76.5
9	C-5[c]	75.5	75.6	75.5	
10	C-2[c]	75.	75.0	75.1	
11	C-6→1	67.1		66.9	66.6
12′	C-6	61.8	61.8	61.9	
12	C-6	61.4	61.6	61.6	61.4

[a]According to the assignments given in the literature (*39–41*).
[b]According to the assignments of Seymour et al. (*42,43*).
[c]Chemical shifts corresponding to the free carbon of the glucose residue (β anomer) located at the reducing end.

Anomeric Region, from 101 to 90 ppm

No signal arises downfield of 100.7 ppm indicating that oligosaccharide B_4 is only composed of α-D-glucosidic linkages. By comparison with the maltose and panose spectra, the resonances at 96.8 ppm (peak 3) and 92.9 ppm (peak 5) were assigned to the C-1β and the C-1α carbons of a glucose residue substituted at C-4. The signal at 100.7 ppm that corresponds to the anomeric carbon of a residue bound through an α-(1→4) linkage also appears on the spectrum. It comes from the maltose molecule used to carry out the acceptor reaction.

Peak 2 at 97.1 ppm, also encountered on the B-1299 dextran spectrum, is characteristic of an α-(1→2)-linked residue. This resonance indicates that oligosaccharide B_4 contains an α-(1→2) glucosidic bond. Peak 4 at 96.5 ppm, which also arises on the spectrum of dextran B-1299, comes from an α-(1→6) linked residue substituted on its C-2 carbon. This is another element that confirms the presence of an α-(1→2) glucosidic bond.

The resolved resonances observed in this region present about equal intensities (the resonances owing to the α and β configurations being added) and thus account for a reducing tetrasaccharide as expected from the HPLC retention time.

Region from 75 to 85 ppm

The resonances associated with the bonded C-2, C-3, and C-4 of the glucose residues as well as the resonances of the C-2, C-3, and C-5 of the β form of the reducing glucose of the molecule arise in this region. By comparison with maltose and panose spectra, peaks 7, 9, and 10 are attributed to carbons 3, 5, and 2, respectively, of the β form of the reducing glucose residue. Although they partially overlap, two resonances, coming from a bonded C-4, are distinguishable at 78.5 and 78.3 ppm (peak 6 and 6'). The splitting of the resonance shows that the carbon is affected by the α and β configurations of the molecule, as in the case of maltose, and confirms that the α-(1→4) linkage (coming from the maltose acceptor) is located at the reducing end of B_4. An additional proof of the presence of the α-(1→2) glucosidic bond in oligosaccharide B_4 is given by peak 8, at 76.5 ppm, which is associated with a bonded C-2 carbon.

Region from 60 to 70 ppm

This region is characteristic of the C-6 carbons. From the analysis of the anomeric region, B_4 should contain one bonded C-6 and three free C-6 atoms. The peaks corresponding to these carbon atoms effectively arise on the spectrum. Peak 11 is assigned to a bonded C-6 atom. From the respective intensities of peaks 12 and 12', we can conclude that they account for three free C-6 carbons.

Only one struture, given in Fig. 6, fits with the ^{13}C NMR analysis of B_4. The oligosaccharide is linear and has an α-(1→2) glucosidic bond at the nonreducing end. This is why it is highly resistant to glucoamylase and dextranase hydrolysis.

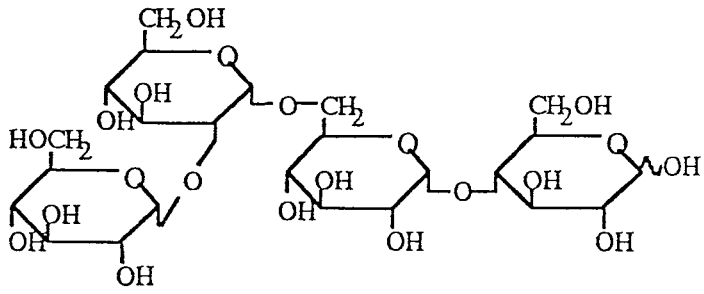

Fig. 6. Structure proposed for oligosaccharide B_4 in accordance with the results obtained from enzyme hydrolysis and ^{13}C NMR.

Comparison of the Insoluble and Soluble Glucosyltransferase Preparations

Maximal glucosyltransferase initial rate was observed at pH values ranging from 5.0 to 6.5 for IGT and from 4.5 to 6.0 for SGT. Acceptor reactions were therefore carried out at pH 5.2 for both preparations. Although a temperature of 40°C was found to be the optimum temperature of the two glucosyltransferase preparations, we carried out oligosaccharide synthesis at 30°C since a better stability of the two catalysts was observed at this temperature.

When maltose was added to the reaction mixture, a fourfold increase in activity was observed with SGT, but almost no change appeared in the initial rate of IGT. When studying the B-512F dextransucrase, Paul et al. (*15*) suggested that in the presence of an efficient acceptor, the transfer of glucose to the growing dextran chain is no longer the limiting step of the reaction; the presence of the acceptor allowing the continuous transfer of glucose and increasing the reaction rate. When maltose was added to the reaction mixture of IGT, there was almost no increase in the reaction rate. In this case, there may be a new rate-limiting step in the reaction mechanism, which is the diffusion of maltose from the bulk solution to the insoluble catalyst. This diffusional limitation could mask the increase of the initial velocity observed with SGT.

A reaction with 100 g/L sucrose and 50 g/L maltose was carried out with 0.3 U/mL SGT and 1 U/mL IGT. After 8 h, almost all the sucrose was consumed. Oligosaccharide analysis (Fig. 7) showed that both enzymes produce the same type of oligosaccharides with differences only in their relative concentrations. The amounts of B_4, B_5, L_4, and L_5 oligosaccharides obtained with SGT and IGT after 1, 4, and 8 h of reaction are reported in Table 4. The B_4 concentration always remained lower than that of L_4 (IGT giving a higher yield of oligosaccharide B_4 than SGT), whereas the B_5 concentration was always higher than that of L_5.

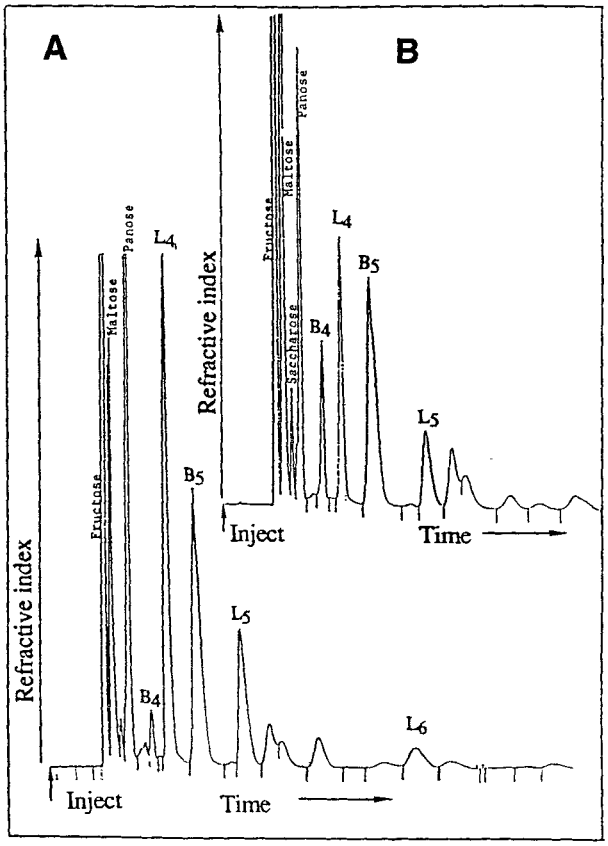

Fig. 7. Comparison of the oligosaccharides synthesized with the SGT (A) and IGT (B). Acceptor reactions were carried out with 100 g/L sucrose, 50 g/L maltose, and 0.3 U/mL SGT or 1 U/mL IGT. HPLC analysis was carried out on C 18 column using ultrapure water as eluent at 0.5 mL/min.

Table 4
Concentration of Oligosaccharides B_4, L_4, B_5, and L_5 After 1, 4, and 8 h of Synthesis with SGT and IGT from *L. mesenteroides* NRRL B-1299[a]

Acceptor reaction	Oligosaccharides synthesized with SGT, g/L				Oligosaccharides synthesized with IGT, g/L			
time, h	B4	L4	B5	L5	B4	L4	B5	L5
1	0.5	4.5	1.2	0.5	1.7	2.8	1.9	0.5
4	1.4	17.6	10.6	5.8	3.9	6.8	9.8	2.8
8	2.1	20.0	17.8	9.4	5.2	8.9	15.2	4.0

[a]Concentrations were determined from the area of each oligosaccharide observed on the HPLC chromatograms.

Table 5
Yield of Maltose Acceptor Reaction Products Synthesized with SGT and IGT

Glucosyltransferase activity	Soluble	Insoluble
Sucrose/Maltose ratio, w/w	2	2
Total oligosaccharide yield, percent[a]	90	70
Glucanase resistant oligosaccharide yield, percent[b]	34	37
High-mol-wt dextran yield, percent[c]	0.2	15

[a]Oligosaccharide yield:
Oligosaccharide (g/L)/[0.474 sucrose (g/L) + maltose (g/L)].
[b]Glucanase Resistant Oligosaccharide yield:
Glucanase Resistant Oligosaccharide (g/L)/[0.474 sucrose (g/L) + maltose (g/L)].
[c]High-mol-wt dextran yield:
[high-mol-wt dextran soluble (g/L) + high-mol-wt dextran insoluble (g/L)]/
[0.474 sucrose (g/L)].

The total oligosaccharide yield was higher with SGT and the high-mol-wt dextran yield was very low, 0.2% (Table 5). With IGT, we observe a higher high-mol-wt dextran yield (15%) probably because of diffusional limitations, which favor polymer synthesis at the expense of maltose glucosylation. By increasing the temperature from 30 to 40°C, it was possible to reduce high-mol-wt dextran production (measured at the end of the reaction) from 15 to 8%. This is another element supporting the hypothesis of diffusional limitations.

Although the acceptor reaction is less efficient with IGT, the yield of glucanase-resistant oligosaccharides (B_n series) is similar to that obtained with SGT (37 and 34%, respectively, Table 5). This shows that the two glucosyltransferase preparations can be efficiently used to produce oligosaccharides of the B_n series that contain α-(1→2) linkages. In addition, this study emphasizes the differences encountered in the acceptor reaction catalyzed by the glucosyltransferases coming from the different strains of *L. mesenteroides*. It was not possible to obtain α-(1→3) branched oligosaccharides having a degree of polymerization inferior to 6 with the B-742 glucosyltransferases (*19*), whereas tetrasaccharides containing α-(1→3) (*12*) or α-(1→2) linkages were synthesized with B-1355 and B-1299 strains, respectively.

CONCLUSION

We describe the production and purification of SGT and IGT from *L. mesenteroides* NRRL B-1299. The two glucosyltransferase preparations differ in their optimal pH and temperature. The presence of an acceptor did not increase the reaction rate of IGT unlike for SGT. Diffusional limita-

tions, appearing with IGT, could explain this as well as the results concerning the different proportions of high-mol-wt dextran synthesized by both fractions.

We demonstrated that the two glucosyltransferase preparations can catalyze the acceptor reaction in the presence of maltose leading to the synthesis of oligosaccharides. At least two series of oligosaccharides were identified: The L_n series, which can be readily hydrolyzed by glucoamylase and dextranase and corresponds to linear oligosaccharides exclusively composed of α-(1→6) linkages with a maltose residue at the reducing end, and the B_n series corresponding to oligosaccharides having an α-(1→2) glucosidic bond. The study revealed that very original and new oligosaccharides of low degree of polymerization can be obtained with a high yield using the glucosyltransferases from *L. mesenteroides* NRRL B-1299. We showed that the specificity of the glucosyltransferases observed during the acceptor reaction is close to that observed for high-mol-wt dextran synthesis.

In addition, oligosaccharides of the B_n series were recently shown to be highly resistant to glycolytic digestive enzymes while they are fermented by beneficial species of the intestinal microflora (44). These very attractive properties, that are a result of the presence of the α-(1→2) linkage, suggest that these oligosaccharides, now under industrial development, may find interesting applications in human or animal nutrition.

REFERENCES

1. Morgan, A. J., Mul, A. J., Beldman, G., and Voragen, A. G. J. (1992), *Agr. Food-Ind. Hi-Tech.* **11/12,** 35.
2. Monsan, P., Paul, F., Remaud, M., and Lopez, A. (1989), *Food Biotech.* **3,** 11.
3. Hidaka, H., Hirayama, M., Tokunaga, T., and Eida, T. (1990), in *New Developments in Dietary Fiber,* Furda, I., and Brine, C. J., eds., Plenum, New York, pp. 105–117.
4. Sherman-Gold, R. (1991), *Genet. Eng. News* **2,** 1.
5. Rademacher, T. W. (1992), *TIBTEC* **10,** 227.
6. Fujii, S. and Komoto, M. (1991), *Zuckerind.* **116,** 197.
7. Ajisaka, K., Nishida, H., and Fujimoto, H. (1987), *Biotech. Lett.* **9,** 387.
8. Horikoshi, K., Nakamura, N., Matsuzawa, N., and Yamamoto, M. (1981), in *Proceedings of the First International Symposium on Cyclodextrins,* Szejtli, J., ed., D. Reidel Publishing Co., Dordrecht, Holland, pp. 25–39.
9. Koepsell, H. J., Tsuchiya, H. M., Hellman, N. N., Kasenko, A., Hoffman, C. A., Sharpe, E. S., and Jackson, R. W. (1952), *J. Biol. Chem.* **200,** 793.
10. Tsuchiya, H. M., Hellman, N. N., and Koepsell, H. J. (1953), *J. Am. Chem. Soc.* **75,** 757.
11. Hehre, E. J. (1953), *J. Am. Chem. Soc.* **75,** 4866.
12. Jeanes, A., Haynes, W. C., Wilham, C. A., Rankin, J. C., Melvin, E. H., Austin, M. J., Cluskey, J. E., Fisher, B. E., Tsuchiya, H. M., and Rist, C. E. (1954), *J. Am. Chem. Soc.* **76,** 5041.
13. Seymour, F. R., Knapp, R. D., and Bishop, S. H. (1976), *Carbohydr. Res.* **51,** 179.

14. Larm, O., Lindberg, B., and Svensson, S. (1971), *Carbohydr. Res.* **20,** 39.
15. Paul, F., Oriol, E., Auriol, D., and Monsan, P. (1986), *Carbohydr. Res.* **149,** 433.
16. Remaud, M., Paul, F., Monsan, P., Heyraud, A., and Rinaudo, M. (1991), *J. Carbohydr. Chem.* **10,** 861.
17. Lopez-Munguia, C. A., Pelenc, V., Remaud, M., Paul, F., Monsan, P., Biton, J., Michel, J. M., and Lang, C. (1990), in *Enzyme Engineering 10,* vol. 613, Laskin, A. I., Mosbach, K., Thomas, D., and Wingard, Jr., L. B., eds., *Ann. NY Acad. Sci.,* New York, pp. 717–722.
18. Pelenc, V., Lopez-Munguia, C. A., Remaud, M., Biton, J., Michel, J. M., Paul, F., and Monsan, P. (1991), *Sci. Aliments* **11,** 465.
19. Remaud, M., Paul, F., Monsan, P., Lopez-Munguia, A., Vignon, M. (1992), *J. Carbohydr. Chem.* **11,** 359.
20. Paul, F., Lopez-Munguia, A., Remaud, M., Pelenc, V., and Monsan, P. (1992), Eur. Patent 0,325,872.
21. Seymour, F. R., Slodki, M. E., Plattner, R. D., and Jeanes, A. (1977), *Carbohydr. Res.* **53,** 153.
22. Seymour, F. R., Chen, E. C. M., and Bishop, S. H. (1979), *Carbohydr. Res.* **68,** 113.
23. Seymour, F. R., Knapp, R. D., Chen, E. C. M., Jeanes, A., and Bishop, S. H. (1979), *Carbohydr. Res.* **71,** 231.
24. Kobayashi, M., Shishido, K., Kikuchi, T., and Matsuda, K. (1973), *Agric. Biol. Chem.* **37,** 357.
25. Kobayashi, M., Shishido, K., Kikuchi, T., and Matsuda, K. (1973), *Agric. Biol. Chem.* **37,** 2763.
26. Kobayashi, M. and Matsuda, K. (1974), *Biochem. Biophys. Acta* **370,** 441.
27. Kobayashi, M. and Matsuda, K. (1976), *J. Biochem.* **79,** 1301.
28. Kobayashi, M. and Matsuda, K. (1975), *Biochem. Biophys. Acta* **397,** 69.
29. Kobayashi, M. and Matsuda, K. (1977), *Agr. Biol. Chem.* **41,** 1931.
30. Smith, E. E. (1970), *FEBS Lett.* **12,** 33.
31. Hehre, E. J. and Suzuki, H. (1966), *Arch. Biochem. Biophys.* **113,** 675.
32. Paul, F., Monsan, P., and Auriol, D. (1984), Eur. Patent 0,125,981.
33. Sumner, J. B. and Howell, S. F. (1935), *J. Biol. Chem.* **108,** 51.
34. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem.* **193,** 265.
35. Bourne, E. J., Hutson, D. H., and Weigel, H. (1963), *Biochem. J.* **86,** 555.
36. Taylor, C., Cheetham, N. W. H., and Walker, G. J. (1985), *Carbohydr. Res.* **137,** 1.
37. Pazur, J. H. and Ando, T. (1960), *J. Biol. Chem.* **235,** 297.
38. Pazur, J. H. and Kleppe, K. (1962), *J. Biol. Chem.* **237,** 1002.
39. Coxon, B. (1980), *Dev. Food Carbohydr.* **2,** 351.
40. Heyraud, A., Rinaudo, M., Vignon, M. R., and Vincendon, M. (1979), *Biopolymers* **18,** 167.
41. Dorman, D. E. and Roberts, J. D. (1971), *J. Am. Chem. Soc.* **93,** 4463.
42. Seymour, F. R., Knapp, R. D., Bishop, S. H., and Jeanes, A. (1979), *Carbohydr. Res.* **68,** 123.
43. Seymour, F. R., Knapp, R. D., and Bishop, S. H. (1979), *Carbohydr. Res.* **72,** 229.
44. Valette, P., Pelenc, V., Djouzy, Z., Andrieux, C., Paul, F., Monsan, P., and Szylit, O. (1993), *J. Sci. Food. Agric.* **62,** 121.