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Production of glucosyltransferases by wild-type *Leuconostoc mesenteroides* in media containing sugars other than sucrose

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Leuconostoc mesenteroides **produces glucosyltransferases (GTFs) and fructosyltransferases (FTFs) which are inducible enzymes which respectively synthesize dextrans and levans from sucrose. Except for a few mutant strains which produce high activities in glucose medium, *L. mesenteroides* is thought not to produce GTFs and FTFs unless sucrose is present. We show here that cultures of eight strains produced low, but detectable GTF activity when glucose, maltose or melibiose replaced sucrose as the growth substrate. Four strains also produced FTFs of approximately 130 kDa in medium with or without sucrose. The GTFs and FTFs produced on sugars other than sucrose could be detected as bands on SDS gels even when not detected by other methods. Except for strain B-523, the number, sizes and relative intensities of the bands were independent of the sugar used for growing the cultures. Alternansucrase from strains B-1355 and B-1501 in glucose or maltose medium was almost entirely associated with the cell fraction, ruling out binding to glucans as the cause of the association.**

Keywords: dextran; dextransucrase; glucan; glucosyltransferase; *Leuconostoc mesenteroides*; inducible enzymes; synthesis; nonsucrose medium

Leuconostoc mesenteroides produces a varied but poorly characterized set of extracellular glucosyltransferases (GTFs) when grown in media containing sucrose [20]. GTFs synthesize dextrans (α-glucopyranosyl-α(1→6) glucopyranoses) from sucrose, by transferring glucosyl units to nascent dextran chains and liberating fructose. The dextrans produced by different GTFs exhibit different degrees of branching via α(1→2), α(1→3) or α(1→4) branch linkages, with the structure of the dextran dependent on the producing strain. Some GTFs synthesize related polysaccharides, such as alternans (α-glucopyranosyl-α(1→6)glucopyranosyl-α(1→3)glucopyranoses) [16] or water-insoluble mutans (α-glucopyranosyl-α(1→3)glucopyranoses) [20, 21]. Dextransucrases (sucrose-1,6-α-glucan glucosyltransferase, E.C. 2.4.1.5), alternansucrases (sucrose-1,6(3)-α-glucan-6(3)-α-glucosyltransferase, E.C. 2.4.1.140), and mutansucrases are GTFs which synthesize dextrans, alternans and mutans, respectively. Some strains of *L. mesenteroides* also produce fructosyltransferases (FTFs, levansucrases or β-2,6-fructan d-glucose 6-fructosyltransferases, EC 2.4.2.10), which synthesize levans (β-2,6-fructans) from sucrose, producing glucose as a byproduct. Some of these GTFs and FTFs might be useful for synthesizing sugars and polysaccharides containing novel structures.

There have been few reports on the regulation of GTF and FTF synthesis by *L. mesenteroides*, but the GTFs and FTFs of *L. mesenteroides* are inducible enzymes requiring sucrose for induction [18], whereas the GTFs from related genera, such as *Streptococcus* sp, are made constitutively during growth on any fermentable sugar [20]. Neely and Nott [18] and others [7–11] did not detect GTF activity in the bacteria-free filtrates or supernatant fractions of cultures grown in media containing sugars other than sucrose and concluded that GTFs were not normally synthesized unless sucrose was present. Mutant strains of *L. mesenteroides* have been described which produce high activities of GTFs in glucose-containing media [7–11,23].

The conclusion that GTFs were not produced in the absence of sucrose contradicted earlier research by Hehre and Sugg [4], who showed that a strain of *L. mesenteroides* produced dextransucrase in glucose medium at approximately 5% of sucrose levels. We observed that *L. mesenteroides* strain B-1355 synthesized polysaccharide after the strain had been grown on glucose agar plates and overlaid with soft agar containing sucrose and streptomycin plus tetracyline to prevent the synthesis of new enzymes [23]. Dols *et al* [3] showed that strain B-1299 produced GTFs when grown with fructose or glucose instead of sucrose as the sole added carbohydrate. However, the possibility that sucrose might have been present in the cultures as an impurity was not rigorously excluded in the first two reports, and strain B-1299 might not be typical of other strains of *L. mesenteroides*. Moreover, the possibility that FTFs might also be produced in media without sucrose has not been investigated for this genus.

We re-examined GTF production by wild-type strains in media without sucrose, because cultures grown in media containing sugars other than sucrose might be an important source of dextran-free FTFs and/or GTFs, and because electrophoretic characterization of the GTFs from new strains should be done where dextrans cannot interfere [9,11]. Dextran-free cultures were needed to confirm the reported specific cellular association of alternansucrase in strain B-1355 [27]. Presently, the only way to obtain dextran-free GTFs is to mutagenize cultures and isolate constitutive mutants [7]. We also wanted to measure the approximate

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levels of GTFs produced in media without sucrose by a representative sample of strains to determine how much variation occurs among strains in the levels of activity.

Materials and methods

Bacterial strains

L. mesenteroides NRRL B-1355, B-1501, B-1299, B-512F, B-742, B-523, B-1118, and B-1149 were obtained from the stock culture collection of the National Center for Agricultural Utilization Research (NCAUR, USDA-ARS, Peoria, IL, USA). Cultures were maintained by weekly transfer on GTF medium [22] containing 1% (wt/vol) glucose.

Chemicals

3,5-Dinitrosalicylic acid, crude (10–25 units mg^{-1}), partially purified (400–800 units mg^{-1}) dextranase (from *Penicillium* sp, EC 3.2.1.11), and invertase (EC 3.2.1.26 from baker's yeast), were purchased from Sigma Chemical Company, St Louis, MO, USA. Protein reagent for the Bradford [2] dye-binding assay was purchased from Bio-Rad, Richmond, CA, USA.

Assays

Assays for GTF activity depended on the release of reducing sugar (fructose), measured using the 3,5-dinitrosalicylic acid (DNS) assay [25], as previously described [22,27]. Whole cultures were assayed by incubating samples (300 μl) at 30°C in 3.0 ml (final volume) of GTF assay solution. GTF assay solution contained (final concentrations): 50 mM sodium acetate, pH 5.6, 2 mM $CaCl_2$, 10% (wt/vol) sucrose, 0.02% (wt/vol) sodium azide and 0.2% (wt/vol) streptomycin sulfate. Sodium azide was included to inhibit the growth of cells. Streptomycin sulfate was omitted when assaying supernatant fractions, because few cells were present to interfere by synthesizing new GTFs and incubation times were short (up to 40 min). The reactions were stopped by removing 300 μl samples of reaction mixture at intervals and adding them to an equal volume of DNS color reagent. The intervals for cultures in sucrose medium 0, 20 and 40 min, and cultures in glucose medium were sampled at 0, 30, and 60 min and 24 h. The longer sampling period for glucose cultures was necessary to obtain a sufficiently high concentration of reducing sugar for accurate measurements. One unit of GTF activity was defined as the amount of activity which released 1 μmole of reducing sugar (fructose) per min from sucrose at 30°C.

Protein in supernatant fractions was assayed by the method of Bradford [2], using Bio-Rad dye-binding protein reagent and bovine serum albumin (Sigma, Fraction V) as the standard. The protein concentration in whole cultures was estimated by adding NaOH to the cultures to 1 N final concentration, boiling the suspension for 5 min, centrifuging it at 15 000 $\times$ *g* for 5 min to remove cells, then assaying the supernatant fractions using the Bradford dye-binding assay and 1 N NaOH as the diluent for the standards and samples.

Enzyme preparations

Supernatant fractions containing GTFs were prepared from overnight (30°C, 100 ml) cultures in GTF medium containing 2% (wt/vol) sugar by centrifuging them at 13 000 $\times$ *g* for 30 min. Protein concentrations in unconcentrated supernatant fractions ranged from 30 to 60 μg ml^{-1}.

Polyacrylamide gel electrophoresis

SDS-PAGE was performed using the Bio-Rad mini-Protean II slab gel system, 7.5% polyacrylamide resolving gels (4.0% stacking gels) of 0.75 mm thickness, and the Laemmli [13] buffer system. Samples (0.1–1 ml) were mixed with an equal volume of SDS/dithiothreitol sample buffer and incubated at 37°C for 1.5–2 h prior to loading onto gels. Final concentrations in samples were 62.5 mM Tris-HCl, pH 6.8, 40 mM dithiothreitol, 2% (wt/vol) sodium dodecylsulfate (SDS), 10% (vol/vol) glycerol, and 0.00125% (wt/vol) bromophenol blue. Samples containing suspended cells were centrifuged at 15 000 $\times$ *g* in a microcentrifuge before applying them to the gels. Approximately 1.0–1.5 milliunits of enzyme (approximately 600 ng of protein), as measured by DNS assay, were loaded onto the gels and electrophoresis was carried out for 1 h. The gels were then stained for GTF activity by washing to remove SDS, incubating the gels overnight with buffer containing 5% (wt/vol) sucrose, then washing and staining them for carbohydrate using the periodic acid-Schiff method [22].

Molecular masses of GTFs and FTFs were estimated from SDS gels that included Bio-Rad protein standards and were stained with Coomassie Blue R250 after staining for GTF activity [27]. The Bio-Rad markers contained myosin (200 kDa), β-galactosidase (116.25 kDa), phosphorylase b (97.4 kDa), bovine serum albumin (66 kDa) and ovalbumin (45 kDa). Electrophoresis was often continued for a period of time sufficient to allow the bovine serum albumin and ovalbumin standards to exit the bottom of the gels in order to provide high resolution and good separation of activity bands in the 90–300 kDa range. Samples from our cultures did not generate protein bands at the loadings used when gels were stained for protein with Coomassie Blue.

Synthesis of oligo-methyl-α-isomaltosides

Oligo-methyl-α-isomaltosides were synthesized from methyl-α-glucoside and sucrose in order to identify the GTFs that were present in cultures grown on different sugars. Samples (300 μl) of cultures of strain B-1355, grown overnight in GTF medium containing 2% (wt/vol) sucrose, glucose or maltose as the growth substrate, were incubated for up to 30 days at 30°C in GTF assay solution containing 200 μg ml^{-1} of streptomycin sulfate (to prevent the induction of enzymes by sucrose). The resulting methyl-α-oligosaccharides were separated and identified by the HPLC method of Lopez-Munguia *et al* [15], as previously described [22,24]. An ISCO (ISCO, Inc, Lincoln, NE, USA) HPLC system equipped with a Waters Dextropak column (length 20 cm, Millipore Inc, Milford, MA, USA) and a mobile phase consisting of water at a flow rate of 1.0 ml min^{-1} were used to separate the oligosaccharides, which were detected using a Waters model 410 differential refractometer. Peaks contributed by culture medium components, reactants (methyl-α-d-glucoside and sucrose), monosaccharide products (glucose, fructose and leucrose), buffers, and metabolic products eluted earlier than 16 min,

while oligo-methyl-α-isomaltosides produced well-resolved peaks eluting later than 16 min.

Results

We examined a diverse (based on the structures of the dextrans produced) sample of strains (strain B-512F, B-742, B-1355, B1501, B-523, B-1299, B-1149 and B-1118), from among the more than 96 dextran-producing strains of *L. mesenteroides* [5], for the ability to synthesize GTFs in medium containing 2% (wt/vol) sucrose, maltose, melibiose or glucose as the sole carbohydrate. These four carbohydrates were chosen because they are the same as those used by Neely and Nott [18], to show that GTFs were produced only in sucrose medium. Cultures were maintained on media containing 1% (wt/vol) glucose for over 1 year prior to inoculation into experimental media.

Sucrase activity in cultures

Table 1 shows that 24-h cultures of all of the strains tested produced GTF activity measured by the DNS method when sugars other than sucrose were provided as the sole energy source; the activity in sucrose medium was higher (usually 5- to 10-fold) than activities in medium containing sugars other than sucrose. Strains B-1501, B-1299, B-1355 and B-742 grew poorly in melibiose medium, and GTF activities were not measured in melibiose medium.

Most of the activity in cultures grown in non-sucrose medium was cell-associated. Supernatant activities, after removing the cells by centrifugation at 16 000 $\times$ *g* for 20 min, ranged from 0.008 to 0.16 units ml^{-1} and represented 20% or less of the total activity, except for cultures of strain B-1501 grown in glucose medium, where it represented approximately 69% of the activity of glucose cultures.

SDS-PAGE

Since DNS assays are not specific for GTFs or FTFs, it was necessary to confirm by SDS-PAGE that GTFs were produced in media containing sugars other than sucrose. Figure 1 (a–e) shows that all of the cultures produced bands of GTF activity on SDS gels regardless of the sugar used for growing the cultures. The number and sizes of the principal activity bands produced by each strain are summarized in Table 2, and except for strain B-523 (Figure 1c), they did not change as result of growing the cultures on sugars other than sucrose. The activity bands produced by the sucrose cultures of strains B-512F, B-1299, B-742, and B-1355 have been identified and reported previously [3,5,7–11,22,23,24], but the bands of GTF activity produced by strains B-1501, B-523, B-1118, and B-1149 are reported here for the first time. SDS gels from strain B-1501 were similar to those produced by strain B-1355, and both strains are known to produce alternansucrase. We did not see any growth substrate-dependent changes, as reported by Dols *et al* [3], in the sizes of activity bands from strain B-1299. Strains B-523, B-1118, and B-1149 exhibited patterns of activity bands on SDS gels that resembled one another in size and number, and they are known to produce similar types of water-insoluble mutan-like glucans, but the patterns of bands were different from patterns produced by cultures of strain B-1501, B-1355, B-1299 or B-742.

To exclude the possibility that our cultures were induced by low concentrations of sucrose which might have been present in our media as an impurity, we carried cultures of strains B-1355 and B-512F for four transfers [1% (vol/vol) inoculum] in glucose medium to which filter-sterilized solutions of invertase had been added to a final concentration of 1000 units (approximately 1.2 mg) ml^{-1}. The invertase was added at least 24 h prior to inoculation, and the medium was incubated at ambient temperature until inoculated (up to 4 days). After four transfers, cultures from the invertase-treated glucose medium produced activity bands on SDS gels following SDS-PAGE that were identical in number, size and intensity to activity bands produced by GTFs from cultures in glucose medium which had not been treated with invertase. We concluded that sucrose impurities did not cause GTF synthesis by our glucose cultures.

Table 1 Effects of growth substrate on the specific activity of GTFs from *L. mesenteroides*[a]

Strain	Specific activity (units mg^{-1} protein) Growth substrate			
	Sucrose	Glucose	Maltose	Melibiose
B-523	17.4	0.2	0.3	1.3
B-1118	4.9	0.3	0.1	2.2
B-1501	10.5	0.4	0.4	NM[b]
B-512F	6.5	ND[b]	0.1	0.1
B-1299	4.4	0.2	0.5	NM
B-1355	3.6	0.2	0.4	NM
B-742	2.8	0.2	0.5	NM
B-1149	2.4	0.2	0.2	0.2

[a]The whole cultures in Table 1 were assayed for GTF activity by the DNS method and protein by the dye-binding assay. The DNS assay buffer contained 0.2% streptomycin sulfate to inhibit protein synthesis. Protein concentrations ranged from 175 to 454 μg ml^{-1}, except where growth was poor (25–48 μg protein ml^{-1}).
[b]ND, not detected; NM, not measured because of poor growth.

Figure 1 SDS-PAGE of sucrases produced by strains grown on sucrose, glucose, maltose, or melibiose as the sole sugar. Samples from 24-h cultures were loaded onto SDS gels and subjected to SDS-PAGE. The gels were then stained for GTF activity. (a) Lane: (1) B-512F on glucose medium; (2) B-512F on maltose; (3) B-512F on melibiose; (4) B-512F on sucrose; (5) B-742 on glucose; (6) B-742 on maltose; (7) B-742 on sucrose. (b) Lane: (1) B-1355 on glucose; (2) B-1355 on maltose; (3) B-1355 on sucrose; (4) B-1501 on glucose; (5) B-1501 on maltose; (6) B-1501 on sucrose. (c) Lane: (1) B-523 on glucose; (2) B-523 on maltose; (3) B-523 on melibiose; (4) B-523 on sucrose; (5) B-1299 on glucose; (6) B-1299 on maltose; (7) B-1299 on sucrose. (d) Lane: (1) B-1149 on glucose; (2) B-1149 on glucose; (3) B-1149 on melibiose; (4) B-1149 on sucrose; (5) B-512F on sucrose; (6) B-742 on sucrose. (e) Lane: (1) B-523 on sucrose; (2) B-512F on sucrose; (3) B-1355 on sucrose; (4) B-1118 on melibiose; (5) B-1118 on maltose; (6) B-1118 on glucose; (7) B-1118 on sucrose. Samples in lanes b-5, d-6, and e-1 to e-3 were included to permit size comparisons.

Table 2 Number of principal activity bands on SDS gels

Strain	Growth substrate				Approx. mol. mass (kDa)
	Suc	Glu	Mal	Mel	
B-512F	2	2	2	2	177, 130
B-742	2	2	2	–	198, 170
B-1355	4	4	4	–	240, 203, 179[a], 171
B-1501	4	4	4	–	240, 203, 183, 173
B-1299	2	2	2	–	240, 197–213
B-523	4	3	3	3	250, 192, 186, 130
B-1149	3	3	3	3	250, 185, 130
B-1118	4	4	4	4	250, 192, 186, 130

[a]Previously considered a minor activity band, but consistently present on SDS gels.
Abbreviations: Suc, sucrose; Glu, glucose; Mal, maltose; Mel, melibiose.

Partial identification of activity bands on SDS gels

We partially identified the activity bands on our SDS gels by comparing gels treated with 10 units ml^{-1} of dextranase prior to staining for carbohydrate with the periodic acid-Schiff reagent to gels not treated with dextranase (Figure 2 a,b). Figure 2a shows the normal pattern of activity bands produced by sucrose cultures of the eight strains of *L. mesenteroides* on SDS gels which had not been treated with dextranase before staining for carbohydrate. Figure 2b is a duplicate gel which was hydrolyzed with dextranase prior to staining for carbohydrate, in order to reveal alternansucrase and other GTFs synthesizing dextranase-resistant polymers containing significant percentages of non-$\alpha(1\rightarrow6)$ linkages. All of the strains except strain B-742 produced at least one activity band that was resistant or partially resistant to hydrolysis with dextranase. The dextranase-resistant bands in lanes 2 and 4 were produced by alternansucrase [22]; that in lane 5 by a GTF which synthesizes highly branched glucans containing $\alpha(1\rightarrow2)$ glucosidic linkages [3,10]. Strain B-512F (lane 1) produced a band of approximately 130 kDa which was resistant to hydrolysis with dextranase, and which we identified (see below) as levansucrase. This band was apparently previously misidentified as dextransucrase [8].

Levansucrases (Figure 2c) were identified by incubating an SDS gel identical to those in Figure 3a and b with 8% (wt/vol) raffinose instead of 5% (wt/vol) sucrose, then staining it using the periodic acid-Schiff method. We also treated our raffinose-incubated gels with dextranase prior to staining them to eliminate any GTF bands that might arise from sucrose impurities in our raffinose solution. Figure 2c shows that strains B-512F, B-1149, B-523, and B-1118 produced levansucrases; the apparent molecular masses were all near 130 kDa. Strain B-523 and B-512F are known to produce levans [6]. The partially dextranase-resistant activity band at 186 kDa seen with cultures of strain B-523 and B-1118 (Figure 2b) were possibly produced by mutansucrases, because the two strains are known to produce mutans and the bands were not levansucrases, as indicated by their absence in Figure 2c.

Identification of GTFs from strain B-1355 by the synthesis of methyl-α-isomaltosides

We confirmed that strain B-1355 produced GTF-1, alternansucrase, and dextransucrase in medium containing maltose, glucose or sucrose as the sole carbohydrate, by showing that cultures grown on each of the three sugars synthesized the same methyl-α-isomaltosides from methyl-α-glucoside and sucrose (Figure 3 a–c). The incubations are described in the Materials and Methods section, and they contained streptomycin to prevent induction of GTFs by sucrose. The presence of GTF-1 was indicated by the presence of 1-O-methyl-α-glucopyranosyl-$\alpha(1\rightarrow2)$ isomaltoside, and alternansucrase by the presence of 1-O-methyl-α-glucopyranosyl-$\alpha(1\rightarrow3)$isomaltoside among the reaction products [15,24]. 1-O-Methyl-α-isomaltoside and 1-O-methyl-α-isomaltotrioside were synthesized by GTF-1, alternansucrase, and dextransucrase [19,24]. In agreement with Dols *et al* [3], we were able to detect a very small peak corresponding to 1-O-methyl-α-glucopyranosyl$\alpha(1\rightarrow3)$isomaltoside after sucrose cultures of strain B-1299 were incubated with sucrose, methyl-α-d-glucoside, and streptomycin, but we did not see a peak corresponding to the trisaccharide when glucose cultures were the source of the GTFs.

Cell-associated alternansucrase activity in glucose-grown cultures

We have previously reported that alternansucrase produced by strain B-1355 was almost entirely a cell-associated GTF, while GTF-1 and dextransucrase were distributed more equally between the cell and supernatant fractions of sucrose cultures than was alternansucrase [27]. However, the apparent cellular association of alternansucrase might have been caused by it binding to dextrans or other glucans of high molecular weight produced in the sucrose cultures. GTFs bind to dextrans, and dextrans of high molecular weight tend to sediment with the cells during centrifugation [11]. To confirm that alternansucrase was truly associated with the cellular fraction of cultures, and not merely binding to dextrans, we grew cultures of strain B-1355 and B-1501 in glucose medium, where dextrans are not produced, and compared the GTFs from whole cultures, using SDS-PAGE, with GTFs from the supernatant fractions. The gels were stained for GTF activity as before. The results (Figure 4) showed that alternansucrase was almost entirely removed from the supernatant fractions of glucose-grown cultures when the cells had been removed by centrifugation at $13\,000 \times g$ for 20 min. We concluded that the cellular association of alternansucrase was a true cellular associ-

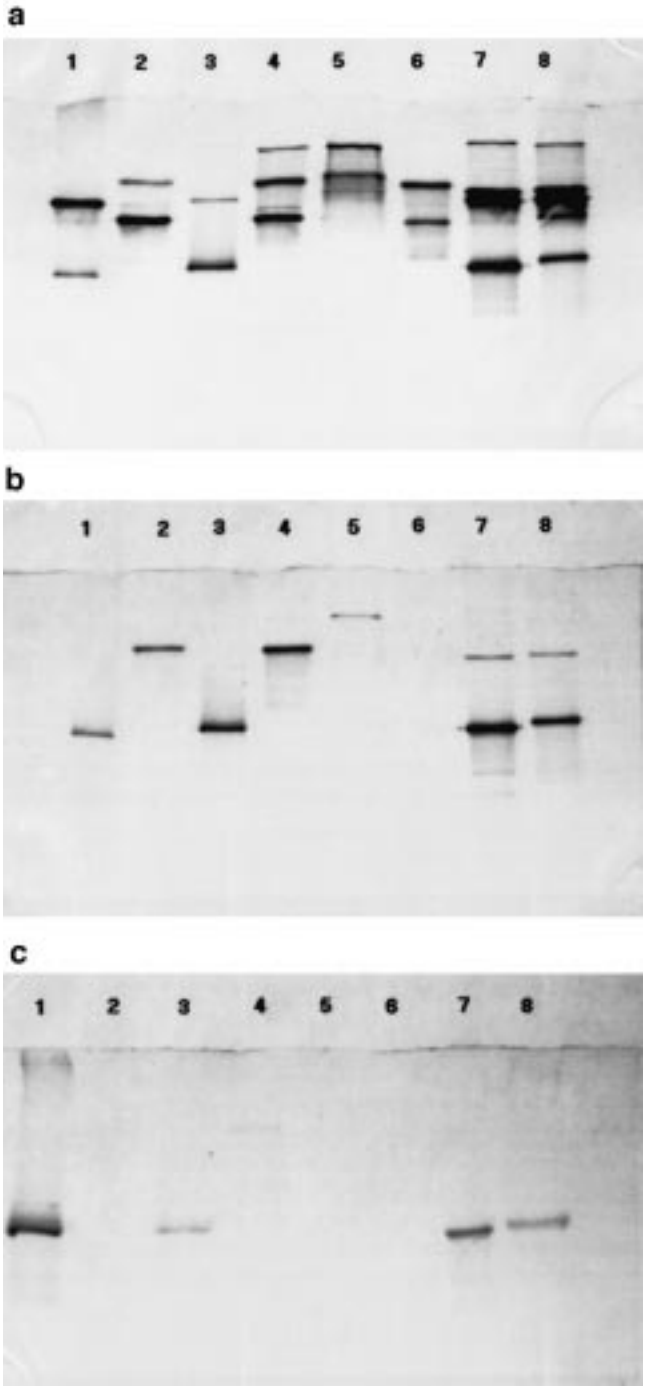

Figure 2 Partial identification of activity bands on SDS gels. Cultures were grown in GTF medium containing 2% (wt/vol) sucrose. Samples from the cultures (3 μl) were loaded onto triplicate SDS gels and subjected to SDS-PAGE. The gels were then incubated with 5% (wt/vol) sucrose or 8% (wt/vol) raffinose to visualize bands of sucrase or FTF activity, respectively. After washing gels to remove carbohydrate, one of the sucrose-incubated gels and the raffinose-incubated gel were treated with 10 units ml^{-1} dextranase. All three gels were then stained for carbohydrate synthesized by the enzymes during incubation with sugar. (a) SDS-gel stained for sucrase activity by incubating with sucrose. (b) SDS-gel stained for sucrase activity by incubating with sucrose, but the gels were treated with dextranase before staining carbohydrate polymers with periodic acid-Schiff reagent. (c) SDS-gel stained for levansucrase activity by incubating it with raffinose followed by dextranase treatment before staining carbohydrates with periodic acid-Schiff reagent. Lanes 1–8 (left to right): B-512F, B-1501, B-1149, B-1355, B-1299, B-742, B-523, B-1118.

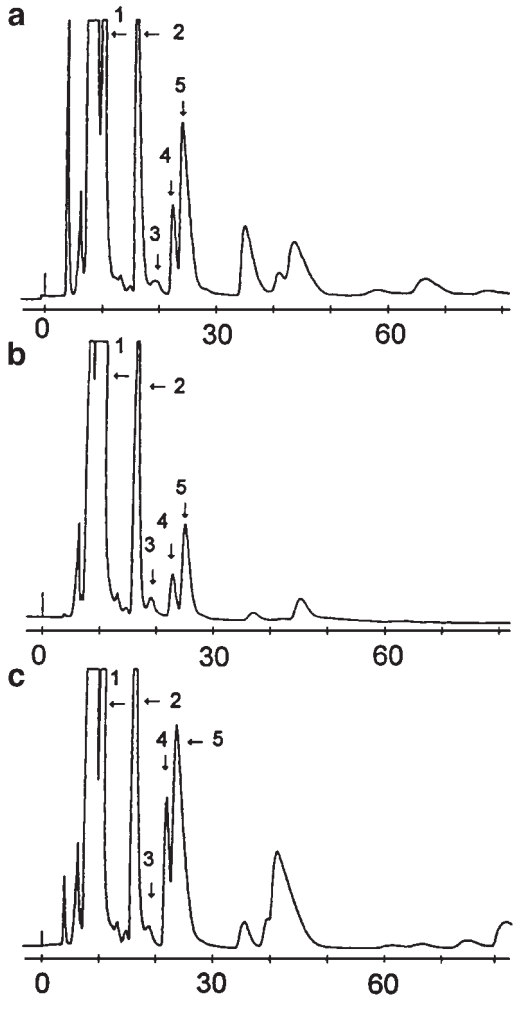

Figure 3 Oligosaccharides synthesized from methyl-α-d-glucoside and sucrose. Cultures of strain B-1355 grown in 2% (wt/vol) sucrose, glucose or maltose were incubated in a solution containing 100 mM methyl-α-d-glucoside, 200 mM sucrose and 200 μg ml^{-1} streptomycin sulfate. The oligosaccharides that were synthesized were separated by HPLC and identified by their relative retention times. Horizontal axes: minutes. (a) sucrose-grown culture; (b) glucose-grown culture; (c) maltose-grown culture. Numbered peaks: (1) methyl-α-d-glucoside; (2) 1-O-methyl-isomaltoside; (3) 1-O-methyl-α-glucopyranoseα(1→2)α-isomaltoside; (4) 1-O-methyl-α-glucopyranoseα(1→3)α-isomaltoside; (5) methyl-α-isomaltotrioside.

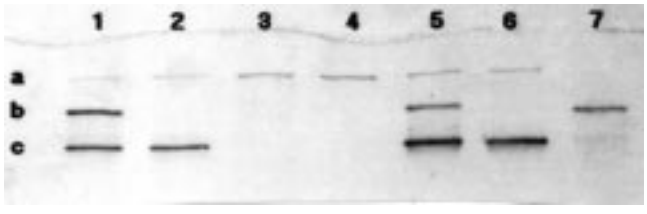

Figure 4 Distribution of alternansucrase activity in cultures of strains B-1355 and B-1501 grown in glucose medium. (a) GTF-1; (b) alternansucrase; (c) dextransucrase. Cultures of strains B-1299 and B-512F grown in sucrose medium were provided for reference. Lanes: (1) B-1355 culture; (2) B-1355 supernatant fraction; (3) B-1299 culture; (4) B-1299 supernatant fraction; (5) B-1501 culture; (6) B-1501 supernatant fraction; (7) B-512F supernatant fraction.

ation, and not the result of alternansucrase binding to glucans.

Discussion

We show for the first time that *L. mesenteroides* produces low levels of GTFs and FTFs in the absence of the inducer sucrose when glucose, maltose or melibiose is substituted for sucrose as the energy source. Bacteria often produce inducible enzymes at low levels in the absence of an inducer, as with β-galactosidase from the lac operon of *Escherichia coli* [1]. *L. mesenteroides* might therefore more closely resemble streptococci in GTF synthesis than formerly thought. GTF and FTF production by uninduced cultures was previously missed, apparently because few strains have been examined, supernatant fractions or bacteria-free filtrates were used as the source of the GTFs [7–11,19], and much of the activity tends to reside with the cellular fraction of cultures. When cell extracts were examined, as in the experiments of Hehre and Sugg [4], the activity of the GTFs in glucose cultures could be detected. The production by wild-type strains of GTFs in glucose medium explains why some researchers [12,23] have reported problems in attempting to use the agar-overlay method of Mizutani *et al* [17] to isolate mutant strains producing high levels of GTFs constitutively in glucose medium.

In spite of its importance to anyone wanting to produce GTFs on a commercial scale, there is little available information concerning regulation of GTF or FTF synthesis by *L. mesenteroides*. Dols *et al* [3] reported energy substrate-dependent changes in the number and types of GTFs produced by strain B-1299. We did not see (except for strain B-523 and B-1299) any substrate-dependent changes in the GTFs produced by our cultures. The GTFs produced by strain B-1355 apparently did not change as result of changes in the energy substrate, because we obtained identical SDS gels from cultures on all of the substrates tested, and cultures grown on the different substrates synthesized the same oligosaccharides from sucrose and methyl-α-d-glucoside. Although we did not strictly show growth substrate-independent GTF production for the other strains tested, especially where the identity of the GTFs is not yet established, it appears likely that the same GTFs and FTFs are produced by most of the strains independently of the substrate, because the activity bands on SDS gels and their relative susceptibilities to hydrolysis with dextranase did not change when the growth substrates were changed. The availability of dextran-free GTFs from wild-type cultures can be useful for characterizing the GTFs of new strains under conditions where interference by dextrans formed in sucrose cultures might be a problem [11], and where constitutive mutants are not available.

Finally, our results confirm for the first time that dextran-free alternansucrase is more strongly cell-associated in strain B-1355 and B-1501 than are the other dextran-free GTFs produced. This is the first time that cellular associations involving specific GTFs have been reported for dextran-free cultures of *L. mesenteroides*. Leathers *et al* [14] reported that strain B-21138, a mutant strain derived from strain B-1355, produced larger, more mucoid colonies on sucrose agar, and less extracellular alternansucrase (but more alternan) in sucrose cultures than did a second mutant strain (B-21297), also derived from strain B-1355. We believe that the apparent discrepancies among measured supernatant alternansucrase activity, colony morphology on sucrose agar, and alternan production result from differences between the mutant strains in the extent to which alternansucrase is associated with the cells. Mutations affecting the cell-association of alternansucrase in strain B-1355 [24], and of GTFs in *Streptococcus gordonii* [26] have been reported previously. Other strains of *L. mesenteroides* produce mixtures of GTFs, some of which might exhibit specific cell associations, and care must be taken to consider the possibility of specific cell associations when drawing conclusions concerning GTF production in cultures.

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