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Enzymatic production of glucooligosaccharides using dextransucrases from strains *Leuconostoc mesenteroides* Lm 22 and *Leuconostoc mesenteroides* Lm 28

ABSTRACT

Dextransucrases are enzymes that transfer the glucosyl moiety from sucrose to other acceptor molecules. In the present study, we have investigated the biosynthesis of dextransucrases by two strains of *Leuconostoc mesenteroides* isolated from Bulgarian fermented products. Extracellular dextransucrase activities of 10.80 U/ml for strain Lm 28 and 9.6 U/ml for strain Lm 22 were measured in a batch culture. The SDS-PAGE analysis showed that the molecular weight of the dextransucrases isolated from both strains was 180 kDa. Enzymes of the studied strains were found to efficiently transfer the glucosyl moiety of sucrose onto maltose acceptor. By increasing the sucrose/maltose ratio, it was possible to catalyze the synthesis of oligosaccharides of increasing degree of polymerization.

Key words: Dextransucrases, glucooligosaccharides, *Leuconostoc mesenteroides*

Introduction

Extracellular glucosyltransferases (GTFs) are mostly produced by lactic acid bacteria belonging to the genera *Leuconostoc, Streptococcus* and *Lactobacillus*. As it was shown previously (Kim & Robyt, 1996; Monsan et al., 2001), glucosyltransferases from *Streptococcus sp*. are produced constitutively, where those from *Leuconostoc sp*. are specifically induced by sucrose. Various strains produce more than one glucosyltransferase. For example, it has been reported that *Streptococcus mutans* 6715 produce three distinct enzymes from this group, *Streptococcus sobrinus* produce four glucosyltransferases and *Leuconostoc mesenteroides* NRRL-1355 also produce three distinct glucosyltransferases (Robyt et al., 1995; Monchois et al., 1999).

GTFs can be classified on the basis of the structure of the glucan that they synthesize: dextransucrases (EC 2.4.1.5), which synthesize a dextran composed mainly of α-(1-6) linkages into the main chain; mutansucrases (EC 2.4.1.5), which produce a mutan only with α-(1-3) linkages in the main chain; alternansucrases (EC 2.4.1.140), which produce

an alternan with 50% α-(1-6) linkages and 50% α-(1-3) linkages in the main chain (Argüello Morales et al., 2001; van Hijum et al., 2006).

GTFs are of great interest as they synthesize specifically branched gluco-oligosaccharides, which are fermented by beneficial species of the intestinal microflora and can be used as prebiotics (Korakli et al., 2002; Monsan et al., 2010).

Koepsell et al. (1953) showed that in the presence of a sugar acceptor molecule, GTFs transfer the glucose coming from sucrose onto the acceptor to produce oligosaccharides. This reaction, named "acceptor-reaction", competes with the α-glucan synthesis. Several authors classified the acceptor molecules according to their capacity to form oligosaccharides and they demonstrated that maltose, isomaltose and α-methyl glucopyranoside are usually the most effective acceptor sugars (Robyt et al., 1995; Argüello Morales et al., 2001).

Recently, different authors focused their attention on the production of oligoalternans and α-(1-3) branched oligosaccharides using the glucosyltransferases produced by *L. mesenteroides* NRRL B-1355 and *L. mesenteroides* NRRL B-742, respectively (Smith et al., 1994; Kim & Robyt, 1995;

RESEARCH ARTICLE

Zahnley & Smith, 1995).

Leuconostoc mesenteroides NRRL B-1299 dextransucrase synthesizes specifically α-1,2 linear and branched glucooligosaccharides, which are highly resistant to glycolytic digestive enzymes while they are fermented by beneficial species of the intestinal microflora (Remaud-Simeon et al., 1994; Dols et al., 1997). The maltose acceptor reaction is used to produce these non-digestible glucooligosaccharides (Paul et al., 1984; Remaud-Simeon et al., 1994; Dols et al., 1998). These glucooligosaccharides are presently marketed for human nutritional and dermocosmetic applications.

During the last years there are many screening programs for isolation of new strains *L. mesenteroides* synthesizing specific glucosyltransferases (Holt et al., 2001; Monsan et al., 2010). These programs are dealing exclusively with the dissolving of the main economic effectiveness of the technology for obtaining glucans and oligosaccharides as isolation of hyper glucosyltransferase producing strains, development of relatively simple purification methods and development of continuous processes for oligosaccharide production using enzyme reactions.

Our laboratory possesses a collection of strains, isolated from traditional Bulgarian fermented products. The main purpose of the study was determination of the dextransucrase activity, its specificity and also the possibility of GTFs to synthesize oligosaccharides.

Materials and Methods

Bacterial strains and culture media

Strains *L. mesenteroides* Lm 22 and *L. mesenteroides* Lm 28 as well as the referent strain *L. mesenteroides* NRRL B-512F were obtained from the Bacterial Culture Collection (BCC) of the Department of Biochemistry & Microbiology, Plovdiv University. One litre of the used standard culture medium consisted of 40 g of sucrose, 20 g of yeast extract, 20 g of K_2HPO_4, 0.2 g of $MgSO_4.7H_2O$, 0.01 g of $MnSO_4.H_2O$, 0.01 g of NaCl, 0.02 g of $CaCl_2$ and 0.01 g of $FeSO_4.7H_2O$. The pH of the phosphate buffer was adjusted to 6.9 with ortophosphoric acid. The sucrose, yeast extract and phosphate with additional salts were sterilized separately. The culture medium (0.2 L) was inoculated with 10 ml of 12 h inoculum and incubated on a rotary shaker (200 rpm) at 27°C. The culture was stopped at the end of the growth when the pH reached the value of 4.8.

Dextransucrase assay

One unit of dextransucrase is defined as the amount of enzyme that catalyzes the production of 1 μmol of fructose per minute at 30°C in 20 mM sodium acetate buffer, pH 5.4, with 100 g of sucrose per litre, 0.05 g of $CaCl_2$ per litre, and 1 g of NaN_3 per litre. It was ascertained that the reducing sugar measured by DNS assay was due to dextransucrase and not to levansucrase, invertase, or sucrose phosphorylase activity as described by Du Bois et al. (1956) and Dols et al. (1997). Glucose concentration was measured enzymatically with glucose oxidase using a Beckman Glucose Analyzer 2. Proteins were assayed by the method of Lowry (Lowry et al., 1951).

Electrophoresis analysis

SDS-PAGE (70x80 mm slab gels, 7% acrilamide gels) was conducted by the method of Laemmli (1970). Protein was stained with Coomassie Brilliant Blue R (Sigma-Aldrich Co.). Dextransucrase activities were detected by incubating the gels in 10% sucrose overnight, followed by staining for polysaccharide by a periodic acid-Schiff procedure (Miller & Robyt, 1986).

Oligosaccharide synthesis and analysis

Oligosaccharide synthesis in the presence of a maltose acceptor was performed by incubating 0.05 U of dextransucrase per milliliter at 25°C in 125 g of total sugar (sucrose and maltose) per litre – 20 mM sodium acetate buffer – 0.05 g of $CaCl_2$ per litre at an sucrose/maltose ratio of 2/7. The oligosaccharides produced were analyzed by HPLC using a C18 column and Hewlett-Packard 1050 series system. Oligosaccharides were detected by using an HP1047A refractometer. The products were identified in the chromatograms as described by Remaud-Simeon et al. (Remaud-Simeon et al., 1994).
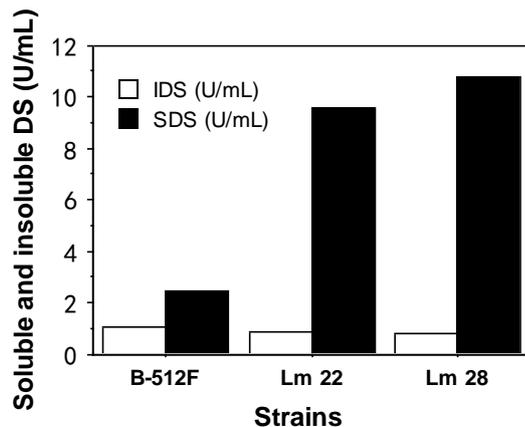
Results and Discussion

We have performed screening of 32 strains of *L. mesenteroides*. Six of the screened strains synthesized dextransucrase more than 2.0 U/ml. It is known that the referent strain of *L. mesenteroides* (NRRL B-512F) produce dextransucrase with activity of 2.5 U/ml (Kim & Robyt, 1996; Quirasco et al., 1999).

Results about the synthesized dextransucrases from the investigated strains with highest activity during the screening procedure in batch fermentation are shown in Figure 1. At the
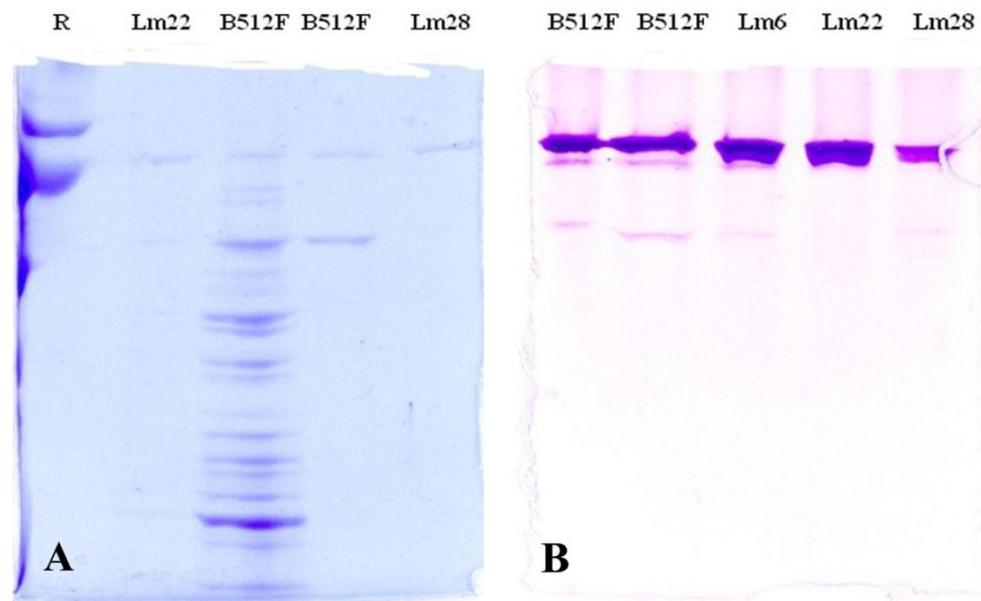
RESEARCH ARTICLE

end of the fermentation, dextransucrase activities were recovered both in the culture supernatant (SGT, soluble glucosyltransferase) and within the cells (IGT, insoluble glucosyltransferase). The activity of the SGT produced from the strains Lm 22 and Lm 28 was 4 times higher in comparison with the referent strain *L. mesenteroides* NRRL B-512F. In the presence of 4% sucrose, the maximum activities (10.8 U/ml and 9.6 U/ml) were determined at the 6-th and 7-th hour of cultivation for the two strains Lm 28 and Lm 22, respectively.

Figure 1 also shows that the investigated strains produce mainly extracellular type glucosyltransferases (more that 90%) and only approximately 10% of the produced dextransucrases were associated with the cells.

Proteins from the studied strains contained in the supernatant of sucrose medium were separated by SDS-PAGE. The SDS was removed from the gels by washing, and dextran-forming activity was detected *in situ*. The results are shown in Figure 2. Both strains Lm 22 and Lm 28 showed only one major band at 180 kDa, which is corresponding to the results from the other authors. It has been shown that the molecular weight of the extracellular glucosyltransferases from different strains could be 180 kDa or 125 kDa (Kim & Robyt, 1995; Kim & Robyt, 1996). In order to determine the type of the obtained GTF activity, the gels were also treated with dextranase after incubation in 10% sucrose. The active band (180 kDa) obtained for both strains Lm 22 and Lm 28 disappeared after the action of dextranase due to the hydrolysis of α-(1→6) glycoside linkages in dextran (data not shown). Probably the investigated strains Lm 22 and Lm 28 produce only one type of GTFs - dextransucrase on sucrose containing media, and these enzymes synthesize glucans from dextran types.

Figure 1. *Dextransucrase activity in L. mesenteroides Lm 22 and L. mesenteroides Lm 28 at the end of fermentation process.*

Figure 2. *SDS-PAGE of dextransucrase of L. mesenteroides strains.* **Gel A**: *Coomassie Brilliant Blue stain for proteins;* **Gel B**: *Enzyme activity was obtained by incubating the gel in 10% sucrose for approx. 16h, followed by periodic acid-Schiff stain.*

RESEARCH ARTICLE
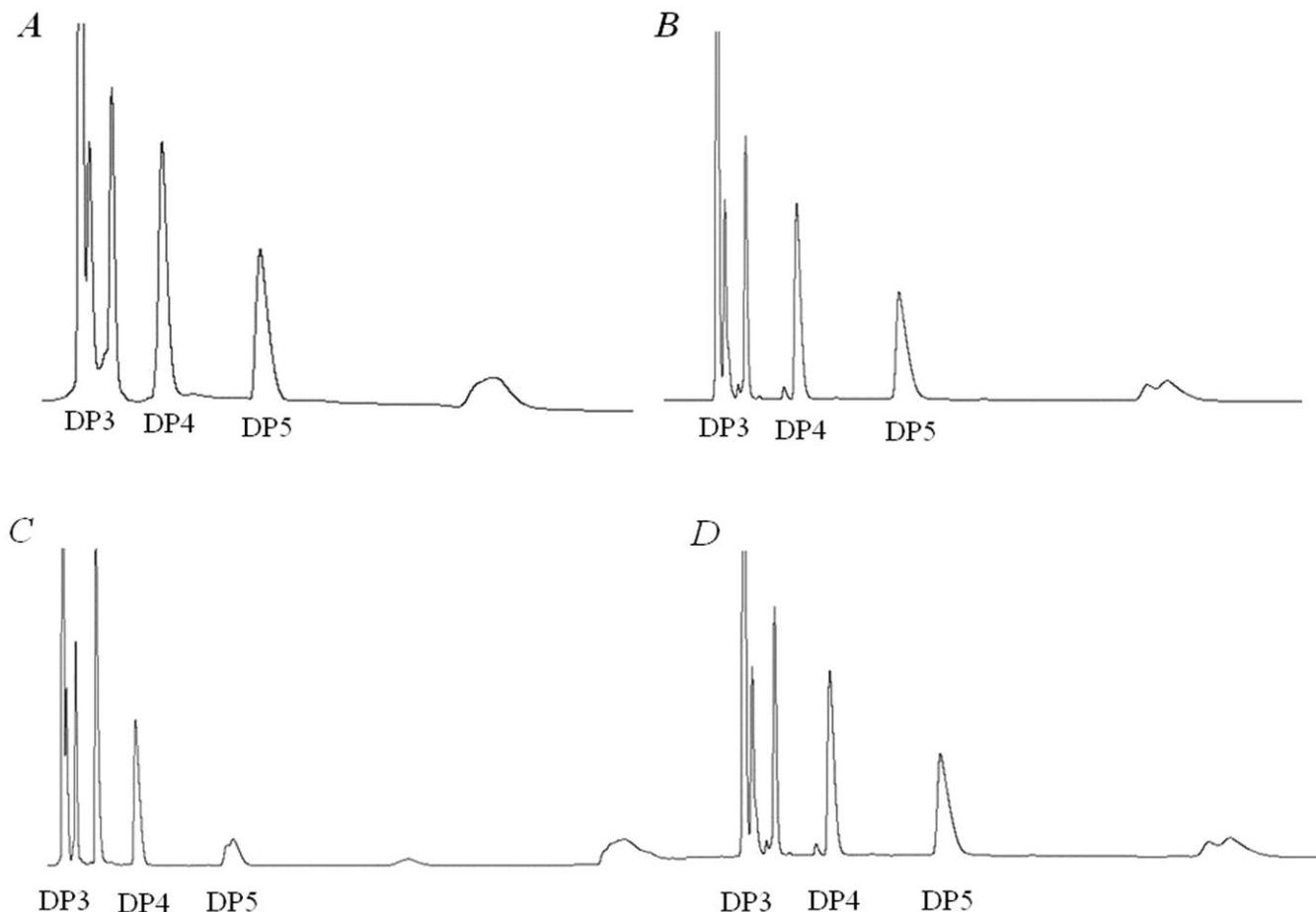
Figure 3. *Chromatograms of the oligosaccharides synthesized in the presence of sucrose and maltose by glucosyltransferases from L. mesenteroides strains Lm 22 and Lm 28.* ***A -*** *Glucosyltransferase from Lm 22 sucrose/maltose ratio 7.* ***B -*** *Glucosyltransferase from Lm 22 sucrose/maltose ratio 2.* ***C -*** *Glucosyltransferase from Lm 28 sucrose/maltose ratio 7.* ***D -*** *Glucosyltransferase from Lm 28 sucrose/maltose ratio 2.*

It is well known that dextransucrases synthesize oligosaccharides when they use maltose as an acceptor (Monsan et al., 2010). The acceptor reaction in the presence of maltose was first carried out using a sucrose/maltose (S/M) ratio of 2.

As shown on the HPLC chromatograms of acceptor reaction (Figure 3), products synthesized with dextransucrases from studied strains displayed six peaks. Oligosaccharides synthesized by glucosyltransferases from the studied strains showed the same retention time as those obtained with the B-512F dextransucrase.

Figure 4 shows the oligosaccharide yield obtained by the two investigated enzymes. Dextransucrases from strains Lm 22 and Lm 28 efficiently synthesized oligosaccharides with an overall yield of 20.70% and 23.50%, respectively. With B-512F dextransucrase a lower yield (14.8%) was obtained (Dols et al, 1997). The synthesized products were: for strain Lm 22 - DP3 (2.17 g/l); DP4 (3.0 g/l) and DP6 (1.7 g/l); for strain Lm 28 - DP3 (1.64 g/l); DP4 (2.67 g/l) and DP5 (1.51 g/l).

By increasing the S/M ratio of the acceptor reaction to 7, the synthesis of oligosaccharides with higher degree of polymerisation (DP) increased for all studied dextransucrases. As shown in Figure 5, dextransucrase from strain Lm 28 produced mainly oligosaccharides with DP 6 (5.19 g/l). Remaud-Simeon et al. (1994) reported similar

RESEARCH ARTICLE banner

RESEARCH ARTICLE

results using dextransucrases from strains B-742 and B512F. Results reported by Côté et al. (2003, 2009) were obtained using reaction conditions, which did not allow the synthesis of oligosaccharides of DP higher than 5. In their study, the acceptor reaction was carried out with an S/M ratio of 0.42 (Côté et al., 2003; Côté et al., 2009).
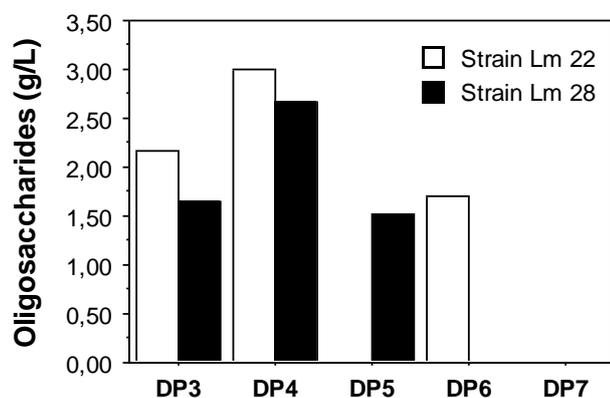
Figure 4. *Oligosaccharide synthesis using soluble dextransucrases from L. mesenteroides Lm 22 and L. mesenteroides Lm 28 (sucrose/maltose ratio = 2).*
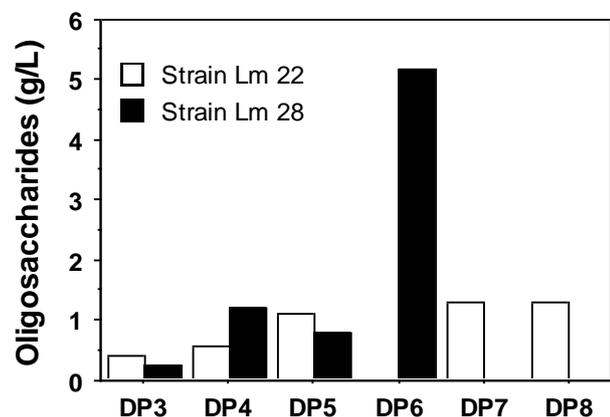
Figure 5. *Oligosaccharide synthesis using soluble glucosyltransferases from L. mesenteroides Lm 22 and L. mesenteroides Lm 28 (sucrose/maltose ratio = 7).*

Conclusion

We have described the production and purification of extracellular dextransucrases from two new strains of *L. mesenteroides*, isolated from Bulgarian traditional fermented foods. As a result of the performed study active producers of dextransucrases were found. They showed four times higher activity in comparison with the already known ones.

We demonstrated that our dextransucrase preparations could catalyze the acceptor reaction in the presence of maltose leading to the synthesis of oligosaccharides. The enzymes from the strains Lm 22 and Lm 28 showed higher efficiency during synthesis for the low molecule glucooligosaccharides (DP 3-5).

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RESEARCH ARTICLE

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