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

ABSTRACT

Dextranucrases are enzymes that transfer the glucosyl moiety from sucrose to other acceptor molecules. In the present study, we have investigated the biosynthesis of dextranucrases by two strains of Leuconostoc mesenteroides isolated from Bulgarian fermented products. Extracellular dextranucrase 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 dextranucrases 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: Dextranucrases, 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: dextranucrases (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;
dextran synthesis was performed using enzyme reactions.

Results about the synthesized dextransucrase

Dextranucrase 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 CaCl₂ per litre, and 1 g of NaCl 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 millilitre at 25°C in 125 g of total sugar (sucrose and maltose) per litre – 20 mM sodium acetate buffer – 0.05 g of CaCl₂ 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 dextranucrase more than 2.0 U/ml. It is known that the referent strain of L. mesenteroides (NRRL B-512F) produce dextranucrase with activity of 2.5 U/ml (Kim & Robyt, 1996; Quirasco et al., 1999).

Results about the synthesized dextranucrases from the investigated strains with highest activity during the screening procedure in batch fermentation are shown in Figure 1. At the
end of the fermentation, dextranucrase 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 dextranucrases 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 - dextranucrase on sucrose containing media, and these enzymes synthesize glucans from dextran types.

![Figure 1](image1.png)

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

![Figure 2](image2.png)

**Figure 2.** SDS-PAGE of dextranucrase 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.
It is well known that dextranucrases 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 dextranucrases 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 dextranucrase.

Figure 4 shows the oligosaccharide yield obtained by the two investigated enzymes. Dextranucrases from strains Lm 22 and Lm 28 efficiently synthesized oligosaccharides with an overall yield of 20.70% and 23.50%, respectively. With B-512F dextranucrase 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 dextranucrases. As shown in Figure 5, dextranucrase from strain Lm 28 produced mainly oligosaccharides with DP 6 (5.19 g/l). Remaud-Simeon et al. (1994) reported similar

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.
Results using dextran sucrases 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).

We demonstrated that our dextran sucrase 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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References


Figure 4. Oligosaccharide synthesis using soluble dextran sucrases 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 dextran sucrases from two new strains of L. mesenteroides, isolated from Bulgarian traditional fermented foods. As a result of the performed study active producers of dextran sucrases were found. They showed four times higher activity in comparison with the already known ones.


