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Influence of ursolic acid on glucooligosaccharides synthesized by dextransucrase from *Leuconostoc mesenteroides* Lm 28

ABSTRACT

A study of modulation of the reactions catalyzed by dextransucrase from *Leuconostoc mesenteroides* Lm 28 strain in the presence of triterpenoid ursolic acid was carried out. This compound showed concentration dependent inhibition of the studied dextransucrase and K_i = 1.9 mM, which is about 5 times higher than K_i value of the known glucansucrase inhibitor acarbose. Ursolic acid affected significantly the acceptor reactions catalyzed by Lm 28 dextransucrase in the presence of maltose and sucrose to maltose ratio 2. Increasing concentrations of ursolic acid shifted concentration and degree of polymerization (DP) distribution of the synthesized glucooligosaccharides (GOS) to acceptor products with DP ≤ 5. The oligosaccharide synthesis scheme applied in this study is a promising approach for production of GOS with controlled length of the chain.

Key words: acceptor reaction, dextransucrase, glucooligosaccharides, ursolic acid

Introduction

Glucosyltransferases (GTFs, glucansucrases) are predominantly extracellular enzymes belonging to Glycoside Hydrolase family 70 (GH70) (CAZy, http://www.cazy.org/GH70.html) (Van Hijum et al., 2006). They are mainly produced by lactic acid bacteria (LAB) from genera *Leuconostoc*, *Weissella* and *Lactobacillus* (Monsan et al., 2001; Korakli & Vogel, 2006). These enzymes use as a substrate sucrose and transfer its glucose moiety to: i) growing glucan chain – transferase reaction; ii) water – hydrolase reaction; iii) suitable acceptor molecules (maltose, isomaltose and etc.) – acceptor reactions (Leemhuis et al., 2013). Depending on the linkages between glucosyl residues in the polymer products, GTFs are divided to: i) dextansucrases (EC 2.4.1.5) synthesizing dextran with α-(1,6) linkages; ii) alternansucrases (EC 2.4.1.140), producing alternan with alternating α-(1,6) and α-(1,3) linkages in the main chain; iii) reuteransucrases (EC 2.4.1.5) synthesizing reuteran containing α-(1,4) linkages in the main chain; iiii) mutansucrases (EC 2.4.1.5) synthesizing mutan with α-(1,3) linkages (Korakli & Vogel, 2006; Andre et al., 2010). Dextransucrases produced by strains of *Leuconostoc mesenteroides* have received significant practical attention in the industrial production of dextran polymers (Naessens et al., 2005), and as a catalytic tool for synthesis of low-molecular weight glucooligosacharides (GOS) with various prebiotic and health-supporting properties (Remaud-Simeon et al., 2000; Palframan et al., 2003; Roberfroid, 2007).

Acceptor properties are reported for many mono-, di-, oligosaccharides, saccharide derivatives and polyphenolic compounds (glucose, fructose, maltose, isomaltose, cellobiose, melibiose, nigerose, D-sorbitol, D-glycerol, luteolin) (Koepsell et al., 1953; Robyt & Walseth, 1978; Robyt & Eklund, 1983; Heincke et al., 1999; Bertrand et al., 2006).

Depending of their ability to participate in the acceptor reaction, the acceptors are divided to two groups: i) strong acceptors – like maltose and isomaltose, which lead to high yield of GOS at the expense of the amount of the synthesized glucan polymer; ii) weak acceptors – like glucose, fructose, melibiose leading to low yield of GOS and predominant production of glucan polymer (Robyt & Walseth, 1978; Robyt & Eklund, 1983; Demuth et al., 2002).

Dols et al. (1997b) reported synthesis of three families of GOS using dextransucrase from *L. mesenteroides* NRRL B-1299 and maltose as an acceptor. The products of two of the families contained terminal and branching α-(1,2) linkages

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with degree of polymerization (DP) ranging from 3 to 10. The oligosaccharides containing α-(1,2) linkages were evaluated as prebiotics and are currently produced on an industrial scale (Remaud et al., 1992; Djouzi et al., 1995). The degree of polymerization of the synthesized GOS as well as their end concentration in relation to the amount of the formed dextran can be modulated by increasing the ratio between sucrose and maltose (S/M) in the reaction mixture (Remaud et al., 1992; Remaud-Simeon et al., 2000). By using engineered dextransucrase DSR-E from *L. mesenteroides* NRRL B-1299, Brison et al. (2009) succeeded to increase the content of α-(1,2) linkages when exogenous dextran from NRRL B-512F strain was used as an acceptor. The authors showed that the content of α-(1,2) linkages as well as the molecular weight of the produced dextran by the enzyme can be controlled by varying the initial molar ratio between the sucrose and dextran (Brison et al., 2009).

It is known that short-chain oligosaccharides have a higher prebiotic potential because their assimilation by healthful gastrointestinal microbiota is more effective (Rastall et al., 2005). In addition, short-chain oligosaccharides act as receptors for most of the intestinal pathogens and thus block their attachment to epithelium cells during colonization of the host (Gibson et al., 2005).

The reactions catalyzed by GTFs can be modulated also by some aminosugar derivatives acting as inhibitors of glycosidehydrolase enzymes (Kim et al., 1999). Such an inhibitor is acarbose: pseudotetrasaccharide containing two glucose units, 4-amino-4,6-dideoxy glucose unit and unsaturated cyclitol unit. The inhibitory effect of acarbose is due to the blocking of transition state for cleavage of glycosidic linkages in the normal glycosidase substrates (Newbrun et al., 1983; Kim et al., 1998). Kim and coworkers found that acarbose inhibits non-competitively dextran formation, acceptor reaction and disproportionation reaction catalyzed by dextransucrase from *L. mesentroides* B-512FMCM and is the strongest non-competitive inhibitor for this type of GTFs (Kim et al., 1998). The authors also found that glucooligosaccharides with DP no more than 5 are formed during the acceptor reaction with maltose, depending on acarbose and maltose concentrations and dextransucrase activity in the reaction mixtures (Kim et al., 1998).

Ursolic acid (3β-hydroxy-urs-12-en-28-oic acid) is a plant triterpenoid distributed widely in the form of free acid or aglycon of saponins (Liu, 1995). Ursolic acid is known as a potent antimicrobial substance inhibiting growth of pathogens such as *Staphylococcus aureus*, *Microsporium lenosum*, and of some bacteria and yeasts associated with food spoiling (Liu, 1995). This triterpenoid inhibits also the glucosyltransferase enzymes produced by plaque-forming bacteria of genera *Streptococcus*, which are associated with cariogenesis (Kozai et al., 1987).

In our previous work we studied the inhibitory effect of ursolic acid and several other plant terpenoids on the activity of dextransucrase from *L. mesenteroides* URE13 strain (Bivolarski et al., 2014). We found that ursolic acid is almost as an effective inhibitor of dextransucrase as acarbose demonstrated by the calculated K_i values for both substances (Bivolarski et al., 2014).

The aim of this work was to study the influence of ursolic acid on the acceptor reaction catalyzed by dextransucrase from *L. mesenteroides* Lm 28 strain in the presence of maltose as an acceptor. The amount of the synthesized oligosaccharides and their distribution on degree of polymerization were compared.

Materials and Methods

Bacterial strains and culture media

Leuconostoc mesenteroides Lm 28 was obtained from the bacterial culture collection of the Department of General and Industrial Microbiology, Sofia University (Bulgaria). For the production of glucansucrase the strain was cultivated 6-8 h in culture media containing sucrose (40 g/L), yeast extract (20 g/L), K_2HPO_4 (20 g/L), $MgSO_4.7H_2O$ (0.2 g/L), $MnSO_4.H_2O$ (0.01 g/L), $CaCl_2$ (0.02 g/L) and $FeSO_4.7H_2O$ (0.01 g/L). The GTF production medium was adjusted to pH 6.9 with *o*-phosphoric acid. The culture medium (200 mL) was inoculated with 10 mL 12 h culture inoculum and incubated at 27°C on a rotary shaker (200 rpm). The culture was stopped at the end of the growth when the pH reached 4.5 (Iliev et al., 2008).

Biomass measurements

Bacterial growth was measured by a turbidimetric method at 620 nm and calibrated against cell dry-weight measurements, as previously described (Dols et al., 1997a).

Enzyme preparation

Compositions The culture medium was centrifuged for 20 min at 7000 g and 4°C. The supernatant was then filtered with a Sartorius membrane (0.2 μm, Goettingen, Germany) to ensure the total absence of cells in the supernatant. GTF was separated from the supernatant and concentrated by using an aqueous two-phase partition between dextran and

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polyethyleneglycol (PEG) (Paul et al., 1984; Goyal & Katiyar, 1994). After addition of PEG 1500 (20%, v/v), the dextran-rich phase containing GTF was separated by centrifugation at 7000 g for 20 min at 4°C, collected in the pellet and diluted in 20 mM/L sodium acetate buffer, pH 5.4.

Protein determination

Proteins were assayed by the method of Bradford by using bovine serum albumin as a standard (Bradford, 1976).

Enzyme activity assays

One unit of glucosyltransferase activity is defined as the amount of enzyme that catalyzes the formation of 1 μmol of fructose per 1 min at 30°C in 20 mM/L sodium acetate buffer (pH 5.4), 0.05 g/L $CaCl_2$, 1 g/L NaN_3 and 100 g/L sucrose (Dols et al., 1997a). The released reducing sugars were determined by 3,5-dinitrosalicylic acid assay (Sumner & Howell, 1935). Additionally, D-fructose and D-glucose were determined with hexokinase, glucose-6-phosphate dehydrogenase, and phosphoglucose isomerase (commercially available kit, Megazyme International Ireland ltd, Wicklow, Ireland), according to the instructions of the manufacturer. All activities were assayed at least in triplicate and average values are given.

Influence of sucrose concentration on the initial velocity of dextransucrase reaction

The influence of substrate concentration on the initial velocity of the enzyme reaction was studied at a range of 1 mM to 1300 mM of sucrose, 30°C in 20 mM/L sodium acetate buffer (pH 5.4), 0.05 g/L $CaCl_2$, 1 g/L NaN_3. D-fructose and D-glucose were determined with hexokinase, glucose-6-phosphate dehydrogenase, and phosphoglucose isomerase (commercially available kit, Megazyme International Ireland ltd, Wicklow, Ireland), according to the instructions of the manufacturer. All the reactions were performed at least in triplicate and the average values were used for determination of the kinetic constants.

Extraction and purification of ursolic acid

Triterpenoidursolic acid was extracted from dried and finely powdered aerial parts of *Lavandula spica* L. with methanol at room temperature for a week. The methanolic solution was concentrated by evaporation to dryness and the residue was chromatographed on silica gel column (Merck, No 7734) as previously described (Papanov et al., 1992). Ursolic acid was dissolved in dimethyl sulfoxide (DMSO) (Calbiochem).

Determination of inhibitory effect of ursolic acid and acarbose

The inhibitory effect of ursolic acid and acarbose (Sigma) was determined at 0.34 mM, 0.68 mM, 1.03 mM 1.71 mM, 2.4 mM and 3.42 mM per mL end concentration in the reaction mixtures. The reactions were performed with 1 U/mL enzyme in the reaction mixtures containing 20 mM/L sodium acetate buffer (pH 5.4), 0.05 g/L $CaCl_2$, 1 g/L NaN_3, 100 g/L sucrose and 20% of DMSO end concentration at 30°C. D-fructose and D-glucose were determined with hexokinase, glucose-6-phosphate dehydrogenase, and phosphoglucose isomerase (commercially available kit, Cat. No. K-FRUGL, Megazyme International Ireland ltd, Wicklow, Ireland), according to the instructions of the manufacturer. The value of inhibitory effect of DMSO was subtracted and the retained dextransucrase activity was used as 100% on which basis the inhibitory effects of ursolic acid and acarbose were calculated in percentage. All the reactions were performed at least in triplicate and average values are given.

Determination of K_i values

The K_i values were determined in reaction mixtures containing between 1 mM and 200 mM of sucrose, ursolic acidoracarbose from 0.34 mM/mL to 3.42 mM/mL. The reactions were performed with 1 U/mL dextransucrase in 20 mM/L sodium acetate buffer (pH 5.4) at 30°C. D-fructose and D-glucose were determined with hexokinase, glucose-6-phosphate dehydrogenase, and phosphoglucose isomerase (commercially available kit, Cat. No. K-FRUGL, Megazyme International Ireland ltd, Wicklow, Ireland), according to the instructions of the manufacturer. All the reactions were performed at least in triplicate and the average values were used for determination of K_i values.

Oligosaccharide synthesis and analysis

Oligosaccharide synthesis in the presence of a maltose acceptor and ursolic acid or acarbose (0.34 mM, 0.68 mM, 1.03 mM, 1.71 mM, 2.4 mM, 3.42 mM) were carried out by incubating 1.0 U/mL of dextransucrase at 25°C in 125 g/L of total sugar (sucrose and maltose) of 20 mM/L sodium acetate buffer, 0.05 g $CaCl_2$, at sucrose / maltose ratios (S/M) of 2 (Remaud et al., 1992). The reactions were stopped after the total consumption of sucrose by heating of the reaction mixtures at 95°C for 5 min. Dextransucrase was eliminated by using ultra-filtration on an Amicon hollow fibre system (membrane cut-off = 100 kDa). The oligosaccharides

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produced were analysed by HPLC using a Symmetry C18 column 4,6x150 mm and a Waters 1525 Binary HPLC Pump series system (GMI Inc., Ramsey, MI, USA), and detected by using an Waters 2414 refractometer. The products were identified in the chromatograms, as described by Remaud-Simeon et al. (1994).

Statistical analysis

In all the cases the software Programmable scientific calculation "CITIZEN" SRP-45N and SigmaPlot 12.0 (Systat Software, Inc.) were used for data analysis.

Results and Discussion

Study of inhibitory effect of ursolic acid and acarbose on the activity of dextransucrase from L. mesenteroides Lm 28

From our previous study is known the strain produce a single dextransucrase with molecular weight about 180 kDa with an enzyme activity two times higher than this one of dextransucrase from *L. mesenteroides* NRRL B-512F strain. Lm 28 dextransucrase synthesizes a dextran-type glucan with linear structure (Iliev et al., 2003; Iliev & Vasileva, 2012).

In order to determine and compare the inhibitory effect of ursolic acid and acarbose on the activity of dextransucrase from *L. mesenteroides* Lm 28, we tested both compounds at concentration range from 0.34 mM to 3.42 mM (Figure 1).

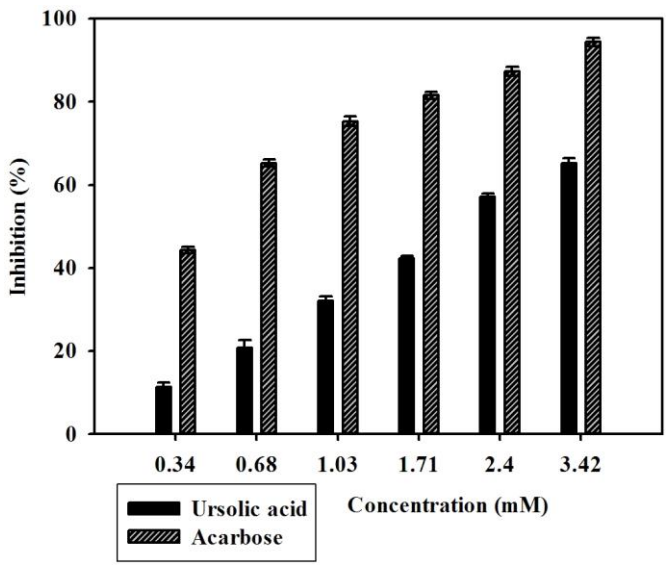

Figure 1. *Inhibition of Lm 28 dextransucrase by different concentrations ursolic acid and acarbose. The reactions were performed with 1 U/mL dextransucrase activity.*

Because of the poor water solubility of the ursolic acid, we dissolved the compound in the biocompatible organic solvent DMSO. We have found that the lowest inhibitory effect (12%) of the organic solvent on dextransucrase activity was observed at 20% concentration of DMSO (Bivolarski et al., 2014). In all the experiments the inhibition of Lm 28 dextransucrase by acarbose was from 28% to 43% higher than this achieved in the presence of ursolic acid in the reaction mixtures. The highest difference between both inhibitors was observed at 0.68 mM and 1.03 mM concentrations (43%) and it decreases to the highest tested concentration – 3.42 mM (28%). Up to 45% inhibition of the studied dextransucrase was determined at 1.71 mM ursolic acid and 0.34 mM acarbose, respectively. This 5-fold lower concentration clearly showed the effectiveness of acarbose as a dextransucrase inhibitor. A full inhibition of the enzyme was not reached at the tested concentration of the compounds. While only 6.5% of the initial dextransucrase activity was retained at 3.42 mM concentration of acarbose, in the presence of ursolic acid the corresponding value is 35% (Figure 1). Higher ursolic acid concentration than 3.42 mM was not tested because of its low solubility in the reaction mixtures, containing 20% DMSO.

In our previous study, we have found that dextransucrase from *L. mesenteroides* URE 13 retained only 13% of its initial activity in the presence of 3.42 mM ursolic acid (Bivolarski et al., 2014). The inhibitory effect of acarbose on Lm 28 dextransucrase did not show significant difference compared to the inhibition of the enzyme from URE 13 strain (Bivolarski et al., 2014)..

Influence of substrate concentration on the activity of dextransucrase from L. mesenteroides Lm 28

The influence of initial sucrose concentration on the velocity of the enzyme reaction catalyzed by Lm 28 dextransucrase was studied (Figure 2). K_m value of 22.08 mM sucrose was determined by non-linear regression approach. The value is comparable with these ones obtained for dextransucrases from other *Leuconostoc* strains, including NRRL B-512F (K_m = 15 mM), IBT-PQ (K_m = 26 mM), and URE 13 (K_m = 18 mM) (Martinez-Espindola & Lopez-Munguia, 1985; Chellapandian et al., 1998; Bivolarski et al., 2013). When the initial sucrose concentration was further increased above 200 mM, an effect of substrate inhibition with K_i = 1353 mM appears (Figure 2). Such kind of inhibition with corresponding K_i values was also previously reported for several dextransucrases (Martinez-Espindola &

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Lopez-Munguia, 1985; Chellapandian et al., 1998; Bivolarski et al., 2013), and is ascribed to decrease of the ratio of free water / dextran in the reaction mixture which benefits the formation of inactive ES2 complexes (Hehre, 1946).

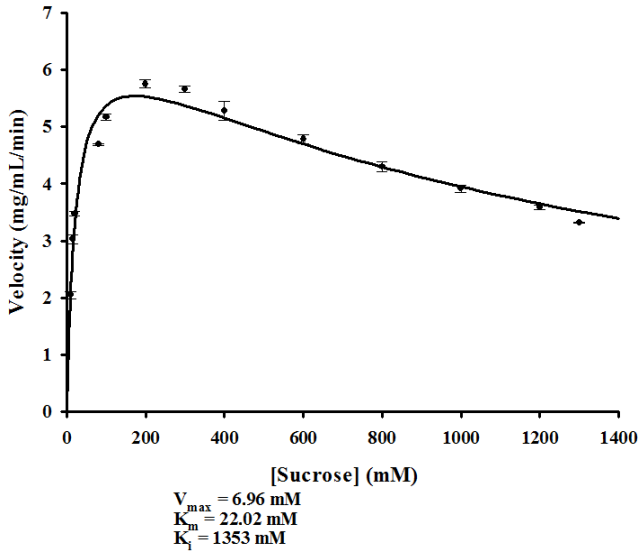

Figure 2. *Determination of substrate dependent kinetic constants of dextransucrase from Lm 28. The reactions were performed with 1 U/mL dextransucrase activity.*

Kinetics of the inhibition of dextransucease from L. mesenteroides Lm 28 by ursolic acid and acarbose

Acarbose is known as an effective inhibitor of several carbohydrase enzymes, including GTFs produced by the cariogenic *Streptococcus mutans* (Strokopytov et al., 1995; Kim et al., 1999). In addition to its medical applications in treatment of diabetes, acarbose has a potential as an anticariogenic agent**,** which is associated with the inhibition of the formation of insoluble glucan by GTFs from cariogenic *Streptococcus* sp. (Newbrun et al., 1983). Such inhibitory effects are also reported for ursolic acid and oleanlocic acid (Kozai et al., 1987).

In order to compare the efficiency of ursolic acid and acarbose as inhibitors of dextransucrase from *L. mesenteroides* Lm 28, we determined K_i values of the reactions in the presence of several concentrations of this compounds (Figure 3). From the reactions performed in the presence of ursolic acid a K_i value of 1.9 mM was calculated (Figure 3 A) which is significantly higher than previously determined value for dextransucrase from *L. mesenteroides* URE 13 strain (K_i = 0.37 mM) (Bivolarski et al., 2013).

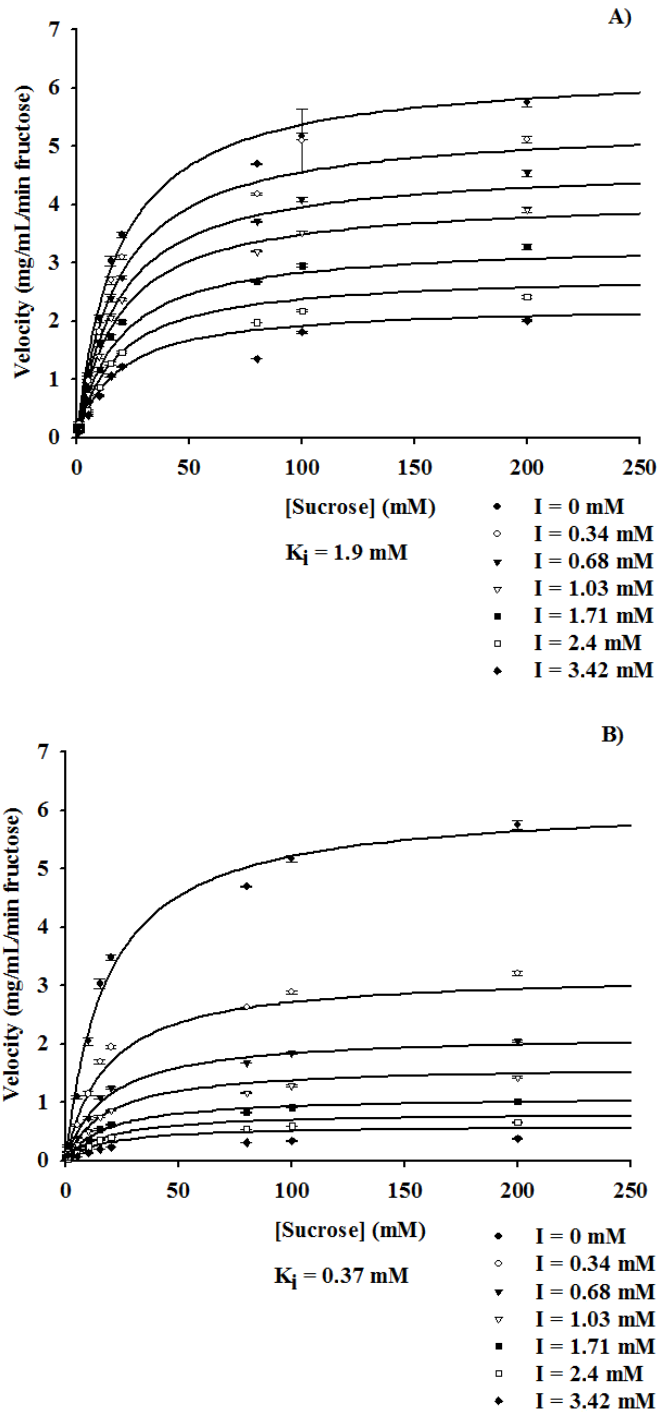

Figure 3. *Determination of K_i of dextransucrase from L. mesenteroides Lm 28 in the presence of ursolic acid **(A)** and acarbose **(B)**. The reactions were performed with 1 U/mL dextransucrase activity.*

In the presence of acarbose, the obtained K_i = 0.37 mM (Figure 3 B) was slightly higher than this calculated for URE

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13 dextransucrase (K_i = 0.29 mM) (Strokopytov et al., 1995).

During the study of the influence of acarbose on the reactions catalyzed by dextransucrase from *L. mesenteroides* B-512FMCM, Kim et al. (1998) found that this compound effectively inhibits the dextran formation in concentration dependent manner. The kinetic study performed by these authors defined acarbose as a non-competitive inhibitor having K_i value of 1.35 mM (Kim et al., 1998).

The results obtained in current study showed that ursolic acid is an effective and comparable inhibitor of Lm 28 dextransucrase, and the variations of the kinetic constants between the different GTFs are strain dependent feature.

Influence of ursolic acid and acarbose on the acceptor reactions

Glucooligosaccharides with low degree of polymerization have a higher prebiotic potential due to their more effective metabolism by beneficial intestinal microflora (Rastall et al., 2005). From our previous study is known that dextransucrase produced by strain *L. mesenteroides* Lm 28 synthesizes low-molecular oligosaccharides at optimal sucrose / maltose (S/M) ratio 2 (Iliev et al., 2003; Iliev et al., 2012).

In the current work, we studied the influence of ursolic acid on the oligosaccharide synthesis performed by dextransucrase from *L. mesenteroides* Lm 28 strain. In the presence of maltose as an acceptor molecule and S/M = 2, the studied dextransucrase synthesizes a total amount of 8.2% oligosacchatides (Iliev et al., 2003). When the acceptor reactions were performed in the presence of different amounts of ursolic acid the total yield of the synthesized oligosaccharides gradually decreases with the increases of the concentration of the studied terpenoid (Figure 4). At concentrations of ursolic acid 0.34 mM and 0.68 mM where the degree of inhibition of the enzyme is between 11% and 21%, the amount of the synthesized oligosaccharides is about 90% and 62% of the total amount of the oligosaccharides produced in the absence of ursolic acid. At 1.71 mM concentration of the studied compound the synthesized oligosaccharides are only 13% of amount of the oligosaccharides obtained after the standard reaction, without ursolic acid. No acceptor products were detected in the presence of 2.40 mM and 3.42 mM of ursolic acid, where the inhibition of the enzyme was over 50% (Figure 4). When the reactions were performed in the same conditions, but in the presence of acarbose instead of ursolic acid, oligosaccharide synthesis was detected only at 0.34 mM concentration of the inhibitor – 12% of the total amount of the oligosaccharides synthesized at standard conditions. At higher acarbose concentrations where the inhibition of dextransucrase is higher than 50% acceptor products are not detected.

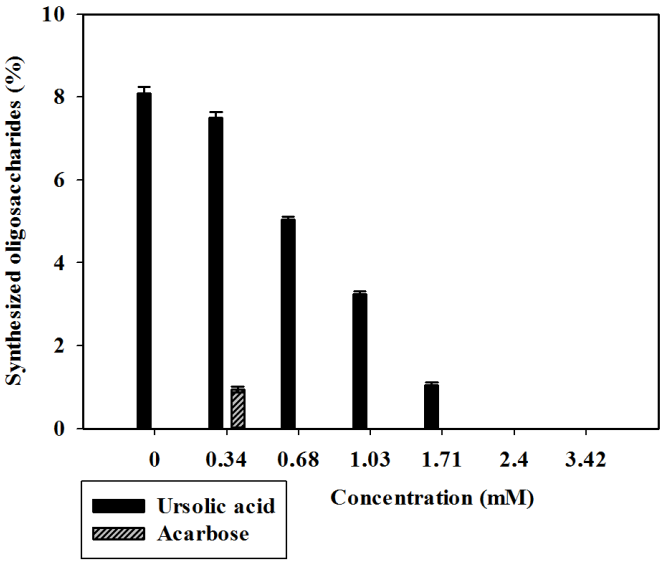

Figure 4. *Total amount of the synthesized oligosaccharides in the presence of different concentrations ursolic acid and acarbose.*

Similar manner of acceptor reaction inhibition by acarbose was observed also by Kim et al. (1998) after synthesis reactions with dextransucrase from *L. mesenteroides* B-512FMCM. The authors also found correlation between the enzyme activity in the reaction mixtures and the amount and DP of the synthesized oligosaccharides which was not dependent of acceptor concentration (Kim et al., 1998).

On the basis of the concentration of released fructose and glucose during the acceptor reaction catalyzed by Lm 28 glucansucrase in the presence of S/M = 2 and uroslic acid (from 0.34 mM to 1.71 mM) or acarbose (0.34 mM), we compared the ratio between transferase and hydrolase activities of the enzyme (Table 1).

As is shown in a Table 1, during the enzyme reaction the transferase activity of the studied enzyme was predominant (above 80%). In the absence of ursolic acid the highest transferase activity was observed at the 3rd hour from the start of the reaction (98.65%). The same tendency was observed in the presence of ursolic acid and acarbose. In general, the ratio between transferase and hydrolase activity decreases with increase of the reaction time and the inhibitor concentration in the reaction mixtures (Table 1).

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Table 1. *Chemical Transferase and hydrolase activity of dextransucrase from L. mesenteroides Lm 28 in the presence of S/M=2 and ursolic acid or acarbose. The reactions were performed with 1 U/mL glucansucrase activity.*

Time (h)	Transferase : hydrolase activity (%)					
	Without ursolic acid	0.34 mM ursolic acid	0.34 mM acarbose	0.68 mM ursolic acid	1.03 mM ursolic acid	1.71 mM ursolic acid
0.5	97.55 : 2.45 (± 0.10)	95.14 : 4.86 (± 0.16)	82.11 : 17.89 (± 0.23)	91.22 : 8.78 (± 0.18)	88.12 : 11.88 (± 0.20)	84.63 : 15.37 (± 0.22)
1	98.18 : 1.82 (± 0.08)	96.15 : 3.85 (± 0.13)	83.56 : 16.44 (± 0.18)	93.64 : 6.36 (± 0.16)	91.23 : 8.77 (± 0.23)	85.78 : 14.22 (± 0.19)
3	98.65 : 1.35 (± 0.04)	96.68 : 3.32 (± 0.15)	84.22 : 15.78 (± 0.20)	94.10 : 5.90 (± 0.14)	93.18 : 6.82 (± 0.17)	86.44 : 13.56 (± 0.20)
5	96.45 : 3.55 (± 0.12)	95.13 : 4.87 (± 0.18)	83.12 : 16.88 (± 0.20)	93.85 : 6.15 (± 0.17)	92.13 : 7.87 (± 0.15)	85.22 : 14.78 (± 0.19)
10	97.12 : 2.88 (± 0.10)	95.55 : 4.45 (± 0.14)	81.75 : 18.25 (± 0.22)	92.48 : 7.52 (± 0.18)	91.45 : 8.55 (± 0.16)	84.28 : 15.72 (± 0.23)
24	95.19 : 4.88 (± 0.16)	92.10 : 7.90 (± 0.20)	80.11 : 19.89 (± 0.21)	90.43 : 9.57 (± 0.22)	89.33 : 10.67 (± 0.23)	83.86 : 16.14 (± 0.22)

Table 2. *Distribution of the oligosaccharides synthesized by dextransucrase from L. mesenteroides Lm 28 according to their degree of polymerization.*

Ursolic acid (mM)	Oligosaccharides (g/L)				
	DP3	DP4	DP5	DP6	DP7
0	2.0 ± 0.02	2.5 ± 0.05	1.5 ± 0.05	1.1 ± 0.05	1.0 ± 0.04
0.34	2.2 ± 0.02	2.8 ± 0.02	1.6 ± 0.02	0.5 ± 0.02	0.2 ± 0.02
0.68	1.8 ± 0.03	2.2 ± 0.03	1.0 ± 0.03	0	0
1.03	1.3 ± 0.03	1.5 ± 0.03	0.3 ± 0.03	0	0
1.71	0.4 ± 0.05	0.6 ± 0.05	0	0	0
Acarbose (0.34 mM)	0.3±0.03	0.5±0.04	0	0	0

The distribution of the synthesized oligosaccharides according to their degree of polymerization is shown in Table 2. In absence of ursolic acid, Lm 28 dextransucrase synthesizes GOS with DP ranging from 3 to 7, which are distributed uniformly. In the presence of ursolic acid the distribution of the oligosaccharides is shifted. The quantity of the oligosaccharides with lower DP to this one with higher DP increases gradually. At the lowest tested concentration of ursolic acid the amount of the oligosaccharides with DP from 3 to 5 (panose, isomatosylmaltose, isomaltotriosylmaltose) is even higher than these of the control reaction, at the expense of the products with DP6 and DP7 (isomaltohexaose and isomaltoheptaose). At 0.68 mM and 1.03 mM of ursolic acid, GOS with DP higher than 5 are not detected, and at 1.71 mM concentration of the inhibitor only GOS with DP3 and DP4 are determined (Table 2). In the presence of acarbose at concentration 0.34 mM, the distribution of the GOS according to DP corresponds to this one determined at 1.71 mM of ursolic acid (Table 2).

In this paper, we describe for the first time that by varying the concentration of ursolic acid, and at S/M=2 is possible to modulate the distribution of the synthesized by Lm 28 dextransucrase oligosaccharides, yielding low DP products as a predominant fraction.

Conclusion

The presence of ursolic acid at concentrations ranging from 0.34 mM to 1.71 mM influences the distribution of GOS synthesized by dextransucrase from *L. mesenteroides* Lm 28. The amount of GOS with DP3 to DP5 is increased in the presence of 0.34 mM ursolic acid. The further increase of ursolic acid concentration leads (0.68 mM and 1.71 mM) to synthesis of GOS with DP no more than 5 and 4,

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respectively. At the lowest tested concentration of acarbose (0.34 mM) the studied dextransucrase synthesized only oligosaccharides with DP3 and DP4, but at lower concentrations. The strongest inhibitory effect of acarbose makes the ursolic acid a better choice for further studies concerning the modulation of glucooligosacharide synthesis performed by Lm 28 dextransucrase. On the basis of these results, the ursolic acid seems promising substance for modulation of the acceptor reactions of dextransucrases in order to obtain higher yields of GOS with defined length of the chain

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References

Andre I, Potocki-Veronese G, Morel S, Monsan P, Remaud-Simeon M. 2010. Sucrose-utilizing transglucosidases for biocatalysis. Top. Curr. Chem., 294: 25-48.

Bertrand A, Morel S, Lefoulon F, Rolland Y, Monsan P, Remaud-Simeon M. 2006. *Leuconostoc mesenteroides* glucansucrase synthesis of flavonoid glucosides by acceptor reactions in aqueous-organic solvents. Carbohyd. Res., 341(7): 855-863.

Bivolarski V, Vasileva T, Bozov P, Iliev I. 2013 Influence of different acceptors on synthesis of glucooligosacharides by purified dextransucrase from *Leuconostoc mesenteroides* URE 13. C. R. Acad. Bulg. Sci., 66(10): 1405-1412.

Bivolarski V, Vasileva T, Bozov P, Iliev I. 2014. Influence of terpenoids and acarbose on glycosyltransferases produced by strain *Leuconostoc mesenteroides* URE 13. Biotechnol. Biotec. Eq., 28(2): 342-349.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Chellapandian M, Larios C, Sanchez-Gonzalez M, Lopez-Munguia A. 1998. Production and properties of a dextransucrase from *Leuconostoc mesenteroides* IBT-PQ isolated from 'pulque', a traditional Aztec alcoholic beverage. J. Ind. Microbiol., Biot., 21(1-2): 51-56.

Brison Y., Fabre E, Moulis C, Portais J-C, Monsan P, Remaud-Simeon M. 2009. Synthesis of dextrans with controlled amounts of α-1,2 linkages using the transglucosidase GBD-CD2. Appl. Microbiol. Biotechnol., 86(2): 545-554.

Demuth K, Jordening H-J, Buchholz K. 2002. Oligosaccharide synthesis by dextransucrase: new unconventional acceptors. Carbohyd. Res., 337(20): 1811-1820.

Djouzi Z, Andrieux C, Pelenc V, Somarriba S, Popot F, Paul F, Monsan P, Szylit O. 1995. Degradation and fermentation of α-gluco-oligosaccharides by bacterial strains from human colon: in vitro and in vivo studies in gnotobiotic rats. J. Appl. Bacteriol., 79(2): 117-127.

Dols M. Remaud-Siméon M, Monsan PF. 1997a. Dextransucrase production by *Leuconostoc mesenteroides* NRRL B-1299 comparison with *L. mesenteroides* NRRL B-512F. Enzyme Microb. Tech., 20(7): 523-530.

Dols M., Remaud-Simeon M, Willemot R, Vignon MR, Monsan P. 1997b. Structural characterization of maltose acceptor-products synthesized by *Leuconostoc mesenteroides* NRRL B-1299 dextransucrase. Carbohyd. Res., 305(3-4): 549-559.

Gibson GR, McCartney AL, Rastall RA. 2005. Prebiotics and resistance to gastrointestinal infections. Brit. J. Nutr., 93(1): S31-S34.

Goyal A, Katiyar SS. 1994. Fractionation of *Leuconostoc mesenteroides* NRRL B-512F dextransucrase by polyethylene glycol: a simple and effective method of purification. J. Microbiol. Meth., 20(3): 225-231.

Hehre EJ. 1946. Studies on the enzymatic systhesis of dextran from sucrose. J. Biol. Chem., 163: 221-233.

Heincke K, Demuth B, Jördening H-S, Buchholz K. 1999. Kinetics of the dextransucrase acceptor reaction with maltose - experimental results and modeling. Enzyme Microb. Tech., 24(8-9): 523-534.

Iliev I, Ivanova I, Remaud M, Monsan P. 2003. Production and use of glycosyltransferases from new strains *Leuconostoc mesenteroides* for the synthesis of glucooligosaccharides. - In: Kniewald Z. (ed), Current studies of Biotechnology,Vol. III – Food. Medicinska naklada: Croatian Society of Biotechnology, Zagreb, Croatia, p. 167-175.

Iliev I., Vassileva T, Ignatova C, Ivanova I, Haertle T, Monsan P, Chobert J-M. 2008. Gluco-oligosaccharides synthesized by glucosyltransferases from constitutive mutants of *Leuconostoc mesenteroides* Lm28. J. Appl. Microbiol., 104(1): 243-250.

Iliev I, Vasileva T. 2012. Enzymatic production of glucooligosaccharides using dextransucrases from strains *Leuconostoc mesenteroides* Lm 22 and *Leuconostoc mesenteroides* Lm 28. J. BioSci. Biotechnol., 1(1): 51-56.

Kim D, Park KH, Robyt JF.1998. Acarbose effect for dexran synthesis, acceptor and disproportionation reactions of *Leuconostoc mesenteroides* B-512FMCM dextransucrase. J. Microbiol. Biotechn., 8(3): 287-290.

Kim MJ, Lee SB, Lee HS, Lee SY, Baek JS, Kim D, Moon TW, Robyt JF, Park KH. 1999. Comparative study of the inhibition of alpha-glucosidase, alpha-amylase, and cyclomaltodextringlucanosyltransferase by acarbose, isoacarbose, and acarviosine-glucose. Arch. Biochem. Biophys., 371(2): 277-283.

Koepsell HJ, Tsuchiya HM, Hellman NN, Kasenko A, Hofman CA, Sharpe EC, Jackson RW. 1953. Enzymatic synthesis of dextran, acceptor specificity and chain initiation. J. Biol. Chem., 200(2): 793-801.

Korakli M, Vogel RF. 2006. Structure/function relationship of homopolysaccharide producing glycansucrases and therapeutic potential of their synthesised glycans. Appl. Microbiol. Biottchnol., 71(6): 790-803.

Kozai K, Miyake Y, Kohda H, Kametaka S, Yamasaki K, Suginaka H, Nagasaka N. 1987. Inhibition of glucosyltransferase from Streptococcus mutans by oleanolic acid and ursolic acid. Caries Res., 21(2): 104-108.

Leemhuis H, Pijning T, Dobruchowska JM, Van Leeuwen SS, Kralj S, Dijkstra BW, Dijkhuizen L. 2013. Glucansucrases: Three-dimensional structures, reactions, mechanism, α-glucan analysis

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and their implications in biotechnology and food applications. J. Biotechnol., 163(2): 250-272.

Liu JJ. 1995. Pharmacology of oleanolic acid and ursolic acid. J. Ethnopharmacol., 49(2): 57-68.

Martinez-Espindola JP, Lopez-Munguia A. 1985. On the kinetics of dextransucrase and dextran synthesis in batch reactors. Biotechnol. Lett., 7(7): 483-486.

Monsan P, Bozzonet S, Albenne C, Joucla G, Willemot R, Remaud-Simeon M. 2001. Homopolysaccharides from lactic acid bacteria. Int. Dairy J., 11(9): 675-685.

Naessens M., Cerdobbel A, Soetaert W, Vandamme EJ. 2005. *Leuconostoc* dextransucrase and dextran: production, properties and applications. J. Chem. Technol. Biot., 80(8): 845-860.

Newbrun E, Hoover CI, Walker GJ. 1983. Inhibition by acarbose, nojirimycin and 1-deoxynojirimycin of glucosyltransferase produced by oral Streptococci. Arch. Oral Biol., 28(6): 531-536.

Palframan R, Gibson G, Rastall R. 2003. Development of a quantitative tool for the comparison of the prebiotic effect of dietary oligosaccharides. Lett. Appl. Microbiol., 37(4): 281-284.

Papanov G, Bozov P, Malakov P. 1992. Triterpenoids from lavandula spica. Phytochemistry, 31(4): 1424-1426.

Paul F., Auriol D, Oriol E, Monsan P. 1984. Production and purification of dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F. Ann. NY Acad. Sci., 434(1): 267-270.

Rastall R, Gibson G, Gill H, Guarner F, Klaenhammer T, Pot B, Reid G, Rowland I, M. Sanders M. 2005. Modulation of the microbial ecology of the human health: an overview of enabling science and potential applications. FEMS Microbiol. Ecol., 52(2): 145-152.

Remaud M, Paul F, Monsan P, Lopez-Mungia A, Vignon M. 1992. Characterisation of α-1,3 branched oligosaccharides synthesised by acceptor reaction with the extracellular glucosyltransferases from Leuconostoc mesenteroides NRRL B-742. J. Carbohyd. Chem., 11(3): 359-378.

Remaud-Simeon M., Lopez-Munguia A, Pepenc V, Paul F, Monsan P. 1994. Production and use of glucosyltransferases from *Leuconostoc mesenteroides* NRRL B-1299 for the synthesis of oligosaccharides containing α-(1,2) linkages. Appl. Biochem. Biotech., 44(2): 101-117.

Remaud-Simeon M, Willemot R, Sarcabal P, De Montalk G, Monsan P. 2000. Glucansucrases: molecular engineering and oligosaccharide synthesis. J. Mol. Catal. B-Enzym., 10(1-3): 117-128.

Roberfroid MB. 2007. Prebiotics: the concept revisited. J. Nutr., 137(3): 830S-837S.

Robyt JF, Eklund SH. 1983. Relative quantitative effects of acceptors in the reaction of *Leuconostoc mesenteroides* B-512F dextransucrase. Carbohyd. Res., 121: 279-286.

Robyt JF, Walseth TF. 1978. The mechanism of acceptor reactions of *Leuconostoc mesenteroides* NRRL B-512F dextransucrase. Carbohyd. Res., 61(1): 433-445.

Strokopytov B, Penninga D, Rozeboom HJ, Kalk KH, Dijkhuizen L, Dijkstra BW. 1995. X-ray structure of cyclodextrin glycosyltransferase complexed with acarbose. Implications for the catalytic mechanism of glycosidases. Biochemistry, 34(7): 2234-2240.

Sumner JB, Howell SF. 1935. A method for determination of saccharase activity. J. Biol. Chem., 108: 51-54.

Van Hijum SA, Kralj S, Ozimek LK, Dijkhuizen L, Van Geel-Schutten GH. 2006. Structure-function ralationships of glucansucrase and fructansucrase enzymes from lactic acid bacteria. Microbiol. Mol. Biol. Rev., 70(1): 157-176.

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