Veselin Bivolarski¹Tonka Vasileva¹Valerie Gabriel²Ilia Iliev¹

¹Department of Biochemistry and Microbiology, Plovdiv University “Paisii Hilendarski”, Plovdiv, Bulgaria

²Laboratory of Food and Environmental Biotechnology (LBAE-EA4565), University Institute of Technology “Paul Sabatier”, Auch, France

Research Article

Synthesis of glucooligosaccharides with prebiotic potential by glucansucrase URE 13–300 acceptor reactions with maltose, raffinose and lactose

In the present work, we report an efficient synthesis of glucooligosaccharides (GOSs) with prebiotic potential by novel glucansucrase URE 13–300 from *Leuconostoc mesenteroides* URE 13 strain. The highest total yield of GOSs with degree of polymerization (DP) from 3 to 6 was obtained with maltose as an acceptor and maltose/sucrose (M/S) ratio 1–136 g/L. An efficient modulation of GOSs composition is achieved by varying the M/S ratio. At M/S = 1, 2, 4 and 7 the content of DP3 products gradually increase from 54.50 to 91.70%. When the M/S ratio was decreased the synthesis of DP>3 GOSs is predominant and reaches 75.60% (M/S = 0.25). In addition, the maltose derived GOSs with DP>3, as well as raffinose and lactose glucosylation products have a branched structure which is prerequisite for increased prebiotic potential. The synthesized GOSs were efficiently metabolized by probiotic strains of *Lb. plantarum* S26, *Lb. brevis* S27 and *Lb. sakei* S16, and the calculated values of specific growth rate (μ) were nearly identical to this on glucose media, when maltose derived GOSs were used as a carbohydrate source. Strain specific features were observed in the utilization of the synthesized GOSs, as well as in the production of lactic acid and acetic acid.

Keywords: Acceptor reactions / Glucansucrase / Glucooligosaccharides / Prebiotics / Probiotics

Additional supporting information may be found in the online version of this article at the publisher's web-site

Received: March 2, 2018; revised: April 17, 2018; accepted: April 18, 2018

DOI: 10.1002/elsc.201800047

1 Introduction

With improving the knowledge and understanding of the complex relationship between the man and the resident microbial flora, the need for accessible means for their healthy modulation becomes more and more pronounced. One way to achieve this is by improving the nutritional quality of food and increase the spread of functional foods, nutraceuticals and dietary

Correspondence: Veselin Bivolarski (morydin@abv.bg), Department of Biochemistry and Microbiology, Plovdiv University “Paisii Hilendarski”, 24 Tzar Assen Str., 4000 Plovdiv, Bulgaria

Abbreviations: DP, degree of polymerization; GH70, glycoside hydrolase family 70; GOSs, glucooligosaccharides; M/S, maltose / sucrose; L/S, lactose / sucrose; R/S, raffinose / sucrose; L., *Leuconostoc*; Lb., *Lactobacillus*; LAB, lactic acid bacteria; μ , specific growth rate; mMRS, modified medium of de Man, Rogosa and Sharpe

supplements [1]. In this regard, the concept of prebiotics is gaining an ever wider realization in the practice of producing healthy foods [2, 3]. On the basis of the scientific knowledge that has accumulated up to now, it is clear that oligosaccharides and polysaccharides of microbial origin have proven prebiotic properties, and are particularly desirable sweeteners in the low-calorie foods [4, 5]. The mechanisms of prebiotic action of oligosaccharides are not fully understood, but their beneficial effects are probably a combination of their specific monosaccharide composition, type of linkages and metabolic pathways for consumption by probiotic bifidobacteria and lactobacilli in the lower intestinal tract of mammals. As a result, the populations of harmful bacteria such as *Salmonella* spp. and *Helicobacter* spp. are reduced, the production of short-chain fatty acids increases, and the overall health status of the organism is improved [4, 6, 7]. In order to quantify the effect of prebiotics on the composition of the microbial population of the intestinal tract have been

developed in vitro approaches determining the prebiotic index (PI) and a measure of the prebiotic effect (MPE), which provide information on the quantitative and functional changes in the major microbial groups, due to the consumption of prebiotics [8,9]. By applying these concepts it is possible to evaluate the effectiveness of various prebiotics, among which the glucooligosaccharides has particularly high prebiotic value [8]. Currently, the production of oligosaccharides is achieved by enzymatic hydrolysis of polysaccharides, or by synthesis using glycosyltransferase enzymes and an inexpensive and substrate such as sucrose [6,10]. Glucansucrases of the glycoside hydrolase family 70 (GH70) produced by LAB of the genera *Lactobacillus*, *Leuconostoc*, *Weissella* and *Streptococcus* are of particular interest as they are capable of synthesizing short-chain GOSs with specific and defined linkages depending on the type of the used enzyme [6,11,12]. In this regard, there is a strong relationship between the structure of the synthesized GOSs, and their prebiotic properties. Currently, it is considered that GOSs containing more α -1,2 or α -1,3-linked glucose residues, i.e. have a branched structure, are able to induce a broad spectrum of glycolytic enzymes in probiotic bacteria without increasing production of gases [13]. Examples of glucansucrases responsible for the formation of specific glycosidic linkages in GOSs are: dextranase DSR-E (EC 2.4.1.5) from *L. citreum* NRRL B-1299; alternansucrase (EC 2.4.1.140) from *L. mesenteroides* NRRL B-21297; reuteransucrase GTF-A (EC 2.4.1.-) from *Lb. reuteri* 121; dextranase DSR-S (EC 2.4.1.5) from *L. mesenteroides* NRRL B-512F [14–17]. Glucansucrases are secreted by LAB in the form of a complex of different enzymes with is a strain-specific composition influencing the overall composition of synthesized polysaccharides and oligosaccharides when partially purified enzymes are used for their production [18,19]. Significant efforts have been made over for purification of glucansucrases from LAB for the purposes of their characterization and application in the synthesis of polysaccharides and oligosaccharides. The applied techniques are mainly limited to the purification of dextranases synthesizing dextran-type linkages in the products [20]. A routine practice providing novel glucansucrases is their identification on a genome level using the growing number of sequenced LAB genomes. The identified novel glucansucrase genes are cloned and expressed in appropriate hosts. This strategy makes possible to extend significantly the range of enzymes used for synthesis of oligosaccharides with specific properties [19,21–24].

The aim of the present work is to investigate and optimize the synthesis of branched oligosaccharides by novel high-molecular weight glucansucrase URE 13–300 from *L. mesenteroides* URE 13 strain in the presence of maltose, raffinose or lactose as acceptors of glucose residues from sucrose. In addition, the ability of probiotic strains of *Lb. plantarum*, *Lb. brevis* and *Lb. sakei* to assimilate the synthesized oligosaccharides is investigated in vitro as an initial measure of their prebiotic potential.

2 Materials and methods

2.1 Bacteria and culture media

L. mesenteroides URE 13 was obtained from the bacterial culture collection of the department of Biochemistry and

Microbiology, Plovdiv University, Bulgaria. The probiotic strains of *Lb. plantarum* S26, *Lb. brevis* S27 and *Lb. sakei* S16 were obtained from the bacterial culture collection of the Department of General and Industrial Microbiology, Sofia University, Bulgaria. The strains were routinely cultivated overnight in de Man, Rogosa and Sharpe (MRS) medium (Biokar Diagnostic, France) at 30°C (*L. mesenteroides* URE13) and at 37°C (*Lactobacillus* strains). For construction of clones the chemically competent cells, *Escherichia coli* 10G (Lucigen Corporation, Middleton, USA) were used and cultivated in Luria-Bertani medium (LB) (10.0 g/L tryptone, 5.0 g/L yeast extract, 5.0 g/L NaCl; Sigma–Aldrich) containing 30 µg/ml kanamycin at 37°C, 250 rpm. For expression of the cloned glucansucrase, *E. coli* BL21(DE3) cells (Lucigen Corporation, Middleton, USA) were grown in Luria-Bertani medium and Terrific Broth medium (TB) (12.0 g/L tryptone, 24.0 g/L yeast extract, 9.4 g/L K₂HPO₄, 2.2 g/L KH₂PO₄; Sigma) supplemented with 30 µg/mL kanamycin on a rotary shaker (250 rpm) at 37°C.

2.2 Cloning the glucansucrase encoding gene

L. mesenteroides total DNA was extracted from the cells using Blood and Tissue kit DNeasy (Qiagen, Hilden, Germany). The following primers containing flanking sequences (in bold) were used for cloning into the pETite N-His SUMO vector (Lucigen Corporation, Middleton, USA): ForSUMOJ188293 5'-CGCGAACAGATTGGAGGTAGAGACAAAAGAATAATATGCG-3'; RevSUMO300J188293 5'-GTGGCGGCCGCTCTATT AATTGTGTATAAAATCAGCATT-3' (Eurogentec, Belgium).

The PCR amplification was performed as described before [25]. The cloning procedure was performed by Expresso™ T7 SUMO Cloning and Expression kit (Lucigen Corporation, Middleton, USA). The amplicon containing the full-size gene was transformed directly into *E. coli* 10G chemically competent cells for construction of clones. Plasmids from the positive transformants were purified using QIAprep Spin Miniprep kit (Qiagen, Hilden, Germany). The positive clones in the pETite™ vectors were transformed into *E. coli* BL21(DE3) cells for expression.

2.3 DNA and protein sequences analysis

DNA sequencing was carried out by Millegene (Toulouse, France). Accession number has been assigned by GenBank to glucansucrase gene (MG869733). DNA sequence information was analyzed by ORFinder (<https://www.ncbi.nlm.nih.gov/orffinder/>), Nucleotide BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), and EMBOSS Transeq (https://www.ebi.ac.uk/Tools/st/emboss_transeq/).

The protein sequence was analyzed by Protein BLAST; HMMER (<https://www.ebi.ac.uk/Tools/hmmer/>); Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>); EMBOSS Needle and RADAR (<https://www.ebi.ac.uk/Tools/pfa/radar/>); ProDom (<http://prodom.prabi.fr/prodom/>). The molecular weight and pI of the protein were predicted using ExPASy software (https://web.expasy.org/compute_pi/) Phylogenetic information was obtained using MAFFT version 7, Archaeopterix (v1.038),

and the neighbor-joining method (<https://mafft.cbrc.jp/alignment/software/>) [26].

2.4 Cultivation of recombinant *E. coli* BL21(DE3) in bioreactor for glucansucrase production

Recombinant *E. coli* BL21(DE3) was cultivated in flasks for preparation of inoculum in TB medium supplemented with 30 $\mu\text{g/mL}$ kanamycin at 37°C on a rotary shaker (250 rpm). When the optical density (OD) of the culture at 600 nm reached 0.6, the inoculum was transferred to 1.5 L TB medium in a benchtop bioreactor (Minifors Benchtop bioreactor, Infors AG, Switzerland) to a final concentration 5% (v/v). The cultivation was performed at 37°C with agitation (180 rpm) until the OD reached 0.6. Inducer (isopropyl- β -D-thiogalactoside) was added to a final concentration of 1.0 mM/L. The protein expression was carried out at 19°C with agitation (180 rpm) for 15 h.

2.5 Isolation of glucansucrase URE 13–300 produced by recombinant *E. coli* BL21(DE3) cells

The cells of *E. coli* BL21(DE3) were collected by centrifugation at $8000 \times g$, 4°C, 15 min. The cell pellet was washed two times with 20 mM sodium acetate buffer, pH 5.3. The cells sonicated in a cold sonication buffer 20 mM sodium acetate buffer, pH 5.3, containing 100 mM NaCl and 20% (v/v) glycerol (Hielscher Ultrasound Technology UP50H, Germany). The cell debris was removed by centrifugation at $8000 \times g$, 4°C, 15 min, and the supernatant was used as a cell-free extract, containing glucansucrase URE 13–300.

2.6 Glucansucrase activity assay

One unit of glucansucrase activity is defined as the amount of enzyme that catalyzes the formation of 1 μM fructose per minute at 30°C in 20 mM sodium acetate buffer, pH 5.3; 0.05 g/L CaCl_2 and 100 g/L of sucrose. Glucansucrase activity was determined by measuring the amount of reducing sugars derived from sucrose by the 3,5-dinitrosalicylic acid method [27]. The released D-fructose was determined enzymatically (Megazyme, International Ireland Ltd., Wicklow, Ireland). The protein concentration was assayed by the method of Bradford [28]. All measurements were performed at least in triplicate from different experiment with standard deviation ($\pm\text{SD}$).

2.7 Electrophoresis analysis (SDS-PAGE analysis)

SDS-PAGE (70 $\times$ 80 mm slab gels, with 3% concentrating and 5% separating acrylamide gels) was performed according to the method of Laemmli [29]. Glucansucrase in situ activity detection was performed by incubation of the gel in 100 g/L sucrose overnight, followed by staining of the synthesized polysaccharide according to a periodic acid-Schiff's procedure (PAS) [30]. The used protein standards (High Molecular Weight

Calibration Kit for SDS Electrophoresis, Amersham, UK) were stained with Coomassie Brilliant Blue R 250 (Sigma–Aldrich, St. Louis, Missouri, USA).

2.8 Preparation and analysis of oligosaccharides

Acceptor reactions for synthesis of oligosaccharides were carried out at 30°C in 20 mM pH 5.3 sodium acetate buffer containing 0.05 g/L CaCl_2 and 0.01% (w/v) sodium azide. Sucrose was used as a donor of glucose units and maltose monohydrate, lactose monohydrate or raffinose pentahydrate (Merck, Bulgaria EAD) as sugar acceptors. Ratios of 0.14, 0.25, 0.5, 1, 2, 4, 7 acceptor / sucrose were used for the reactions with maltose or raffinose, and ratios of 0.5, 1, and 2 with lactose as an acceptor. The final substrate concentration for each reaction mixture was 300 g/L. Each reaction was started by addition of 1.0 U/mL enzyme activity. The progress of the reactions was monitored by HPLC, and they were stopped at 48 h when all the sucrose was consumed. To each reaction was added 1 volume of 96% ethanol in order to precipitate the synthesized polysaccharide. The glucan fraction was pelleted by centrifugation at 8000 g, and the supernatant fraction containing GOSs was subjected to evaporation under vacuum. The quantities of the synthesized GOSs and their DP values were evaluated by HPLC using KONIK HPLC System (KONIK HPLC System, Barcelona, Spain); 250 $\times$ 4.6 mm HPLC column YMC-Pack™ Polyamine II (YMC™, Co., Ltd., Japan) at 30°C; elution with 1.0 mL/min 60:40% (v/v) acetonitrile:water mobile phase. The results were analyzed using Konikrom Plus v2010 software (KONIK, Barcelona, Spain) against appropriate carbohydrate standards.

The available monosaccharides and acceptors were removed using Bio-Gel P2 (fine mesh) (Bio-Rad Laboratories, Inc., USA) packed in 5 $\times$ 150 cm column [31]. Fractions of 20 mL were collected and analyzed by HPLC. Fractions containing synthesized GOSs were combined, freeze-dried (FreeZone® Plus™ 4.5 Liter Cascade, Labconco Corporation, Kansas City, USA) and used in the utilization and enzyme hydrolysis experiments.

The reactions for GOSs synthesis were carried out in duplicate. The HPLC analyses of the oligosaccharide composition and quantity were performed in duplicate from two different experiments with standard deviation ($\pm\text{SD}$).

2.9 Enzyme hydrolysis of GOSs

Purified GOSs, dissolved (1% w/v) in 20 mM sodium acetate buffer (pH 5.4) were incubated with 1.0 U/mL dextranase (EC 3.2.1.11) from *Penicillium* sp. (Sigma–Aldrich Chemie, Germany) for 24 h at 30°C. As a control was used 1% (w/v) isomaltose (Sigma–Aldrich Chemie, Germany). One unit dextranase is defined as the amount of enzyme catalyzing the hydrolysis of μM isomaltose per minute at 30°C and pH 5.4 [32]. The released glucose was determined by HPLC.

The hydrolysis reactions of GOSs were performed in triplicate, and HPLC analyses were performed in duplicate from different experiments with standard deviation ($\pm\text{SD}$).

2.10 Utilization of the synthesized oligosaccharides from probiotic strains *Lb. plantarum* S26, *Lb. brevis* S27 and *Lb. sakei* S16

Overnight grown cells of *Lb. plantarum* S26, *Lb. brevis* S27 and *Lb. sakei* S16 were washed twice in 0.85% NaCl (w/v) saline solution, and were used to inoculate (10%, v/v) modified MRS (mMRS) broth medium containing (g/L): glucose or GOSs (filter sterilized) – 10 g/L; Tween 80 – 1.0; ammonium citrate – 2.0; $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ – 55.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ – 0.1; MnSO_4 – 0.05; K_2HPO_4 – 2.0; meat extract – 10.0; peptone – 10.0; yeast extract – 5.0; pH – 6.8. The anaerobic fermentations with the strains were performed in 100 mL glass bottles at 37°C for 48 h (BBL® Gas Pak anaerobic system Envelopes, Becton Dickinson Microbiology Systems, Sparks, USA) under non-pH-controlled conditions.

GOSs utilization was determined by measuring the bacterial growth using turbidimetric method at 600 nm against a cell dry-weight standard curve (Beckman Coulter DU 800 Spectrophotometer, Beckman Coulter, Inc., Brea, California, USA) [31]. After a logarithmic conversion of the obtained values a specific growth rates (μ) of the strains were calculated. Growth curves which reflect the relation between the logarithm of the living cells and the time were built for all used growth media. [33].

Acid production from GOSs was determined by measuring pH (W.T.W. Sen. Tix 97T, Germany) during the cultivation of each strain of lactobacilli. The amounts of lactate, acetate and ethanol formed during the cultivation of the strains in mMRS media supplemented with GOSs were measured enzymatically with commercially available kits (Boehringer, Mannheim, Germany).

Each GOSs utilization test was performed in triplicate. The bacterial growth, pH, and metabolites were measured in duplicate from different experiments with standard deviation ($\pm$ SD).

2.11 Statistical analysis

In all the cases the programmable scientific calculator “CASIO” fx-991ES Plus, statistical software package SigmaPlot v12.0 (Systat Software, Inc., Chicago, USA) and Microsoft Excel were used for data analysis and graphical representation.

3 Results and discussion

3.1 Cloning of URE 13–300 encoding gene and sequence analysis

The cloned sequence from *L. mesenteroides* URE 13 contains 8526 kb-long coding region which encodes a glucansucrase of 2841 amino acids. The protein has a predicted pI of 4.4 and a theoretical molecular mass of 311.85 kDa. The analyses of the glucansucrase (called URE 13–300) sequence revealed that it consists of 42 aa signal peptide, specific for secretory proteins [34]. A catalytic domain of 799 aa (CD1) with the characteristic structure of GH70 family of enzymes was identified. The catalytic triad consists of catalytic nucleophile (Asp543), acid/base catalyst (Glu581) and transition state stabilizer (Asp654) (URE

13–300 numbering) [19]. A second GH70-type catalytic domain (CD2) was identified too. CD2 contains a catalytic triad – Asp2215, Glu2253 and Asp2327 (URE 13–300 numbering). The identity between the amino acid sequences of CD1 and CD2 is 45.4% suggesting differences in catalytic specificity of these two domains.

The phylogenetic analysis showed that glucansucrase URE 13–300 is clustered together with enzymes from *L. mesenteroides* ATCC 8293 and J18 strains which also have two catalytic domains and share more than 96% identity with the enzyme of study (Fig. 1). Glucansucrases from *L. citreum* having two catalytic domains are grouped in a separate clade, and share 69% identity with glucansucrase URE 13–300 (Fig. 1).

From glucansucrases possessing a second catalytic domain, the best studied is DSR-E of *L. citreum* NRRL B-1299. This enzyme is able to introduce into synthesizing polysaccharides and oligosaccharides α -1,6-glycosidic linkages through its CD1 domain and α -1,2 branches of glucose units by its CD2 domain which is a quite rare property [14, 35]. On the other hand, the presence of a second catalytic domain with a potential branching activity does not guarantee such a functional realization, as is the case with the two high-molecular-weight glucansucrases produced by *L. citreum* LBAE C11 and E16 strains which synthesize only a α -1,6-linkages containing dextran [21]. From this point of view, a too little is known about the catalytic potential of glucansucrases containing two catalytic domains and produced by strains of *L. mesenteroides* [36, 37].

3.2 In situ analysis of glucansucrase URE 13–300

The in situ analysis of recombinant *E. coli* cell-free extract showed an activity band with an expected molecular weight of about 300 kDa which correlates well with the detection of glucansucrase URE 13–300 in the supernatant fraction of the native producer *L. mesenteroides* URE 13 (Fig. 2). It is known that this strain produces a complex of glycosyltransferase enzymes [38]. Such a glycosyltransferase complex is observed by Olvera and coworkers for *L. mesenteroides* ATCC 8293 strain which also produce natively high-molecular-weight glucansucrase [37]. This required cloning of glucansucrase URE 13–300 encoding gene in a conventional *E. coli* based expression system which, moreover, does not produce other glucansucrases. Therefore, recombinant cell-free extracts of induced cells were directly used as a source of glucansucrase activity for synthesis of GOSs.

3.3 Synthesis of GOSs by glucansucrase URE 13–300 in the presence of maltose, raffinose or lactose

In order to modulate the production and composition of GOSs synthesized by glucansucrase URE 13–300, we carried out series of synthesis reactions using various ratios of acceptor/sucrose. A summary of the obtained yields of GOSs and their distribution according to DP is depicted in Fig. 3.

The highest yields of GOSs were obtained at acceptor/sucrose ratio 1. During the acceptor reactions with maltose, series of GOSs are synthesized with DP ranging from 3 to 7. At increased M/S ratios, the proportion of GOSs with DP > 3 gradually

Figure 1. Phylogenetic analysis of glucansucrases containing one and two catalytic domains. Note: The analysis was performed with MAFFT version 7 by the neighbor-joining method [26]. Bar: substitutions/nucleotide position. Numbers indicate bootstrap percentages per 1000 repeats.

Figure 2. In situ analysis of crude glucansucrase URE 13-300 expressed in *E. coli* BL21 cells. Note: M: protein molecular-weight size marker (Amersham, UK); 1 – supernatant fraction from native strain *L. mesenteroides* URE 13; 2 – recombinant extract of induced *E. coli* BL21 cells.

decreases from 45.5% (M/S = 1) to 8.3% (M/S = 7). The decreased M/S ratios causes elevated synthesis of GOSs with DP > 3 and gradually diminished the yields of DP3 products from 28.1% (M/S = 0.5) to 21.2% (M/S = 0.14) (Fig. 3). These effects could be explained with the specific features of the acceptor reaction which are defined by the concentration of the acceptor molecule and its strength. The maltose was identified as one of the strongest acceptors for glucansucrase reactions [39, 40]. At a higher concentration of maltose, a less amount of the synthesized GOSs is directed to accept glucose units and to generate products with higher DP. On the other hand, at lower concentrations of maltose and a plenty of available sucrose, the studied glucansucrase catalyzes the addition of glucose residues to DP3 and the higher products reaching DP7 (Fig. 3). At M/S = 1, the tendency of each next synthesis product to be less potent to accept glucose units is clearly observed (Fig. 3, Supporting Information Fig. 1A).

In the presence of raffinose, glucansucrase URE 13-300 give rise only of DP4 products, independently of R/S ratio used (Fig. 3, Supporting Information Fig. 1B). The highest yields of GOSs were obtained at R/S ratio 1 and the further change of the acceptor concentration has not produced any improvement of the yield or DP-composition of the obtained GOSs (Fig. 3). The glucosylation of raffinose by dextransucrase DSR-S produces only GOSs with DP4, and Cote and coworkers reported synthesis of tetrasaccharides and a minor amount of pentasaccharides by alternansucrase [40, 42].

Figure 3. Yields of GOSs synthesized by glucansucrase URE 13–300 in the presence of maltose, raffinose or lactose as acceptors. Note: Total concentration of sugars at the start of each reaction – 300 g/L. The calculated yields are means ($\pm$ SD) from duplicate measurements of different experiments, before removal of the residual monosaccharides and acceptor molecules. DP – degree of polymerization from 3 to 7.

When lactose was applied as an acceptor, a mixture of GOSs with DP3 and DP4 was obtained (Fig. 3, Fig. S1-C). The highest yield of GOSs was achieved again at acceptor/sucrose ratio 1, and predominant formation of products with DP3. The further increase of lactose concentration leads to reduction of the yield of products with DP4 (Fig. 3). The ambiguous signal for the products with DP4 corresponds to the information for synthesis GOSs with mixed composition by alternansucrase and *Lb. reuteri* glucansucrases, respectively. For dextranase DSR-S a synthesis of lactose derived GOSs with DP4 is not reported to date [30, 40–42] (Supporting Information Fig. 1C).

According to the obtained results, the most efficient synthesis of GOSs is achieved in the presence of maltose as an acceptor of glucose residues. At $M/S > 1$, the percent of glucose available in sucrose that is transferred to maltose or higher GOSs was over 70%. As less efficient acceptors were determined lactose and raffinose (Table 1). The obtained values for efficiency of transfer of glucose units from sucrose to the used acceptors and their derivatives are in good correlation with the available empiric data concerning the strength of different sugar acceptors [40]. The ability to modulate the productive properties of glucansucrase URE 13–300 and the DP-specter of the synthesized GOSs by changing the ratio acceptor /donor appears to be an efficient tool for obtaining of oligosaccharide compositions with more defined structure. This is especially well demonstrated in the maltose acceptor reactions where as a predominant products were obtained GOSs with DP3 ($M/S = 2, 4$ and 7) (Fig. 3).

3.4 Enzymatic hydrolysis of GOSs synthesized by glucansucrase URE 13–300

In order to determine the content of α -1,6 linkages in the synthesized GOSs, we performed dextranase hydrolysis (Table 2).

The highest percent of α -1,6 linkages in maltose acceptor products were determined at $M/S = 2$ (90.77%), $M/S = 4$ (92.50%) and $M/S = 7$ (95.83%) ratios, respectively. This corresponds to the increased proportion of GOSs with DP3 at these M/S ratios, and suggests that glucose is attached to maltose

mostly by of α -1,6 linkages. In contrast, with decrease of M/S ratio, the determined content of α -1,6-linkages lowers – $M/S = 0.5$ (73.43%), $M/S = 0.25$ (75.25%), and $M/S = 0.14$ (74.14%) (Table 2). This suggests that glucansucrase URE 13–300 incorporates linkages which are different from α -1,6. The obtained results are in good correlation with the available data for synthesis of branched oligosaccharides containing α -1,3- or α -1,2-linked glucose units by dextranases from *L. citreum* NRRL B-742 and NRRL B-1299 strains [32, 43, 44]. It could be concluded that the varied M/S ratios influences not only the DP values of the obtained products, but also their content α -1,6 linkages.

The dextranase hydrolysis of raffinose and lactose acceptor products did not produced any glucose, suggesting the absence of α -1,6 linkages between the acceptor and the attached glucose residues (Table 2). In correlation with these results, Robyt and Eklund (1983) reported that dextranase DSR-S attaches the glucose residue to the reducing-end glucose of lactose disaccharide forming a α -1,2 linkage in the DP3 product [40]. Similarly, Shi and coworkers (2016) have reported synthesis of branched lactose derived oligosaccharides with dextranase from *Weissella confusa* VTT E-90392 strain in which the added by the enzyme glucose residue is α -1,2-linked to the reducing end of lactose [45]. Recently, Pham and coworkers (2017) reported successful glucosylation of lactose by glucansucrases from *Lb. reuteri*, which attach the glucose at position α -1,3 or α -1,4 of the non-reducing galactose moiety of lactose [41].

Cote and coworkers (2009) reported glucosylation of raffinose by alternansucrase, and obtaining a mixture of tetrasaccharides in which the glucose residue is attached with α -1,3; α -1,4 or a α -1,6 linkage to the galactose residue of raffinose. In the same work, the authors also reported synthesis of a raffinose acceptor product with DP4 by dextranase DSR-S in which the glucose is α -1,4-linked to the galactose residue of raffinose [42].

3.5 Utilization and prebiotic potential of GOSs synthesized by glucansucrase URE 13–300

The utilization and prebiotic potential of GOSs synthesized by glucansucrase URE 13–300 was investigated by cultivation of *Lb.*

Table 1. Efficiency of glucose transfer for synthesis of GOSs catalyzed by glucansucrase URE 13–300

Maltose acceptor reactions							
M/S ratio	1	2	4	7	0.5	0.25	0.14
Transferred glucose to GOSs, (%)	76.72 ± 1.16	75.84 ± 1.21	72.71 ± 0.95	71.47 ± 2.32	55.52 ± 1.52	48.85	13.96 ± 0.65
Raffinose acceptor reactions							
R/S ratio	1	2	4	7	0.5	0.25	0.14
Transferred glucose to GOSs, (%)	32.97 ± 1.35	44.70 ± 2.18	38.34 ± 1.12	47.04 ± 2.14	18.68 ± 0.95	10.89 ± 1.1	8.17 ± 0.60
Lactose acceptor reactions							
L/S ratio	1	2			0.5		
Transferred glucose to GOSs, (%)	55.29 ± 1.90	59.02 ± 2.20			32.18 ± 1.45		

Note: The calculated yields are means (±SD) from duplicate measurements of different experiments.

Table 2. Hydrolysis of GOSs synthesized by glucansucrase URE 13–300 with dextranase

Sample of GOSs	Glucose included in GOSs (mM/L) ^{a)}	Glucose released by dextranase (mM/L)	Presumed content of α-1,6 linkages (%)
M/S = 1	23.50 ± 1.17	19.10 ± 1.00	81.27 ± 4.06
M/S = 0.5	27.10 ± 1.30	19.90 ± 1.00	73.43 ± 3.67
M/S = 2	20.60 ± 1.00	18.70 ± 0.93	90.77 ± 4.53
M/S = 0.25	29.10 ± 1.50	21.90 ± 1.00	75.25 ± 3.76
M/S = 4	20.00 ± 1.00	18.50 ± 0.92	92.50 ± 4.62
M/S = 0.14	30.20 ± 1.50	22.40 ± 1.12	74.14 ± 3.77
M/S = 7	19.20 ± 0.96	18.40 ± 0.92	95.83 ± 4.79
R/S = 1	13.80 ± 0.69	N/A	N/A
R/S = 0.5	13.80 ± 0.69	N/A	N/A
R/S = 2	13.80 ± 0.69	N/A	N/A
R/S = 0.25	13.80 ± 0.69	N/A	N/A
R/S = 4	13.80 ± 0.69	N/A	N/A
R/S = 0.14	13.80 ± 0.69	N/A	N/A
R/S = 7	13.80 ± 0.69	N/A	N/A
L/S = 1	20.60 ± 1.00	N/A	N/A
L/S = 0.5	20 ± 1.00	N/A	N/A
L/S = 2	19.10 ± 1.00	N/A	N/A
Isomaltose (control)	29.20 ± 1.46	29.10 ± 1.45	98.64 ± 4.93

^{a)} Note: – glucose transferred from sucrose to GOSs by glucansucrase URE 13–300 and calculated for 1% (w/v) initial solution. M/S – maltose/sucrose ratio; R/S – raffinose / sucrose ratio; L/S – lactose/sucrose ratio. The calculated yields of glucose are means (±SD) from duplicate measurements of different experiments.

plantarum S26, *Lb. brevis* S27 and *Lb. sakei* S16 strains, which are known to have probiotic properties [46]. Glucooligosaccharides synthesized at acceptor/donor ratios 1 and 0.5 of maltose, raffinose or lactose were selected for this study. A strain-dependant specificity was determined, concerning the cell growth during cultivation of the strains in media containing the corresponding GOSs. The specific growth rates (μ) of the strains cultivated in the presence of maltose derived GOSs are nearly identical to this of glucose containing control fermentation (Table 3). The obtained values of μ for *Lb. strain* S26 were two times lower when the strain was cultivated in media containing raffinose or lactose derived GOSs, while for *Lb. brevis*

S27 strain the calculated values are about 50 and 20% lower, respectively. Uniform values of μ were determined when *Lb. sakei* S16 strain was cultivated in media with GOSs or glucose (Table 3).

The obtained results correlate well with the data for media acidification and production of lactate, acetate and ethanol by the strains which metabolize the sugars heterofermentatively (Table 4). After 48 h of cultivation in the presence of GOSs, strain specificity is detected in the lactate/acetate ratio. It is 4:1 for *Lb. plantarum* S26, and 2:1 and 1:1 for the other two strains respectively. This could be explained with different velocity of assimilation of the products released after hydrolysis of the

Table 3. Specific growth rates of *Lb. plantarum* S26, *Lb. brevis* S27, *Lb. sakei* S16 strains during their cultivation in media supplemented with maltose, raffinose or lactose derived GOSs

Carbohydrate source ^{a)}	μ (h ⁻¹) of <i>Lb. plantarum</i> S26	μ (h ⁻¹) of <i>Lb. brevis</i> S27	μ (h ⁻¹) of <i>Lb. sakei</i> S16
Glucose	0.42 ± 0.01	0.56 ± 0.05	0.26 ± 0.04
GOSs, M/S = 1	0.43 ± 0.10	0.48 ± 0.12	0.31 ± 0.14
GOSs, M/S = 0.5	0.43 ± 0.04	0.46 ± 0.09	0.27 ± 0.08
GOSs, R/S = 1	0.24 ± 0.50	0.28 ± 0.08	0.23 ± 0.05
GOSs, R/S = 0.5	0.16 ± 0.19	0.25 ± 0.03	0.24 ± 0.05
GOSs, L/S = 1	0.20 ± 0.90	0.43 ± 0.13	0.26 ± 0.10
GOSs, L/S = 0.5	0.21 ± 0.60	0.41 ± 0.10	0.27 ± 0.40

^{a)}Note: 1% (w/v) of glucose or GOSs. The calculated specific growth rates are means (± SD) from duplicate measurements of different experiments.

studied GOSs, and differences in the activity of lactate dehydrogenase and acetate kinase catalyzing the last phases of the sugar metabolism.

According to the available data, the ability of lactobacilli to utilize various oligosaccharides as a source of energy and building blocks is at the expense of reduction of the size of their genomes [47]. The enzymes responsible for assimilation of oligosaccharides by lactobacilli are intracellular only. This property clearly differentiates the genus from other microorganisms, such as *Bifidobacteria* which are adapted to the intestinal habitats and have an “ingenious” specter of glycosylolases and carbohydrate transporters [47]. Our results demonstrate the need to establish the specificity of the metabolism of oligosaccharides having different structures by probiotic lactobacilli which is an important condition for obtaining of effective synbiotic products. In addition, our results correlate well with thes of Grimoud and coworkers (2010), and Cecchini and coworkers (2013) [48, 49].

4 Concluding remarks

In the current work, we describe synthesis of GOSs by novel glucansucrase URE 13–300 from *L. mesenteroides* URE 13. Since the enzyme of interest is produced from the native strain in a complex with other enzymes, the corresponding gene was cloned into an *E. coli* expression system. This allowed the synthesis of GOSs with a more defined and specific structure. The enzyme contains two catalytic domains and is the second of this type reported to perform an efficient synthesis of branched-chain oligosaccharides having prebiotic potential. We report an efficient production of GOSs in the presence of maltose, raffinose or lactose as acceptors, achieving between 30 and 75% inclusion of the available glucose in oligosaccharide products. Additionally, we have achieved effective modulation of the spectrum of oligosaccharide products synthesized by glucansucrase URE 13–300, according to their degree of polymerization and content of α -1,6 linkages. At M/S ratios 0.5, and 0.25, the reaction mixtures were enriched with GOSs having DP > 3, and containing between 25% and 27% less α -1,6 glycosidic linkages. At M/S ratios 2, 4 and 7, the production of GOSs with DP3 is predominant, and the content of α -1,6 linkages is between 90% and 95%. This tendency is less pronounced in the reactions with lactose where only GOSs with DP3 and DP4 are detected. The acceptor reactions with raffinose give rise of only DP4 oligosaccharides. However, glucansucrase URE 13–300 has a high efficiency in the glucosylation of these two acceptors, achieving yields of about 100 g/L and 120 g/L GOSs, respectively. The ability of probiotic lactobacilli strains to digest the synthesized oligosaccharide mixtures was investigated too. The probiotic strains of *Lb. plantarum* S26, *Lb. brevis* S27 and *Lb. sakei* S16 utilize maltose derived GOSs with specific growth rates nearly identical to this of glucose. The utilization of maltose, raffinose and lactose derived GOSs follows a strain specific manner and varies. The highest values of μ were obtained for *Lb. brevis* S27. These results correlate well with the values of lactate, acetate and ethanol produced by the strains, contributing to the

Table 4. Values of pH, production of lactate, acetate and ethanol by *Lb. plantarum* S26, *Lb. brevis* S27 and *Lb. sakei* S16 strains on media containing GOSs

Strain	Carbohydrate source ^{a)}	pH ^{b)}	Lactate, (mg/mL) ^{b)}	Acetate, (mg/mL) ^{b)}	Ethanol, (mg/mL) ^{b)}
<i>Lb. plantarum</i> S26	Glucose	4.02 ± 0.02	6.05 ± 0.10	1.43 ± 0.20	0.10 ± 0.04
	GOSs, M/S = 0.5	4.04 ± 0.03	6.11 ± 0.30	1.46 ± 0.30	0.10 ± 0.05
	GOSs, R/S = 0.5	4.23 ± 0.04	5.42 ± 0.30	1.28 ± 0.20	0.15 ± 0.02
	GOSs, L/S = 0.5	4.46 ± 0.07	4.78 ± 0.20	1.56 ± 0.30	0.20 ± 0.03
<i>Lb. brevis</i> S27	Glucose	3.85 ± 0.10	4.15 ± 0.20	2.90 ± 0.40	0.10 ± 0.01
	GOSs, M/S = 0.5	4.11 ± 0.08	4.12 ± 0.30	2.86 ± 0.30	0.08 ± 0.01
	GOSs, R/S = 0.5	4.00 ± 0.05	4.10 ± 0.30	2.96 ± 0.20	0.07 ± 0.01
	GOSs, L/S = 0.5	4.62 ± 0.06	3.57 ± 0.30	3.04 ± 0.30	0.08 ± 0.01
<i>Lb. sakei</i> S16	Glucose	4.65 ± 0.08	2.95 ± 0.20	3.52 ± 0.20	0.13 ± 0.03
	GOSs, M/S = 0.5	4.55 ± 0.05	3.01 ± 0.20	3.46 ± 0.20	0.12 ± 0.02
	GOSs, R/S = 0.5	4.24 ± 0.06	4.16 ± 0.30	2.95 ± 0.30	0.05 ± 0.01
	GOSs, L/S = 0.5	4.44 ± 0.05	3.11 ± 0.15	2.46 ± 0.20	0.03 ± 0.01

^{a)}Note: 1% (w/v) of glucose or GOSs.

^{b)}pH, lactic acid, acetic acid and ethanol at 48 h of the fermentation. The measured values of pH, lactate, acetate and ethanol are means (±SD) from duplicate measurements of different experiments.

prebiotic potential of the synthesized by glucansucrase URE 13–300 glucooligosaccharides.

Greek symbols

μ	$[\text{h}^{-1}]$	specific growth rate
-------	-------------------	----------------------

Practical application

Glucansucrase URE 13–300 has the ability to synthesize efficiently glucooligosaccharides in the presence of maltose, raffinose or lactose as acceptors of glucose residues from sucrose donor. Yields of 136, 98 and 111 g/L glucooligosaccharides were achieved, at 300 g/L initial carbohydrate concentration in the reaction mixtures. In addition, a possibility to modulate the proportion of the products regarding their degree of polymerization and content of α -1,6-linked glucose residues by varying acceptor/donor ratio is available. This is effectively applicable for the acceptor reactions with maltose and lactose. The studied enzyme introduces glycosidic linkages differing from α -1,6 in its maltose, raffinose and lactose acceptor products. This property of the enzyme represents potential interest for production of prebiotic oligosaccharides.

This work was supported by the National Scientific Fund, Bulgaria, grant ДМ01/7-17.12.2016.

The authors have declared no conflict of interest.

5 References

- [1] Cencic, A., Chingwaru, W., The role of functional foods, nutraceuticals, and food supplements in intestinal health. *Nutrients* 2010, 2, 611–625.
- [2] Manning, T. S., Gibson, G. R., Prebiotics. *Best Practice Res. Clin. Gastroenterol.* 2004, 18, 287–298.
- [3] Moreno, F. J., Corzo, N., Montilla, A., Villamiel, M. et al., Current state and latest advances in the concept, production and functionality of prebiotic oligosaccharides. *Curr. Opin. Food Sci.* 2017, 13, 50–55.
- [4] Rajendran, S. R. C. K., Okolie, C. L., Udenigwe, C. C., Mason, B., Structural features underlying prebiotic activity of conventional and potential prebiotic oligosaccharides in food and health. *J. Food Biochem.* 2017, e12389, 1–19, 10.1111/jfbc.12389.
- [5] Al-Shereji, S. H., Ismail, A., Manap, M. Y., Mustafa, S. et al., Prebiotics as functional foods: a review. *J. Funct. Foods.* 2013, 5, 1542–1553.
- [6] Kim, Y.-M., Kang, H.-K., Moon, Y.-H., Nguyen, T. T. H. et al., Production and bioactivity of glucooligosaccharides and glucosides synthesized using glucansucrases, in: Moreno, F. J., Sanz, M. L. (Eds.), *Food Oligosaccharides – Production, Analysis and Bioactivity*, Wiley Blackwell, Chichester 2014, pp. 168–183.
- [7] Goh, Y., Klaenhammer, T. R., Genetic mechanisms of prebiotic oligosaccharides metabolism in probiotic microbes. *Annu. Rev. Food Sci. Technol.* 2015, 6, 137–156.
- [8] Palframan, R., Gibson, G. R., Rastall, R. A., Development of a quantitative tool for the comparison of the prebiotic effect of dietary oligosaccharides. *Lett. Appl. Microbiol.* 2003, 37, 281–284.
- [9] Vulevic, J., Rastall, R. A., Gibson, G. R., Developing a quantitative approach for determining the in vitro prebiotic potential of dietary oligosaccharides. *FEMS Microbiol. Lett.* 2004, 236, 153–159.
- [10] Patel, S., Goyal, A., Functional oligosaccharides: production, properties and applications. *World J. Microbiol. Biotechnol.* 2011, 27, 1119–1128.
- [11] Monsan, P., Remaud-Simeon, M., Andre, I., Transglucosidases as efficient tools for oligosaccharide and glucoconjugate synthesis. *Curr. Opin. Microbiol.* 2010, 13, 293–300.
- [12] Andre, I., Potocki-Veronese, G., Morel, S., Monsan, P. et al., Sucrose-utilizing transglucosidases for biocatalysis. *Top. Curr. Chem.* 2010, 294, 25–48.
- [13] Monsan, P. F., Ouarne, F. Oligosaccharides derived from sucrose, in: Charalampopoulos, D., Rastall, R. A. (Eds.), *Prebiotics and Probiotics Science*, Springer Science+Media, New York 2009, pp. 293–337.
- [14] Fabre, E., Bozonnet, S., Arcache, A., Willemot, R.-M. et al., Role of two catalytic domains of DSR-E dextranucrase and their involvement in the formation of highly α -1,2 branched dextran. *J. Bacteriol.* 2005, 187, 296–303.
- [15] Cote, G. L., Sheng, S., Dunlap, C. A., Alternansucrase acceptor products. *Biocatal. Biotransfor.* 2008, 26, 161–168.
- [16] Kralj, S., van Geel-Schutten, G. H., van der Maarel, M. J. E. C., Dijkhuizen, L., Biochemical and molecular characterization of *Lactobacillus reuteri* 121 reuteransucrase. *Microbiology.* 2004, 150, 2099–2112.
- [17] Monchois, V., Remaud-Simeon, M., Russell, R. R. B., Monsan, P. et al., Characterization of *Leuconostoc mesenteroides* NRRL B-512F dextranucrase (DSRS) and identification of amino-acid residues playing a key role in enzyme activity. *Appl. Microbiol. Biotechnol.* 1997, 48, 465–472.
- [18] Korakli, M., Vogel, R. F., Structure/functional relationship of homopolysaccharide producing glycansucrases and therapeutic potential of their synthesised glycans. *Appl. Microbiol. Biotechnol.* 2006, 71, 790–803.
- [19] van Hijum, S. A. F. T., Kralj, S., Ozimek, L. K., Dijkhuizen, L. et al., Structure-function relationships of glucansucrase and fructansucrase enzymes from lactic acid bacteria. *Microbiol. Mol. Biol. R.* 2006, 70, 157–176.
- [20] Majumder, A., Purama, R. K., Goyal, A., An overview of purification methods of glycoside hydrolase family 70 dextranucrase. *Indian J. Microbiol.* 2007, 47, 197–206.
- [21] Amari, M., Gabriel, V., Herve, R., Morel, S. et al., Overview of the glucansucrase equipment of *Leuconostoc citreum* LBAE-E16 and LBAE-C11, two strains isolated from sourdough. *FEMS Microbiol. Lett.* 2014, 362, 1–8.
- [22] Passerini, D., Vuillemin, M., Ufarte, L., Morel, S. et al., Inventory of the GH70 enzymes encoded by

- Leuconostoc citreum* NRRL B-1299 – identification of three novel α -transglucosylases. *FEBS J.* 2015, 282, 2115–2130.
- [23] Skory, C. D., Cote, G. L., Secretes expression of *Leuconostoc mesenteroides* glucansucrase in *Lactococcus lactis* for the production of insoluble glucans. *Appl. Microbiol. Biotechnol.* 2015, <https://doi.org/10.1007/s00253-015-6854-0>
- [24] Vuillemin, M., Claverie, M., Brison, Y., Severac, E. et al., Characterization of the first α -(1-3) branching sucrose of the GH70 family. *J. Biol. Chem.* 2016, 291, 7687–7702.
- [25] Iliev, I., Vasileva, T., Bivolarski, V., Salim, A. et al., Optimization of the expression of levansucrase L17 in recombinant *E. coli*. *Biotechnol. Biotechnol. Eq.* 2018, 32, 477–486.
- [26] Han, M. V., Zmasek, C. M., PhyloXML: XML for evolutionary biology and comparative genomics. *BMC Bioinformatics.* 2009, 10, 356.
- [27] Miller, G. L., Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 1959, 31, 426–428.
- [28] Bradford, M., A rapid and sensitive for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 1976, 72, 248–254.
- [29] Laemmli, U. K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970, 227, 680–685.
- [30] Miller, A. W., Robyt, J., Detection of dextransucrase and levansucrase on polyacrylamide gels by the periodic acid-Schiff stain: staining artifacts and their prevention. *Anal. Biochem.* 1986, 156, 357–363.
- [31] Cote, G. L., Holt, S. M., Miller-Fosmore, C., Prebiotic oligosaccharides via alternansucrase acceptor reactions, in: Eggleston, G., Cote, G. L. (Eds.), *Oligosaccharides in Food and Agriculture*, ACS Symposium Series, Washington 2003, pp. 76–89.
- [32] Remaud, M., Paul, F., Monsan, P., Lopez-Munguia, A. et al., Characterization of α -(1,3) branched oligosaccharides synthesized by acceptor reaction with the extracellular glucosyltransferases from *L. mesenteroides* NRRL B-742. *J. Carbohydr. Chem.* 1992, 11, 359–378.
- [33] Najafpour, G. D. (Ed), *Biochemical engineering and biotechnology*, Elsevier, Amsterdam 2015.
- [34] Bensing, B. A., Siboo, I. R., Sullam, P. M., Glycine residues in the hydrophobic core of GspB signal sequence route export toward the accessory Sec pathway. *J. Bacteriol.* 2007, 189, 3846–3854.
- [35] Bozonnet, S., Dols-Laffargue, M., Fabre, E., Pizzut, S. et al., Molecular characterization of DSR-E, an α -1,2 linkage-synthesizing dextransucrase with two catalytic domains. *J. Bacteriol.* 2002, 184, 5753–5761.
- [36] Makarova, K., Slesarev, A., Wolf, Y., Sorokin, A. et al., Comparative genomics of the lactic acid bacteria. *PNAS* 2006, 103, 15611–15616.
- [37] Olvera, C., Centeno-Leija, S., Lopez-Munguia, S., Structural and functional features of fructansucrases present in *Leuconostoc mesenteroides* ATCC 8293. *Antonie van Leeuwenhoek.* 2007, 92, 11–20.
- [38] Vasileva, T., Iliev, I., Amari, M., Bivolarski, V. et al., Characterization of glucosyltransferase activity of wild-type *Leuconostoc mesenteroides* strains from Bulgarian fermented vegetables. *Appl. Biochem. Biotechnol.* 2012, 168, 718–730.
- [39] Koepsell, H. J., Tsuchiya, H. M., Hellman, N. N., Kazenko, A. et al., Enzymatic synthesis of dextran acceptor specificity and chain initiation. *J. Biol. Chem.* 1952, 200, 793–801.
- [40] Robyt, J. F., Eklund, S. H., Relative quantitative effects of acceptors in the reaction of *Leuconostoc mesenteroides* B-512F dextransucrase. *Carbohydr. Res.* 1983, 121, 279–286.
- [41] Pham, H. T. T., Dijkhuizen, L., van Leeuwen, S., Structural characterization of glucosylated lactose derivatives synthesized by the *Lactobacillus reuteri* GtfA and Gtf180 glucansucrase enzymes. 2017. *Carbohydr. Res.* 449, 59–64.
- [42] Cote, L. G., Dunlap, C. A., Vermillion, K. E., Glucosylation of raffinose via alternansucrase acceptor reactions. *Carbohydr. Res.* 2009, 344, 1951–1959.
- [43] Remaud-Simeon, M., Lopez-Munguia, A., Pelenc, V., Monsan, P., Production and use of glucosyltransferases from *Leuconostoc mesenteroides* NRRL B-1299 for the synthesis of oligosaccharides containing α -(1,2) linkages. *Appl. Biochem. Biotechnol.* 1994, 44, 101–117.
- [44] Remaud-Simeon, M., Willemot, R-M., Sarcabal, P., de Montalk, G. P. et al., Glucansucrases: molecular engineering and oligosaccharide synthesis. *J. Mol. Catal. B Enzym.* 2000, 10, 117–128.
- [45] Shi, Q., Juvonen, M., Hou, Y., Kajala, I. et al., Lactose- and cellobiose-derived branched trisaccharides and a sucrose-containing trisaccharide produced by acceptor reactions of *Weissella confuse* dextransucrase. *Food Chem.* 2016, 190, 226–236.
- [46] Ananieva, M., Tzenova, M., Iliev, I., Ivanova, I., Gene expression of enzymes involved in utilization of xylooligosaccharides by *Lactobacillus* strains. *Biotechnol. Biotechnol. Eq.* 2014, 28, 941–948.
- [47] Motherway, M., Kinsella, M., Fitzgerald, G. F., van Sinderen, D., Transcriptional and functional characterization of genetic elements involved in galacto-oligosaccharide utilization by *Bifidobacterium breve* UCC2003. 2012. *Microb. Biotechnol.* 2013, 6, 67–79.
- [48] Grimoud, J., Durand, H., Courtin, C., Monsan, P. et al., In vitro screening of probiotic lactic acid bacteria and prebiotic glucooligosaccharides to select effective synbiotics. *Anaerobe*, 16, 493–500.
- [49] Cecchini, D. A., Laville, E., Laguerre, S., Robe, P. et al., Metagenomics reveals novel pathways of prebiotic breakdown by human gut bacteria. *PLoS ONE.* 2013, 8, e72766.