Characterization of structure/prebiotic potential correlation of glucans and oligosaccharides synthetized by glucansucrases from fructophilic lactic acid bacteria from honey bee *Apis mellifera*

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Keywords:	Apis mellifera, fructophilic LAB, glucansucrases, glucans, prebiotic GOSs
Abstract:	In this work, we reported production of glucansucrases with molecular weights of about 300 kDa by strains of fructophilic lactic acid bacteria (FLAB) Lactobacillus pentosus AG8, Lactobacillus kunkeei AG9, AG10 and AG11 isolated from the gut of honey bee Apis mellifera. From sucrose, these enzymes synthesize average to high-molecular-weight dextran-type glucans ranging from 8.5 × 105 to >4 × 106 Da and containing 16 to 22% α-(1→3)-linked glucose units, with 5 to 8% branching in their structures. Enzyme preparations from the strains were applied in the synthesis of glucooligosaccharides (GOSs) with degrees of polymerization (DP) from 3 to 7 using maltose/sucrose ratios (M/S) from 8 to 0.12. The yields and composition of the obtained oligosaccharide products were strongly influenced by the ratios of the acceptor and donor of glucose units in the reactions. The highest yields of GOSs (>50 g/L) were achieved at M/S=1, and the lowest content of α-(1→6) linkages (71%) combined with increased proportion of the products with

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	DP>3 were determined at M/S=0.5. GOSs preparations from these two ratios were able to maintain the growth and acids production of three probiotic strains of lactobacilli, suggesting potential application as prebiotics.

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ARTICLE

Characterization of structure/prebiotic potential correlation of glucans and oligosaccharides synthetized by glucansucrases from fructophilic lactic acid bacteria from honey bee *Apis mellifera*

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Abstract

In this work, we reported production of glucansucrases with molecular weights of about 300 kDa by strains of fructophilic lactic acid bacteria (FLAB) *Lactobacillus pentosus* AG8, *Lactobacillus kunkeei* AG9, AG10 and AG11 isolated from the gut of honey bee *Apis mellifera*. From sucrose, these enzymes synthesize average to high-molecular-weight dextran-type glucans ranging from 8.5×10^5 to $>4 \times 10^6$ Da and containing 16 to 22% α-(1→3)-linked glucose units, with 5 to 8% branching in their structures. Enzyme preparations from the strains were applied in the synthesis of glucooligosaccharides (GOSs) with degrees of polymerization (DP) from 3 to 7 using maltose/sucrose ratios (M/S) from 8 to 0.12. The yields and composition of the obtained oligosaccharide products were strongly influenced by the ratios of the acceptor and donor of glucose units in the reactions. The highest yields of GOSs (>50 g/L) were achieved at M/S=1, and the lowest content of α-(1→6) linkages (71%) combined with increased proportion of the products with DP>3 were determined at M/S=0.5. GOSs preparations from these two ratios were able to maintain the growth and acids production of three probiotic strains of lactobacilli, suggesting potential application as prebiotics.

Keywords: *Apis mellifera*, fructophilic LAB, glucansucrases, glucans, prebiotic GOSs

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Introduction

In the recent years, the microbial communities associated with economically important insects such as the western honey bee *Apis mellifera* are showing significant scientific interest. This could be attributed to the better understanding of the importance of the symbiotic relationships between the honey bees and their gastrointestinal microflora which play a substantial role in the well-being of these natural pollinators. On the other hand, there is a growing demand for isolation of new niche-specific microorganisms having beneficial properties that could be applied as probiotics, source of enzymes and other bio-active substances benefiting both humans and bees [1–5]. One important group of microorganisms associated with the gut and the ecological niche of honey bee *Apis mellifera* is this of lactic acid bacteria (LAB) [6,7]. The substrates rich in sugars and mainly in fructose, such as fruits and flowers, play a selective role for differentiation of a specific group of LAB called fructophilic lactic acid bacteria (FLAB) [8].

Fructophilic LAB have heterofermentative metabolism and on the basis of specific differences in their fermentative and 16S rDNA profiles, they were divided into obligate and facultative fructophilic lactic acid bacteria. To the first subgroup were included *Lactobacillus kunkeei* and several *Leuconostoc* spp. which were reclassified into the new genus *Fructobacillus*, namely *F. fructosus*, *F. durionis*, *F. ficulneus* and *F. pseudoficulneus* [9]. *Lb. florum* and *Lb. brevis* isolated from honey bees and flowers were classified into the facultative FLAB subgroup [10,11]. A characteristic property that distinguishes the facultative FLAB is their ability to grow anaerobically on glucose media in the absence of external electron acceptors, and to produce one order of magnitude more ethanol in comparison to obligate FLAB such as *Lb. kunkeei* [10,12].

Lactic acid bacteria belonging to genera *Lactobacillus*, *Leuconostoc*, *Weissella* and *Streptococcus* are well-known for their beneficial properties as probiotics and starter cultures in the production and preservation of foods. In addition, many species of these bacteria are able to produce exopolysaccharides (EPSs) which find application as prebiotics, immunomodulators, blood volume expanders, main compounds or additives to new materials [13–17]. An especially important group of LAB-derived EPSs are the glucose homopolysaccharides synthesized by a special family of α-retaining non-Leloir-type sucrases called glucansucrases which transfer glucose residues from sucrose to the growing chain of the synthesizing α-glucan polysaccharide [13,18]. Currently, there are more than 60 glucansucrase enzymes which were characterized biochemically and classified in the glycoside hydrolase family 70 (http://www.cazy.org) on the basis of their structure and mechanism of action [19,20]. Depending on the linkages between the residues in the synthesized glucans, there are four well-studied types of glucansucrases, namely: i) dextransucrases (EC 2.4.1.5), producing dextran containing >95% α-(1→6) linkages; ii) mutansucrases (EC 2.4.1.5), synthesizing mutan polysaccharide composed of >50% α-(1→3)-linked glucose units; iii) reuteransucrases (EC 2.4.1.5), synthesizing reuteran containing >50% α-(1→4) linkages in the main chain; iv) alternansucrases (EC 2.4.1.140), synthesizing a polysaccharide composed of alternating α-(1→6) and α-(1→3)-linked glucose units called alternan [13,18,20].

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3 In addition to their ability to synthesize high-molecular-weight glucans, glucansucrases from
4 LAB are able to transfer competitively the glucose moiety of sucrose to suitable low-molecular-
5 weight acceptors, such as maltose, isomaltose, lactose, raffinose, glucose and fructose. Thus they
6 generate a series of short-chain GOSs having a prebiotic potential, which depends on their
7 structure and composition, and is tightly related to the specificity of the enzymes used in the
8 synthesis reactions [21–23]. Presently, the low-molecular-weight GOSs having branched
9 structures and α-(1→2) or α-(1→3)-linked glucose residues are considered to have more
10 pronounced prebiotic effects due to their ability to induce a broader spectrum of glycoside
11 hydrolase enzymes in the probiotic bacteria inhabiting the host's gut [24]. Some of the well-
12 studied glucansucrases from LAB which are able to synthesize GOSs containing specific
13 glyosidic linkages and have a practical application in their production are dextransucrases DSR-S
14 and DSR-E from *L. mesenteroides* NRRL B-512F and *L. citreum* NRRL B-1299, respectively;
15 alternansucrase from *L. citreum* NRRL B-21297 and reuteransucrase GTF-A from *Lb. reuteri*
16 121 [25–28].
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22 In our previous study, we reported the isolation of two new strains of FLAB *Lb. kunkeei* which
23 produce high-molecular-weight glucansucrases that are able to synthesize branched glucans form
24 sucrose [29]. This and the other research mentioned above underline the importance of the honey
25 bee's gut microbiota both as means by which this economically important insect adapts to its
26 ecological niche, and as a new source of biologically active molecules such as the glucansucrase
27 enzymes. In addition, there is a growing demand for new molecular tools by which to achieve a
28 cost-effective production of polysaccharides, prebiotic GOSs and glycoconjugates with new
29 properties. This makes the habitats of the honey bees, which are rich in sugars, as well as their
30 gastrointestinal tract a tempting source of useful carbohydrate-acting biocatalysts.
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35 In the present study, we report the production of glucansucrases by four FLAB strains isolated
36 from the gut of honey bees *Apis mellifera*. In addition, partially purified enzyme preparations
37 from the strains were applied in the synthesis of glucan polysaccharides, whose molecular
38 weights and structures were determined. The ability of the enzymes to catalyze acceptor reactions
39 synthesizing GOSs and their prebiotic potential was also studied.
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43 **Materials and methods**
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45 ***Bacterial strains and cultural conditions***
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47 Fructophilic LAB strains *Lb. pentosus* AG8, *Lb. kunkeei* AG9, AG10 and AG 11 were obtained
48 from the culture collection of the Department of Biochemistry and Microbiology, Plovdiv
49 University (Plovdiv, Bulgaria). The strains were identified prior to this study on the basis of their
50 carbohydrate fermentation profiles (API CH50®) and 16S *rDNA* sequencing and comparison
51 [30]. The strains were maintained weekly on fructose yeast extract polypeptone (FYP10) broth,
52 containing: 100 g/L D-fructose; 10 g/L yeast extract; 5 g/L polypeptone; 2 g/L anhydrous sodium
53 acetate; 0.5 g/L Tween 80; 0.2 g/L $MgSO_4.7H_2O$; 0.01 g/L $MnSO_4.4H_2O$; 0.01 g/L $FeSO_4.7H_2O$;
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0.01 g/L NaCl, pH 6.8 at 30 ºC, and were also stored in 20% glycerol (*v/v*) at –20 ºC [8]. For production of glucansucrases, the studied FLAB strains and the reference strain of *L. mesenteroides* subsp. *mesenteroides* NCIMB 8023 (purchased from National Bank for Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria, and known also as ATCC 8293) were cultivated aerobically on a rotary shaker (200 rpm) in Dols medium containing: 40 g/L sucrose; 20 g/L yeast extract; 20 g/L K_2HPO_4; 0.2 g/L $MgSO_4.7H_2O$; 0.01 g/L $MnSO_4.H_2O$; 0.01 g/L NaCl; 0.01 g/L $CaCl_2$; 0.01 g/L $FeSO_4.7H_2O$; pH 6.9 at 30 ºC for 10 to 12 h [31]. Probiotic strains *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, and were routinely grown in de Man, Rogosa and Sharpe (MRS) medium (Biokar Diagnostics, France) at 37 ºC.

Quantitative determination of metabolites

The amounts of lactic acid, acetic acid and ethanol produced during the anaerobic cultivation of probiotic strains *Lb. plantarum* S26, *Lb. brevis* S27 and *Lb. sakei* S16 in media supplemented with GOSs/glucose were determined enzymatically with the following commercially available kits (Megazyme, International Ireland Ltd., Wicklow, Ireland): i) K-DLATE – for determination of lactate; ii) K-ACET – for determination of acetate; iii) K-ETOH – for determination of ethanol. All the analyses for determination of metabolites were performed in duplicate from different experiments with standard deviations (±SD).

Partial purification and concentration of glucansucrases

The bacterial cells were separated from culture media by centrifugation (10000 *g*, 4 ºC, 20 min). The cell pellet was washed twice in 20 mmol/L acetate buffer (pH 5.4) and re-suspended in the initial volume of buffer for further measurement of the cell-wall-bound glucansucrase activity and *in situ* analysis. To ensure the total absence of cells, the cultural liquids were filtered through 0.2 µm membranes. Extracellular glucansucrases were then concentrated and partially purified by addition of polyethylene glycol with molecular mass 1500 (PEG 1500) to a final concentration of 200 g/L. This approach achieved a two-phase partitioning between the native polysaccharides and the PEG-rich phase containing glucansucrases [32]. The enzyme fraction was then pelleted by centrifugation (8500 *g*, 4 ºC, 20 min). The obtained enzyme fraction from each isolate was used in the *in situ* analysis, polysaccharide and GOSs synthesis.

Assay of glucansucrase activity

One unit of glucansucrase activity is defined as the amount of enzyme catalyzing the release of 1.0 µmol fructose per minute at 30 ºC in 20 mmol/L acetate buffer, pH 5.4; 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 using 3,5-dinitrosalicylic acid method [31,33]. Additionally, released fructose was determined enzymatically (Cat. K-FRUGL, Megazyme, International Ireland Ltd., Wicklow, Ireland). Protein concentration was measured by the method of Bradford [34] against

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bovine serum album standard curve. All the analyses for determination of residual sugars and protein concentrations were performed in duplicate from different experiments with standard deviations (±SD).

Electrophoresis analysis (SDS-PAGE)

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis using polyacrylamide 70 × 80 slab gels (3% concentrating; 5% separating gel) was performed according to the method of Laemmli [35]. *In situ* activity detection of glucansucrases was performed by incubation of the gels in 100 g/L sucrose, 20 mmol/L acetate buffer, 0.05 g/L $CaCl_2$ overnight, followed by staining the synthesized polysaccharides according to a periodic acid-Schiff's protocol [36]. The protein standards (High Molecular Weight Calibration Kit for SDS Electrophoresis, Amersham, UK) were stained with 1.0 g/L Coomassie Brilliant Blue R 250 in 5% (*v/v*) acetic acid.

Synthesis and analysis of glucan polysaccharides

Glucansucrase preparation from a FLAB strain was added to 100 g/L sucrose in 20 mmol/L acetate buffer (pH 5.4) containing 0.05 g/L $CaCl_2$ to a final enzyme activity of 0.5 U/mL. To each reaction mixture was added 0.01% (*w/v*) sodium azide to prevent microbial growth. Reactions were incubated at 30 ºC with shacking (100 rpm) for 48 h. The depletion of sucrose was monitored by high performance liquid chromatography (HPLC) as described previously [37]. The reactions were stopped by addition of equal volume of 96% ethanol to each mixture which led to denaturation of the enzyme and precipitation of the synthesized glucan. The polysaccharides were pelleted (9000 *g*, 4 ºC, 20 min), washed two more times with ethanol, dissolved in ultra-pure water and freeze-dried (FreeZone® Plus™ 4.5 Liter Cascade, Labconco Corporation, Kansas City, USA).

The molecular mass of the purified glucans was determined by size-exclusion chromatography (HPSEC) using column Shodex OHpak SB-805, 8 × 300 mm, equipped with OH SB-G 6B guard column. The mobile phase was ultra-pure water containing 0.45 mmol/L $NaNO_3$; 0.6 mL/min flow rate; 30 ºC; 20 µL injection volume. The detection was performed with a refractive index detector RID-560 (KONIK, Barcelona, Spain). The molecular mass of the glucans was calculated against a standard curve built with dextran standards of 12, 50, 70, 150, 500, 670 and 1360 kDa (Sigma). All the analyses for molecular mass determination of the glucans were performed in triplicate and average values are given.

The linkages in the purified polysaccharides and their distribution were determined by Nuclear magnetic resonance (NMR) spectroscopy on Avance II (Bruker, Billerirrica, Massachusetts, USA) as described before [38]. Samples (10 mg) were dissolved in 0.5 mL D_2O and specters were recorded at 500 MHz and 125 MHz for ^{1}H and ^{13}C NMR analyses, respectively using 5 mm *z*-gradient TBI (triple resonance broadband inverse) probe. ^{1}H and ^{13}C chemical shifts (δ) were expressed in ppm by reference to internal acetone (δ=2.225 ppm) and the methyl-carbon of

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internal acetone (δ=31.08 ppm), respectively. The temperature of the analysis was 298 K. The data acquisition and processing was performed using TopSpin 3 software. The percentages of the linkages in glucans were calculated on the basis of the relative intensities of the anomeric protons or carbons.

Synthesis and analysis of GOSs

The reactions for synthesis of GOSs were performed at 30 ºC in 20 mmol/L acetate buffer (pH 5.4) supplemented with 0.05 g/L $CaCl_2$ and 0.01% (*w/v*) sodium azide. Sucrose served as a donor of glucose units and maltose monohydrate (Merck, Bulgaria EAD) as a sugar acceptor. The following ratios of maltose/sucrose were used: 1, 2, 4, 6, 8, 0.12, 0.16, 0.25 and 0.5. The final concentration of sugar substrates was 100 g/L, and each reaction was started by addition of glucansucrase preparation to 1.0 U per milliliter of reaction mixture. The course of the reactions was monitored by HPLC, and they were stopped when all sucrose was depleted (~48 h). The further steps of processing and analysis of the synthesized GOSs were described previously [37]. Briefly, polysaccharide fractions were precipitated with 96% ethanol, and GOSs fractions were subjected to vacuum evaporation, and then evaluated by HPLC analysis using YMC-Pack™ Polyamine II column (YMC™, Co., Ltd., Japan), 60:40% acetonitrile:water mobile phase at 30 ºC. The remaining monosaccharides and maltose were removed by gel-filtration (Bio-Gel P2, fine mesh, Bio-Rad Laboratories, Inc., USA) through 5 × 150 cm column [39]. GOSs preparations were then subjected to freeze-drying and used in the bacterial utilization and hydrolysis experiments. Synthesis reactions, HPLC analyses and the purification were carried out in duplicate and the results from different experiments are presented with a standard deviation (±SD).

Enzymatic hydrolysis of GOSs

Hydrolysis reactions of purified GOSs were performed as described before [37]. Briefly, 1% (*w/v*) GOSs solution in 20 mmol/L acetate buffer (pH 5.4) was incubated with 1.0 U/mL endodextranase (EC 3.2.1.11) (Sigma – Aldrich, Chemie, Germany) for 24 h at 30 ºC. As a control was used 1% (*w/v*) isomaltose. One unit endodextranase activity is defined as the amount of enzyme catalyzing the hydrolysis of 1.0 μmol isomaltose per minute at the above-mentioned reaction conditions [39]. The reaction products were analyzed by HPLC. The hydrolysis of GOSs was carried out in triplicate and HPLC analyses were performed in duplicate, and the results from different experiments with standard deviations (±SD) are presented.

Utilization of GOSs by probiotic lactobacilli

The overall experimental settings were described in full before [37]. Briefly, washed *Lb. plantarum* S26, *Lb. brevis* S27 and *Lb. sakei* S16 cells were inoculated into modified MRS medium containing 10 g/L glucose or filter-sterilized GOSs. The fermentations were carried out in BBL® Gas Pack anaerobic system (Becton Dickinson, Sparks, USA) at 37 ºC for 48 h, without pH control. GOSs utilization was estimated by measurement of bacterial growth, calculation of

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the specific growth rate (μ), changes in pH and production of lactate, acetate and ethanol by the strains. Each GOSs utilization experiment was performed in triplicate, and the results from different experiments with standard deviations (±SD) are presented.

Statistical analysis

Programmable scientific calculator “CASIO” fx-991ES Plus, statistical software package SigmaPlot v12.0 (Systat Software, Inc., Chicago, USA) and Microsoft Excel (version 2010, SP2 …) were used for data analysis and graphical representation. Data from the performed experiments were subjected to one-way analysis of variance (ANOVA) and the statistical significance was set at a level of confidence $p<0.05$.

Results

Detection of glucansucrases by in situ SDS-PAGE

In order to detect and determine the molecular weights and distribution of glycansucrases produced by FLAB strains AG8, AG9, AG10 and AG11 on sucrose media, *in situ* SDS–PAGE of extracellular and cell-associated fractions was performed. As controls were used enzyme preparations from a reference strain *L. mesenteroides* ATCC 8293, which is a well-known producer of several types of glycansucrase enzymes (Figure 1) [40].

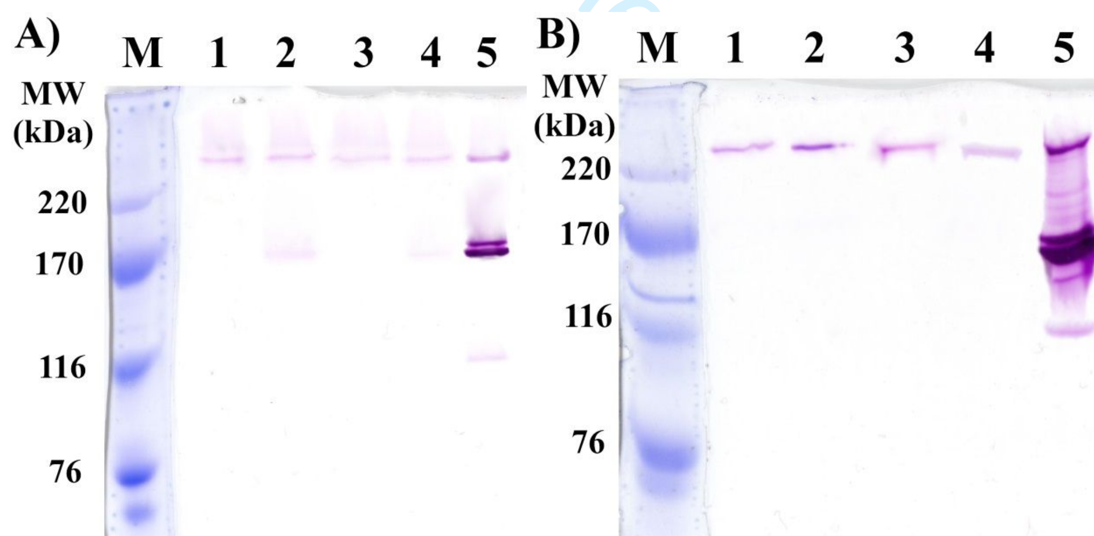

Figure 1. *In situ* SDS-PAGE analysis of extracellular and cell-associated glycansucrases from FLAB strains AG8, AG9, AG10 and AG11. M, protein standard (Amersham High Molecular Weight Calibration Kit for SDS Electrophoresis, GE Healthcare. Cat. 17-0615-01); (**A**) Lane 1, extracellular fraction of AG8; Lane 2, extracellular fraction of AG9; Lane 3, extracellular fraction of AG10; Lane 4, extracellular fraction of AG11; Lane 5, extracellular fraction of *L. mesenteroides* ATCC 8293. (**B**) Lane 1, cell-associated fraction of AG8; Lane 2, cell-associated fraction of AG9; Lane 3, cell-associated fraction of AG10; Lane 4, cell-associated fraction of AG11; Lane 5, cell-associated fraction of *L. mesenteroides* ATCC 8293.

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The *in situ* analysis of enzyme preparations from strains AG8, AG9, AG10 and AG11 showed that all of them produce extracellular and cell-associated glucansucrases with molecular weights of about 300 kDa (Figure 1). In addition, activity bands of about 180 kDa were detected only in the extracellular fractions of strain AG9 and AG11 (Figure 1A, lanes 2 and 4). The referent strain *L. mesenteroides* ATCC 8293 also showed such bands in both extracellular and cell-associated enzyme fractions, and they correspond to dextransucrase. This strain is also known as a producer of fructansucrase with a molecular weight of about 120 kDa, but such activity was not detected in the extracellular and cell-associated enzyme profiles of the studied fructophilic strains (Figure 1).

Analysis of glucans synthesized by FLAB

The size-exclusion chromatography analysis of the glucans revealed that strains *Lb. pentosus* AG8 and *Lb. kunkeei* AG10 produce polymers with molecular weights of about 9.4×10^5 Da and 1.1×10^6 Da, respectively. For the polysaccharide preparations produced by glucansucrases from strains *Lb. kunkeei* AG9 and AG11, fractions with molecular weights of about 8.5×10^5 and 9.8×10^5, respectively, were detected. Additionally, these two strains produced glucan fractions that exceeded the exclusion limit of the column used, so they have molecular weights greater than 4×10^6. These high-molecular polysaccharide fractions, which represent about 8% of the area of the signals, correlate well with the detection of additional 180 kDa activity bands in the extracellular enzyme preparations from strains *Lb. kunkeei* AG9 and AG11.

The analyses of the ^{1}H part of NMR spectra showed chemical shifts at 5.31 ppm and 4.94 ppm corresponding to D-Glc*p* residues bound with α-(1→3) and α-(1→6) linkages, respectively. Anomeric protons with these chemical shifts were detected in the glucan samples from the four fructophilic strains (Figure 2). These results were further confirmed by the obtained ^{13}C spectra of the analyzed glucans (Figure 3). The signal at 101.18 ppm was assigned to the anomeric carbon involved in the formation of α-(1→3) linkages, and the chemical shift at 98.98 ppm was assigned to the anomeric carbon involved in the formation of α-(1→6) linkages between D-Glc*p* residues. In addition, the presence of a signal at 84.47 ppm, corresponding to C3 involved directly in the formation of α-(1→3) linkages, further confirms the explanation of the results (Figure 3). The presence of ^{1}H NMR signal at 4.98 ppm and of ^{13}C NMR signal at 98.73 ppm indicated the presence of 3,6-di-O-substituted α-D-Glc*p* residues and branched structure of the analyzed glucans, ranging from 5 to 8%. The obtained spectra of the studied glucans correlate well with the ones reported previously by Bounaix et al. [41]. On the basis of the obtained NMR spectra, the highest content of α-(1→3) linkages was calculated in the glucan of *Lb. pentosus* AG8 (22%), followed by these of *Lb. kunkeei* AG10 (20%), *Lb. kunkeei* AG9 (18%) and *Lb. kunkeei* AG11 (16%), respectively.

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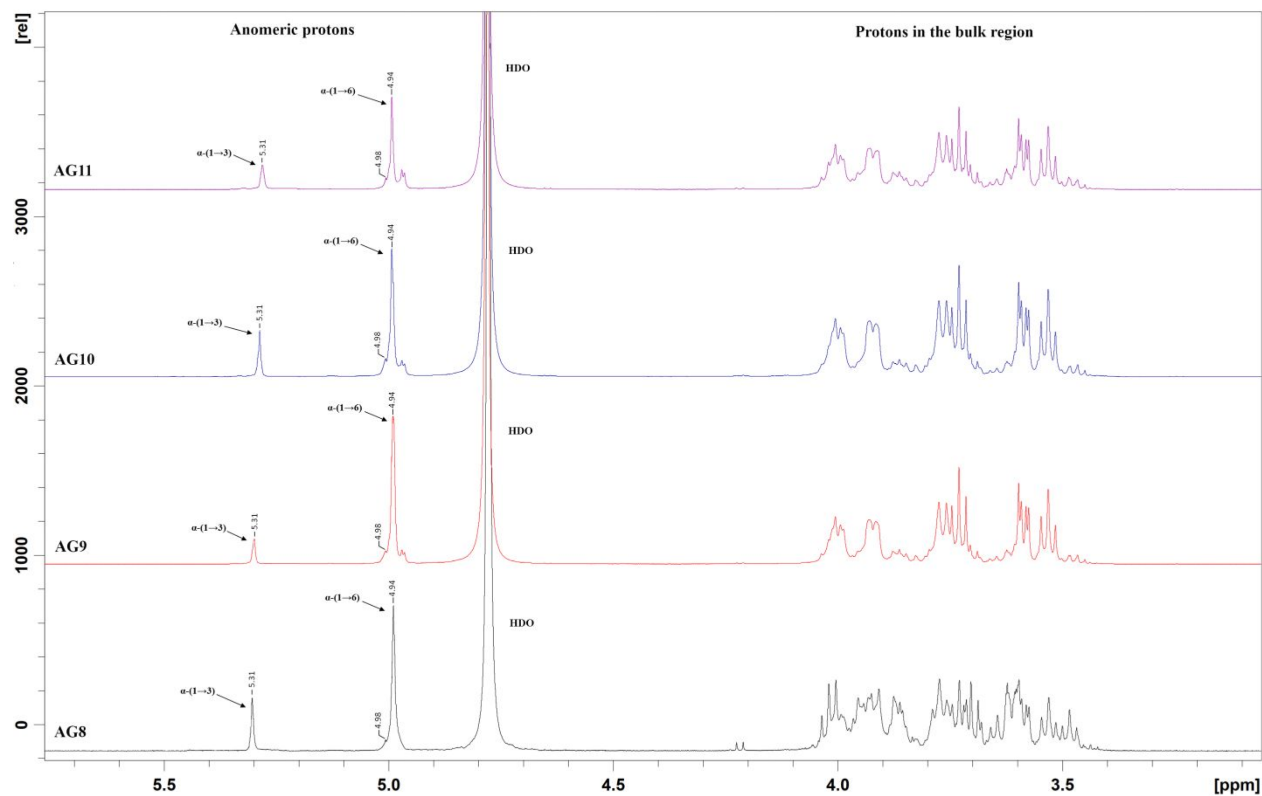

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Figure 2. ^{1}H NMR spectra of glucans produced by glucansucrases from FLAB *Lb. pentosus* AG8, *Lb. kunkeei* AG9, *Lb. kunkeei* AG10 and *Lb. kunkeei* AG11. The peaks were recorded at 298 K in D_2O, and they were referenced to an internal standard acetone (^{1}H=2.225 ppm).

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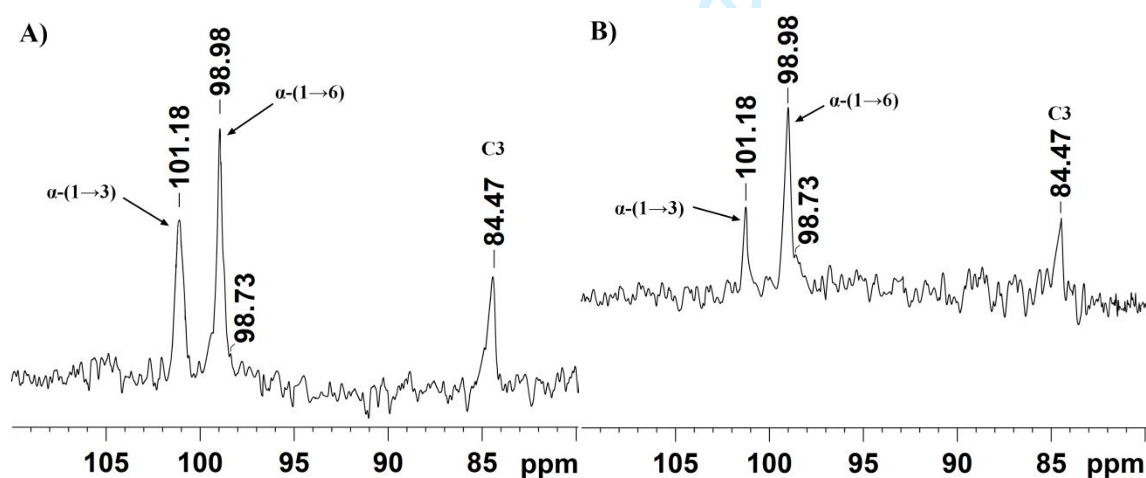

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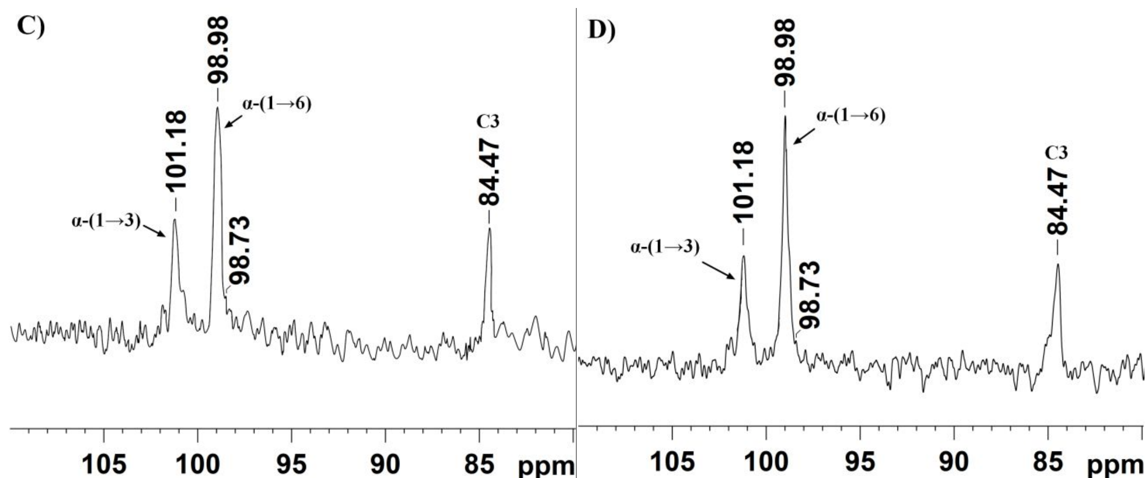
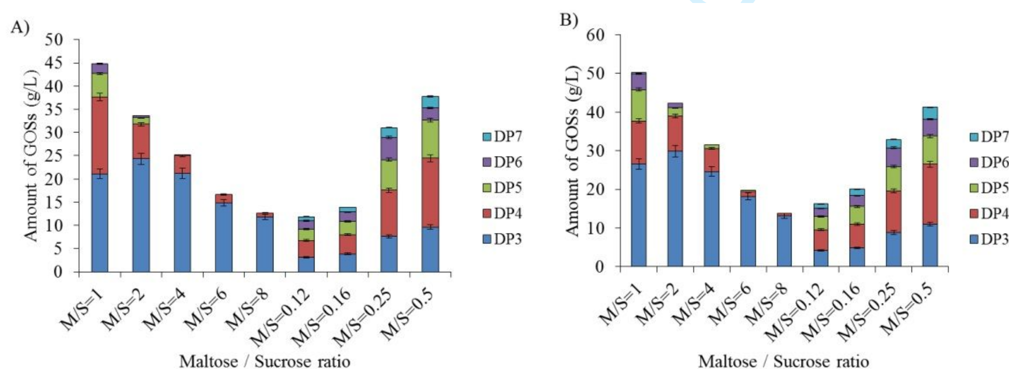


Figure 3. ^{13}C NMR spectra of glucans produced by glucansucrases from FLAB *Lb. pentosus* AG8 (**A**), *Lb. kunkeei* AG9 (**B**), *Lb. kunkeei* AG10 (**C**) and *Lb. kunkeei* AG11 (**D**). The spectra were recorded at 125 MHz, 298 K in D_2O. The peaks were referenced to an internal standard acetone (31.03 ppm).

Synthesis of GOSs by glucansucrases from FLAB

In order to evaluate the ability of glucansucrases from the studied strains to produce GOSs, we performed a series of synthesis reactions using various maltose/sucrose ratios. A summary of the obtained yields, their distribution by degree of polymerization (DP) and the overall profiles that differentiate the expressed glucansucrase inventory of strains *Lb. pentosus* AG8 and *Lb. kunkeei* AG9, AG10 and AG11 is presented in Figure 4.



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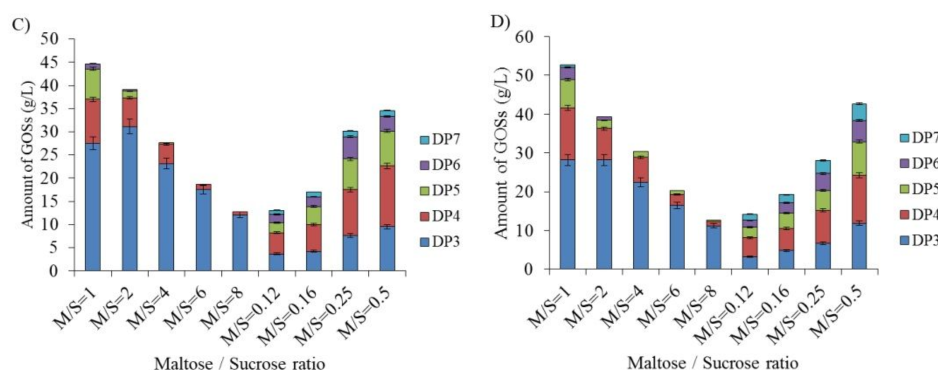

Figure 4. Synthesis of GOSs by extracellular glucansucrases from strains AG8 (**A**), AG9 (**B**), AG10 (**C**) and AG11 (**D**) in the presence of different maltose/sucrose ratios.

The synthesis reactions generated a series of GOSs with DP ranging from 3 to 7 and distribution of the yields that is strongly affected by the maltose/sucrose (M/S) ratios. In all the cases, the highest yields of GOSs were obtained at M/S=1, and the products with DP3 were the predominant oligosaccharide fraction – 47.15, 52.9, 61.8 and 53.43% of the total amounts of the synthesized GOSs by the enzymes from strains AG8, AG9, AG10 and AG11, respectively (Figure 4). The increase in the M/S ratios resulted in a progressive decrease in the proportion of GOSs having DP>3 and also their total quantities, which at M/S=8 were between 70 and 75% lower compared to those synthesized at M/S=1. On the other hand, at lower M/S ratios where sucrose is the prevalent sugar at the start of the reactions, we recorded a more even distribution of the fractions according to their DP. Under these conditions, DP3 products served as efficient acceptors of glucose units from sucrose leading to a decrease in their proportion at the expense of the accumulation of DP4 GOSs, which are the largest fraction at M/S ratios from 0.12 to 0.5 (Figure 4), and representing between 29% and 39% of the total amount of the synthesized oligosaccharides. In contrast to the reactions with increasing M/S ratios, where maltose competes more efficiently for the glucose residues in the less available sucrose, which leads to gradual elimination of GOSs with DP>3, all the oligosaccharides synthesized at M/S ratios <1 contained products with DP from 3 to 7. Their amounts and the overall production of GOSs increased towards M/S=0.5 where the highest production of GOSs was achieved with DP>3 – 15.77, 21.84, 31.67 and 20.41%, respectively for the reactions driven by glucansucrases from strains AG8, AG9, AG10 and AG11, compared to the yields at M/S=1.

An interesting feature that distinguished the oligosaccharide profiles of strains AG9 and AG11 is the production of DP7 GOSs at M/S=1: respectively, 0.5 and 1.14% of the total amount of oligosaccharides produced by the strains. In addition, DP5 products at M/S=6 were detected only in the profiles of these two strains: 1.54 and 4.68% of the total amount of GOSs, respectively. These differences, compared to the oligosaccharide profiles of strains AG8 and AG10 correlate well with the detection of two extracellular glucansucrases produced by strains AG9 and AG11 (Figure 1).

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Enzymatic hydrolysis of GOSs synthesized by glucansucrases from FLAB

The content of α-(1→6) linkages in the GOSs synthesized by glucansucrases from strains AG8, AG9, AG10 and AG11 was evaluated by subjecting the obtained preparations to endodextranase hydrolysis and determination of the amounts of the released glucose (Figure 5).

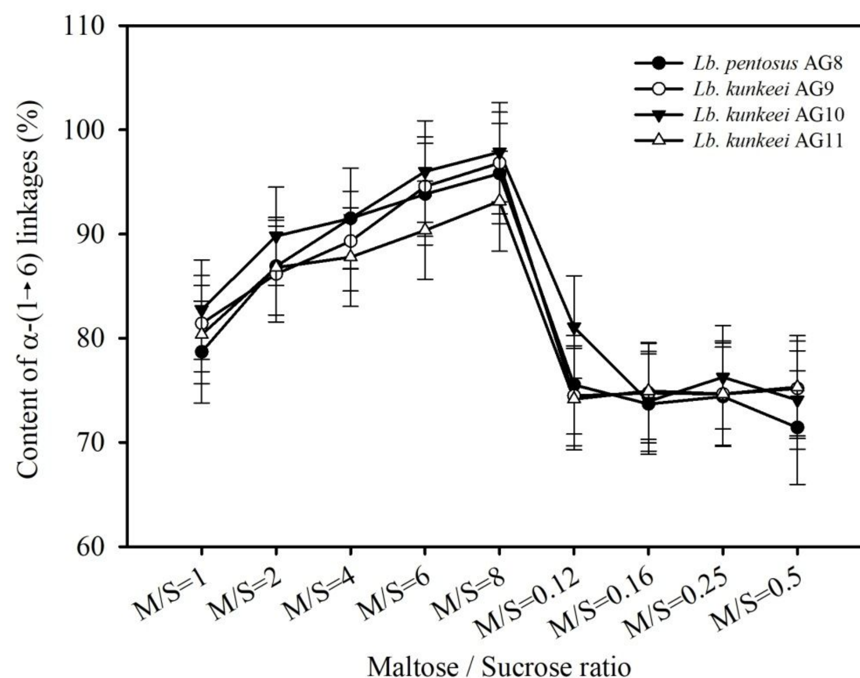

Figure 5. Distribution profiles of α-(1→6) linkages in GOSs synthesized by glucansucrases from strains *Lb. pentosus* AG8, *Lb. kunkeei* AG9, AG10 and AG11.

The highest content of α-(1→6)-linked glucose units in the synthesized acceptor products was determined at M/S=8 and ranges from 93.16% (AG11) to 96.86% (AG10). This correlates well both with the decrease of sucrose concentration from M/S=1 to MS=8 and the fractions of GOSs having DP>3. In addition, this suggests that at increasing M/S ratios where maltose is the predominant acceptor sugar in the reaction mixtures, glucose residues are transferred to it forming mostly α-(1→6) linkages, instead to GOSs having DP>3 which in turn are less efficient and less competitive acceptors of the of glucose units available in the scarce amounts of sucrose. At M/S=0.12 where the amount of maltose was the lowest tested, there was a significant decrease in the content of α-(1→6) linkages in the synthesized GOSs (Figure 5). For the enzyme preparations tested at this M/S ratio, the content of α-(1→6) linkages ranged from 74.15% to (AG11) to 81.60% (AG10). The further increase in the M/S ratios to 0.5 while keeping the concentration of sucrose prevalent, led only to an increase in the overall yield of GOSs with no significant change in the content of α-(1→6) linkages in the products (Figures 5 and 4). At M/S = 0.5, the lowest content of α-(1→6) linkages was detected in the GOSs preparations of isolate AG8 – 71.42 % (Figure 5). All in all, at decreasing M/S ratios where the maltose was less abundant, more of the available glucose units in the sucrose were transferred to acceptor products

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having DP>3. This led to a decrease in the amount of α-(1→6)-transferred glucose units, suggesting formation of different linkages in the acceptor GOSs products. This trend correlated well with the observation that, at M/S ratios from 0.12 to 0.5, the amount of GOSs with DP3 was lower than that of DP4 products, suggesting a competitive transfer of glucose units to DP3 acceptors, which diminishes their amount in favor of the accumulation of DP4 GOSs (Figure 4).

As seen, the profiles of the content of α-(1→6) linkages in GOSs synthesized by enzyme preparations from the four FLAB strains were very similar. This suggested more general effects of M/S ratios on the linkages specificity of the studied glucansucrases (Figure 5).

Utilization and prebiotic potential of GOSs synthesized by glucansucrases from FLAB

Strains of *Lb. plantarum* S26, *Lb. brevis* S27 and *Lb. sakei* S16, which are known to exhibit probiotic properties [42,43], were grown anaerobically in media supplemented with GOSs preparations synthesized at M/S=1 and 0.5 (Table 1). All the strains demonstrated nearly identical specific growth rates (μ) during their cultivation in media with GOSs to the ones calculated in glucose media. The observed growth of the probiotic lactobacilli in the GOSs supplemented media showed strain-dependent features. The specific growth rates of *Lb. sakei* S16 were on average 39% lower than the ones of the other two probiotic strains independently of the carbohydrates supplemented in the media (Table 1).

Table 1. Specific growth rates of *Lb. plantarum* S26, *Lb. brevis* S27 and *Lb. sakei* S16 cultivated in media supplemented with maltose derived GOSs.

GOSs from FLAB isolate [a]	M/S ratio	μ (h^{-1}) *Lb. plantarum* S26	μ (h^{-1}) *Lb. brevis* S27	μ (h^{-1}) *Lb. sakei* S16
AG8	1	0.43 ± 0.01	0.46 ± 0.01	0.28 ± 0.02
	0.5	0.45 ± 0.02	0.45 ± 0.02	0.27 ± 0.03
	Glucose (control)	0.44 ± 0.02	0.45 ± 0.03	0.29 ± 0.02
AG9	1	0.50 ± 0.03	0.52 ± 0.02	0.30 ± 0.02
	0.5	0.46 ± 0.01	0.50 ± 0.02	0.29 ± 0.01
	Glucose (control)	0.48 ± 0.02	0.49 ± 0.01	0.28 ± 0.02
AG10	1	0.44 ± 0.02	0.48 ± 0.02	0.26 ± 0.01
	0.5	0.45 ± 0.01	0.49 ± 0.02	0.27 ± 0.03
	Glucose (control)	0.46 ± 0.02	0.49 ± 0.01	0.28 ± 0.02
AG11	1	0.49 ± 0.03	0.47 ± 0.01	0.28 ± 0.01
	0.5	0.47 ± 0.02	0.50 ± 0.04	0.26 ± 0.02
	Glucose (control)	0.43 ± 0.01	0.53 ±0.02	0.28 ± 0.01

[a] – 1% (*w/v*) of glucose or GOSs. The calculated specific growth rates are means (±SD) from duplicate measurements in independent experiments.

The calculated values of the specific growth rates during cultivation in media with GOSs or glucose correlated well with the heterofermentative profiles of the strains associated with

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3 acidification of the media due to the production of lactic acid, acetic acid and very little amounts of ethanol (Table 2). At the end of the cultivation of the strains (48 h), strain specific rations of the amounts of lactic acid to acetic acid produced were observed. For *Lb. plantarum* S26, the ratio is 4.1, and for the other two strains they are 1.4 and 0.8, respectively. This decrease of the amounts of the produced lactic acid at the expense of acetic acid was associated with the specific metabolic properties of the strains and was not directly related to the composition of the oligosaccharide preparations tested in the current study.

Table 2. Values of pH, lactate, acetate and ethanol after cultivation of *Lb. plantarum* S26, *Lb. brevis* S27 and *Lb. sakei* S16 in media containing GOSs.

Strain	Carbohydrate [a]	pH [b]	Lactate (g/L) [b]	Acetate (g/L) [b]	Ethanol (g/L) [b]
***Lb. plantarum* S26**	AG8, GOSs, M/S = 1	4.05 ± 0.12	6.01 ± 0.19	1.41 ± 0.03	0.11 ± 0.03
	AG8, GOSs, M/S = 0.5	4.03 ± 0.16	6.09 ± 0.14	1.48 ± 0.07	0.08 ± 0.01
	AG9, GOSs, M/S = 1	4.12 ± 0.16	5.90 ± 0.18	1.51 ± 0.06	0.10 ± 0.02
	AG9, GOSs, M/S = 0.5	4.10 ± 0.08	6.14 ± 0.19	1.56 ± 0.07	0.13 ± 0.03
	AG10, GOSs, M/S = 1	4.04 ± 0.12	6.18 ± 0.14	1.46 ± 0.05	0.09 ± 0.02
	AG10, GOSs, M/S = 0.5	4.15 ± 0.12	6.20 ± 0.18	1.49 ± 0.04	0.12 ± 0.03
	AG11, GOSs, M/S = 1	4.09 ± 0.16	6.14 ± 0.27	1.44 ± 0.04	0.11 ± 0.02
	AG11, GOSs, M/S = 0.5	4.11 ± 0.13	6.07 ± 0.12	1.46 ± 0.07	0.07 ± 0.01
	Glucose (control)	4.03 ± 0.10	5.95 ± 0.21	1.48 ± 0.05	0.08 ± 0.03
***Lb. brevis* S27**	AG8, GOSs, M/S = 1	4.18 ± 0.13	4.32 ± 0.13	2.88 ± 0.11	0.13 ± 0.02
	AG8, GOSs, M/S = 0.5	4.14 ± 0.17	4.41 ± 0.13	2.93 ± 0.09	0.10 ± 0.01
	AG9, GOSs, M/S = 1	4.22 ± 0.10	4.13 ± 0.14	2.95 ± 0.06	0.14 ± 0.02
	AG9, GOSs, M/S = 0.5	4.05 ± 0.14	4.18 ± 0.12	2.90 ± 0.06	0.12 ± 0.01
	AG10, GOSs, M/S = 1	4.19 ± 0.18	4.21 ± 0.16	2.86 ± 0.08	0.08 ± 0.01
	AG10, GOSs, M/S = 0.5	4.18 ± 0.15	4.20 ± 0.12	2.91 ± 0.09	0.11 ± 0.02
	AG11, GOSs, M/S = 1	4.22 ± 0.14	4.16 ± 0.09	2.81 ± 0.10	0.10 ±0.01
	AG11, GOSs, M/S = 0.5	4.05 ± 0.14	4.18 ± 0.12	2.83 ± 0.06	0.12 ± 0.02
	Glucose (control)	3.88 ± 0.10	4.24 ± 0.13	2.88 ± 0.10	0.07 ± 0.01
***Lb. sakei* S16**	AG8, GOSs, M/S = 1	4.46 ± 0.14	2.91 ± 0.09	3.38 ± 0.07	0.08 ± 0.01
	AG8, GOSs, M/S = 0.5	4.51 ± 0.18	3.00 ± 0.09	3.35 ± 0.09	0.05 ± 0.01
	AG9, GOSs,	4.58 ± 0.09	2.88 ± 0.12	3.41 ± 0.09	0.06 ± 0.01

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M/S = 1				
AG9, GOSs, M/S = 0.5	4.44 ± 0.09	2.93 ± 0.12	3.51 ± 0.17	0.07 ± 0.01
AG10, GOSs, M/S = 1	4.48 ± 0.13	3.02 ± 0.09	3.44 ± 0.15	0.12 ± 0.02
AG10, GOSs, M/S = 0.5	4.55 ± 0.14	3.05 ± 0.13	3.42 ± 0.09	0.11 ± 0.01
AG11, GOSs, M/S = 1	4.31 ± 0.12	3.11 ± 0.11	3.51 ± 0.13	0.09 ± 0.01
AG11, GOSs, M/S = 0.5	4.61 ± 0.10	3.09 ± 0.12	3.46 ± 0.14	0.10 ± 0.02
Glucose (control)	4.73 ± 0.13	3.02 ± 0.10	3.48 ± 0.12	0.13 ± 0.02

[a] – 1% (*w/v*) glucose or GOSs from FLAB strains AG8, AG9, AG10 or AG11.

[b] – pH, lactate, acetate and ethanol measured at 48 h of the fermentation. The measured values are means (±SD) from duplicate measurements in independent experiments.

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Discussion

Previously, we have reported isolation of two *Lb. kunkeei* strains from the gastrointestinal tract of honey bee *A. mellifera* that produce extracellular glucansucrases synthesizing branched glucans [29]. Here, we report isolation of four more FLAB strains producing glucans in sucrose media. According to the description made by Endo et al. [12], *Lb. kunkeei* does not produce dextran from sucrose suggesting the lack of dextransucrase activity. In a previous study, we isolated two *Lb. kunkeei* strains producing glucansucrases with molecular weights of about 300 kDa and synthesizing branched dextrans [29]. Vasquez et al. [44] described the formation of EPS-containing biofilms in the honey stomach of honey bees that maintain the presence of FLAB and *Lb. kunkeei* as a predominant species in this organ. The analysis of the genome of *Lb. kunkeei* MP2 also revealed the presence of a gene encoding glucosyltransferase GtfC which is expressed in the presence of carbohydrates such as sucrose and glucose, and is responsible for the synthesis of a mixture of soluble and insoluble glucans playing a role in the biofilm formation [45]. A genome-wide screening of *Lb. kunkeei* EFB6 identified glucansucrase BRS-D which catalyzes α-(1→2)-branching of external dextran using sucrose. Such branching sucrases are also identified in *L. citreum* NRRL B-1299 (BRS-A), *L. citreum* NRRL B-742 (BRS-B) [46] and *Lb. fallax* KCTC3537 (BRS-C) [5]. Here, we reported the isolation of four glucan-synthesizing FLAB producing glucansucrases with molecular weights of about 300 kDa. Currently, production of enzymes with such a high molecular mass is reported only for members of genus *Leuconostoc*. The most well studied among them is a 312 kDa dextransucrase DSR-E produced by *L. citreum* NRRL B-1299 which was cloned and characterized. This enzyme contains two catalytic domains: one responsible for formation of α-(1→6) linkages, and a second one that introduces α-(1→2) branches in synthesized glucans [26,47]. Production of enzymes of a similar size has been reported also for *L. mesenteroides* ATCC 8293 [40] and *L. mesenteroides* URE 13 [38]. The gene for glucansucrase URE13-300 from the second strain was cloned and employed in the synthesis of branched GOSs [37].

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Extracellular glucansucrases from *Lb. pentosus* AG8, *Lb. kunkeei* AG9, AG10 and AG11 synthesize branched medium to high-molecular-weight dextrans containing 16–22% α-(1→3) linkages and 5–8% 3,6-branches. Depending on their source and specificity, glucansucrases from LAB produce glucans with different molecular weights and content of α-(1→6) linkages. Dextrans containing >50% α-(1→6) linkages usually display molecular mass in the range 10^6–10^8 Da [14]. Dextransucrase DSR-S from *L. mesentroides* NRRL B-512F, which is used for production of commercial dextrans, synthesizes up to 10^6 Da dextran containing 95% α-(1→6) linkages and about 5% α-(1→3) linkages. Dextransucrase Gtf180 from *Lb. reuteri* 180 synthesizes branched α-glucan with molecular weight of 3×10^7 Da, and about 30% α-(1→3) linkages [20]. Alternansucrase ASR from *L. citreum* NRRL B-1355 synthesizes two populations of glucans containing about 40% α-(1→3) linkages: i) around 10^7 Da, and ii) medium size polymers of 10^3–10^4 Da [48]. Dextrans with low and average molecular weights have found significant application as volume expanders, infusion fluids, anticoagulants, iron carriers and vaccine adjuvants, and because of that the discovery of new glucansucrases able to produce directly such glucan fractions are of interest [14]. In this regard, two dextransucrases from the genome of *L. citreum* NRRL B-1299 were cloned: 145 kDa DSR-M and 229 kDa DSR-DP synthesizing dextrans with molecular weights ranging from 580 to 27×10^3 Da. Most strikingly, DSR-DP was reported as the first dextransucrase introduced in the genome of LAB by phage-mediated horizontal gene transfer [49].

An additional catalytic feature of the non-Leloir glucansucrases from LAB is the so-called acceptor reaction leading to the synthesis of low-molecular-weight GOSs having prebiotic properties [49,50]. In the present work, we reported for the first time employment of glucansucrases from FLAB isolated from honey bee *A. mellifera* in acceptor reactions for synthesis of GOSs. The highest yields of GOSs were obtained at M/S=1, and the observed variations in the composition and quantity of the oligosaccharide preparations could be explained with the specific features of the acceptor reactions, which are function of the used catalyst, concentration of the acceptor molecule and its strength. Maltose was reported as one of the most potent sugar acceptors that is able to redirect significant part of the glucose units in sucrose to synthesis of low-molecular-weight GOSs, and thus suppressing the production of glucan polysaccharides [51–53]. Robyt and Eklund [52] reported synthesis of a homologous series of GOSs with DP up to 7 using dextransucrase DSR-S from *L. mesentroides* NRRL B-512F and M/S=1. They observed a gradual decrease in the amount of GOSs correlating with the increase in their DP values. Notable exceptions were the DP4 products, which showed the highest yields, suggesting that the DP3 product (panose) is a better glucose acceptor compared to the products with higher DP. At equimolar initial concentrations of maltose and sucrose in the reactions, the authors reported that >75% of the glucose available in sucrose is included in GOS products [52]. In the present work, between 74% (AG10) and 92% (AG11) of the glucose units available in sucrose were included in GOS products at M/S=1, and these results are comparable to those discussed above, suggesting similar efficiency of the acceptor reactions catalyzed by the enzymes studied here.

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Maltose was the strongest acceptor for synthesis of GOSs by alternansucrase from *L. citreum* NRRL B-1355. Using M/S=0.42, Cote and Robyt [54] reported the synthesis of GOSs with DP up to 4. The authors found that the first added glucose residue is α-(1→6)-linked to maltose, and α-(1→3), and more α-(1→6) products appear at DP4. The authors also reported yields of 84% GOSs [54]. In comparison, at M/S=0.5, we achieved lower yields of GOSs ranging from 52% (AG10) to 66% (AG11), but with more even distribution of the products up to DP7. Later studies of the maltose acceptor reactions by alternansucrase from *L. citreum* NRRL B-21297 revealed that at M/S=1 the enzyme produces non-homogenous series of alternan-type GOSs with DP up to 8 [27,55]. Using glucansucrase preparation from *L. citreum* NRRL B-742 and M/S ratios 0.5 and 0.14, Remaud et al. [39] reported the synthesis of α-(1→3)-branched GOSs with DP up to 7. They observed an increase in the proportion of branched GOSs with the increase of sucrose concentration [39]. This correlates with our observation of decreased content of α-(1→6)-linked glucose units in GOSs synthesized at M/S<1, but the differences between M/S ratios 0.12 to 0.5 are in the range of only 2 to 4%. Knowing that the enzyme preparations from the studied FLAB strains introduce α-(1→3)-branches in the synthesizing polysaccharides, we could presume retaining of this specificity in the acceptor products too. At M/S=0.5, using cloned dextransucrase DSR-E, Bozonnet et al. [47] reported the synthesis of α-(1→2)-branched R5 fraction with DP5. In previous studies concerning the synthesis of GOSs with dextransucrase preparation from *L. citreum* NRRL B-1299, Remaud-Simeon et al. [49] revealed that the sucrose concentration and its ratio to maltose are crucial factors affecting the yields of α-(1→2) branched GOSs. The authors reported the highest yields of α-(1→2)-branched GOSs at M/S=0.25, where the concentration of sucrose is prevalent. This also led to an increase in the proportion of the longer GOSs products containing α-(1→2) linkages [49]. These findings correlate well with the results obtained in the present study and also with the ones for glucansucrase URE 13-300 from *L. mesenteroides* URE 13. Using maltose as an acceptor, this enzyme incorporates between 55% (M/S=0.5) and 76% (M/S=1) of the available glucose units from sucrose into GOSs [37].

The ability of lactobacilli to metabolize prebiotic GOSs differs significantly from this of *Bifidobacterium* because their enzyme machinery for carbohydrate utilization is mostly intracellular and depends strongly on the presence and efficiency of the available mechanisms for transport of sugars [56,57]. In addition, the ability to utilize and ferment a certain type of GOSs is not tightly bound to a given *Lactobacillus* species but also to specific strains, and is often a result of acquisition of plasmids or horizontal gene transfer [58,59]. Despite that the evolution of lactobacilli was accompanied by reduction in the sizes of their genomes and tight specialization to ecological niches, such as the upper parts of the intestinal tract of humans and animals, they demonstrate remarkable abilities to utilize a variable range of specific carbohydrates which allow them to compete with other genera in the given habitat [57,60]. An interesting example of the effectiveness of the habitat adaptation on a genetic level are the *Lb. plantarum* strains which are referred to as 'natural metabolic engineers' due to the cassette organization of their carbohydrate utilization genes. These genes can be acquired, shuffled, substituted or deleted depending on the conditions of the particular ecological niche [61].

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Practically all lactobacilli are able to utilize α-glucosides, and amylopullulanase and amylase were identified as the only extracellular glycoside hydrolases participating in the metabolism of these substrates by hydrolysis resulting in the release of maltodextrins and other α-glucooligosaccharides [57]. A particularly important role in the utilization of α-GOSs substrates is attributed to the four-component ATP-binding cassette (ABC) transport system MalEFG/MsmK, which is well characterized in *Lb. acidophilus*, *Lb. sanfranciscensis* and other lactobacilli [62]. This transport system seems to be responsible for the uptake not only of maltose but also of longer α-GOSs, because a phosphotransferase system that could facilitate the transport of these carbohydrates has not been identified in lactobacilli [57]. In addition, in *Lb. acidophilus* and *Lb. casei* strains intracelular amylopullulanases and maltose phosphorylase were identified, which were expressed from the same operon as MalEFG/MsmK ABC transporter and β-phosphoglucomutase, which together ensure entering of maltose and maltodextrins in the corresponding metabolic pathways. The identification of intracellular dextranase DexB in lactobacilli further contributes to the knowledge about the ability of these LAB to utilize GOSs containing α-(1→6) linkages, both natural or obtained by planned acceptor reactions using glucansucrase enzymes [57,63]. In the present work, we reported a strain-specific utilization of GOSs containing between 71% and 80% α-(1→6) linkages suggesting the presence of additional intracellular glycoside hydrolase enzymes that contribute to the hydrolysis of oligosaccharides different from the dextran-derived isomaltooligosaccharides. As we have shown above, the enzyme preparations from the studied FLAB strains produce natively glucans containing >10% α-(1→3) linkages, suggesting their presence in the maltose acceptor products too. In a previous work, Cote et al. [53], and Sanz et al. [64] reported poor utilization of α-(1→3)-containing GOSs by pure cultures of lactobacilli. Later studies on utilization of alternansucrase oligosaccharide preparations by microbiota of fecal samples and estimation of their prebiotic indices revealed that DP7 products contribute significantly to the growth of lactobacilli population [65]. According to the authors, this could be due to a cooperative degradation of GOSs by other bacterial groups of the gut community or to the intrinsic properties of the given population of lactobacilli and its overall inventory of carbohydrate-acting enzymes.

The presented results about utilization of oligosaccharides with prebiotic potential by *Lb. plantarum* S26, *Lb. brevis* S27 and *Lb. sakei* S16 correlate well with our recent studies concerning the utilization of maltose, raffinose, lactose and xylose-derived oligosaccharides by these strains [37,43].

Conclusions

Considering the increasing demand for obtaining of new materials, the discovery of new natural biocatalysts that are able to fit the desired purpose of transforming comparatively cheap substrates into valuable commodities is trending. In the presented work, we reported application of four FLAB strains from the gut of honey bee *A. mellifera* for production of extracellular high-molecular-weight glucansucrases. This suggested that these inherently important insects, responsible for production of honey, bee bread and other beneficial products, and most

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significantly – pollinating the flowering crops all over the world, could be viewed also as a source of proven biotechnologically important enzymes such as glucansucrases. These enzymes were able to synthesize glucans containing >10% α-(1→3) linkages from sucrose, and also were employed into the synthesis of GOSs with DP up to 7 using maltose as an acceptor of glucose units. To the best of our knowledge, this work reports for the first time application of glucansucrases from fructophilic lactobacilli in the production of glucooligosaccharides via maltose acceptor reactions, and also testing of their prebiotic potential. In addition, the yields and composition of the acceptor products containing >20% assumed α-(1→3) linkages are influenced by the acceptor/donor ratio which is a promising opportunity for modulation of the acceptor reactions. Still, further studies employing the studied enzymes and other acceptor molecules remain to be performed in order to characterize the synthetic potential of these glucansucrases in full.

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Disclosure statement

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Data availability statement

All data that support the findings reported in this study are available from the corresponding author upon reasonable request.

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