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Effect of Dextransucrase Cellobiose Acceptor Products on the Growth of Human Gut Bacteria

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ABSTRACT: The selective fermentation by human gut bacteria of gluco-oligosaccharides obtained from the reaction between the glucosyl group of sucrose and cellobiose, catalyzed by dextransucrases (DSR) from *Leuconostoc mesenteroides*, has been evaluated. Oligosaccharides were fractionated according to their molecular weight, and their effect on the growth of different bacterial groups was studied. To determine the structure (position and configuration of glycosidic linkages)−function relationship, their properties were compared to those of DSR maltose acceptor products (DSRMal) and of recognized prebiotic carbohydrates (fructo-oligosaccharides, FOS). Cellobiose acceptor products (DSRCel) showed bifidogenic properties similar to those of FOS. However, no significant differences related to molecular weight or isomeric configurations were found for DSRCel and DSRMal products.

KEYWORDS: dextransucrase cellobiose acceptor oligosaccharides, structure−function relationship, gut microbiota, prebiotic, bifidobacteria

INTRODUCTION

The search for and manufacture of new prebiotics, which are nondigestible oligosaccharides that are selectively fermented by health-positive bacteria in the human gut, are targets of researchers and food companies. Besides those oligosaccharides commonly accepted as prebiotics (galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS), and lactulose), some carbohydrates such as β-(1→6)-gluco-oligosaccharides (also called gentio-oligosaccharides)[1,2] arabinoxylo-oligosaccharides,[3] pectic-oligosaccharides,[4] oligosaccharides derived from lactulose,[5] etc., have recently been proposed as potential functional products that may helpfully affect the host by selectively stimulating the growth and/or activity of beneficial bacteria.[6]

Enzymatic transfer reactions from disaccharides such as sucrose and lactose[7−9] are one of the main sources of production of new potential prebiotic carbohydrates. Series of oligosaccharides derived from the reaction between the glucosyl group of sucrose and low molecular weight acceptor carbohydrates, catalyzed by extracellular glucansucrases (e.g., dextransucrases, DSR) from *Leuconostoc mesenteroides*, have been widely studied.[10,11] In these reactions, there is a competition between the formation of oligosaccharides containing one, two, three, or more D-glucopyranosyl groups more than the acceptor and the normal high molecular weight glucan product. A wide range of acceptor molecules have been used to form oligosaccharides, and a wide variety of products have been obtained. However, the effect of most of these carbohydrates on the gut microbiota has not yet been assessed.

One such group of oligosaccharides is that from the reaction between sucrose and cellobiose (an easily available and nondigestible disaccharide[12]). The product is a trisaccharide obtained from the transfer of the glucosyl residue from sucrose onto the reducing end of a cellobiose acceptor through the formation of an α-(1→2)-glucosidic linkage.[13] The structure proposed for the DSR cellobiose acceptor product is α-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-D-glucopyranose.[14,15] Cellobio-oligosaccharides have been found to be effective as antifungal agents and for preventing bacterial adherence to teeth and dental caries by inhibiting the formation of glucans in bacterial plaque.[16]

However, the search for new prebiotic products is still limited by the lack of basic understanding of the mechanisms by which oligosaccharides exert a selective influence on the gut microbiota. Little is understood about the influence of oligosaccharide structure on selectivity of fermentation. The metabolism of FOS and GOS has been investigated;[17] however, more studies are needed to establish a structure−function relationship for new prebiotics.

The effect of glycosidic linkages and monosaccharide composition of a wide range of disaccharides on the selectivity of fermentation has been recently reported.[18] (1→2)-Linked glucobioses (kojibiose and sophorose) were highly selective for bifidobacteria compared with other disaccharides, including lactulose. Koji-oligosaccharides are not digestible in the small intestine, and pure culture studies have shown that these carbohydrates promote the growth of some beneficial bacteria.[19] Moreover, these products containing α-(1→2) bonds are being

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increasingly used by the food industry.[10] Gómez de Segura et al.[20] investigated the synthesis of gluco-oligosaccharides containing α-(1→2) bonds using DSR from *Leuconostoc mesenteroides* NRRL B-1299 and methyl 1-*O*-α-D-glucopyranoside as acceptor. These carbohydrates are capable of promoting the development of the beneficial cutaneous flora to the detriment of less desirable microorganisms. On the other hand, some studies indicate that oligosaccharides with 1→6 glycosidic linkages such as gentio-oligosaccharides and isomalto-oligosaccharides could not be hydrolyzed in the stomach or small intestine[7,21] and reach the colon intact. In addition, some in vitro studies have demonstrated that they have bifidogenic activity higher than that of FOS.[1,22]

The molecular weight of the fermented carbohydrates is likely to have a significant effect on fermentation selectivity. There is interest in the production of oligosaccharides with greater colonic persistence, which can reach the most distal regions where most of the chronic intestinal disorders originate.[23] Oligosaccharides with high degrees of polymerization (DP) may be more slowly fermented by gut bacteria; however, most polysaccharides are not selectively fermented in the colon. Although previous studies with isomalto-oligosaccharides (IMO) and FOS have suggested the optimal DP was in the range of 3−4, other studies have revealed a relatively high selectivity toward beneficial bacteria of gluco-oligosaccharides with DP 5−7.[2] Nevertheless, this behavior was not followed by DSR maltose acceptor products, which did not show a clear influence of molecular weight on the growth of beneficial bacteria. Therefore, more studies with different carbohydrate sources of different structures are needed to clarify this behavior.

In this work we have evaluated for the first time the effect of dextransucrase cellobiose acceptor oligosaccharides on the growth of human gut bacteria. The influence of molecular weight and the position and configuration (α or β) of the glycosidic linkages of these carbohydrates as compared to those of dextransucrase maltose acceptor products, kojibiose (α-(1→2)-glucosyl-glucose), and a recognized prebiotic (FOS) was also studied.

MATERIALS AND METHODS

Dextransucrase Purification. Dextransucrase NRRL B-512F was prepared according to the method described by Tsuchiya and Koepsell[24] and stored as a lyophilized powder. The cell-free culture fluid was concentrated by ultrafiltration using a 100 000 nominal molecular weight cutoff membrane and dialyzed against 20 mM (pH 5.4) sodium acetate.

Standards. Kojibiose and acetic, butyric, lactic, and propionic acids were purchased from Sigma Co. (St. Louis, MO) and fructo-oligosaccharides (FOS, Beneo P-95) from Orafti (Tienen, Belgium).

Acceptor Reaction Conditions. Acceptor reactions were carried out at room temperature using 25 g of sucrose in 75 mL of 20 mM (pH 5.4) sodium acetate buffer containing 0.01% (w/v) sodium azide with 75 units of B-512F dextransucrase as described previously for alternansucrase acceptor reactions.[25] Reactions were terminated when all of the sucrose was consumed, typically after 24−48 h.

Purification of Dextransucrase Acceptor Products. Both dextransucrase cellobiose acceptor products (DSRCel) and maltose acceptor products (DSRMal) were separated in fractions using a Bio-Gel P2 (fine mesh) column (5 × 150 cm), eluted with water under gravity flow. Fractions of the same molecular weight were pooled according to results obtained by thin layer chromatography (TLC) as previously described by Côté and Robyt.[26]

Characterization of Oligosaccharides. *Determination of Molecular Weight.* The molecular weight of oligosaccharide fractions was confirmed by matrix-assisted laser desorption/ionization coupled to time-of-flight mass spectrometry (MALDI-ToF MS) analyses using a Bruker Daltonics Omniflex spectrometer. Aqueous solutions of oligosaccharides were mixed with an equal volume of saturated 2,5-dihydroxybenzoic acid solution in acetonitrile and allowed to dry on the probe as previously described.[2] The instrument was set in reflective mode with positive polarity. An average of 60−100 shots per sample at 60−75% laser intensity was collected and averaged. Ion source 1 was set at 19 kV, ion source 2 at 13.4 kV, the lens at 1.2 kV, and the reflector at 20 kV.

Study of Chemical Structure. The chemical structure of DSRCel was confirmed using a methylation analysis as follows. Powdered sodium hydroxide (20 mg) and 0.1 mL of methyl iodide were added to a solution of oligosaccharide (5 mg) in 0.5 mL dimethyl sulfoxide (DMSO) and left at room temperature for 1 h. Samples were washed with water (1 mL) and chloroform (1 mL) five times, and the organic phase was evaporated to dryness under a stream of nitrogen. Hydrolysis was carried out with 2 N trifluoroacetic acid (1 mL) at 120 °C for 90 min. Samples were evaporated to dryness, and aldononitrile acetate derivatives were obtained with 0.5 mL of 4.5% hydroxylamine chloride in pyridine at 60 °C for 60 min. After cooling, acetic anhydride (0.5 mL) was added and heated to 60 °C for 30 min. Samples were washed with water (2 mL) and ethyl acetate (1.5 mL), and the organic phase was stored in a sealed vial for further GC-MS analysis on a polycarborane−siloxane HT-5 column (SGE Europe Ltd., U.K.) at 100 °C for 20 min working in split mode (1:20). The injector temperature was 300 °C, and the transfer line was thermostated at 280 °C. Helium at ∼1 mL min^{-1} was used as carrier gas. Acquisition was done using HP ChemStation software (Hewlett-Packard, Palo Alto, CA). Assignment of glycosidic linkages was carried out using commercial standards.

In Vitro Fermentations. In vitro fermentations in small-scale batch cultures were carried out in duplicate. Ten milligrams of carbohydrates was dissolved in autoclaved nutrient basal medium (2 g L^{-1} peptone water (Oxoid), 2 g L^{-1} yeast extract (Oxoid), 0.01 g L^{-1} NaCl (BDH), 0.04 g L^{-1} K_2HPO_4 (BDH), 0.04 g L^{-1} KH_2PO_4 (BDH), 0.01 g L^{-1} $MgSO_4 \cdot 7H_2O$ (BDH), 0.01 g L^{-1} $CaCl_2 \cdot 2H_2O$ (BDH), 2 g L^{-1} $NaHCO_3$ (BDH), 1:500 (v/v) Tween 80 (BDH), 0.05 g L^{-1} Haemin (Sigma, Dorset, U.K.), 1:10^5 (v/v) vitamin K_1 (Sigma), 0.5 g L^{-1} cysteine−HCl, 0.5 g L^{-1} Bile Salts (Oxoid), 1 mg L^{-1} Resazurin (Sigma)) to give a final concentration of 1% (w/v). Samples were then inoculated with 100 μL of slurry, which was prepared by homogenizing fresh human feces from healthy donors (10%, w/v) in phosphate-buffered saline using a stomacher (model 6041; Seward Scientific, U.K.) for 120 s. Three donors who did not have any history of gastrointestinal disorders and had avoided probiotics, prebiotics, and antibiotics for at least 3 months prior to the study were chosen. Two samples were additionally prepared without any carbohydrate addition as a control for 0 h and for 5 and 24 h, respectively. All additions, inoculations, and incubations were conducted inside an anaerobic cabinet at 37 °C. Samples (375 μL) were removed after 5 and 10 h of fermentation for enumeration of bacteria and short-chain fatty acid (SCFA) analysis.

Enumeration of Bacteria. Bacteria were counted using fluorescent in situ hybridization (FISH). Samples (375 μL) were fixed overnight at 4 °C with 4% (w/v) filtered paraformaldehyde (pH 7.2) in a ratio of 1:3 (v/v). Samples were then centrifuged and washed twice with filtered PBS (0.1 M, pH 7), resuspended in 150 μL of a mixture of PBS/ethanol (1:1, v/v), and stored at −20 °C until further analysis. Hybridization of the samples was carried out as described previously[1] using appropriate genus-specific 16S rRNA-targeted oligonucleotide probes labeled with the fluorescent dye Cy3 (MWG Biotech, Germany) for the different bacteria or with the nucleic acid stain 4′,6-diamidino-2-phenylindole (DAPI; Sigma) for total cell counts. Probes used for each

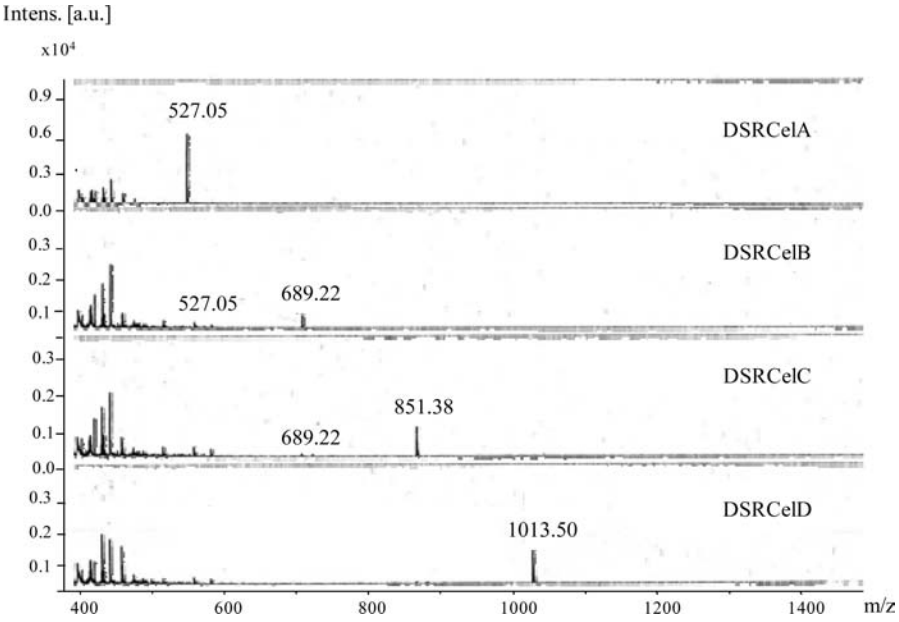

Figure 1. MALDI-ToF MS analysis of dextransucrase cellobiose acceptor (DSRCelA-D) oligosaccharide fractions obtained by SEC.

Table 1. Percentages of Structural Elements Present in Dextransucrase Cellobiose Acceptor Products (DSRCel) Determined by Methylation Followed by GC-MS Analysis

structural element (as derivative from methylation analysis)	linkage position	DSRCelA	DSRCelB	DSRCelC	DSRCelD
2,3,4,6-tetra-*O*MeGlc	1	67	35	20	25
2,3,6-tri-*O*MeGlc	4	3	10	6	5
2,3,4-tri-*O*MeGlc	6	6	50	72	68
2,4-di-*O*MeGlc	3, 6	0	0	1	1
3,6-di-*O*MeGlc	2, 4	24	5	1	1

of the bacteria, previously validated by different authors, were BIF164, specific for *Bifidobacterium* spp.;[27] BAC303, specific for *Bacteroides* spp.;[28] HIS150, for *Clostridium* spp. (*histolyticum* subgroup);[27] EREC482 for *Eubacterium* spp. (*Clostridium coccoides*−*Eubacterium rectale* group);[29] LAB158, for *Lactobacillus* spp./*Enterococcus* spp.;[30] and ATO291 for *Atopobium* spp. (*Coriobacterium* group).[31]

Analysis of Short-Chain Fatty Acids and Lactic Acid. Analysis of SCFA (acetic, propionic, and butyric acids) and lactic acid was carried out as previously indicated by Sanz et al.[2] Samples were centrifuged at 13000*g* for 5 min. and 20 μL was injected onto the HPLC system (Hewlett-Packard HP1050 series) equipped with a refractive index detector and an automatic injector. The column was an anion-exclusion REZEX-ROA organic acid column (Phenomenex, Chester, U.K.) maintained at 50 °C. The eluent was 0.005 mM sulfuric acid in HPLC-grade water, and the flow was 0.6 mL min^{-1}. Quantification of the samples was carried out using calibration curves for acetic, propionic, butyric, and lactic acids at concentrations ranging from 0.5 to 100 mM.

Statistical Analysis. Statistical analysis was performed using SPSS for Windows version 15.0. Univariate analysis of variance (ANOVA) and LSD post hoc test were also used to determine significant differences among bacteria populations using the different oligosaccharides. Differences were considered to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

DSRCel oligosaccharides were fractionated into four samples by size exclusion chromatography (SEC) and analyzed by MALDI-ToF MS. DSRCelA, DSRCelB, DSRCelC, and DSRCelD were high-purity fractions with DP3, DP4, DP5, and DP6, respectively (Figure 1). Only minor amounts of oligosaccharides with other DPs were observed in each fraction. This is in good agreement with the results previously observed by Kim and Day,[12] who reported the formation of gluco-oligosaccharides up to DP7 in the reaction catalyzed by DSB-512F. On the contrary, other studies[13,32] found that only trisaccharides were produced during incubation of cellobiose and sucrose with these enzymes. This discrepancy may be the result of different concentrations of sucrose and cellobiose in the reaction, as this is known to affect product distribution.[12]

Table 1 shows the results obtained from methylation analysis of DSRCel fractions. The main structural elements of DSRCelA were terminal glucopyranosyl units and 2,4-disubstituted glucopyranosyl units, which confirmed the presence of the trisaccharide α-D-Glc*p*-(1→2)-[β-D-Glc*p*-(1→4)]-D-Glc (DSRCelA1), previously described by Bailey et al.[15] and Yamauchi et al.[14] in cellobiose acceptor reactions catalyzed by dextransucrase from *L. mesenteroides* NRRL B-512F. Minor amounts of 4- and 6-monosubstituted glucopyranosyl units were also detected. This is in good agreement with Arguello-Morales et al.,[13] who found the presence of low amounts of another trisaccharide with α-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→4)-D-Glc structure (DSRCelA2) in this kind of reaction mixture. DSRCelB, DSRCelC, and DSRCelD mixtures were mainly constituted by terminal

Table 2. Main Structures of Dextransucrase Cellobiose (DSRCel) and Maltose (DSRMal) Acceptor Oligosaccharides

sample	DP	structure
DSRCel	3	α-Glc-(1→2)-[β-Glc-(1→4)]-Glc
	3	α-Glc-(1→6)-β-Glc-(1→4)-Glc
	4	α-Glc-(1→6)-α-Glc-(1→6)-β-Glc-(1→4)-Glc
	5	α-Glc-(1→6)-α-Glc-(1→6)-α-Glc-(1→6)-β-Glc-(1→4)-Glc
	6	α-Glc-(1→6)-α-Glc-(1→6)-α-Glc-(1→6)-α-Glc-(1→6)-β-Glc-(1→4)-Glc
DSRMal	3	α-Glc-(1→6)-α-Glc-(1→4)-Glc
	4	α-Glc-(1→6)-α-Glc-(1→6)-α-Glc-(1→4)-Glc
	5	α-Glc-(1→6)-α-Glc-(1→6)-α-Glc-(1→6)-α-Glc-(1→4)-Glc
	6	α-Glc-(1→6)-α-Glc-(1→6)-α-Glc-(1→6)-α-Glc-(1→6)-α-Glc-(1→4)-Glc
	7	α-Glc-(1→6)-α-Glc-(1→6)-α-Glc-(1→6)-α-Glc-(1→6)-α-Glc-(1→6)-α-Glc-(1→4)-Glc
	8	α-Glc-(1→6)-α-Glc-(1→6)-α-Glc-(1→6)-α-Glc-(1→6)-α-Glc-(1→6)-α-Glc-(1→6)-α-Glc-(1→4)-Glc

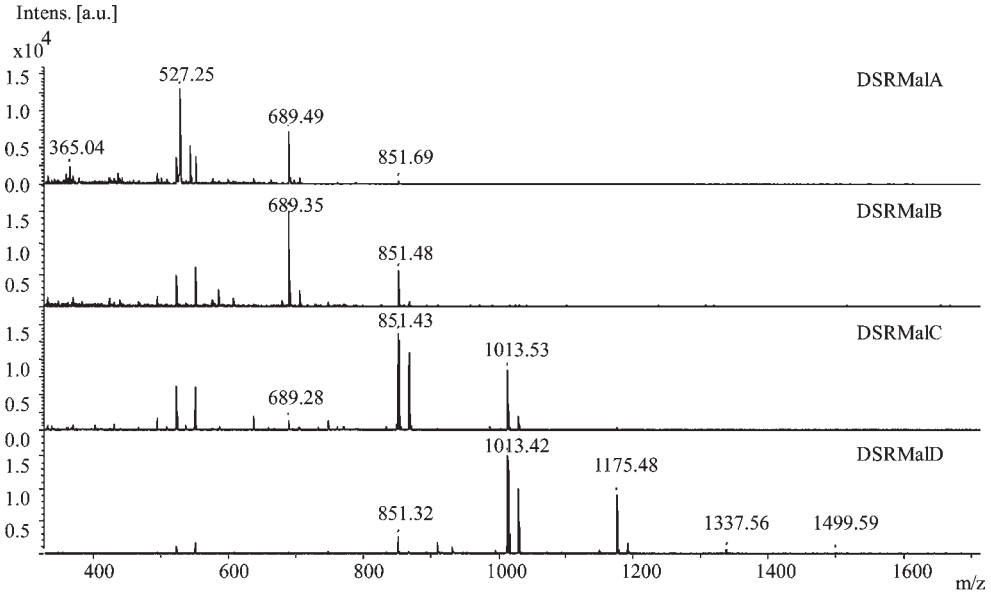

Figure 2. MALDI-ToF MS analysis of dextransucrase maltose acceptor (DSRMalA-D) oligosaccharide fractions obtained by SEC.

glucopyranosyl and 6-monosubstituted glucopyranosyl residues. Therefore, the structures with higher DP fractionated by SEC arise from DSRCelA2. This trisaccharide is in turn glucosylated to produce the tetrasaccharide DSRCelB (α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-cellobiose), which is also glucosylated to give the pentasaccharide DSRCelC and similarly the hexasaccharide DSRCelD. The α-(1→2) linkage in the DSRCelA1 molecule prevents its binding to the enzyme and, therefore, its glucosylation.[13] Table 2 shows the resulting structures of DSRCel.

For comparison, dextransucrase maltose acceptor products (DSRMal) were also analyzed by MALDI-ToF MS (Figure 2). Structures as previously reported by Killey et al.[33] are shown in Table 2. Fraction DSRMalA was mainly composed by oligosaccharides of DP3 and DP4. DSRMalB and C were composed of DP4 and DP5 and DP5 and DP6, respectively, whereas DP6, DP7, and DP8 were the main constituents for DSRMalD.

Table 3 shows the bacterial population counted by FISH after 0, 5, and 10 h of incubation of dextransucrase acceptor oligosaccharides with fecal inoculum. Kojibiose and a FOS mixture were also included in this study for comparison. Different letters indicate significant differences ($P < 0.05$) among all of the substrates under study for each bacterial group and for each time of incubation, including 0 h. For instance, significant differences were observed for *Atopobium* spp. population between 0 and 5 h for all of the substrates studied (letters a and b in Table 3), whereas nonsignificant differences were detected in this population among all of the carbohydrates incubated for 5 h (letter b in Table 3).

Levels of total bacteria were maintained after 5 h of incubation except for DSRCelA, DSRCelB, and DSRCelC, which significantly increased compared to the control sample taken at 0 h. Additionally, at 10 h of incubation total bacteria of DSRMalA also showed significant differences against the sample taken at 0 h. In general, an increase of *Bifidobacterium* spp. population was observed at 5 h of incubation for all of the samples under study, this increase being significant at 10 h of incubation in all of them. Therefore, all of the DSRCel products could be considered

Table 3. Changes in Bacterial Populations (Log Cell per Milliliter) after 5 and 10 h of Small-Scale in Vitro Incubation with Dextransucrase Acceptor Oligosaccharides[a]

bacterial group	time (h)	control	kojibiose	FOS	DSRCelA	DSRCelB	DSRCelC	DSRCelD	DSRMalA	DSRMalB	DSRMalC	DSRMalD
total bacteria	0	9.21(0.03) ae										
	5	9.09(0.13) be	9.47(0.06) acd	9.38(0.28) acd	9.50(0.14) cdf	9.52(0.11) cdf	9.54(0.11) cd	9.23(0.22) ae	9.27(0.16) aef	9.30(0.13) cde	9.41(0.17) acd	9.42(0.03) acd
	10	9.20(0.18) a	9.33(0.07) ac	9.43(0.21) abc	9.51(0.06) bc	9.57(0.25) bc	9.58(0.26) bc	9.43(0.05) abc	9.60(0.21) b	9.43(0.08) abc	9.25(0.09) a	9.43(0.07) abc
Atopobium spp.	0	7.58(0.57) a										
(*Coriobacterium* group)	5	8.02(0.20) b	8.05(0.03) b	8.16(0.21) b	8.15(0.15) b	8.21(0.09) b	8.28(0.14) b	8.15(0.18) b	8.13(0.31) b	8.17(0.12) b	8.14(0.19) b	8.01(0.13) b
	10	7.90(0.22) ac	8.27(0.10) bc	8.18(0.22) bc	8.37(0.11) bc	8.09(0.54) bc	8.16(0.33) bc	8.08(0.33) bc	8.32(0.15) bc	8.20(0.25) bc	8.14(0.11) bc	8.40(0.08) b
Bacteroides spp.	0	8.44(0.36) ad										
	5	8.60(0.15) a	8.76(0.28) a	8.75(0.21) a	8.76(0.22) a	8.78(0.48) a	8.81(0.19) a	8.71(0.28) a	8.78(0.29) a	8.75(0.07) a	8.72(0.26) a	8.63(0.21) a
	10	8.46(0.28) ad	8.65(0.32) abc	8.32(0.25) bd	8.94(0.12) ac	8.61(0.50) abc	8.59(0.32) abc	8.73(0.12) abc	8.59(0.11) abc	8.62(0.22) abc	8.99(0.13) c	8.56(0.20) abc
Bifidobacterium spp.	0	8.03(0.19) ac										
	5	8.03(0.32) ac	8.38(0.16) abcd	8.58(0.39) d	8.50(0.17) bd	8.33(0.10) abcd	8.48(0.25) bd	8.34(0.12) abcd	8.52(0.25) d	8.53(0.30) d	8.47(0.28) bd	8.32(0.22) abcd
	10	7.92(0.30) a	8.60(0.26) b	8.51(0.40) b	8.76(0.10) b	8.69(0.12) b	8.72(0.06) b	8.75(0.22) b	8.60(0.32) b	8.76(0.18) b	8.71(0.15) b	8.78(0.20) b
Eubacterium spp.	0	8.10(0.11) a										
(*Clostridium coccoides*—*Eubacterium rectale* group)	5	8.12(0.11) a	8.28(0.30) a	8.04(0.17) a	7.97(0.71) a	8.11(0.30) a	8.09(0.22) a	8.02(0.32) a	8.03(0.32) a	8.15(0.08) a	8.40(0.10) a	8.09(0.19) a
	10	7.96(0.14) a	7.83(0.32) a	7.71(0.20) a	7.90(0.50) a	7.85(0.23) a	7.91(0.45) a	7.61(0.47) a	7.84(0.23) a	7.97(0.37) a	8.04(0.29) a	8.02(0.27) a
Clostridium spp.	0	7.17(0.11) ae										
(*histolyticum* subgroup)	5	7.07(0.06) a	6.72(0.58) a	7.18(0.23) a	7.19(0.34) a	7.10(0.16) a	7.20(0.22) a	7.13(0.11) a	6.96(0.18) a	6.93(0.28) a	6.94(0.26) a	7.18(0.37) a
	10	7.27(0.14) a	7.16(0.18) ac	7.22(0.31) a	7.05(0.21) ad	7.17(0.18) ac	7.31(0.28) a	7.18(0.14) ac	6.87(0.04) bd	6.81(0.09) bd	6.92(0.14) cde	7.03(0.03) ad
Lactobacillus spp./	0	7.72(0.11) a										
Enterococcus spp.	5	7.81(0.08) abcd	7.86(0.06) abcd	7.96(0.14) cd	7.85(0.14) abcd	7.75(0.02) ab	7.75(0.10) ab	7.93(0.09) bc	7.90(0.17) abcd	7.84(0.14) abcd	7.91(0.08) bc	7.97(0.09) cd
	10	7.71(0.14) a	7.93(0.14) a	7.99(0.30) a	7.88(0.21) a	7.76(0.25) a	7.86(0.18) a	7.86(0.27) a	7.72(0.20) a	7.90(0.02) a	7.83(0.29) a	7.86(0.28) a

[a] Samples without a carbohydrate source, with kojibiose, and with FOS are also included. Standard deviation is given in parentheses. Different letters indicate significant differences ($P < 0.05$) among all of the substrates for each bacterial group and for each time of incubation including 0 h.

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Table 4. SCFA and Lactic Acid Concentrations Determined by HPLC Produced in Dextransucrase Cellobiose and Dextransucrase Maltose Oligosaccharide Mixtures after 5 and 10 h of in Vitro Incubation with Human Gut Bacteria*

	lactic acid		acetic acid		propionic acid		butyric acid	
	5 h	10 h	5 h	10 h	5 h	10 h	5 h	10 h
control (0 h)	2.46(0.19) a		2.36(0.59) ab		1.06(0.09) a		0.76(0.24) a	
control	3.06(0.50) a	3.12(1.28) abc	6.68(1.85) bci	9.16(2.38) bf	2.70(0.28) b	2.98(0.29) ad	1.09(0.23) a	1.49(0.86) a
kojibiose	3.97(1.48) ab	5.26(2.16) abc	10.39(2.35) cdf	18.55(4.93) df	3.66(0.47) c	8.60(3.78) c	0.88(0.37) a	2.61(3.00) a
FOS	12.21(4.30) e	22.44(5.14) e	13.10(4.69) de	22.28(9.38) de	3.42(1.27) bc	4.64(2.01) bd	0.72(0.22) a	0.76(0.16) a
DSRCelA	4.53(1.71) abc	9.23(5.23) abcd	8.51(3.74) cfgh	22.61(0.81) de	3.23(0.13) bc	6.05(2.18) bc	1.14(0.58) a	1.65(1.58) a
DSrCelB	4.04(0.68) ab	9.03(4.33) abcd	10.09(1.28) cdf	25.60(3.81) de	3.50(0.58) c	5.91(0.46) bc	1.01(0.51) a	1.76(1.27) a
DSRCelC	3.62(0.26) ab	10.29(2.21) bcfg	9.94(1.30) cdf	27.21(2.47) de	3.46(0.23) bc	6.06(0.85) bc	1.00(0.55) a	1.61(1.23) a
DSRCelD	3.36(0.42) ab	6.33(3.25) acf	8.16(1.98) cfi	17.42(8.17) df	2.97(0.34) bc	5.00(2.25) bd	0.97(0.42) a	1.10(0.66) a
DSRMalA	7.25(2.42) cd	13.00(1.47) dfg	12.53(3.42) deh	23.36(8.72) de	3.21(0.31) bc	5.27(1.12) bd	0.84(0.39) a	1.00(0.71) a
DSRMalB	5.93(2.19) bc	17.39(10.43) eg	13.35(3.73) de	30.00(7.98) ce	3.18(0.20) bc	4.58(0.89) bd	0.95(0.37) a	1.15(0.54) a
DSRMalC	4.64(1.09) abd	15.86(6.71) edg	11.23(2.24) def	30.07(7.75) ce	2.91(0.49) bc	4.77(1.10) bd	0.92(0.23) a	1.27(0.77) a
DSRMalD	3.68(0.49) ab	6.46(1.50) abcf	10.60(1.78) cdef	25.00(6.32) de	3.47(0.30) c	6.21(1.55) bc	1.15(0.28) a	1.50(1.13) a

*Different letters indicate significant differences ($P < 0.05$) among all of the substrates for each acid and for each time of incubation including 0 h. Standard deviation is given in parentheses

bifidogenic under the studied conditions, with cell values even higher than those produced by FOS at 10 h. Similar results have previously been reported for gentio-oligosaccharides (with β-(1→6)-glycosidic linkages,[1,22]), isomalto-oligosaccharides (with α-(1→6)-glycosidic linkages),[1,21] and alternansucrase gentiobiose oligosaccharides,[22] which promoted the effect upon the growth of bifidobacteria. Moreover, oligosaccharides with 1→4 linkages and 1→2 linkages have also been shown to produce this effect.[2,19]

Atopobium significantly increased at both 5 and 10 h of incubation for all samples under study. However, *Bacteroides* spp., *Clostridium* spp., and *Eubacteria* spp. populations did not exhibit significant differences with the sample taken at 0 h. A similar behavior was observed for the growth of clostridia during the incubation with isomalto-oligosaccharides.[1] On the contrary, gentio-oligosaccharides resulted in a high increase of both clostridia and bacteroides populations[22] and isomalto-oligosaccharides in a significant increase of bacteroides at 5 h of incubation.[1] Only a slight increase of *Lactobacillus* spp. population was observed for FOS, DSRCelD, DSRMalC, and DSRMalD at 5 h of incubation.

Although a higher selective growth of beneficial bacteria produced by the action of α-glucosylglucose disaccharides than by the β-isomers was observed by Sanz et al.,[18] in this work no significant differences between bacterial growth produced by maltose and cellobiose acceptor products were detected.

With regard to the effect of molecular weight, no significant differences were observed in either maltose or cellobiose DSR acceptor products, except for total bacteria population of incubations with DSRCelA, DSRCelB, and DSRCelC against that of DSRCelD at 5 h and for total bacteria population of DSRMalA against DSRMalC at 10 h. Similar results have been obtained in previous works for other carbohydrates for which no significant differences were detected among the different DPs,[2,18] despite the variable results regarding their global effect on the gut microbiota calculated as selectivity index (SI) for bifidobacteria, *Lactobacillus*/*Enterococcus* group, and *Eubacterium rectale* group[4] (eq 1)

$$SI = ((Bif\ 12/Bif\ 0) + (Lac\ 12/Lac\ 0) + (EREC\ 12/EREC\ 0) - (Bac\ 12/Bac\ 0) - (His\ 12/His\ 0))/(total\ count\ 12/(total\ count\ 0)) \quad (1)$$

where Bif 12 = bifidobacterial count at 12 h, Bif 0 = bifidobacterial count at 0 h, etc.

In this study, SI values were calculated only as a means to determine the global effect of the different DPs of DSRCel products on the gut microbiota. A similar behavior was observed at 5 and 10 h: DP6 cellobiose-derived oligosaccharides showed the highest SI values (i.e., DSRCelD SI = 2.77 at 10 h) followed by DP3 carbohydrates (DSRCelA SI = 2.12). DP4 and DP5 cellobiose-derived oligosaccharides showed the lowest values (DSRCelB and DSRCelC SI = 1.29 and 1.58, respectively). The presence of the α-(1→2) glycosidic linkage in DSRCelA could explain its SI value, considering that kojibiose resulted in a positive relative selectivity toward groups generally considered to be health-positive (SI at 10 h = 2.77). These results are supported by the beneficial effects found by Sanz et al.[18] for (1→2) disaccharides.

SCFA and lactic acid concentrations determined by HPLC are shown in Table 4. In general, a significant increase of lactic, acetic, and propionic acids was observed for all of the samples analyzed after 10 h of incubation. However, no noticeable changes in butyric acid were observed for these samples. This is in good agreement with the results found for bifidobacteria, clostridia, and eubacteria populations (Table 3), considering that acetic and lactic acids are typical fermentation products of the bifidus pathway and this bacteria population increases during incubations, whereas butyric acid is an end product of clostridia and eubacteria pathway (which did not vary during incubations).[34] No significant differences were found for acetic and propionic acid values between samples incubated for 10 h with DSRCel products and FOS. However, lactic acid of this reference mixture was significantly higher than the rest.

In conclusion, the significant growth of the *Bifidobacterium* spp. population shown in this work by incubation with acceptor oligosaccharides arising from dextransucrase reactions with cellobiose and sucrose, both derived from biomass, proves the utility of these carbohydrates as potential prebiotics for the food and feed industry. With regard to the structure−function relationship, no significant differences were found in the selective fermentation properties of similar oligosaccharides with α or β glycosidic linkages (DSRCel and DSRMal products) or with different molecular weights. The content of short-chain fatty acids was in agreement with the bifidogenic effect observed on gut microbiota for DSRCel products here studied. Thus, cellobiose from lignocellulosic biomass could serve equally as well as maltose from starch for the production of prebiotic oligosaccharides via glucansucrase acceptor reactions.

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