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Original Article

Manufacture of gluco-oligosaccharide prebiotic by *Gluconobacter oxydans* NCIMB 4943

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Abstract

This study investigated the optimal environmental factors for gluco-oligosaccharide production by *Gluconobacter oxydans* NCIMB 4943 from a range of commercial maltodextrins, and the influence of reaction parameters on the molecular weight distribution and chemical structure of the gluco-oligosaccharide products. *G. oxydans* gave significantly ($p<0.01$) higher gluco-oligosaccharide yield in maltodextrin complex medium (30.4%) than using cell suspensions (19.6%). Cell concentration had a significant ($p<0.05$) effect on gluco-oligosaccharide yields at 24 h. The optimal pH was found to be 4.5 for the cell suspension method. Temperature had a significant ($p<0.05$) effect and the optimal temperature was found to be 30°C. Maximum yield (30.0%) was obtained with high dextrose equivalent (DE) maltodextrin in maltodextrin complex medium. Low DE substrates gave the lowest gluco-oligosaccharide yields. Substrate concentration affected gluco-oligosaccharide formation significantly ($p<0.05$). Low molecular weight (approximately 1 kDa) maltodextrin was converted to higher molecular weight gluco-oligosaccharide (7.8-65.6 kDa) with ratios of α-1,6-, α-1,4- and α-1,4,6-D-glucosidic linkages in the ranges of 1.37-3.99, 1.48-4.30 and 0.29-0.73 respectively, depending on the manufacturing conditions. Gluco-oligosaccharides contained at least 88 glucose residues.

Keywords: gluco-oligosaccharide, prebiotic, functional food, *Gluconobacter oxydans*

1. Introduction

Much interest in the use of prebiotic oligosaccharides as functional food to improve health has developed over the last decade worldwide (Gibson and Roberfroid, 1995; Rastall and Gibson, 2002; Siro *et al*., 2008). Technologies for production of commercial prebiotics are categorized into 3 approaches; chemical synthesis, extraction from natural sources and enzymatic approach (either via transferase or hydrolysis reaction). Lactulose is only one prebiotic produced by chemical synthesis, whereas inulin and soybean oligosaccharides are produced by direct extraction from natural sources. Prebiotics which are produced by enzymatic approach are oligofructose, fructooligosaccharides, lactosucrose, galactooligosaccharides, maltooligosaccharides, isomaltooligosaccharides and xylooligosaccharides (Casci and Rastall, 2006).

Examples of newly developed candidate prebiotics using as enzymatic approach are dextran and oligodextran (10,000 to 70,000 Daltons). They could selectively enrich

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bifidobacteria in a mixed faecal culture and they also produced elevated levels of butyrate (Olano-Martin *et al*., 2000). Gentio-oligosaccharide, chitooligosaccharide, pectic-oligosaccharide, abino-galacto-oligosaccharides, rhamno-galacto-oligosaccharide, galacturonic oligosaccharide and sialic acid oligosaccharide are emerging prebiotics developed by complex synthesis for specific targets such as pathogen decoy and immune stimulant (Crittenden, 2006).

The gluco-oligosaccharide from *G. oxydans* has recently been shown to be a candidate prebiotic (Wichienchot *et al*., 2006a). There is some evidence from *in vitro* model systems that this material with its molecular weight of approximately 7.8-65.6 kDa will display better persistence to the distal colon (Wichienchot *et al*., 2006b). The aim of this work was to investigate enzymatic synthesis of gluco-oligosaccharide by *G. oxydans* NCIMB 4943 from commercially available maltodextrin substrates to a functional food ingredient, which previously was proven as a prebiotic.

2. Materials and Methods

2.1 Microorganism, medium, maltodextrin substrates

Gluconobacter oxydans NCIMB 4943 was obtained from the National Collections of Industrial Food and Marine Bacteria, Aberdeen, UK. Unless otherwise stated, all chemicals and reagents were obtained from Sigma-Aldrich Co. Ltd. (Poole, UK) and bacteriological growth media supplements from Oxoid Ltd. (Basingstoke, UK). The glucose yeast extract-chalk (GYC) agar contained (g l^{-1}); glucose, 50, yeast extract, 10, $CaCO_3$, 30 and agar, 25, without pH adjustment (Mountzouris *et al*., 1999). The glucose complex medium (GY) contained glucose 10 g l^{-1} and yeast extract 10 g l^{-1}, without pH adjustment (Mountzouris *et al*., 1999). The maltodextrin complex medium contained the same components as the glucose complex medium except that glucose was replaced by maltodextrin. Maltodextrins were supplied by two companies; Goldex 15, 20, 30 and 37 were supplied by ABR Foods Ltd., UK and Glucidex 12, 19 and 29 were supplied by Roquette Ltd, France.

2.2 Cell growth characteristics

The stock cell suspensions were prepared by inoculating one loopful of *G. oxydans* NCIMB 4943 (prepared by growing on GYC agar for 72 h) into 100 ml of the GY medium in 250 ml Erlenmeyer flask. Cultivation was performed on a rotary shaker (175 rev min^{-1}) for 24 h at 30°C. Cells were harvested by centrifugation at 17,000xg for 15 min at 4°C, then washed three times with sterilized 0.025 mol l^{-1} sodium acetate buffer, pH 4.5 and filtered sterilized on 0.2 μm nylon membrane filter. The wet cell pastes were mixed with the above buffer to yield stock cell suspension for production of gluco-oligosaccharide by cell suspension method. Growth of *G. oxydans* was tested using maltodextrin complex medium containing various maltodextrins. Cultivation was performed in shake-flasks on a rotary shaker (175 rev min^{-1}) at 30°C for 72 h (Mountzouris *et al*., 1999). Samples were taken at 0, 4, 8, 16, 24, 32, 48 and 72 h of cultivation. Cell growth (absorbance was measured at 620 nm) and pH were measured in triplicate samples.

2.3 Comparison of gluco-oligosaccharide production by the culture medium method and the cell suspension method

In the culture medium method, the microorganism was grown on GYC agar for 72 h at 30°C, then one loopful of the microorganism was transferred into a 250 ml Erlenmeyer flask containing 100 ml maltodextrin complex medium. Cultivation was carried out for 72 h at 30°C and 175 rev min^{-1} on a rotary shaker. Samples were taken at 0, 4, 8, 16, 24, 32, 48 and 72 h. Bacterial cells were removed from the viscous culture by centrifugation at 17,000xg for 15 min at 4°C. The yield of gluco-oligosaccharides in the cell-free supernatant was determination by HPSEC (Mountzouris *et al*., 1999).

For the cell suspension method, cell suspensions were prepared as described above. The substrate was dissolved in 0.025 mol l^{-1} sodium acetate buffer (pH 4.5) and filter-sterilized on a 0.2 μm nylon membrane filter. Substrate solution (90 ml) was mixed with stock cell suspension (10 ml) to yield 10 and 5 g l^{-1} of the initial substrate and cell concentrations, respectively. Reactions were performed in 250 ml Erlenmeyer flasks at 30°C and 200 rev min^{-1} on a rotary shaker for 72 h. Samples were taken at the same time intervals as before and gluco-oligosaccharide production determined by HPSEC (Mountzouris *et al*., 1999). The experiments were performed in triplicate.

2.4 Effect of cell concentration, buffer pH and temperature

Experiments were performed in 100 ml reaction volumes in 250 ml Erlenmeyer flasks using cell suspension conditions as described previously. Effects of cell concentrations (5, 10, 15, 30 g l^{-1}), sodium acetate buffer (0.025 mol l^{-1}) pH (3.5, 4.5, 5.5) and temperature (25, 30, 35°C) were tested. Samples were taken at 0, 4, 8, 16, 24, 32, 48 and 72 h of reaction time. Gluco-oligosaccharide production and substrate utilization were evaluated by HPSEC.

2.5 Effect of substrate type and its concentration

Experiments were performed as described above. The maltodextrins tested were Goldex 15, 20, 30 and 37 and Glucidex 12, 19 and 29 (the numbers refer to the DE value) at concentrations of 10, 25, 50, 100 g l^{-1}. Samples (100 ml) were taken at 0, 4, 8, 16, 24, 32, 48 and 72 h of reaction time. Gluco-oligosaccharide production and substrate utilization were determined by HPSEC.

2.6 Determination of molecular weight distribution of gluco-oligosaccharide

After the cells were removed by centrifugation, the supernatant fluids were analyzed by HPSEC with refractive index detection. The analytical system consisted of two TSK G4000 PW columns and one TSK G6000 PW_{XL}, connected in series with decreasing pore size. The flow rate was 0.6 ml min^{-1}, the eluent was 0.02% (w/v) sodium azide in HPLC water and the operating temperature was 30°C. Samples and standards (dextran with different molecular weight) were filtered through a 0.45 mm syringe filter and the injection volume was 100 µl. The system was calibrated with at least six standards consisting of dextrans with average molecular weights of 38000 kDa, 2000 kDa, 464 kDa, 68.4 kDa, 42 kDa, 19.5 kDa, 9.5 kDa, maltoheptaose (1.15 kDa) and glucose (0.18 kDa). External standards of dextran (9.5 kDa) at a concentration of 1-10 mg ml^{-1} were used to establish the area response for quantification of the products. Gluco-oligosaccharide yield was calculated by the area of gluco-oligosaccharide divided by the respective curve area of its maltodextrin substrate and multiplying by 100. The molecular weight (MW) of gluco-oligosaccharide was determined by comparing sample peak K_{av} values with those of the standards (Mountzouris *et al*., 1999).

2.7 Separation and purification of gluco-oligosaccharides

Gluco-oligosaccharides were recovered by gel filtration chromatography using a Sephadex G-50 column (2.5 cm x 50 cm) with a nominal molecular weight cut off of 1,000 to 10,000 Da and a gel bead pore size of 50-150 µm. Eluent (water) flow rate was 0.40 ml min^{-1}. Freeze dried samples were prepared at 0.5% (w/v) and 12.3 ml were loaded onto the column. Fractions were collected in a fraction collector. The high molecular weight fraction from the Sephadex G-50 column was further purified on a Sephadex G-200 column (0.5 cm x 25 cm) with bead pore size of 40-120 µm. Eluent (water) flow rate was 0.2 ml min^{-1}. Carbohydrate samples were prepared at 0.5% (w/v) and 2 ml were loaded. Each fraction was lyophilized prior to further linkage analysis by methylation.

2.8 Methylation analysis

The purified products were lyophilized and the linkage positions of the sugar residues were determined by methylation analysis (Mountzouris *et al*., 1999). Samples (5 mg) were dispersed in 0.4 ml of dry dimethylsulfoxide (DMSO) then 20 mg of powdered sodium hydroxide and 0.1 ml of methyl iodide were added. The mixture was then vortex-mixed in a closed vial for 5 minutes and left at room temperature for 1 hour (Ciucano and Kerek, 1984). Water (1 ml) and chloroform (1 ml) were added and this mixture was vortex-mixed again. After phase separation, the water phase was removed with a micropipette and discarded. The chloroform layer was washed five times with water. After the final wash, any remaining water was removed by micropipette followed by evaporation to dryness under a steam of nitrogen. The partially methylated sample was hydrolyzed in 5 M trifluoroacetic acid (0.3 ml) by heating to 100°C for 15 hours in a sealed vial (Blakeney *et al*., 1983). The hydrolyzed sample was cooled before being evaporated to dryness under stream of nitrogen. The dried hydrolyzed sample was reduced by adding sodium borohydride (1 ml of 0.5 M sodium borohydride in 2 M ammonia), and heating at 60°C for 1 hour (Albersheim *et al*., 1967). Acetone (0.5 ml) was added to stop the reaction and the sample was evaporated to dryness under stream of nitrogen. The methylated and reduced products were acetylated by dissolving the dried sample in 0.2 ml of 18 M acetic acid, followed by addition of 1 ml of ethyl acetate, 3 ml of acetic anhydride, and 100 µl of 70% perchloric acid. The reaction mixture was vortex-mixed and allowed to stand for 5 minutes. After this, the mixture was cooled on ice before 5 ml of water were added, which was followed by addition of 200 µl of 1-methylimidazole (Albersheim *et al*., 1967). The reaction mixture was again allowed to stand for 5 minutes. Finally, 1 ml of dichloromethane was added and mixed on a vortex-mixer. After phase separation, the organic phase was removed with a micropipette and stored in a sealed vial prior to analysis by gas chromatography and mass spectrometry.

The partially methylated alditol acetates (PMAAs) were analyzed by GC-MS on a cross-bonded 50% cyanopropyl methyl- 50% phenyl methyl polysiloxane column (Thames Chromatography, Maidenhead, UK) using a flame ionization detector and a temperature program: 55°C (2 min), + 45°C min^{-1} (1.9 min), 140°C (2 min), +2°C min^{-1} (35 min), 210°C (40 min). The PMAAs were identified by measuring their retention times relative to *myo*-inositol hexaacetate, and comparing the relative retention times with those of external standards. Peak areas were represented as relative molar quantities compared to the terminal residues. Identities of PMAAs were confirmed by their electron-ionization mass spectra using a Fisons Analytical Trio 1S mass spectrometer, using a source temperature of 200°C and ionization potential of 70eV.

2.9 NMR analysis

Spectra were obtained on a Bruker ARX 400 spectrometer operating at 400 MHz for ^{1}H. Chemical shifts were expressed in ppm from TMS (tetramethyl silane) as internal reference for ^{1}H. Samples (1-4 mg) were dissolved in 0.6 ml D_2O, placed in 5 mm o.d. NMR tubes, and the temperature was regulated at room temperature (25°C). The ^{1}H chemical shifts for the anomeric protons were compared to previous published data (terminal-residue, $\delta = 5.22$; α-1-6-D-glucose, $\delta = 4.96$; α-1-4-D-glucose, $\delta = 5.35$, (Jodelet *et al*., 1998).

3. Results

3.1 Cell growth characteristics

The growth of *G. oxydans* on the various maltodextrin substrates is shown in Figure 1. Higher DE substrates such as Glucidex 29, Goldex 30 and Goldex 37 gave better growth during the first 18 h compared to lower DE substrates. The culture pH rapidly declined (from 5.5 to around 3.0) within the first 24 h of cultivation.

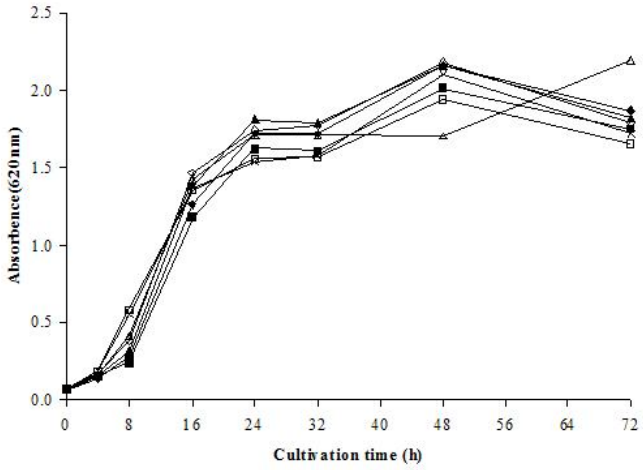

Figure 1. Effect of various commercial maltodextrins on growth of *G. oxydans* NCIMB 4943 cultivated in maltodextrin complex medium on shake-flask batch culture (200 rev min^{-1}, 30°C) using culture medium method. ◆, Glucidex 12; ■, Goldex15; ▲, Glucidex19; ◇, Goldex20; □, Glucidex29; △, Goldex30; ×, Goldex37.

3.2 Comparison of gluco-oligosaccharide production by the culture medium method and the cell suspension method

Gluco-oligosaccharide production was affected by the production method. The cell suspension method had significantly lower ($p<0.01$) gluco-oligosaccharide yields compared to the culture medium method despite a similar production rate during the first 16 h of cultivation. Gluco-oligosaccharide yields (mean ± SD) obtained from the maltodextrin complex medium and by using cell suspensions at 48 h were 30.41±1.56% and 19.64±1.76%, respectively.

3.3 Effects of cell concentration, buffer pH and temperature

Gluco-oligosaccharide yields (mean ± SD) obtained at the cell concentrations (g l^{-1}) of 5, 10, 15 and 30 at 48 h were 25.47±1.32%, 26.23±1.49%, 25.73±1.50% and 24.80±1.44%, respectively. The maximum gluco-oligosaccharide yield (26.66%) was reached at 72 h using 5 g l^{-1} cell concentration.

The pH of the reaction mixtures affected gluco-oligosaccharide production from *G. oxydans* NCIMB 4943. The maximum yields were obtained at 72 h reaction time, giving the gluco-oligosaccharide yields (mean ± SD) of 24.00±1.39%, 24.60±1.22% and 22.90±1.48%, at pH 3.5, 4.5, 5.5, respectively. The maximum gluco-oligosaccharide yield (25.94%) was obtained at the reaction temperature of 30°C at 72 h reaction time whereas the values were 25.30% and 23.40% at 25C and 35°C, respectively.

3.4 Effects of substrate and substrate concentration

Gluco-oligosaccharide yields depended on the type of maltodextrins used with the maximum gluco-oligosaccharide yield of 30.02% obtained using Goldex 30 in maltodextrin complex medium. In addition, Glucidex 29 and Goldex 37 also gave high gluco-oligosaccharide yields whereas low DE substrates such as Glucidex 12 and Goldex 15 gave the lowest gluco-oligosaccharide yields. Gluco-oligosaccharide yields (mean ± SD) are shown in Figure 2. Each substrate type significantly ($p<0.05$) affected the gluco-oligosaccharide yield. All of the maltodextrins tested with the cell suspension method gave significantly lower ($p<0.05$) gluco-oligosaccharide yields than with the maltodextrin complex medium (Figure 3). Substrate concentration had a significant effect ($p<0.05$) on gluco-oligosaccharide formation except at 10 g l^{-1} and 25 g l^{-1}. The highest gluco-oligosaccharide yield (20.50%) was obtained at 48 h using either 10 g l^{-1} or 25 g l^{-1} of Glucidex 19 and 0.5 g l^{-1} *G. oxydans* cells in buffer pH 4.5 at 30°C on shake-flask culture at 200 rev min^{-1}. The optimal concentration of maltodextrin to give the maximum gluco-oligosaccharide yield was 10 g l^{-1}. High concentrations (50, 100 g l^{-1}) of maltodextrin gave lower gluco-oligosaccharide yields.

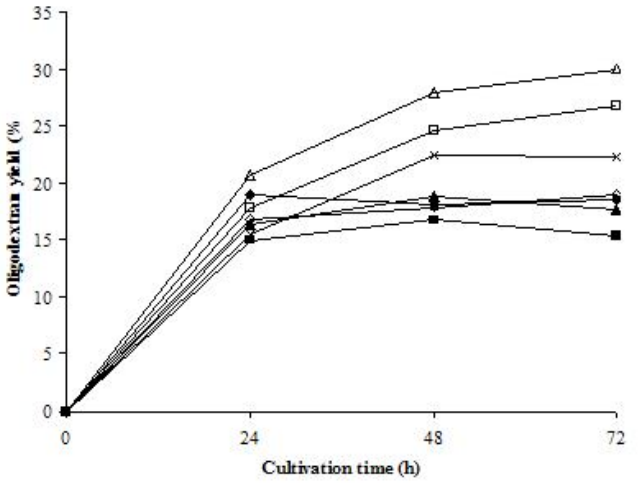

Figure 2. Effect of substrate on gluco-oligosaccharide yields from *G. oxydans* NCIMB 4943 cultivated by shake-flask batch culture (200 rev min^{-1}, 10 g l^{-1} maltodextrin, 30°C) using the culture medium method. ◆, Glucidex12; ■, Goldex 15; ▲, Glucidex19; ◇, Goldex20; □, Glucidex29; △, Goldex30; ×, Goldex37.

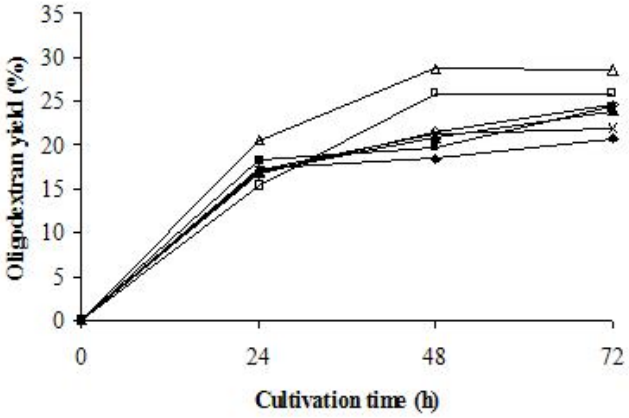

Figure 3. Effect of substrate on gluco-oligosaccharide yields from *G. oxydans* NCIMB 4943 cultivated by shake-flask batch culture (200 rev min^{-1}, 10 g l^{-1} maltodextrin, 5 g l^{-1} *G. oxydans* cells, buffer pH 4.5, 30°C) using the cell suspension method. ◆, Glucidex12; ■, Goldex15; ▲, Glucidex 19; ◇, Goldex20; □, Glucidex29; △, Goldex30; ×, Goldex37.

3.5 Molecular weight distribution of gluco-oligosaccharides

The profiles of molecular weight distribution varied as cultivation time increased. The maltodextrin substrates contained large amounts of low molecular weight material and this fraction was reduced dramatically as cultivation time increased with the concomitant increase of the high molecular weight fraction (Figure 4). The molecular weight distribution of gluco-oligosaccharides and Glucidex 19 substrate using the cell suspension method displayed a similar pattern to those obtained using the maltodextrin complex medium (Figure 5) although the reaction rates were lower in the cell suspension method.

The reaction mixtures had two fractions. Results showed that low molecular weight fraction which remained from inconvertible maltodextrin substrate had a molecular weight of approximately 1 kDa and the high molecular weight gluco-oligosaccharide-containing fraction had a molecular weight of 7.8-65.6 kDa. Gluco-oligosaccharides produced by either the culture method or the cell suspension method had exactly the same molecular weight (7.8-65.6 kDa).

3.6 Structural analysis

Gluco-oligosaccharide products from several maltodextrins made using the culture medium method consisted of α-1,4, α-1,6 and α-1,4,6-D-glucosidic linkages (Table 1). Use of Glucidex 19 as a substrate resulted in a product which contained α-1,6-D-glucosidic linkages at 3.99 mol mol^{-1} of terminal-D-glucose. This substrate was also suitable for gluco-oligosaccharide production using the cell suspension method, although the ratio of α-1,6-D-glucosidic linkage was lower (Table 2).

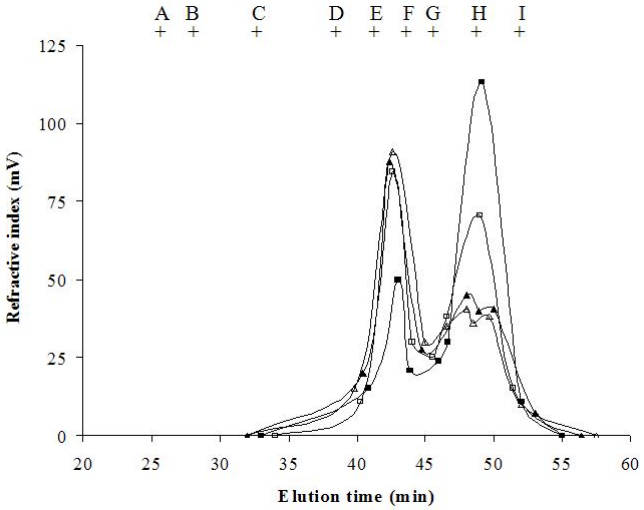

Figure 4. Molecular weight distributions of products formed by *G. oxydans* NCIMB 4943 cultivated in maltodextrin complex medium by shake-flask batch culture (200 rev min^{-1}, 10 g l^{-1} Glucidex 19, 30°C) using the culture medium method at 0 (■), 24 (□), 48 (▲), 72 (△) h cultivation.
Crosses (+) labeled with the letter A, B,…, I; represent the elution time at peak height of the standards used to calibrate the chromatographic system. A: dextran 38000 kDa for V_0; B: dextran 2000 kDa; C: dextran 464 kDa; D: dextran 68.4 kDa; E: dextran 42 kDa; F: dextran 19.5 kDa; G: dextran 9.5 kDa; H: maltoheptaose 1.15 kDa; I: glucose 0.18 kDa.

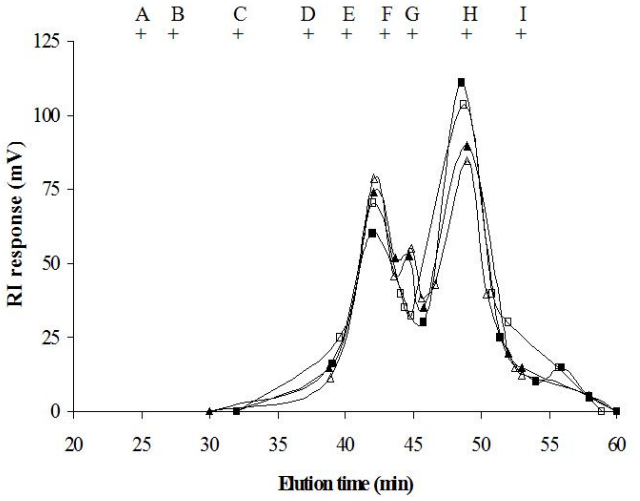

Figure 5. Molecular weight distributions of products formed by *G. oxydans* NCIMB 4943 cultivated by shake-flask batch culture (200 rev min^{-1}, 10 g l^{-1} Glucidex 19, 5 g l^{-1} *G. oxydans* cells, pH buffer 4.5, 30C) using the cell suspension method at 0 (■), 24 (□), 48 (▲), 72 (△) h cultivation.
Crosses (+) labeled with the letter A, B,…, I; represent the elution time at peak height of the standards used to calibrate the chromatographic system. A: dextran 38000 kDa for V_0; B: dextran 2000 kDa; C: dextran 464 kDa; D: dextran 68.4 kDa; E: dextran 42 kDa; F: dextran 19.5 kDa; G: dextran 9.5 kDa; H: maltoheptaose 1.15 kDa; I: glucose 0.18 kDa.

Table 1. Effect of maltodextrin type on the molar ratio of linkage residues of gluco-oligosaccharide from *G. oxydans* NCIMB 4943 cultivated in maltodextrin complex medium using the culture medium method, as determined by methylation analysis.

Linkage type	Maltodextrin (DE)				
	11-14	14-16	18-20	28-31	36-38
t-Glc	1.00	1.00	1.00	1.00	1.00
1,6	2.96	3.29	3.99	2.51	1.43
1,4	4.27	3.28	3.40	2.17	1.48
1,4,6	0.73	0.68	0.93	0.59	0.31

t-Glc: terminal glucose

Table 2. Effect of maltodextrin type on the molar ratio of linkage residues of gluco-oligosaccharide from *G. oxydans* NCIMB 4943 using the cell suspension method, as determined by methylation analysis.

Linkage type	Maltodextrin (DE)				
	11-14	14-16	18-20	28-31	36-38
t-Glc	1.00	1.00	1.00	1.00	1.00
1,6	2.73	3.26	3.76	2.45	1.37
1,4	4.36	3.87	3.49	2.23	1.53
1,4,6	0.65	0.65	0.77	0.53	0.29

t-Glc: terminal glucose

Table 3. Effect of maltodextrin type on the molar ratio of linkage residues of gluco-oligosaccharide from *G. oxydans* NCIMB 4943 using the culture medium method as determined by ^{1}H-NMR.

Linkage Type	Goldex20		Glucidex19	
	t=0	t=48	t=0	t=48
t-Glc	1.00	1.00	1.00	1.00
1,6	0	44.91	0	48.60
1,4	6.73	40.57	7.39	43.83

t-Glc: terminal glucose

The results of methylation analysis were confirmed by ^{1}H-NMR. Glucidex 19 and Glucidex 20 were found to contain mostly α-1,4-D-glucosidic linkages and had an average degree of polymerization (DP) of 6-8 glucose residues. Gluco-oligosaccharides obtained from both maltodextrins had similar linkage ratios, however, the gluco-oligosaccharide derived from Glucidex 19 had slightly higher content of α-1,6-D-glucosidic residues (Table 3). The ratios of α-1,4- and α-1,6- were similar to those obtained by methylation analysis. The chemical structure of gluco-oligosaccharide elucidated by NMR was proposed to contain approximately 43 α-1,4-D-glucosidic residues and more than 45 α-1,6-D-glucosidic residues. This means the gluco-oligosaccharide had at least 88 glucose residues of both linkage types.

4. Discussion

The gluco-oligosaccharides from *Gluconobacter oxydans* NCIMB 4943 have recently been shown to be candidate prebiotic (Wichienchot *et al*., 2006a; Wichienchot *et al*., 2006b). Moreover, some strains of this microorganism have been used for vitamin C and dihydroxyacetone production (Mishra *et al*., 2008; Schleyer *et al*., 2008). In this study, a culture-based method and a cell suspension method gave gluco-oligosaccharide yields ranged from 24.67-30.41% and 19.64-24.57%, respectively, compared to dextran yields of 1.21-41.69% reported by Mountzouris *et al*. (1999) using a cell suspension method.

Gluco-oligosaccharide production reached higher yield when *G. oxydans* NCIMB 4943 was grown in maltodextrin complex medium suggesting that DDase activity was correlated with growth of cell biomass. However, DDase expression was found to be non-growth associated in a previous study Mountzouris *et al*. (1999).

Using purified DDase, dextran yields on maltotriose, maltotritol, maltotetraose, maltotetritol, maltopentaose, maltohexaose, short chain amylase and soluble starch were 11.0, 22.4, 13.4, 36.8, 25.0, 30.2, 57.6 and 21.4%, respectively (Yamamoto *et al*., 1992; Yamamoto *et al*., 1993b). This showed that dextran yield was largely dependant on chain length of the substrate. The linkage composition of the dextran was, however, largely independent of substrate chain length and contained an average of 83 mol% α1,6-, 8 mol% α1,4-, 6 mol% terminal-glucose and 3 mol% α-1,4,6-D-glucose linkages (Yamamoto *et al*., 1994). Gluco-oligosaccharide produced in this study contained 48% α1,6-, 40% α1,4- and 11% α-1,4,6-D-glucose linkages, consistent with the use of maltodextrin substrate with a DP higher than 28 (Sims *et al*., 2001).

NMR analysis confirmed the linkage composition in the gluco-oligosaccharide products. In addition, NMR analysis showed that the gluco-oligosaccharide had a DP of at least 88 glucose residues, almost half of which were linked by α-1,4-linkages and half by α-1,6-D- linkages.

This study showed that low DE (high DP) maltodextrin had lower conversion rates to gluco-oligosaccharide compared to low DE maltodextrin. This result is consistent with the mode of action of DDase which catalyses the transglucosylation non-reducing terminal residue of a donor substrate to an acceptor (Yamamoto *et al*., 1993a). Long glucose chains have lower concentrations of non-reducing terminal residues than an equivalent mass of short glucose chains.

Gluco-oligosaccharide yield and the ratio of 1-4 to 1-6 linkages largely depended on substrate type and concentration, cell concentration and temperature. The molecular

weight was 7.8-65.6 kDa, similar to that reported by Mountzouris *et al*. (1999) of 6.6-38 kDa. However, the product contained both fractions of gluco-oligosaccharide and maltodextrin substrate and these cannot be separated using HPSEC. Thus, the product contained a mixture of gluco-oligosaccharide and maltodextrin substrate, which is the likely source of α-1,4 linkages in the product.

Acknowledgements

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