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# Andean Yacon Root (*Smallanthus sonchifolius* Poepp. Endl) Fructooligosaccharides as a Potential Novel Source of Prebiotics

ROMINA PEDRESCHI,† DAVID CAMPOS,‡ GIULIANA NORATTO,‡ ROSANA CHIRINOS,‡ AND LUIS CISNEROS-ZEVALLOS*,†

Department of Horticultural Sciences, Texas A&M University, College Station, Texas 77843, and Instituto de Biotecnologia (IBT), Universidad Nacional Agraria−La Molina, Lima 12, Peru

The ability of three known probiotic strains (two lactobacilli and one bifidobacterium) to ferment fructooligosaccharides (FOS) from yacon roots (*Smallanthus sonchifolius* Poepp. Endl) was compared to commercial FOS in this study. Results indicate that *Lactobacillus acidophilus* NRRL-1910, *Lactobacillus plantarum* NRRL B-4496, and *Bifidobacterium bifidum* ATCC 15696 were able to ferment yacon root FOS. FOS consumption apparently depended on the degree of polymerization and the initial FOS composition. *L. plantarum* NRRL B-4496 and *L. acidophilus* NRRL B-1910 completely utilized 1-kestose molecules, while *B. bifidum* was able to utilize 1-kestose molecules as well as molecules with a higher degree of polymerization.

**KEYWORDS: Yacon roots; Fructooligosaccharides; prebiotics; *Lactobacillus*; *Bifidobacterium***

## INTRODUCTION

Yacon (*Smallanthus sonchifolius* Poepp. Endl) is a root crop native to the Andean region of South America, grown mainly in Peru and Bolivia in an altitude range of 1000−3200 m above sea level (*1*). The root has a sweet taste and crisp texture and is consumed raw, boiled, baked, or used for making a refreshing drink (*2*). There have been recent studies in relation to its health-promoting properties such as the characterization of its antioxidant activity associated with the phenolic compounds (*3*, *4*) and the lowering of blood glucose levels, which is related to the fructooligosaccharides (FOS) present (*5*). These yacon FOS are of a low degree of polymerization (DP) and are present in high amounts in the tissue (∼60 to 70%, dry basis) (*6*−*9*).

In general, FOS may decrease blood glucose levels and different health-related serum lipids in humans and animal models (*10*−*13*) and are considered to work as prebiotics by improving the intestinal microflora balance and by promoting the growth of probiotic organisms (*14*, *15*). Prebiotics are defined as nondigestible food ingredients that beneficially affect the host selectively by stimulating the growth and activity of a limited number of bacteria in the bowel and improving the health of the host (*14*, *15*). FOS fulfill all of the classification criteria as prebiotics (*14*). For example, it should be neither hydrolyzed nor absorbed in the upper part of the gastrointestinal tract; it must be selectively fermented by one or a limited number of potentially beneficial bacteria in the colon; it should alter the composition of the colonic microbiota toward a healthier composition; and it must preferably induce effects that are beneficial to the host health (*14*). FOS can be prepared by fructan partial hydrolysis from vegetable sources or enzymatic synthesis from sucrose solutions. Depending on the chain length or DP, fructans are named FOS (DP < 9, average DP = 4.8) or inulin (DP up to 60, average DP = 12) (*16*). Both polymers are composed of $\beta$-D-fructafuranoses (F) joined by a $\beta$ (2→1) bond. A glucose molecule (G) is present at the end of the fructose chain joined by an $\alpha$ (1,2) bond, as in the case of a sucrose molecule (*17*).

The human intestinal microflora are composed of more than 400 species (*18*, *19*); the colon is the gastrointestinal part with the highest microbial population (∼$10^{10}$ to $10^{12}$ cfu/g). Lactobacilli constitute only 0.07−1% of the bowel bacteria population (*19*) while bifidobacteria constitute 25−30% of the total population of the bowel (*20*−*22*). Some strains of bifidobacteria and lactobacilli are considered important probiotics for human health (*23*−*26*). Probiotics are defined as live microorganisms added to the diet that benefit the development of the colon microbiota. The reported benefits include inmunopotentiation, competition with putrefactive and pathogenic bacteria (bacteroids, *Escherichia coli*, and anaerobic cocci) for nutrients and attachment sites, and production of volatile fatty acids that provide metabolic energy for the host, acidification of the bowel, and growth inhibition of many pathogens (*27*, *28*). For all of these reasons, there is a growing interest in determining different ways to increase the number of these bacteria in the bowel (*29*). The use of FOS as prebiotics is one method to achieve this. For example, Gibson and Roberfroid (*14*) demonstrated that FOS are selectively fermented by most strains of bifidobacteria. Even more, when these strains grow in FOS, bacteroids, clostridia,

* To whom correspondence should be addressed. Tel: (979)845-3244. Fax: (979)845-0627. E-mail: lcisnero@taexgw.tamu.edu.
† Texas A&M University.
‡ Universidad Nacional Agraria−La Molina.

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and coliforms are inhibited (*29*−*32*). Other studies have shown that some strains of lactobacilli are able to ferment FOS as well (*27*, *33*, *34*). To our knowledge, there have not been studies of yacon FOS fermentation. Because yacon roots are a rich source of FOS, there is the potential to use them to stimulate the growth of health-promoting bacteria and to be a potential novel source of prebiotic nutraceuticals for the food and dietary supplement industry. Yacon roots may be an alternative to more traditional FOS sources such as Jerusalem artichokes and chicory roots.

In this investigation, we hypothesized that yacon FOS have the potential to be fermented by probiotic bifidobacteria and lactobacilli species. In our research approach, we characterized the fermentative use of yacon FOS by three selected probiotic strains and determined the chain length or DP of the FOS being consumed.

## MATERIALS AND METHODS

**Carbohydrate Sources.** Yacon extracts were obtained from the Instituto de Biotecnologia, Universidad Nacional Agraria−La Molina (Peru) with the following composition: 6% moisture, 11.3% reducing sugars (dry basis), 11% sucrose (dry basis), 63.7% FOS (dry basis), and 6.9% ash matter (dry basis). Neosugar (Nutraflora) was generously given by GTC Nutrition Company (Colorado) with the following composition: $GF_2$ (33.4%), $GF_3$ (54.6%), $GF_4$ (10.4%), glucose plus fructose (0.8%), and sucrose (0.8%). Glucose was purchased from Merck (Darmstadt, Germany).

**Strains and Culture Conditions.** The strains of *Lactobacillus plantarum* NRRL-B-4496 isolated from a fermented cucumber and *Lactobacillus acidophilus* NRRL-B-1910 isolated from human bowel were obtained from the ARS Culture Collection (NRRL). The strain of *Bifidobacterium bifidum* ATCC 15696 isolated from human bowel was a gift from the Bioindustry Unity of Gembloux University (Belgium). These strains were selected since they are widely used as probiotics and most of them are naturally present in the colon (*30*, *31*). *Lactobacillus* strains were reactivated in MRS medium containing 1% glucose while *B. bifidum* was reactivated in RCM medium with 1% glucose. The precultures were done at 37 °C during 18 h under agitation. Nitrogen was injected for 20 min in *B. bifidum* precultures to ensure anaerobic conditions. All preculture strains had $10^6$ cfu/mL measured by using a Neubauer chamber (MediaTech Cellgro, Herdon, Virginia). Precultures were added to the culture medium to a final concentration of 5% v/v and $5 \times 10^4$ cfu/mL (absorbance $\sim$ 0.1).

High oxygen cultures were done in 250 mL Erlenmeyer flasks containing 100 mL of culture medium while low oxygen cultures were done in 12 mL flasks containing 10 mL of culture medium. Both types of culture conditions were only used for *Lactobacillus* strains. Anaerobic cultures were done in 100 mL penicillin flasks containing 80 mL of culture medium. Nitrogen was injected for 20 min. The culture media contained 1% of commercial FOS, and the culture media with yacon extract contained $\sim$1% carbon source (equivalent to $\sim$1.27% yacon extract), while the controls contained either 0% carbon source or 1% glucose. Media with *Lactobacillus* strains and *B. bifidum* were incubated for 24 and 48 h, respectively, at 37 °C under constant agitation. The incubation times were selected previously based on the maximum growth reached by each microorganism and the observation of no further carbohydrate use.

Bacterial growth was measured by optical density (OD), defined as absorbance readings times the dilution of the culture medium. Absorbance readings were done at 600 nm using a Genesys-5 UV−vis spectrophotometer (Milton Roy, New York) in the range of 0−0.9.

**Fermentation of Carbohydrates.** The use of carbohydrate sources by the selected probiotics was evaluated by measuring changes in the pH of the medium (*35*) using a digital Hanna Instruments model 8417 pH meter (Weiheim, Germany) and by determining changes in the media bacterial mass or growth (*36*). Additionally, the amount of FOS consumed in the culture media was determined as well as the chain length or DP of the FOS being consumed.

*Amount of FOS Consumed in the Culture Media.* FOS was determined by the method adapted from Jaime et al. (*37*). This method is based on an enzymatic hydrolysis of the sample with inulinase (Novo Nordisk Ferment Ltd., Bagsvaerd, Denmark). A 0.1 mL inulinase solution (enzyme activity 330 units/g inulin) was added to 0.9 mL of sample. The solution was mixed and incubated at 60 °C for 30 min, and the total glucose and fructose released was determined with the Bioquant enzymatic kit (Merck). The initial amounts of free glucose, fructose, and sucrose in the samples were previously determined, and the amounts obtained were subtracted from the sugars released during FOS determination by the enzymatic hydrolysis.

The initial amount of free glucose and fructose was determined by using the same enzymatic kit, while the initial free sucrose was done using an adaptation of the AOAC method (*38*). This method is based on an enzymatic treatment with invertase (Novo Nordisk Ferment Ltd.). A 0.25 mL invertase solution of 20 U enzyme activity (1 U was equivalent to 1 $\mu$mol of reducing sugars released at 60 °C, pH 4.5, per min) was added to 0.25 mL of sample. The solution was mixed and incubated at 60 °C for 20 min. The amount of glucose plus fructose was determined with the Bioquant enzymatic kit, and the water loss during hydrolysis was corrected by a 0.95 factor.

The concentration of total FOS and/or inulin was calculated according to the method of Prosky and Hoebregs (*39*):

$$G = G_t - S/1.9 - G_f$$

$$F = F_t - S/1.9 - F_f$$

where G = glucose from FOS or inulin, $G_t$ = total glucose, F = fructose from FOS or inulin, $F_t$ = total fructose, S/1.9 = glucose or fructose from sucrose, $G_f$ = initial free glucose, and $F_f$ = initial free fructose. The total FOS or inulin content is the sum of G and F and corrected for the water loss during hydrolysis. Thus

$$\text{total FOS or inulin} = k(G + F)$$

where $k = 0.925$ (for FOS, average DP = 4) and $k = 0.91$ (for inulin, average DP = 10).

*FOS Chain Length or DP.* To determine the DP of the FOS being consumed in the culture media, we initially separated FOS using gel filtration chromatography (*9*) and identified the corresponding peaks obtained using thin-layer chromatography (TLC) (*40*).

The separation of FOS by gel filtration chromatography was done using Biogel P-2 (Bio-Rad, California), which was rehydrated in distilled water at room temperature during 24 h. The 210 cm × 1.2 cm column was filled, maintaining a 0.22 mL/min flow with 20 mM phosphate buffer at pH 7.0. After passing 3 volumes of buffer, the column was ready for the separation of the FOS, which was performed by injecting 0.5 mL of sample. The elution was carried out with 20 mM phosphate buffer at pH 7.0 maintaining a constant 0.1 mL/min flow with a Masterflex L/S peristaltic pump (Cole Parmer Instrument Co., Illinois). A total of 180 fractions of 1 mL were collected with a fraction collector. Then, each fraction was analyzed in order to determine the presence of FOS. Because FOS do not absorb at a specific wavelength, FOS analysis was done by acid hydrolysis with HCl at 100 °C during 5 min to release the reducing sugars (fructose and glucose). The amount of FOS is directly proportional to the amount of reducing sugars. The sugars released were determined by absorbance readings at 550 nm using Miller's method (*41*).

The identification of the corresponding FOS peaks was performed by TLC. A small amount (5 $\mu$L) of sample was applied to the silica gel 60 $F_{254}$ plates (Merck). An ascending two development chromatography was performed using propanol:water (85:15 v/v) as the solvent and diphenylamine−aniline−phosphate as the revealing agent. Diphenylamine and phosphoric acid were purchased from Riedel de Häen (Seelze, Germany), and aniline was purchased from Carbo Erba (Milan, Italy). The plates were submerged in the revealing solution in order to identify the FOS according to Toba and Adachi (*40*). Results were compared with standards of glucose, fructose, sucrose, and commercial FOS (Nutraflora) consisting of 1-kestose ($GF_2$), nystose ($GF_3$), and $1^F$-fructofuranosyl nystose ($GF_4$).

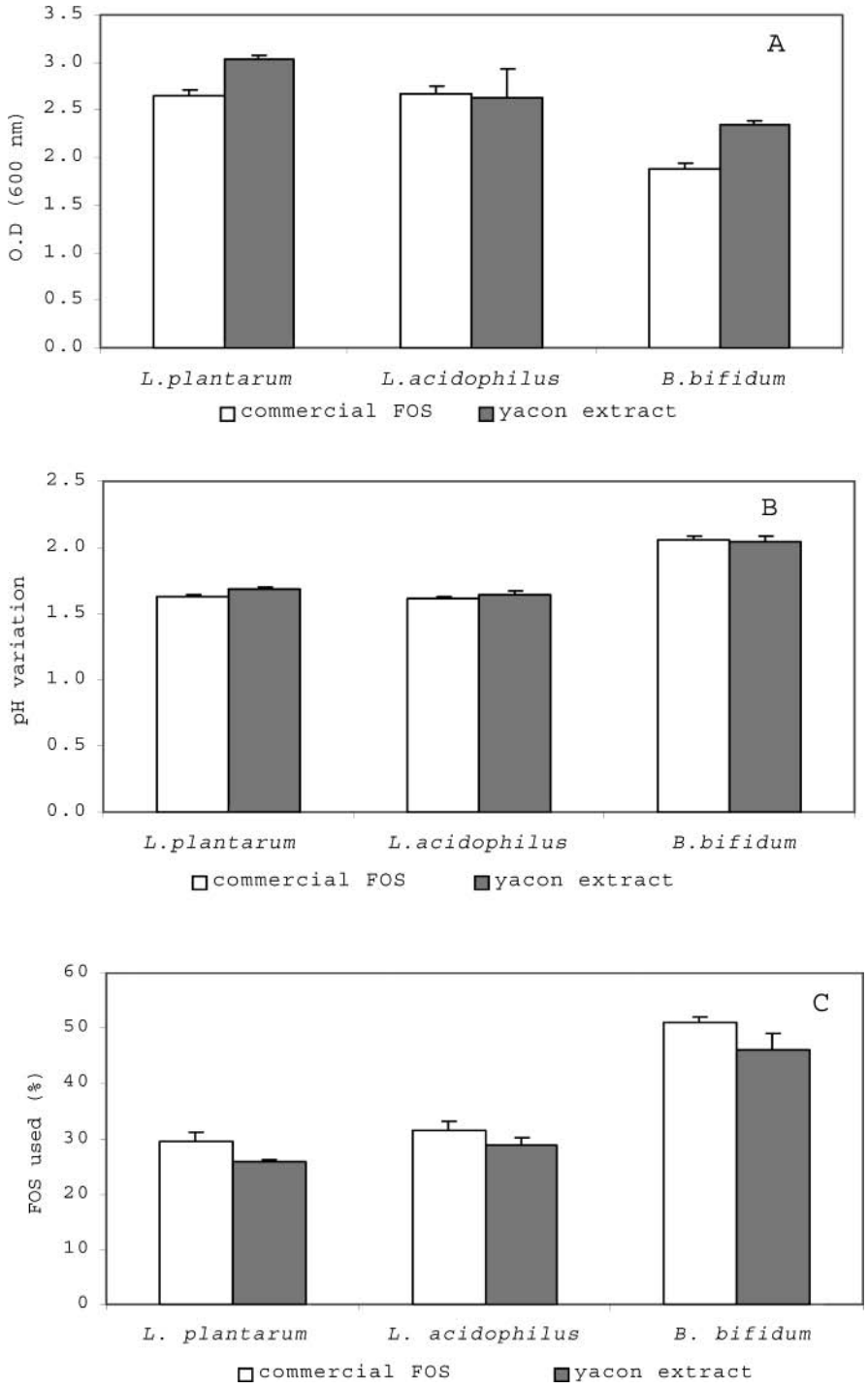


**Figure 1.** Use of commercial FOS and yacon extract by *L. plantarum* NRRL B-4496, *L. acidophilus* NRRL B-1910, and *B. bifidum* ATCC 15696 under anaerobic conditions. (A) Growth expressed as OD values, (B) pH decrease, and (C) FOS used (%) by lactobacilli and *B. bifidum* after 24 and 48 h, respectively. The line on top of the vertical bar represents one-sided standard deviation ($n = 6$).

In general, all experiments were repeated twice with similar results. All treatments were replicated three times within each experiment, and means and standard errors were calculated from pooled data.

## RESULTS AND DISCUSSION

**Fermentation of Yacon FOS by Bifidobacterium and Lactobacillus Probiotics.** *L. plantarum* NRRL B-4496, *L. acidophilus* NRRL B-1910, and *B. bifidum* were cultured on commercial FOS and yacon extract under anaerobic conditions. These conditions simulate the bowel environment and are preferred by intestinal bacteria for their growth (*42*, *43*). The fermentation of FOS was evaluated by measuring microbial growth (changes in OD), pH, or acidification of the media and by determining the amount of FOS consumed. Previous studies have used either one or a combination of these tools to characterize FOS fermentation (*32*, *34*, *44*).

Culture media with both *Lactobacillus* strains and *B. bifidum* showed increased OD after 24 and 48 h, respectively, indicating microbial growth and suggesting utilization of the FOS carbon source (**Figure 1A**). In general, the increase in OD for each type of probiotic was very similar for both commercial FOS and the yacon extract. In relation to the pH medium measurements, it is considered that a compound is fermented when there is a pH decrease ($\Delta pH > 1$), with respect to the noninoculated

**Table 1.** Comparative Use of FOS Sources by *Lactobacillus* Strains under Different Oxygen Conditions after a 24 h Incubation at 37 °C

| | | commercial FOS[a] | | | yacon extract[b] | | |
|---|---|---|---|---|---|---|---|
| strain | oxygen tension | $OD_{600\ nm}$ | pH | FOS used (%) | $OD_{600\ nm}$ | pH | FOS used (%) |
| *L. acidophilus* NRRL B-1910 | high oxygen | NG[c] | 6.31 ± 0.01 | 0.00 | NG | 6.23 ± 0.01 | 0.00 |
| | low oxygen | 0.69 ± 0.06 | 5.26 ± 0.01 | 20.93 ± 0.30 | 1.00 ± 0.02 | 5.00 ± 0.02 | 9.27 ± 0.26 |
| | anaerobic | 2.67 ± 0.07 | 4.89 ± 0.02 | 31.43 ± 1.72 | 2.64 ± 0.29 | 4.85 ± 0.02 | 28.91 ± 1.17 |
| *L. plantarum* NRRL B-4496 | high oxygen | 2.41 ± 0.05 | 4.96 ± 0.02 | 44.05 ± 2.80 | 1.74 ± 0.03 | 4.94 ± 0.05 | 31.80 ± 0.77 |
| | low oxygen | 2.34 ± 0.13 | 4.91 ± 0.04 | 37.30 ± 0.79 | 1.63 ± 0.10 | 4.90 ± 0.01 | 18.87 ± 1.14 |
| | anaerobic | 2.64 ± 0.05 | 4.88 ± 0.01 | 29.45 ± 1.67 | 3.03 ± 0.04 | 4.83 ± 0.01 | 25.93 ± 0.39 |

[a] From $GF_2$ to $GF_4$. [b] From $GF_2$ to $GF_{n>4}$. The ± indicates standard deviations for $n = 6$. [c] NG is nonsignificant growth.

medium (pH ∼ 6.5) (*35*). Results indicated that all three probiotic culture media had a ΔpH > 1, with *B. bifidum* showing a larger decrease in pH (ΔpH ∼ 2.0) as compared to both lactobacilli (ΔpH ∼ 1.5) (**Figure 1B**). Control samples with only inoculum and control samples with inoculum and glucose showed culture media with ΔpH < 0.7 and ΔpH > 1.6, respectively, for all strains tested. In general, the observed ΔpH media for each type of probiotic was similar for both commercial FOS and the yacon extract. The larger decrease in pH for *B. bifidum* suggests that this strain is utilizing more carbon source than both *Lactobacillus* strains.

The analysis of the amount of FOS consumed confirms that *B. bifidum* and *Lactobacillus* probiotics utilize yacon FOS by anaerobic fermentation (**Figure 1C**). The *B. bifidum* ATCC 15696 strain utilized higher amounts of yacon FOS than both lactobacilli strains. Both commercial FOS and yacon FOS seemed to be consumed similarly by each type of probiotic strain. These results suggest that yacon FOS have potential prebiotic properties and can be utilized by probiotic *Bifidobacterium* and *Lactobacillus* species.

*Yacon FOS Utilized by Lactobacillus Probiotics under Oxygen Tension.* The ability of probiotics to withstand the presence of oxygen tension may be a desired characteristic if these strains will be incorporated alone or mixed with prebiotics into processed products (e.g., dairy products). We characterized the ability of *L. plantarum* NRRL B-4496 and *L. acidophilus* NRRL B-1910 to grow in vitro in MRS media containing yacon extract and commercial FOS under high and low oxygen conditions. Results indicated that *L. plantarum* NRRL B-4496 was able to ferment yacon extract in both high and low oxygen conditions (ΔpH > 1.5). However, *L. acidophilus* NRRL B-1910 was only able to ferment commercial FOS and yacon extract under low oxygen conditions (ΔpH ∼ 1.5) and not under high oxygen conditions (ΔpH < 0.5), suggesting that this strain is sensitive to high oxygen tensions (**Table 1**). In all cases, media with yacon extract showed similar ΔpH to culture media with commercial FOS. These results were also confirmed by measuring OD values. For yacon extracts and commercial FOS, *L. plantarum* NRRL B-4496 had increased microbial growth (OD > 1.6) at both oxygen tensions, while for *L. acidophilus* NRRL B-1910 (OD ≤ 1) microbial growth only occurred at low oxygen conditions.

The yacon FOS consumed under high and low oxygen conditions by *L. plantarum* NRRL B-4496 ranged from ∼18 to 31%, while for *L. acidophilus* NRRL B-1910 the FOS consumed ranged from 0 to 9%. These numbers were higher when the carbon source was commercial FOS, suggesting that lactobacilli efficiently utilized commercial FOS under oxygen tension as compared to yacon FOS. In general, FOS consumption will be different under aerobic or anaerobic conditions and will depend on the *Lactobacillus* strain. For example, for *L. plantarum* NRRL B-4496, aerobic consumption > anaerobic consumption while for *L. acidophilus* NRRL B-1910, anaerobic consumption > aerobic consumption (**Table 1**).

Our results suggest that *L. plantarum* NRRL B-4496 may be mixed with yacon FOS in a carrier processed product under high and low oxygen tensions and still maintain viable probiotics as well as yacon FOS available for its use in the bowel. On the other hand, *L. acidophilus* NRRL B-1910 may be mixed with yacon FOS under low oxygen tensions.

**Yacon FOS Chain Length and Probiotic Selective Consumption.** Previous studies have shown that different probiotic strains utilize FOS according to chain length or DP (*32*, *34*). Thus, the overall FOS composition will define how efficiently a FOS source will be used as a prebiotic. The commercial FOS used contains $GF_2$, $GF_3$, and $GF_4$ molecules while yacon root is known to contain $GF_2$ to $GF_9$ molecules (*44*). The efficiency of yacon root as a FOS source was determined by measuring the selective consumption of the yacon extract FOS composition using gel filtration chromatography and TLC for identification of the individual FOS constituents. According to the standards used, our results indicated that yacon extracts contained $GF_2$, $GF_3$, $GF_4$, and $GF_{n>4}$ molecules, as well as glucose, fructose, and sucrose (**Figure 2A**).

After they were incubated for 24 h at 37 °C, *L. plantarum* NRRL B-4496 and *L. acidophilus* NRRL B-1910 completely utilized sucrose, reducing sugars, and the $GF_2$ molecules from yacon extract, while a small decrease in the peaks corresponding to $GF_3$, $GF_4$, and $GF_{n>4}$ molecules was observed (**Figure 2B**,**C**). These strains showed similar selective consumption behavior on commercial FOS (data not shown). It is known that the $GF_2$ molecules represent 34% of the commercial FOS composition (**Table 2**). Thus, the observed ∼30% of commercial FOS consumed by both *Lactobacillus* strains (**Figure 1C**) would correspond basically to $GF_2$ molecules. Similarly, because both *Lactobacillus* strains consumed ∼27% of yacon FOS (**Figure 1C**), this value would correspond to the amount of $GF_2$ molecules present in the yacon extract.

The *B. bifidum* strain incubated for 48 h at 37 °C completely utilized sucrose, reducing sugars, and the $GF_2$, $GF_4$, and $GF_{n>4}$ molecules from the yacon extract. There was also a small decrease in the peak corresponding to $GF_3$ molecules (**Figure 2D**). Again, we saw a similar selective consumption behavior on commercial FOS for this strain (data not shown). We know that $GF_2$ and $GF_4$ molecules represent 44.6% of the commercial FOS composition (**Table 2**). Because ∼51% of commercial FOS was consumed by *B. bifidum* (**Figure 1C**), this would mean that only 6.4% of the commercial FOS consumed by the strain corresponded to $GF_3$ molecules. Similarly, because *B. bifidum* consumed ∼46% of yacon FOS (**Figure 1C**), this value would correspond basically to the combined amounts of $GF_2$, $GF_4$, and $GF_{n>4}$ molecules in yacon extracts.

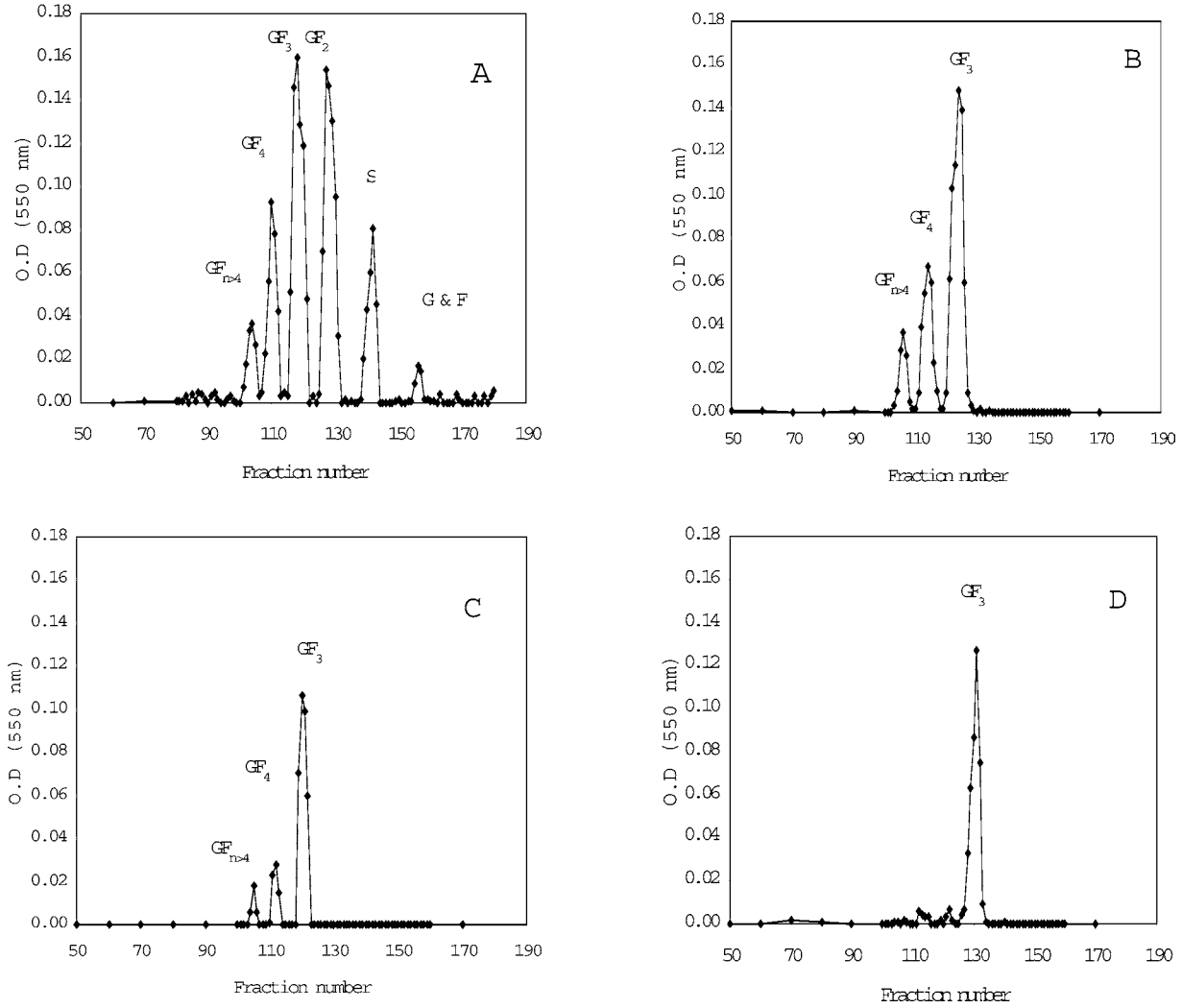


**Figure 2.** Gel filtration chromatography of (A) a 1.27% yacon extract noninoculated medium, (B) after a 24 h fermentation by *L. plantarum* NRRL B-4496, (C) after a 24 h fermentation by *L. acidophilus* NRRL B-1910, and (D) after a 48 h fermentation by *B. bifidum* ATCC 15696, under anaerobic conditions.

**Table 2.** Commercial FOS and Yacon Extract Composition (Weight %)

| type of FOS molecule | commercial FOS[a] | yacon extract[b] |
|---|---|---|
| $GF_2$ | 33.9 | 27 |
| $GF_3$ | 55.5 | 54 |
| $GF_4$ | 10.6 | |
| $GF_4$ and $GF_{n>4}$ | | 19 |

[a] On the basis of FOS composition provided by the manufacturer of Nutraflora Commercial FOS. [b] Estimated from the selective FOS consumption assay.

According to the results obtained from the selective consumption assay of yacon FOS by both probiotic genera, we infer the FOS composition to be ~27, 54, and 19% for $GF_2$, $GF_3$, and the combined $GF_4$ and $GF_{n>4}$ molecules, respectively. This FOS composition distribution follows a similar trend to the commercial FOS used (**Table 2**). However, for commercial FOS, the $GF_3$ molecules are present in higher amounts.

Several factors could explain the difference in FOS peak profiles observed from the selective consumption assays between *Lactobacillus* strains and *Bifidobacterium*. Most likely, the resulting peaks are due to the combined effects of the initial FOS composition (**Table 2**), the difference in consumption rate of the individual FOS chain length molecules, and the difference in the overall consumption rate of FOS between both types of probiotic genera.

For example, Perrin et al. (*32*) showed that the consumption rate of individual FOS molecules had the following descending order: $GF_2 > GF_3 > GF_4$ molecules. This would explain in part why in our assay $GF_2$ molecules disappear first, since there was an initial low content of these molecules. On the other hand, $GF_3$ still remained because there was an initial higher content of these molecules. For $GF_4$ and $GF_{n>4}$ molecules, there was a tendency to decrease or disappear and this would be associated with the initial low content of these molecules. This explanation was verified with a TLC assay, where the observed decrease in individual FOS chain length molecules through time was influenced by the initial FOS composition of the commercial FOS and the yacon extract. Additionally, the difference in the overall consumption rate of FOS between both types of probiotic genera would also play a role. For example, the observed higher consumption of FOS (**Figure 1C**) and of individual FOS chain length molecules (**Figure 2D**) by *B. bifidum* ATCC 15696 suggests that it has an overall higher rate of FOS consumption than *Lactobacillus* strains.

In general, our results indicate that *L. plantarum* NRRL B-4496, *L. acidophilus* NRRL B-1910, and *B. bifidum* ATCC 15696 efficiently utilized yacon FOS. Individual FOS chain length molecules and the FOS composition of the yacon extract apparently influence yacon FOS consumption. The *Lactobacillus* strains mostly utilized $GF_2$ molecules, while the *Bifidobacterium* strain utilized $GF_2$ molecules and also higher FOS chain length

molecules. Yacon FOS may be considered a potential novel source of prebiotics for the food and dietary supplement industry.

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**Supporting Information Available:** Thin-layer chromatography of 1% commercial FOS and 1.27% yacon extract during a 48 h fermentation by *B. bifidum* ATCC 15696 under anaerobic conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

## LITERATURE CITED

(1) Fernández, C. E.; Lipavska, H.; Milchl, J. Determination of saccharides content in different ecotypes of yacon (*Polymixia sonchifolia* poepp. and endlicher) cultivated under conditions of Czech Republic. *Agricultura Trópica et Subtrópica. Universitas Agriculturae Praga*. **1997**, *30*, 79−89.

(2) Anonymus. In *Lost Crops of the Incas. Little-Known Plants of the Andes with Promise for Worldwide Cultivation*; The National Academic Press: Washington, DC, 1989; pp 124−128.

(3) Yan, X.; Suzuki, M.; Ohnishi-Kameyama, M.; Sada, Y.; Nakanishi, T.; Nagata, T. Extraction and identification of antioxidants in the roots of yacon (*Smallanthus sonchifolius*). *J. Agric. Food Chem*. **1999**, *47*, 4711−4713.

(4) Takaneka, M.; Yan, X.; Ono, H.; Yoshida, M.; Nagata, T.; Nakanishi, T. Caffeic acid derivatives in the roots of yacon (*Smallanthus sonchifolius*). *J. Agric. Food Chem.* **2003**, *51*, 793−796.

(5) Mayta, P.; Payano, J.; Peláez, J.; Pérez, M.; Pichardo, L.; Puylán, L. *Efecto Hipoglicerimiante de la Raiz del Smallanthus sonchifolius en Adultos Jóvenes Clínicamente Sanos (Estudios Preliminares)* (text in spanish); Resumen II Simposio Latinoamericano de raíces y tubérculos; UNALM, CIP: Perú, 2001.

(6) Ohyama, T.; Ikarashi, T.; Baba, A. Determination of the structure of oligofructans in the tulip bulb. *Soil Sci. Plant Nutr*. **1985**, *31*, 293−295.

(7) Wei, B.; Hara, M.; Yamauchi, R.; Ueno, Y.; Kato, K. Fructooligosaccharides in the tubers of Jerusalem artichoke. *Res. Bull. Fac. Agric., Gifu Univ*. **1991**, *56*, 133−138.

(8) Goto, K.; Fukai, K.; Hikida, J.; Nanji, F.; Hara, Y. Isolation and structural analysis of oligosaccharides from yacon (*Polymnia sonchifolia*). *Biosci., Biotechnol., Biochem*. **1995**, *59*, 2346−2347.

(9) Nuñez, R.; Chirinos, R.; Campos, D.; Arbizú, C.; Cisneros-Zevallos, L. In *Caracterización de 10 Entradas y Evaluación de los Fructooligosacáridos de la Raíz de Yacón, Smallanthus sonchifolius, Durante el Almacenaje* (text in spanish); Resúmenes II Simposio Latinoamericano de raíces y tubérculos; UNALM, CIP: Peru, 2001.

(10) Yamashita, K.; Kawai, K.; Itakura, M. Effects of fructooligosaccharides on blood glucose and serum lipids in diabetic subjects. *Nutr. Res*. **1984**, *4*, 961−966.

(11) Fiordaliso, M.; Kok, N.; Desager, J. P.; Goethals, F.; Deboyser, D.; Roberfroid, M.; Delzenne, N. Dietary oligofructose lowers triglycerides, phospholipids and cholesterol in serum and very low-density lipoproteins of rats. *Lipids* **1995**, *30*, 163−167.

(12) Luo, J.; Riskalla, S. W.; Alanowitch, C.; Boussari, A.; Blayo, A.; Barry, J. L.; Laffitte, A.; Guyon, F.; Bornet, F. R.; Slama, G. Chronic consumption of short-chain fructooligosaccharides by healthy subjects decreased basal hepatic glucose production but had no effect on insulin-stimulated glucose metabolism. *Am. J. Clin. Nutr*. **1996**, *63*, 939−945.

(13) Kok, N.; Roberfroid, M.; Delzenne, N. Dietary oligofructose modifies the impact of fructose on hepatic triacylglycerol metabolism. *Metabolism* **1996**, *45*, 1547−1550.

(14) Gibson, G.; Roberfroid, M. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *J. Nutr*. **1995**, *125*, 1401−1412.

(15) Ruperez, P. Bifidogenic oligosaccharides. *Int. J. Food Sci. Technol*. **1998**, *4*, 237−243.

(16) Gibson, R. Dietary modulation of the human gut microbiota using the prebiotics oligofructose and inulin. *J. Nutr*. **1999**, *129*, 1438S−1441S.

(17) Niness, K. Inulin and oligofructose. What are they? *J. Nutr*. **1999**, *129*, 1402−1406.

(18) Brock, T.; Smith, D.; Madigan, M. *Microbiologia*, 4th ed.; Prentice Hall Hispanoamericana: Mexico, 1987.

(19) Balows, A.; Trüper, H.; Schlegel, H. *The Prokaryotes. A Handbook on the Biology of Bacteria, Physiology, Isolation, Identification and Application*, 2nd ed.; Springer-Verlag Editorial: New York, 1990; Chapter 70.

(20) Hartemink, R. Prebiotic effects of nondigestible oligo and polysaccharides. Thesis, Wageningen University, The Netherlands, 1999.

(21) Mitsuoka, T. Taxonomy and ecology of bifidobacteria. *Bifidobact. Microflora* **1984**, *3*, 11−28.

(22) Scardovi, V. Genus *Bifidobacterium. Bergey's Manual of Systematic Bacteriology*; New York, 1986; Vol. 2, pp 1418−1434.

(23) Chen, M.; Chen, K.; Lin, C. Optimization of the viability of probiotics in a new fermented milk drink by the genetic algorithms for response surface. *J. Food Sci*. **2003**, *68*, 632−638.

(24) Kurmann, J.; Rasic, J. The health potential of products containing bifidobacteria. In *Therapeutic Properties of Fermented Milks*; Robinson, R. K., Ed.; Elsevier Applied Science Publishers, Ltd.: London, England, 1991.

(25) Macfarlane, G.; Cummings, J. Probiotics and prebiotics: Can they regulate the activities of intestinal bacteria benefit health? *Br. Med. J*. **1999**, *318*, 999−1003.

(26) Modler, H.; McKellar, R.; Yaguchi, M. Bifidobacteria and bifidogenic factors. *Inst. Food Sci. Technol. J*. **1990**, *23*, 29−41.

(27) Sghir, A.; Chow, J. M.; Mackie, R. I. Continuous culture selection of bifidobacteria and lactobacilli from human fecal samples using fructooligosaccharides as selective substrate. *J. Appl. Microbiol*. **1998**, *85*, 769−777.

(28) Gmeiner, M.; Kneifel, W.; Kulbe, K. D.; Wouters, R.; De Boever, P.; Nollet, L.; Verstraete, W. Influence of a synbiotic mixture consisting of *Lactobacillus acidophilus* 74-2 and a fructooligosaccharide preparation on the microbial ecosystem (SHIME Reactor). *Appl. Microbiol. Biotechnol*. **2000**, *53*, 219−223.

(29) Hopkins, M. J.; Cumming, J. H.; Macfarlane, G. T. Inter-species differences in maximum specific growth rates and cell yields of bifidobacteria cultured on oligosaccharides and other simple carbohydrate sources. *J. Appl. Microbiol*. **1998**, *85*, 381−386.

(30) Sghir, A.; Chow, J.; Mackie, R. Continuous culture selection of bifidobacteria and lactobacilli from human faecal samples using fructooligosaccharides as selective substrate. *J. Appl. Microbiol*. **1998**, *85*, 769−777.

(31) Mc. Kellar, R. C.; Modler, H. W.; Mullin, J. Characterization of growth and inulinase production by *Bifidobacterium* spp. on fructooligosaccharides. *Bifidobact. Microflora* **1993**, *12*, 75−86.

(32) Perrin, S.; Warchol, J.; Grill, J. P.; Schneider, F. Fermentation of fructooligosaccharides and their components by *Bifidobacterium infantis* ATCC 15697 on batch culture in semi-synthetic medium. *J. Appl. Microbiol*. **2001**, *90*, 859−865.

(33) Kneifel, W.; Rajal, A.; Kulbe, K. D. In vitro growth behaviour of probiotic bacteria in culture media with carbohydrates of prebiotic importance. *Microb. Ecol., Health Dis*. **2000**, *12*, 27−34.

(34) Kaplan, H.; Hutkins, R. W. Fermentation of fructooligosaccharides by lactic acid bacteria and Bifidobacteria. *Appl. Environ. Microbiol*. **2000**, *66*, 2682−2684.

(35) Mazur, A. W.; Mohlenkamp, M. J.; Hiller, G. Digestability of selected carbohydrates by anaerobic bacteria. *J. Agric. Food Chem*. **1993**, *41*, 1925−1930.

(36) McKellar, R. C.; Modler, H. W. Metabolism of fructooligosaccharides by *Bifidobacterium* spp. *Appl. Microbiol. Biotechnol*. **1989**, *31*, 537−541.
(37) Jaime, L.; Martin, M. A.; Mollá, E.; López-Andréu, F.; Esteban, R. M. Effects of storage on fructan and fructooligosaccharide of onion (*Allium cepa* L.). *J. Agric. Food. Chem*. **2001**, *49*, 982−988.
(38) AOAC. Measurement of total fructans in foods. *Official Method 999.03*; AOAC: Gaithersburg, MD, 2000.
(39) Prosky, L.; Hoebregs, H. Methods to determine food inulin and oligofructose. *J. Nutr*. **1999**, *129*, 1418S−1423S.
(40) Toba, T.; Adachi, S. Specific colour reaction for the detection of 1,2 linked reducing disaccharides on paper and thin-layer chromatography. *J. Chromatogr. A* **1978**, *154*, 106−109.
(41) Miller, G. Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Anal. Chem*. **1959**, *31*, 426−428.
(42) Arihara, K.; Ota, H.; Kondo, I.; Sameshima, T.; Yamanaka, M.; Akimoto, M.; Kanai, S.; Miki, T. *Lactobacillus acidophilus* group lactic acid bacteria applied to meat fermentation. *J. Food Sci*. **1998**, *63*, 544−547.
(43) Rolfe, R.; Hentges, D.; Barrett, J.; Campbell, B. Oxygen tolerance of human intestinal anaerobes. *Am. J. Clin. Nutr*. **1977**, *30*, 1762−1769.
(44) Van Laere, K.; Hartemink, R.; Bosveld, M.; Schols, H.; Voragen, A. Fermentation of plant cell wall derived polysaccharides and their corresponding oligosaccharides by intestinal bacteria. *J. Agric. Food. Chem*. **2000**, *48*, 1644−1652.

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