Acemannan and Fructans from Aloe vera (Aloe barbadensis Miller) Plants as Novel Prebiotics

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ABSTRACT: The nutraceutical properties of Aloe vera have been attributed to a glucomannan known as acemannan. Recently information has been published about the presence of fructans in Aloe vera but there are no publications about acemannan and fructans as prebiotic compounds. This study investigated in vitro the prebiotic properties of these polysaccharides. Our results demonstrated that fructans from Aloe vera induced bacterial growth better than inulin (commercial FOS). Acemannan stimulated bacterial growth less than fructans, and as much as commercial FOS. Using qPCR to study the bacterial population of human feces fermented in a bioreactor simulating colon conditions, we found that fructans induce an increase in the population of Bifidobacterium spp. Fructans produced greater amounts of short chain fatty acids (SCFA), while the branched-chain fatty acids (BCFA) did not increase with these polysaccharides. Acemannan increased significantly acetate concentrations. Therefore, both Aloe vera polysaccharides have prebiotic potentials.

KEYWORDS: Aloe vera, fructans, prebiotics, bifidobacterium, qPCR, SCFA, BCFA

INTRODUCTION

According to Ajose, the World Health Organization states that 80% of the world population uses medicinal plants for the treatment of a large number of diseases. Many components derived from plants are currently used in drug preparations by pharmaceutical companies. One of the most used plants is Aloe barbadensis Miller, also known as Aloe vera, a crassulacean acid metabolism (CAM) plant. As a CAM plant, Aloe vera is adapted to arid and semiarid environments and is well-known for its potential health-promoting properties, such as immunostimulation and cell regeneration. These properties are mainly attributed to its mucilaginous leaf gel, which has pharmacological, cosmetic, and food industrial importance with great economic projections. This gel contains various polysaccharides, including an acetylated glucomannan known as acemannan, which is the most abundant polysaccharide. Mannose constitutes the backbone of the polysaccharide, which is only a recent publication on Aloe vera leaf gel as a prebiotic. The effects of prebiotic compounds have been widely described in the literature. Prebiotics promote the growth of beneficial bacterial populations such as Lactobacillus and Bifidobacterium species in the colon, accompanied by the production of short-chain fatty acids (SCFAs) through fermentation processes. These events have been associated with a lower risk of nontransmissible chronic diseases, including some types of cancer such as colorectal cancer.

The aim of this study was to determine the prebiotic potential of acemannan and fructans (alone and combined) isolated from water-stressed Aloe vera plants on the growth of beneficial colon bacteria in pure cultures. We also determined, using qPCR the effect of these polysaccharides on the bacterial populations of human feces and in the synthesis of SCFAs produced during fermentation of human feces.

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MATERIALS AND METHODS

Plant Material. All the experiments were performed with plants of Aloe barbadensis Miller (Aloe vera) cultivated in Parcela Piedra Redonda, located in La Higuera, Coquimbo Region, IV Region of Chile. Plants were irrigated at 25% field capacity, submitted to a severe water deficit. They were one year old when used for polysaccharide extraction. Mature leaves from four different plants were removed and stored at −80 °C.

Polysaccharide Extraction. For the extraction of polysaccharides, whole leaves were thawed at 4 °C and cleaned with a disinfectant solution of sodium hypochlorite (50 mg/L). It was necessary to process at least 10 leaves (obtained from 4 different plants) to get the amount of polysaccharides required for this study. The photosynthetic cortex of the leaf was separated from the internal pulp or gel corresponding to the parenchyma under a laminar flow hood using sterilized material. Once the parts were separated, fructans were extracted from the cortex and acemannan from the gel, using different extraction protocols.

Fructan Extraction and Depigmentation. Fructan extraction was carried out according to the methodology described by Cairns and Pollock,24 with modifications. Five grams of cortex were boiled with 10 mL 80% ethanol (v/v) for 5 min in a water bath. The tissue was homogenized in a UV pre-sterilized blender for 20–25 min, the homogenate was centrifuged at 5000 rpm for 5 min at room temperature and the supernatant withdrawn and saved. The pellet was resuspended in 80% ethanol, heated again at 70 °C under agitation for 5 min and centrifuged at 5000 rpm for 5 min. The supernatants were mixed and kept frozen at −20 °C. The pellet was then resuspended in 10 mL deionized water, heated at 60 °C for 15 min and centrifuged at 5000 rpm for 5 min. The supernatant was withdrawn and this step was repeated once. The supernatants were mixed and depigmented by adding 5% (w/v) activated carbon and maintaining the solution under agitation at room temperature for 15 h. Then the mix was centrifuged at 9000 rpm for 1 h at 5 °C and the supernatant filtered with Whatman N°1 and N°3 filters under vacuum. Celite 560 (calcined diatomaceous earth) from Sigma-Aldrich at 5% (w/v) was added to the filtrate and left under agitation at room temperature for 15 h. After centrifugation at 9000 rpm for 60 min at 5 °C, the supernatant was collected and kept frozen at −20 °C until used.

Acemannan Extraction and Depigmentation. The frozen gel was cut into small pieces of approximately 4 × 3 cm and placed in a solution of 0.5 M KCl under gentle stirring at room temperature for 4 h. The pigmented solution was discarded and the pieces of gel were homogenized in a blender (previously sterilized under UV light for 20–30 min) at maximum speed. The gel solution was centrifuged at 8000 rpm for 30 min at 4 °C and the supernatant collected and stored.

Figure 1. Polysaccharide structures from Aloe vera plants. (I) Acemannan. An acetyl group is highlighted inside a red line. (II) Fructans found in water-stressed plants of Aloe vera. The terminal sucrose (glucose + fructose) unit is shown in red.
at 4 °C. The pellet was resuspended in 50 mL distilled water and stirred for 3 h at room temperature. The solution was then centrifuged under the same conditions. The supernatants were mixed and deglycosylated by incubating with 5% Celite 560 (w/v) under agitation at room temperature for 15 h. The mixture was centrifuged at 9000 rpm for 1 h at 4 °C, the supernatant was withdrawn and mixed with activated carbon 5% (w/v) and left under agitation at room temperature for 15 h, then centrifuged at 9000 rpm for 60 min at 5 °C. The supernatant was recovered and filtered with Whatman N°1 and N°3 filters under vacuum. The Celite and filtration steps were repeated until the gel solution remained pigmented. Two volumes of 100% cold ethanol were added to the filtered acemannan solution and the mixture was incubated at −20 °C overnight. The solution was centrifuged at 9000 rpm at 5 °C for 1 h and the pellet was resuspended in deionized water (1:10, v/v).

The fructans and acemannan solutions were evaporated with a rotary evaporator to their minimum volume and lyophilized.

**Fructans and Acemannan Quantification.** Fructans were quantified using a modified anthrone method optimized to increase sensitivity to ketoses.25 Fructans were also analyzed and quantified by GC-MS analysis as has been previously described by Salinas et al.11 For this, sugars standards of fructose, arabinose, xylose, mannose, galactose, and glucose (from Merck and Sigma-Aldrich) were derivatized to alditol acetates and used to carry out the respective calibration curves, going from 0 to 600 μg. The sugar standards were prepared by serial dilutions ranging from 4.6667 μg/μL to 0.03733 μg/μL. Myo-inositol (50 μg) was added as an internal standard. The extracted polysaccharide was hydrolyzed to monosaccharides in 2N HCl for 60 min at 100 °C, and derivatized to per-O-acetylated alditols under the same conditions. The supernatants were mixed and stirred for 3 h at room temperature. The solution was then centrifuged at 10 000 rpm at 5 °C for 1 h. The supernatant was evaporated with a rotary evaporator under reduced pressure (3 g/L) from water-stressed Aloe vera plants. Acemannan was limited to 3 g/L because higher concentrations were insoluble. Inulin oligofructose, referred to as commercial FOS (10 g/L, Beneo P95, Orafti, Pemuco, Chile) was used as a positive control, instead of fructans from well-irrigated plants. This was due to the fact that fructan isolation and purification is very time-consuming. Besides, the commercial FOS has a 95% linear inulin structure, plus 4% fructose, and 1% sucrose+glucose.13 Growth curves for Lactobacillus species were performed in sterile 96-well microplates, in a Synergy HT microplate reader (BioTek Instruments, VT, USA) by automatically measuring changes in optical density at 600 nm (OD) every 30 min for 18 h. The plates were incubated at 37 °C. The growth curves for *Bifidobacterium* strains were performed for 72 h under anaerobic conditions (GENbox/GENbag, bioMérieux, Santiago, Chile), measuring the OD every 12 h with the Synergy HT, also incubated at 37 °C. All measurements were made in triplicate.

**In Vitro Fermentation of Aloe vera Polysaccharides with Human Fecal Bacteria.** Fresh stools from three healthy volunteers (23–26 years old; body mass index between 18 and 24.9 kg/m² and without previous intake of antibiotics, prebiotics or probiotics) were collected in sterile, plastic, hermetic recipients which were refrigerated at 4 °C under anaerobic conditions (GasPak, BD, Santiago, Chile) until their delivery to the laboratory. Five grams of stool were homogenized in reduced culture medium (10% w/v) with a stomacher and the suspension was added to the vessel of a bioreactor (B-Braun Scientific M200 PRO). The culture medium contained 5 g/L yeast extract (MObio), 10 g/L ascorbic acid, 10 g/L sodium acetate, 5 g/L (NH₄)₂SO₄, 3 g/L urea, 0.2 g/L MgSO₄, 0.01 g/L FeSO₄, 0.007 g/L MnSO₄, 0.01 g/L NaCl, 1 mL/L Tween 80, 0.035 g/L hemin, 0.5 g/L cysteine (all from Merck, Santiago, Chile), and the tested Aloe vera polysaccharides (10 g/L fructan or 3 g/L acemannan), pH 7.0.28 The suspension was incubated under a nitrogen atmosphere (anaerobic conditions) at 37 °C in the dark under constant stirring for 48 h. The pH of the cultured suspension was measured regularly. Samples were obtained at the end of the fermentation period and stored at −20 °C. The samples were measured in triplicate.

**Microbiota Quantification from Human Feces by qPCR.** From the stool samples incubated in the bioreactors of each individual, the bacterial genomic DNA was extracted using the commercial kit QIAamp DNA Stool Mini Kit, Qiagen, Hilden, Germany, following the manufacturer recommendations. The concentration of total DNA was determined by the absorbance at 260 nm in a spectrophotometer Infinite M200 PRO NanoQuant (Tecan). The purity of the DNA was determined through the absorbance ratio 260 nm/280 nm. The samples obtained were maintained at −80 °C. The bacterial populations of *Bifidobacterium* spp., *Lactobacillus* spp. and total bacteria were determined. For this, 10 ng of DNA was amplified using the corresponding primers of each bacterial population using the kit LightCycler FastStart DNA Master SYBR Green I in a thermocycler LightCycler (Roche Diagnostics, Mannheim, Germany).

The qPCR reactions were performed with 10 ng of DNA, 2 μL LightCycler FastStart DNA Master SYBR Green I 10X (Roche Diagnostics), 1 μL of each primer (sense and antisense for the 16S rRNA gene) in a concentration of 0.5 μM each. Ultrapure water was added to a final volume of 10 μL. The PCR reaction for *Bifidobacterium* spp. was done with the following thermal program: 1 preincubation cycle at 95 °C (10 min.), 45 amplification cycles consisting of an initial dissociation at 95 °C (10 s), hybridization at 66 °C (25 s), and extension at 72 °C (25 s), followed by one cycle for the melting curve at 95 °C, 65 °C (15 s) and 95 °C, and 1 cooling cycle at 40 °C (30 s).

For *Lactobacillus* spp. the thermal program was: a preincubation cycle at 95 °C (10 min.), 45 cycles of amplification with 95 °C of
Table 1. Recovery of Aloe vera Fructans and Acemannan after Extraction and Purification 

<table>
<thead>
<tr>
<th>polysaccharide</th>
<th>leaf samples</th>
<th>amount obtained (mg)</th>
<th>mg polysaccharide/g FW</th>
<th>mg polysaccharide/g DW</th>
<th>% recovered from FW</th>
<th>% recovered from DW</th>
</tr>
</thead>
<tbody>
<tr>
<td>fructans</td>
<td>4</td>
<td>7749 ± 67.9</td>
<td>5.25 ± 0.32</td>
<td>27.36 ± 1.69</td>
<td>0.53 ± 0.03</td>
<td>2.74 ± 0.17</td>
</tr>
<tr>
<td>acemannan</td>
<td>6</td>
<td>4282 ± 5.8</td>
<td>3.25 ± 0.18</td>
<td>93.58 ± 5.14</td>
<td>0.32 ± 0.02</td>
<td>9.36 ± 0.51</td>
</tr>
</tbody>
</table>

*The polysaccharides were quantified by GC-MS analysis as described in Materials and Methods Section. The figures represent the average of four samples, in the case of fructans and of six samples in the case of acemannan ± standard deviations. FW = fresh weight, DW = dry weight.*

Table 2. Sugar Composition of Aloe vera Fructans and Acemannans by GC-MS 

<table>
<thead>
<tr>
<th>polysaccharide sample</th>
<th>fucose (Fuc)</th>
<th>arabinose (Ara)</th>
<th>xylose (Xyl)</th>
<th>mannose (Man)</th>
<th>galactose (Gal)</th>
<th>glucose (Glc)</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>fructans</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>41.73 ± 2.17</td>
<td>n.d.</td>
<td>58.27 ± 2.17</td>
<td>100 ± 4.34</td>
</tr>
<tr>
<td>acemannans</td>
<td>n.d.</td>
<td>0.39 ± 0.54</td>
<td>n.d.</td>
<td>86.87 ± 2.53</td>
<td>0.05 ± 0.07</td>
<td>12.68 ± 2.98</td>
<td>99.99 ± 0.12</td>
</tr>
</tbody>
</table>

*Both polysaccharides extracted from Aloe vera were analyzed by GC-MS to determine their respective sugar composition. Results are given in molar percentage (molar %) with its respective standard deviation (±S.D.). n.d.: the corresponding monosaccharide was not detected in the sample.*

Figure 2. TLC analyses of the fructans obtained from water-stressed Aloe vera plants (A) and commercial FOS inulin (B). Both plates are observed under UV light (366 nm). Standards (St) of fructose (F) and sucrose (S). K3, K4, K5: fructan standards of the inulin series. K3: 1-kestose (trisaccharide), K4: tetrasaccharide, K5: pentasaccharide. DP: Degree of polymerization of Aloe vera fructans and commercial FOS inulin. N: Neo-kestose, trisaccharide of neo-fructans; P: pigments. In (A) a: fructans from water-stressed plants without depigmentation. b: Fructans from water-stressed plants after depigmentation with AC and Celite 560. c: Fructans from water-stressed plants purified with a Dowex ion-exchange resin column, used as a control of depigmentation. In (B) lanes a, b, c, and d were loaded with 5, 10, 20, and 40 μg of commercial FOS inulin, respectively. Key shows the TLC area were the pigments appear.
Sugar Composition of Fructans and Acemannan.

Table 2 shows the sugar components of fructan and acemannan from leaves of water-stressed Aloe vera plants. The results indicate that the fructans are free of cell wall contaminants and the analysis reveals only mannose and glucose as components, due to tautomerization of fructose as glucose and mannose.13

The acemannan sugar components reveal that there are very little sugar contaminants, probably from cell walls. Mannose is the major sugar component being 86.87% and glucose 12.68%, followed by arabinose, 0.38% and galactose 0.05% all percentages are expressed in molar percent. Mannose is 6.85 times the amount of glucose (Table 2).

The linkage analyses of both polysaccharides from Aloe vera were performed by GC-MS of the partially methylated alditol acetates. The fructan linkages were reported previously in Salinas et al.11 Respect to acemannan, the linkage analyses indicated the presence of glucose linked to mannose by (1→4) glycosidic bonds. A polysaccharide analysis by carbohydrate gel electrophoresis (PACE) of acemannan showed that galactose was not present as a sugar component (data not shown).

TLC Analyses of the Extracted and Purified Fructans.

Figure 2 shows a thin layer chromatography performed to corroborate that the purified polysaccharides were free of pigments. Figure 2A, lane a shows fructans obtained from leaves of plants subjected to water deficit previous to the depigmentation procedure. Figure 2A, lane b shows depigmented fructans using Celite 560 and activated carbon (AC). In this lane no pigments can be observed in the upper part of the plate, which can be seen in lane a before depigmentation. Figure 2A, lane c shows a control sample of Aloe vera fructans purified with Dowex, an ion-exchange resin. The isolated fructans are less pigmented than those purified by Dowex ion exchange column chromatography.

Figure 2B shows a TLC analysis with increasing concentrations of commercial FOS. Sucrose is present in all lanes and fructose was also detected in three lanes of higher concentrations.

Results published by our group,11 indicate that the sum of sucrose and fructose present in the depigmented fructans is 42%. Lower percentages of sucrose and fructose were detected in commercial FOS, estimated at about 10%.

In Vitro Fermentation of Aloe vera Polysaccharides with Pure Bacterial Cultures. The effect of Aloe vera fructans and acemannan on the growth of pure cultures of Lactobacillus and Bifidobacterium species was evaluated in vitro. The growth was compared to the widely used prebiotic commercial FOS. The growth kinetics of the four Lactobacillus strains is shown in Figure 3. When grown only with glucose the strains grew rapidly, reaching a plateau at about 13 h. The bacterial growth of the four Lactobacillus strains was more progressive in the presence of Aloe vera fructans and reached a plateau at higher OD values than those observed with glucose (except for L. plantarum L46-1-12). Less bacterial growth was observed with acemannan (except for L. fermentum L55-2-35); interestingly, bacterial growth in the presence of commercial FOS was much less than with Aloe vera fructans, but commercial FOS has less soluble sugars (fructose and sucrose).

Table 2 demonstrates that glucose is the best carbon source for in vitro growth of Lactobacillus species. The table also compares commercial FOS (inulin oligofructan) with Aloe vera fructans extracted from water-stressed plants. Aloe vera fructans increased bacterial growth 2.58−3.12 times, while commercial FOS increased it 0.8−0.9 times.
Figure 4 shows the growth kinetics of the Bifidobacterium species cultivated in the presence of all the carbon sources used. Since Bifidobacteria are anaerobic bacteria, their growth is slower than that of the Lactobacillus species; for this reason, growth kinetics was monitored for 72 h. As shown in Figure 4, the tested strains did not grow in the medium with glucose, while their growth was moderate with commercial FOS and Aloe vera acemannan, and maximum with Aloe vera fructans. Table 3 shows that growth increment was species-dependent. No significant differences in fold increment were detected between B. longum and B. animalis with fructans, the combination of fructan + acemannan or commercial FOS. For B. bifidum, the greatest increase was with fructans + acemannan, with a 3.76-fold increment. The greatest increase for B. catenulatum was 3.56 fold with commercial FOS. However, the final counts of the Bifidobacterium species were greater with fructans and the combined mixture of fructan and acemannan (Figure 4).

Table 3. Comparison of the Fold Increment of the Lactobacillus Species in Three Carbon Sources, Glucose, Commercial FOS, and Fructans, After 18 h of Culture at 37 °C

<table>
<thead>
<tr>
<th>Lactobacillus species</th>
<th>Glucose</th>
<th>Commercial FOS</th>
<th>Aloe vera fructans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus plantarum</td>
<td>7.32 a</td>
<td>0.90 c</td>
<td>2.58 b</td>
</tr>
<tr>
<td>Lactobacillus casei</td>
<td>6.43 a</td>
<td>0.82 c</td>
<td>3.12 b</td>
</tr>
<tr>
<td>Lactobacillus fermentum</td>
<td>6.59 a</td>
<td>0.83 c</td>
<td>2.74 b</td>
</tr>
</tbody>
</table>

The table shows the folds of growth increment. Significant differences between 0 h and 18 h of culture for the same strain of Lactobacillus within the same carbon source, are given by asterisks (*, **, ***; Student’s t test, * = P < 0.05, ** = P < 0.01, *** = P < 0.001). Significant differences between different carbon sources among the same strains are indicated by different letters according to Tukey’s Test. Values at 0 and 18 h are the readings at OD 600 nm.

Quantification of Bacterial Populations from Human Feces by qPCR. The results in Figure 5A show that the total bacterial population does not change after 48 h of in vitro fermentation with any carbon source used. Similar results were obtained for Lactobacillus spp., Figure 5B. In the case of Bifidobacterium spp., there is a significant increase only with fructans of 13.73% with a P = 0.044, Figure 5C.

Quantification of Short Chain Fatty Acids by in Vitro Fermentation of Human Feces in the Presence of Aloe vera Polysaccharides. Figure 6A shows the concentration of total SCFAs at 0 and 48 h of fermentation. It also shows the concentration of the linear SCFA (Figure 6B) and BCFA (Figure 6C). Due to the small sample size, the figure has a high dispersion of values with large standard errors. Figure 6A shows the total concentration of SCFA with glucose, Aloe vera fructans, and acemannan, commercial FOS, and without a carbon source, which is the negative control. The highest concentration means were obtained with Aloe vera fructans.
with $P = 0.0395$ and acemannan with $P = 0.0607$, followed by commercial FOS with $P = 0.0176$. Again, in the case of total linear SCFA (Figure 6B), significant differences were found using Aloe vera fructans and commercial FOS, with $P$ values of
constituted less than 1% of total SCFAs and did not show a total SCFAs with fructans and acemannan, respectively. In contrast, propionate was 3.8% and 5.5% of the fermentation; however, the percentages of butyrate generated than with commercial FOS (86%). Butyrate increased with all acemannan 73% of the total SCFAs, both values were lower than with fructans, acetate constituted 71% and with fermentation. Figure 7A and B show that acetate is the major SCFA present with all carbon sources among the same strains are indicated by different letters according to Tukey’s Test. Values at 0 and 18 h are the readings at OD 600 nm.

The structure of fructans from Aloe vera plants subjected to water stress (shown in Figure 1) has been recently characterized by Salinas and Salinas et al. In these publications we reported that with water deficit fructans increased in concentration and in their DP, detecting new glycosidic linkages. These modifications are probably due to the osmotic adjustment that Aloe vera plants undergo when suffering water deficit. Water-stressed plants also contain increased concentrations of acemannan. Changes in DP, a higher degree of ramification, and the presence of new glycosidic linkages in fructans from Aloe vera would certainly make the fermentation of these polysaccharides by intestinal bacteria more difficult.

The table shows the folds of growth increment. Significant differences between 0 h, and 18 h of culture for the same strain of Bifidobacterium within the same carbon source are given by asterisks (*). (Student’s t test, * P < 0.05, ** P < 0.01, *** P < 0.001, ns, not significant). Significant differences between different carbon sources among the same strains are indicated by different letters according to Tukey’s Test. Values at 0 and 18 h are the readings at OD 600 nm.

Table 4. Comparison of the Fold Increment of the Bifidobacterium Species in Three Carbon Sources, Commercial FOS, Fructans+Acemannan, and Fructans, After 72 h of Culture at 37 °C

<table>
<thead>
<tr>
<th>Bifidobacterium species</th>
<th>Carbon source</th>
<th>OD 0 h</th>
<th>OD 72 h</th>
<th>Fold increase</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bifidobacterium catenatum N 173-2</strong></td>
<td>commercial FOS</td>
<td>0.24</td>
<td>0.35</td>
<td>3.56 a</td>
</tr>
<tr>
<td></td>
<td>Aloe vera fructans + acemannan</td>
<td>0.67</td>
<td>0.78</td>
<td>2.33 b</td>
</tr>
<tr>
<td></td>
<td>Aloe vera fructans</td>
<td>0.71</td>
<td>0.78</td>
<td>2.33 b</td>
</tr>
<tr>
<td><strong>Bifidobacterium bifidum N 364-3</strong></td>
<td>commercial FOS</td>
<td>0.28</td>
<td>0.28</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>Aloe vera fructans + acemannan</td>
<td>0.73</td>
<td>0.78</td>
<td>2.33 b</td>
</tr>
<tr>
<td></td>
<td>Aloe vera fructans</td>
<td>0.71</td>
<td>0.78</td>
<td>2.33 b</td>
</tr>
<tr>
<td><strong>Bifidobacterium longum N 180-3</strong></td>
<td>commercial FOS</td>
<td>0.27</td>
<td>0.27</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>Aloe vera fructans + acemannan</td>
<td>0.73</td>
<td>0.78</td>
<td>2.33 b</td>
</tr>
<tr>
<td></td>
<td>Aloe vera fructans</td>
<td>0.71</td>
<td>0.78</td>
<td>2.33 b</td>
</tr>
<tr>
<td><strong>Bifidobacterium animalis, spp. lactis BB-12</strong></td>
<td>commercial FOS</td>
<td>0.28</td>
<td>0.28</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>Aloe vera fructans + acemannan</td>
<td>0.73</td>
<td>0.78</td>
<td>2.33 b</td>
</tr>
<tr>
<td></td>
<td>Aloe vera fructans</td>
<td>0.71</td>
<td>0.78</td>
<td>2.33 b</td>
</tr>
</tbody>
</table>

DISCUSSION

The structure of fructans from Aloe vera plants subjected to water stress (shown in Figure 1) has been recently characterized by Salinas and Salinas et al. In these publications we reported that with water deficit fructans increased in concentration and in their DP, detecting new glycosidic linkages. These modifications are probably due to the osmotic adjustment that Aloe vera plants undergo when suffering water deficit. Water-stressed plants also contain increased concentrations of acemannan. Changes in DP, a higher degree of ramification, and the presence of new glycosidic linkages in fructans from Aloe vera would certainly make the fermentation of these polysaccharides by intestinal bacteria more difficult.

To test the prebiotic properties of the polysaccharides, we used pure cultures of Lactobacillus and Bifidobacterium species, and human fecal microbiota anaerobically cultured in bioreactors. The Aloe vera fructans and acemannan used in the study were free of pigment contaminants, as shown by TLC analyses (Figure 2A). These results suggest that the prebiotic activity reported here was not due to the pigment contaminants present in the samples. However, the depigmented fructan extracts did have free fructose and sucrose present in them.

Our results indicate that the greater amounts of ramified fructans of higher DP obtained from Aloe vera water-stressed plants are more efficient than acemannan and commercial FOS in stimulating the growth of different Lactobacillus and Bifidobacterium spp. In Lactobacillus spp. there was a significant 2.6–3.1 fold increase with fructans, and these fold increases were greater than those obtained with commercial FOS. In Bifidobacterium spp. there was also a significant 2.0–3.3 fold increase with fructans. In this case, the fold increases were similar to those produced by commercial FOS (see Tables 3 and 4).

Lactobacillus spp. can grow with soluble sugars such as glucose, fructose, and sucrose, and unfortunately our fructan extracts contain fructose and sucrose in greater amounts than commercial FOS. Therefore, under these conditions we suggest that the greater growth of Lactobacillus spp. is caused mainly by
fructans without neglecting that the free sugars present may also induce growth.

With *Bifidobacterium* spp, the best carbon sources to induce growth were Aloe vera fructans, followed by the combination of fructans and acemannan. Unlike *Lactobacillus* our *Bifidobacterium* species did not grow with glucose. Therefore, the growth of *Bifidobacteria* cannot be attributed to the free fructose, glucose, and sucrose present in the fructan extracts. While acemannan by itself only slightly stimulated the growth of *Lactobacillus* spp, in *Bifidobacterium* the effect was as much as commercial FOS. It is most likely that the high molecular weight of Aloe vera acemannan makes it difficult to be digested by *Lactobacillus* species. This idea is supported by previous reports that indicate unhydrolyzed konjac glucomannan has a less prebiotic effect than the hydrolyzed konjac glucomannan. In addition, when acemannan was combined with Aloe vera fructans it decreased the growth of *Lactobacillus* spp induced by fructans. We can speculate that the high viscosity of Aloe vera acemannan decreases the solubility of fructans in the medium, therefore, decreasing the prebiotic properties of fructans. This does not happen in *Bifidobacterium*, which indicates that *Bifidobacterium* species can ferment the mixture of fructans+acemannan more easily. This may be due to the presence of more efficient mannannases. Indeed the *Bifidobacterium* strains ferment the acemannan better than the *Lactobacillus* strains, as shown in the growth kinetic curves.

The *Lactobacillus* species reached their stationary phase more rapidly with glucose than with fructans, even though fructans isolated from Aloe vera have sucrose, glucose, and fructose as contaminants. The media with only glucose is more easily absorbed and metabolized by *Lactobacillus* than fructans. Fructans are complex polysaccharides made of different glycosidic linkages that need to be hydrolyzed by bacterial enzymes. Our results are similar to those of the previous study by Roberfroid et al. They observed that different *Lactobacillus* and *Bifidobacterium* species grew significantly more in the presence of glucose followed by oligofructose, and to a lesser extent with inulin. The necessary enzymes are only present in some bacterial populations. Therefore, polysaccharide digestion slows down bacterial growth. In contrast, glucose is depleted earlier when used as the sole energy source. Bacterial growth stagnation may be due to the depletion of essential nutrients, the accumulation of toxic products, because the culture reaches an excessive number of cells for the available space, or a combination of these factors. Cardarelli et al. quantified by qPCR the total bacteria and *L. amylovorus* DSM 16698 in human and pig feces fermented with oligofructans and glucomannan oligosaccharides. They reported that the fermentation process was shorter with oligofructans than with glucomannan oligosaccharides, confirming that *Lactobacillus* spp. grow more easily in the presence of fructans compared to acemannan.

Fructans from water-stressed Aloe vera stimulated *Bifidobacterium* growth more efficiently than commercial FOS and better than Aloe vera acemannan. But it is important to point out that fructans were added to the culture medium at a higher concentration than acemannan in our experiments. This is because acemannan in higher concentrations shows a tendency to form a gel which makes bacterial growth in vitro more difficult. However, the mixture of the two polysaccharides induces growth almost as well as with just fructans in *Bifidobacterium* spp. Therefore, both polysaccharides combined make a good prebiotic preparation; the mixture was better than the commercial FOS when evaluated with these specific *Bifidobacterium* species.

These results were partially corroborated by the qPCR analysis of bacterial population. The population of *Bifidobacterium* spp, present in human feces increases significantly in the presence of fructans. Fructans also increase, not significantly, the population size of *Lactobacillus* spp with a *P* = 0.0537. Acemannan and glucose, on the other hand, did not increase significantly the population sizes of either probiotic genus. But, since our sample size was small (*n* = 3), by increasing the number of individuals in further studies, we could probably find significant population increments with acemannan and/or glucose.

Here we also present preliminary results of short-chain fatty acids (SCFAs) produced by human stool fermentation in the presence of Aloe vera polysaccharides. Aloe vera fructans and acemannan seem to increase the production of total SCFAs during the stool fermentation. The high dispersion of values, due to a low number of samples, did not allow us to demonstrate significant differences among the carbon sources used in the analyses. However, significant increments were found with fructans (*P* = 0.0365) and commercial FOS (*P* = 0.0176). With acemannan, there was only a tendency to increase total SCFA (*P* = 0.0612). The greatest variation in total SCFA production was found with acemannan followed by fructans (145% increase with acemannan and 98% increase with fructans).

The SCFA composition percentages show that butyrate and propionate increased in all conditions, with and without carbon sources. After 48 h of fermentation, butyrate composition was higher with fructans and acemannan. The figure of composition percentages does not show that the acetate concentration increases significantly with acemannan. The acetate produced from commercial FOS was 86% of the total SCFAs, which was the highest value detected under all the conditions used. Acetate is important since it has been shown to help reduce obesity and body fat.

The BCFAs are the fermentation products of some amino acids. There were no significant differences in the total amounts of BCFAs with any carbon source. Indeed, the BCFAs produced by the negative control were similar to those generated by the other carbon sources, confirming that purified Aloe vera polysaccharides are free of amino acid contaminants.

Fermentation of complex polysaccharides and eventually some amino acids can be used as an energy source by the beneficial colon bacteria generating SCFAs and BCFAs. Among the SCFAs, propionate decreases bowel inflammatory diseases and is associated with the production of lipogenic enzymes. Acetate, in contrast, enters the peripheral circulation and is metabolized by the peripheral tissues. Butyrate is the main substrate and principal energy source of the colonocytes, promoting the normal phenotypes of these cells avoiding the formation of polyps and, therefore, preventing colorectal cancer. Butyrate with propionate induce apoptosis of colorectal cancer cells. These benefits would potentially be induced by Aloe vera polysaccharides. Indeed in our results, fructans and acemannan stimulate the production of acetate, propionate, and butyrate, which in turn possibly prevents the formation of polyps. Further studies are needed to corroborate these health benefits from Aloe vera polysaccharides.

The results of this study confirm that fructans and acemannan from Aloe vera are very promising prebiotics, that
had not been studied previously in detail. The growth kinetics of single bacterial strains, corroborated by bacterial population qPCR, and SCFA quantifications, indicate that ramified fructans are better prebiotics than commercial FOS which is a linear fructan.

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