LWT - Food Science and Technology 92 (2018) 380–387

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

In vitro evaluation of prebiotic activity, pathogen inhibition and enzymatic metabolism of intestinal bacteria in the presence of fructans extracted from agave: A comparison based on polymerization degree

Ricardo García Gamboa[a], Rosa Isela Ortiz Basurto[b], Montserrat Calderón Santoyo[b], Jorge Bravo Madrigal[a], Blanca Elizabeth Ruiz Álvarez[a], Marisela González Avila[a,*]

[a] Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C. Av. Normalistas No.800, col Colinas de la Normal, C.P. 44270, Guadalajara Jalisco, Mexico

[b] Instituto Tecnológico de Tepic Laboratorio Integral de Investigación en Alimentos, División de Estudios de Posgrado, Av. Tecnológico No. 2595, Col. Lagos del Country, C. P. 63175, Tepic, Nayarit, Mexico

ARTICLE INFO

Keywords:
Prebiotic potential
Bacterial growth
Growth inhibition
Enzyme activity

ABSTRACT

The prebiotic effect of fructans is well known, including their beneficial influence on health. This study shows agave fructans impact as potential prebiotics, depending on their structure and polymerization degree (PD). The growth of seven probiotics and three pathogens was estimated by turbidimetric analysis and the latter (*Salmonella typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*) were submitted to growth inhibition tests in the presence of metabolites produced by probiotics. *Lactobacillus casei* and *L. paracasei* growth was optimal when supplemented with agave fructans. *L. casei* was grown in the presence of the extracted fractions obtained from *Agave salmiana* spp. *crassipina* (optical density (O.D.) 1.09 ± 0.02, 0.98 ± 0.03, 0.98 ± 0.07, low, medium and high PD, respectively), *A. salmiana* var. *liso* (0.85 ± 0.13), *A. atrovirens* (0.79 ± 0.03) and *A. tequilana* spp. (0.89 ± 0.03). The growth of *L. paracasei* was optimal when supplemented with those fractions obtained from the *A. salmiana* (O.D. 1.12 ± 0.02, 1.18 ± 0.02, 1.13 ± 0.007, low, medium and high PD, respectively) and *A. tequilana* var. *cenizo* (1.18 ± 0.01 and 1.15 ± 0.02, medium and high PD, respectively) species. Both bacteria were tested in order to assess enzyme activity using API ZYM galleries after they were grown on agave fructans. The results show an increase of five enzymes (cysteine-arylamidase, α-chymotrypsin, β-galactosidase, N-acetyl-β-glucosaminidase and α-fucosidase).

1. Introduction

The prebiotic effect of fructans has been documented by some authors because they possess the ability to significantly modify intestinal microbiota (Krumbeck, Maldonado-Gomez, Ramer-Tait, & Hutkins, 2016). Fructans are molecules bonded by fructosyl linkages and generally they have a terminal glucose moiety. They may be present in approximately 15% of all flowering plants. Regarding their structure, five different types may be distinguished: inulin (β 2→1 linkage), levan (β 2→6 linkage) graminan (β 2→1 and β 2→6 linkages), inulin neoseries and levan neoseries (fructans possessing an internal glucose residue) (Peshev & Van den Ende, 2014; Ritsema & Smeekens, 2003). In Mexico, *Agave* is used as an important source of fructans. However, it has been reported that these plants contain more than one fructan structure. *Agave* contains branched graminan fructans, also known as "agavins". These contain a complex mixture of fructans possessing different polymerization degrees (PD) (Mellado-Mojica & López, 2012; Praznik, Löppert, Rubio, Zangger, & Huber, 2013; Velázquez-Martínez et al., 2014). Because of their β linkages, fructans are not hydrolyzed by the human digestive enzymes and they are fermented only by some bacterial species found as intestinal colonic microbiota. This fermentation is beneficial to health as it may be translated into improved results from clinical blood tests, i.e. it regulates glucose and triglyceride levels, it enhances resistance against both intestinal and extra-intestinal pathogens, it modulates the immune response and it attenuates allergies. It has also been reported that fructans prevent body weight increases as it impacts on food intake and it regulates the satiety-related hormones (Delgado, Tamashiro, & Pastore, 2010; Márquez-Aguirre et al., 2013; Santiago-Garcia & Lopez, 2014). Nevertheless, it was demonstrated that fructans possessing several PD display a different prebiotic activity (González-Avila et al., 2014; Mueller et al., 2016). Bacteria from the *Lactobacillus* and *Bifidobacterium* genera metabolize

* Corresponding author. CIATEJ, Unidad de Biotecnología Médica y Farmacéutica, Guadalajara, Jal, Mexico.
E-mail address: mgavila@ciatej.mx (M. González Avila).

https://doi.org/10.1016/j.lwt.2018.02.051
Received 20 April 2017; Received in revised form 14 February 2018; Accepted 18 February 2018
Available online 21 February 2018
0023-6438/

fructans exhibiting low PD, whereas bacteria residing at both proximal and distal colon metabolize those with high PD (Mueller et al., 2016; Van De Wiele, Boon, Possemiers, Jacobs, & Verstraete, 2007). A recent study demonstrated that low PD fructans obtained from *Agave tequilana* prevent weight gain, hyperglycemia and fatty liver disease. Moreover, fructans showing several PD are involved in short-chain fatty acids biosynthesis, playing an important role on gastrointestinal health (Kleessen, Hartmann, & Blaut, 2001; Koenen, Rubio, Mueller, & Venema, 2016). Among the approximately 300 described *Agave* species, nearly 75% are found on Mexican territory. However, less than 5% of these species are used during the production of alcoholic beverages and many others are not used for industrial purposes. The aim of this study was to evaluate *in vitro* the prebiotic activity displayed by those fructans obtained from seven agave species, which are characterized by different PD.

2. Materials and methods

2.1. Agave fructans

In this study we used fructans extracted from Mexican agave. Two of them originated from Guanajuato (*A. salmiana* var. *liso* and *A. salmiana* var. *chino*), 3 of them from Veracruz (*A. salmiana* spp. *crassipina*, *A. atrovirens* and *Agave* spp.), and the last 2 from Jalisco (*A. tequilana* var. *cenizo* and *A. tequilana* spp.). Aqueous preparations of these extracts were used and their preparation was carried out as previously described (Lopez, Mancilla-Margalli, & Mendoza-Diaz, 2003). Briefly, fructans were submitted to fractioning using ultrafiltration membranes, according to Aldrete-Herrera, 2013. These extracts were freeze-dried and kept frozen (−20 °C) until further evaluation.

2.2. Microorganisms

Seven probiotics strains (*Lactobacillus casei* ATCC 393, *L. paracasei* Lpc-37, *L. rhamnosus* NH001, *L. plantarum* Lp-115, *L. gasseri* Lg-36, *Pediococcus acidilactici* MA18/5M and *Saccharomyces boulardii* CNCM I-1079) and three pathogenic microorganisms (*Staphylococcus aureus* ATCC 6538, *Salmonella typhimurium* ATCC 14028, and *Listeria monocytogenes* ATCC 19114) were used. They were obtained from the collection kept at CIATEJ.

2.3. Growth media

Both, probiotics and pathogens strains were grown in Difco™ MRS (Man-Rogosa-Sharpe) Medium at 37 °C during 16 h. A modified MRS medium was prepared by substituting dextrose with the respective agave fructans characterized by different PD. In all cases concentration was 20 g/L.

2.4. Assessment of prebiotic activity

One hundred μL-aliquots containing 1×10^6 cells from the respective activated bacteria were inoculated in 5 mL MRS (containing dextrose) or in modified MRS medium, accordingly, and they were incubated at 37 °C during 24 h. Two hundred μL containing 1.2×10^6 Colony Forming Units (CFU) were taken from precultures of either modified or control (regular MRS) media and they were transferred to a 96-well micro-plate as triplicate. Optical density (OD) was measured every hour during 10 h using a micro-plate reader (EspectraMax 340, Molecular devices®). The equipment settings were 490 nm, 37 °C and micro-plate high-speed shaking for 10 s before each reading. The experimental results observed for bacterial growth were fitted to an exponential model according to the following expression (Equation (1)) (Arrizon, Hernández-Moedano, Toksoy-Oner, & González-Avila, 2014):

$$y(t) = a \exp^{(bt)} \quad (1)$$

Where, y is the number of cells (N_t expressed as CFU/mL), a is the initial value (N_0 expressed as CFU/mL), b is growth rate (expressed as h^{-1}), and t is time (expressed as h).

2.5. Determination of growth inhibition elicited by extracellular metabolites produced by probiotics

A preliminary small-scale experiment was performed in order to identify the carbon source concentration required to stimulate the production of bacterial metabolites. Three concentrations were evaluated (0.5, 1 and 2%). The samples did not show significant differences, thus we used the 2% concentration as we did in previous tests. Seven probiotic strains were used in order to evaluate growth inhibition on three pathogenic bacteria mediated by their secreted metabolites. This assay was carried out according to Kirby-Bauer (1960). Two fructans were selected for this test (they displayed intermediate and high PD and they were extracted from *A. tequilana* var. *cenizo*). Each probiotic bacteria was grown in MRS and MRS modified media containing the respective fructans at 37 °C during 16 h and they were further kept as stock. Each bacterial stock was centrifuged (5000 rpm, 5 min, 4 °C) and filtered through a 0.45 μm membrane (CORNING®) in order to obtain the extracellular metabolites produced by the respective probiotic. The final suspension was labelled as cell free supernatant and it was used for antimicrobial tests. Subsequently, 1×10^6 CFU of each pathogen suspended in 100 μL were homogeneously spread on Petri dishes containing nutritive agar. The latter was dried under sterile air during 5 min. Six 5-mm diameter holes were pierced and they were filled with 50 μL containing cell-free supernatant. The supernatant obtained from each probiotic bacteria was tested as triplicate (Arrizon et al., 2014).

2.6. Enzyme activity

The APY ZYM® system (BioMérieux) was used in order to evaluate the enzyme activity displayed by all microorganisms. This is a laboratory kit intended to perform a semi-quantitative analysis of enzymes. This kit evaluates 19 different enzyme activities, including β-glucosidase. Two probiotic bacteria were selected to carry out this test: *L. casei* and *L. paracasei*. Fructans possessing intermediate and high PD obtained from *A. tequilana* var. *cenizo* were used as carbon source. The activity displayed by extracellular and intracellular enzymes was assayed in both supernatants and pellets, previously obtained from 16 h-cultures. The test was performed according to the instructions provided by the manufacturer. Results were scored from 0 to 5 (API ZYM units), according to the reaction color shown on a scale chart. A value of “0” is a negative reaction, whereas “5” describes a reaction showing the highest intensity.

2.7. Statistical analysis

The Statgraphics centurion XVI software was used to carry out a two-way ANOVA test, followed by a Tuckey post-hoc test using a 0.05 significance level (p-value). The pathogen inhibition test was conducted by correspondence analysis to determine if there were any significant differences by the effect of the substrates.

3. Results

3.1. Bacterial growth in presence of different fructans

Eleven fructan samples extracted from 7 agave species were evaluated in this study (Table 1). They were obtained from Guanajuato, Veracruz and Jalisco and they showed different PD. Only two species (*A. tequilana* var. *cenizo* and *A. salmiana* spp. *crassipina*) contained fructans possessing low, intermediate and high PD. The other species contained fructans with a PD averaging between 8 and 12 fructan residues. Those samples were labeled as Native Agave Fructans (NAF).