LWT - Food Science and Technology 84 (2017) 151–159

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Potential use of *Agave salmiana* as a prebiotic that stimulates the growth of probiotic bacteria

Fidel Martinez-Gutierrez [a, *], Stefan Ratering [b], Bertha Juárez-Flores [c], Cesar Godinez-Hernandez [c], Rita Geissler-Plaum [b], Florian Prell [d], Holger Zorn [d], Peter Czermak [d, e], Sylvia Schnell [b]

[a] Facultad de Ciencias Quimicas, Universidad Autonoma de San Luis Potosi, Mexico
[b] Institute for Applied Microbiology, IFZ, Justus Liebig University Giessen, Germany
[c] Instituto de Investigacion de Zonas Deserticas, Universidad Autonoma de San Luis Potosi, Mexico
[d] Institute of Food Chemistry and Food Biotechnology, Justus Liebig University Giessen, Germany
[e] Institute of Bioprocess Engineering and Pharmaceutical Technology, University of Applied Sciences Mittelhessen, Giessen, Germany

ARTICLE INFO

Article history:
Received 14 February 2017
Received in revised form
20 May 2017
Accepted 22 May 2017
Available online 26 May 2017

Keywords:
Prebiotic
Agave salmiana
Probiotic strains
Short-chain fatty acids

ABSTRACT

Inulin and other fructans that provide health benefits are accumulated in *Agave salmiana*. Currently, *Agave* species are only exploited for the production of alcoholic beverages. Few reports of beneficial effects of fructans obtained from *A. salmiana* have been presented and therefore the goal of this study was to examine the specific stimulation of probiotic bacteria by *A. salmiana* powder in comparison to commercial prebiotic products with different bacteria.

The composition of *A. salmiana* that includes fructooligosaccharides (FOS) supports the growth of probiotic bacteria. Inulin Orafti GR showed almost the same stimulation to probiotic bacteria in comparison to *A. salmiana*. *Lactobacillus acidophilus* showed the best growth with *A. salmiana*, decreased the final pH-value to the lowest level and produced the highest concentration of lactate.

The structural heterogeneity of fructans from *A. salmiana* is useful as prebiotic and to maintain persistence of probiotic strains *in vivo*. The variation of gut microbiota composition that might be caused by microbiota-targeted therapies might also be influenced. Future research should work towards optimizing the FOS content and profile in the plants by selection of plant varieties or changing agronomic and postharvest practice to develop their innovative applications for the food industry and the health promotion.

1. Introduction

Intestinal microorganisms closely interact with the host and have an important potential to influence host health (Van den Abbeele, Van de Wiele, Verstraete, & Possemiers, 2011). Generally, a high microbial diversity is thought to be associated with a healthy gut microbiota, while loss of diversity seems to correlate with disease (Scott, Antoine, Midtvedt, & van Hemert, 2015). Nowadays over 25 diseases or syndromes have been linked to an altered intestinal microbiome (de Vos & de Vos, 2012). The most studied disease conditions in relation to intestinal microbiota are obesity, metabolic syndrome, type II diabetes and bowel diseases (Crohn's disease, ulcerative colitis, irritable bowel syndrome) (Scott et al., 2015).

A food-based strategy to modulate the composition and/or metabolic activity of the intestinal microbiota, for the purpose of imparting beneficial effects, is a route that is increasing the dietary intake of functional foods containing prebiotics, probiotics or symbiotics (Watson et al., 2013). Prebiotics are defined as: “The selective stimulation of growth and/or activity(ies) of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host” (Roberfroid, 2007; Roberfroid et al., 2010). Several prebiotic substrates, in particular fructooligosaccharides (FOS), galacto-oligosaccharides (GOS), inulin and lactulose, have already obtained scientific credibility due

* Corresponding author. Laboratorio de Microbiología, Facultad de Ciencias Químicas, UASLP, Av. Dr. Nava #6 Zona Universitaria, San Luis Potosi, SLP 78210, Mexico.
E-mail address: fidel@uaslp.mx (F. Martinez-Gutierrez).

http://dx.doi.org/10.1016/j.lwt.2017.05.044
0023-6438/

to their inclusion in human trials where they have demonstrated prebiotic effects (Davis, Martínez, Walter, & Hutkins, 2010). The *Agave* proliferate under arid and semiarid conditions because of the special metabolism known as Crassulacean Acid Metabolism (CAM) (Garcia-Moya, Romero-Manzanares, & Nobel, 2011) and agaves were second only to maize (corn) in the development of agriculture in Mesoamerica. They are used for beverages, food, poultices for wounds, fiber, and for soil stabilization to prevent desertification (Garcia-Moya et al., 2011). Currently, beverages from the stems of *Agave salmiana* include the sweet drink aguamiel, the fermented pulque, and the distilled mezcal (Wang & Nobel, 1998). However, every day more plants are dedicated to high fructose syrup and agave fructans production as ingredients for healthier food and feed (Garcia-Aguirre et al., 2009).

The FOS are classified as prebiotics and are used as food ingredients because of their beneficial effects in proliferating bifidobacteria in the human colon (Roberfroid, 2007). Recent studies of the FOS from *A. salmiana* showed their prebiotic effect when Wistar rats were fed (Jasso-Padilla et al., 2017). The prebiotics have presented evidence on physiological and antagonist of some pathological effects, which can be described as an improvement and stabilization of the gut microbiota composition, modulation of the gastrointestinal peptides production, like the glucagon-like peptide-1 (GLP-1), energy metabolism and satiety, obesity risk reduction, type 2 diabetes, metabolic syndrome, intestinal inflammation and colon cancer, among others (Morris & Morris, 2012).

The gut microbiota contributes to energy metabolism through the production of short-chain fatty acids (SCFA), that are produced by colonic fermentation which involves the anaerobic breakdown of dietary fiber, protein and peptides (Baothman, Zamzami, Taher, Abubaker, & Abu-Farha, 2016). SCFA are bacterial products that are utilized by the human colonic epithelial cells, stimulating their growth as well as salt and water absorption, thus increasing the humidity of fecal bolus through osmotic pressure, and consequently improving the intestinal motility (Qin et al., 2010). As a whole, SCFA acidify the luminal pH which suppresses the growth of pathogens and they also influence intestinal motility (Qiang, YongLie, & QianBing, 2009). Therefore, is important to describe how dietary fibers, as well as FOS, alter SCFA profiles and the intrinsic and extrinsic effects of prebiotics on the host metabolism (Jakobsdottir, Nyman, & Fåk, 2014). The goal of this research was to study the combination of the *A. salmiana* compounds with different probiotic strains to evaluate if this novel prebiotic is capable of developing a specific stimulation to some of them.

2. Materials and methods

2.1. Plant material and dehydration

The harvest of the agave pineapple of *A. salmiana* was conducted in the area of Laguna Seca mezcal factory, which is located in the town of Charcas, San Luis Potosi, Mexico. The pineapple maguey of *A. salmiana* has a state of maturity in which the maguey contains a high concentration of fructans; this occurs immediately before the plant emits the scape, and in turn the stem emerges from the center of the maguey, presenting only flowers at the apex (Aguirre Rivera et al., 2001). The stem of the pineapple head was removed, then the stem was cut into 1.5 cm thick cubes and the juice extracted (Model EXS Series 6/18/02 Volt; Lyon, France). Samples of dried extract of stem of *A. salmiana* were obtained by lyophilization (Free Dry System, model FreeZone 6: Labconco Corporation, Kansas City, USA).

2.2. Identification of FOS

Carbohydrate analysis was performed by high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) on a Dionex ICS-3000 (Thermo Scientific, Waltham, Massachusetts, USA), equipped with a pulsed amperometric detector. Elution of carbohydrates was performed at 30 °C on a Dionex Carbopac PA200 column (Thermo Scientific, Waltham, USA), at a flow rate of 0.3 mL min-1. The elution program was adapted from the method used by Ronkart et al. (2007).

As standards, glucose, fructose, sucrose, 1-kestose (GF2), nystose (GF3), and 1-fructofuranosyl-D-nystose (GF4) (Wako Chemicals, Germany) were used in a concentration of 200 μM each. Samples of *A. salmiana* and Inulin Orafti GR, respectively were dissolved in distilled water (100 mg 10 mL-1) and filtered (membrane, 0.2 μm pore size, Whatman, Dassel, Germany) before injection.

2.3. Analysis of the composition of the powder

The total amount of carbohydrates of the *A. salmiana* powder was analyzed by the method of Luff-Schoorl (Schoorl, 1929) after total hydrolysis with 2 M hydrochloric acid, and crude protein was determined by Kjeldahl analysis. The moisture content was determined by measuring the loss of weight. Therefore, the powder was dried at 103 °C after using the sea sand disruption method (Manhita, Teixeira, & da Costa, 2006). The fat content was analyzed using a soxhlet extraction and ash content by heating the powder to 550 °C and determination of the weight of the residual mass.

2.4. Bacterial strains and culture media

Six reference strains of bacteria (*Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium longum* subsp. *infantis*, *Bifidobacterium longum* subsp. *longum*, *Bacteroides vulgatus*, *Akkermansia muciniphila*) documented to colonize the gastrointestinal tract were studied (Rajilic-Stojanovic & de Vos, 2014). Modifications were made to the Man-Rogosa-Sharpe (mMRS) (in 1 L): casein peptone (tryptic digest) 5 g, meat extract 10 g, yeast extract 3.5 g. The pH was adjusted to 6.2 in the mMRS, which was used as a basal growth medium to study the ability of *L. casei* (ATCC 334) and *L. acidophilus* (DSM 20079) to evaluate them develop. Modifications were made to the Bifidobacterium medium (mBM) (per 1 L): casein peptone (tryptic digest) 1.5 g, yeast extract 1.5 g, meat extract 1.5 g, bacto soytone 1.5 g, the pH was adjusted to 6.8. The mBM was updated for the study of both species of *B. longum* subsp. *infantis* (DSM 20088) and *B. longum* subsp *longum* (DSM 20219). Modifications were made to Clostridial broth (mCB) (per 1 L): peptone 5 g, beef extract 5 g, yeast extract 1.5 g, the pH was adjusted to 6.8. The mCB was updated for the study of *Ba. vulgatus* (DSM 1447) and *Ak. muciniphila* (DSM 22959). The bottles with 50 mL of each autoclaved medium were filled up anoxically under an N_2–CO_2 mixture using the construction described by Widdel and Bak (1992, pp. 3352–3378) and closed with metallic screw caps.

2.5. Prebiotics and carbohydrates

Three powders with prebiotic activity were tested: *Agave salmiana*, Orafti GR (granulated inulin powder, average degree of polymerization > 10, BENEO GmbH, Obrigheim, Germany) and Orafti HP (high performance inulin powder, average degree of polymerization > 23, BENEO GmbH, Obrigheim, Germany). On the other hand, a solution of glucose with a 2 mM concentration and the respectively modified medium without glucose were evaluated. The powders were prepared to different concentrations according to their solubility. For *A. salmiana* powder and Orafti GR, a solution