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Growth of selected probiotic strains with fructans from different sources relating to degree of polymerization and structure

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ABSTRACT

Fructans as prebiotics are an important factor in the functional food industry due to their beneficial effect on health. However, the influence of structure and degree of polymerization (dp) on their prebiotic effect is not fully elucidated so far. Unbranched inulin-type (β-2,1 linked) fructans from chicory and branched mixed-type (β-2,1 and β-2,6 linked) fructans from agaves were separated into fractions with different dps using preparative size exclusion chromatography, and the growth curves of selected probiotic strains were determined. All fructans exerted a significant growth enhancement, being higher with lower dp and with branching. *Lactobacillus acidophilus* and *L. paracasei* CRL431 quickly used fractions independent of the dp, whereas other strains (i.e. *L. reuteri*) did not use or only slowly used high dp fractions. Most strains cleaved fructans into smaller units before uptake into the cells. Our findings may contribute significantly to the development of improved prebiotic formulations.

1. Introduction

Due to their beneficial effects on health, fructans as prebiotics are an important factor in the functional food industry. Prebiotics are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health (Gibson & Roberfroid, 1995). Fructans are unbranched or branched fructose oligo- or polymers, either β-2,1-linked inulin-type, β-2,6-linked levan-type or β-2,1 and β-2,6-linked mixed-type, and indigestible for the human gut (Roberfroid & Delzenne, 1998). Fructose polymers are accumulated by a great variety of plants, including composites (e.g. chicory, Jerusalem artichoke), liliales (e.g. garlic, onion), asparagales (asparagus) or agavaceae (div. agave species) (Praznik & Loeppert, 2016; Praznik, Löppert, & Huber, 2007; van Loo, Coussement, de Leenheer, Hoebregs, & Smits, 1995).

Only a few fructan-containing plants are currently used in the functional food industry, including chicory (*Cichorium intybus*), Jerusalem artichoke (*Helianthus tuberosus*) and agave (*Agave tequilana*). Chicory and Jerusalem artichoke – as composites – contain inulin-type fructan being predominantly

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unbranched. Fructan from agaves possesses a mixed-type structure containing β-2,1 and β-2,6 linkages with branching characteristics (Praznik & Loeppert, 2016; Roberfroid & Delzenne, 1998). Until now the scientific focus was more concentrated on unbranched inulin-type fructan isolated from chicory and Jerusalem artichoke and not so much on the effect of mixed-type branched fructan from agaves.

Fructan can exert its beneficial effect via direct or indirect mechanisms (Vogt et al., 2015). Indirect mechanisms involve a stimulation of the growth of probiotic bacteria and can be caused by their fermentation products such as short chain fatty acids. A direct effect was suggested for inulin-type fructans on lipid profile (dos Reis, da Conceição, Rosa, dos Santos Dias, & do Carmo Gouveia Peluzio, 2015) or on immunomodulation via activation of Toll-like receptors, nucleotide oligomerization domain containing proteins, C-type lectin receptors, or galectins, eventually inducing pro- and anti-inflammatory cytokines (Capitán-Cañadas et al., 2014; Vogt et al., 2015). A direct effect for agave fructooligosaccharide (FOS) on metabolic parameters (Márquez-Aguirre et al., 2013) and as immunomodulator (Moreno-Vilet et al., 2014) was suggested as well.

The dp of fructans has a major impact on the kinetics of fermentation by probiotic bacteria and thus on the beneficial effect. For most probiotic strains, inulin-type fructans with lower dp lead to an earlier growth of lactobacilli and bifidobacteria than those with higher dp. Longer chain inulins, however, showed a more pronounced prebiotic effect affecting not only probiotics in the proximal colon, but also in the distal colon (Ito et al., 2011; Pompei et al., 2008; Stewart, Timm, & Slavin, 2008; van de Wiele, Boon, Possemiers, Jacobs, & Verstraete, 2007). Furthermore, the range of dp from inulin-type fructan has an impact on the short chain fatty acid profile, in such a way that increase of butyrate can only be found in faecal samples from rats fed with inulin of high dp (Kleessen, Hartmann, & Blaut, 2001).

For mixed-type branched fructans from agave, a similar dependency was shown. Probiotic strains prefer fructan sources with different dps and grow only or faster with fructans of low dp (Velázquez-Martínez et al., 2014; Mueller et al, 2016). The effect of fructans from agave and chicory on food intake and weight gain was shown to be dependent on the dp and structure of fructan as well. In fact, agave fructan had a significant effect on weight reduction and on the increased secretion of peptides involved in appetite regulation, whereas inulin from chicory did not significantly show such an effect (Santiago-García & López, 2014).

Recently we showed the dependency of dp and structure of fructan on the formation of short chain fatty acids playing a major role in the gut's health. Lactate and butyrate production was higher from fructans with lower dp. Branched fructan with high dp led to a higher butyrate formation than unbranched fructans with high dp (Koenen, Cruz Rubio, Mueller, & Venema, 2016).

An elucidation of the degradation pattern of fructans may help to explain the ability of probiotics to use fructans with different dp. Inulin-type fructans may be degraded either extracellularly or taken up by the bacteria and metabolized intracellularly (Tsujikawa, Nomoto, & Osawa, 2013). The presence of fructanase in the cell wall of several probiotic strains was found previously. The expression of the enzyme was enhanced using inulin as sole carbon source for *Lactobacillus paracasei*, even more than for FOS or fructose (Goh, Lee, & Hutkins, 2007). However, the influence of mixed-type branched fructans on the induction of fructanase expression was not shown so far.

In general the influence of structure and dp on the prebiotic effectivity of fructan for developing of probiotic strains is not fully elucidated yet. Thus, we compared the growth enhancement of selected probiotic strains on different samples of dps from unbranched chicory inulin and agave fructan with mixed-type and branching characteristics. In this manner we obtained information about degradation of low and high molecular fructans during bacteria development and allowed an evaluation of their metabolism.

2. Materials and methods

2.1. Materials

Yeast extract was obtained from Oxoid (Hampshire, UK). All other chemicals were obtained from Sigma-Aldrich (St. Louis, MA, USA). The source for inulin-type chicory fructan was Raftiline® or Raftilose® (Orafti, Oreye, Belgium) and for mixed-type agave fructans Metlin® or Metlos® (Nekutli, Guadalajara, Mexico). The prebiotic effect of fructan was tested on seven selected probiotic strains, namely *Lactobacillus paracasei ssp. paracasei* CRL 431 (ATCC 55544), *Lactobacillus paracasei ssp. paracasei* DN114001, *Lactobacillus paracasei ssp. paracasei* (DSM20315), *Lactobacillus rhamnosus* GG (ATCC 53103), *Lactobacillus reuteri* (ATCC 55730), *Lactobacillus acidophilus* LA-5 (DSM 13241) and *Bifidobacterium animalis ssp. lactis BB12* (DSM 15954).

Man-Rogosa-Sharpe (MRS) liquid medium was prepared by dissolving 10 g of peptone from casein, 8 g of meat extract, 4 g of yeast extract, 1 g di-potassium-hydrogen phosphate, 2 g Tween 80, 2 g of di-ammonium-hydrogencitrate, 5 g of sodium acetate, 0.2 g of magnesium sulphate, 0.5 g cysteine hydrochloride, and 0.04 g manganese sulphate per litre. The medium was autoclaved and stored at 4 °C until further usage. MRS plates were prepared with the same components as MRS broth and additionally agar (Sigma Aldrich).

2.2. Determination of growth curves of probiotic strains

Growth curves were derived using Bioscreen C (Oy Growth Curves Ab Ltd, Helsinki, Finland) based on a turbidity measurement as indicated by an increased OD_{600} value. A fresh overnight culture was prepared and cell density was determined. The cell amount for a final starting OD_{600} of 0.1 was calculated. The bacteria were washed three times in phosphate buffered saline (PBS) supplemented with 0.5 g/L cysteine hydrochloride and resuspended in MRS-medium without carbohydrate source. The negative control was incubated without sugar and the samples in the presence of 1% carbohydrates in honeycomb plates with a final volume of 200 μL. The plates were incubated at 37 °C for 48 hours and the OD_{600} was measured every hour after mixing by shaking for 15 sec. All samples were tested in triplicates at various concentrations, and the average was calculated and plotted in the graphs.