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Growth of selected probiotic bacterial strains with fructans from Nendran banana and garlic

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A B S T R A C T

This work assesses the utilization of fructan from garlic cultivars and *Nendran* banana as prebiotics. Fructans from both sources significantly stimulated the growth of four strains of lactic acid bacteria (LAB) to varying degrees. In case of garlic, 1-kestose (≥60%) was utilized more compared to nystose (≥38%). *Nendran* banana showed the highest prebiotic activity score, even higher than commercial FOS and inulin. In all three foods, presence of FOS stimulated better growth than inulin. *Lactobacillus plantarum* alone produced butyrate. These results show that *in vitro* prebiotic activity of fructan from the two sources is directly related to its structure and the specific strains of LAB. Therefore, fructans from hill and country garlic and *Nendran* banana cultivars can be explored as functional ingredients targeted towards modulation of the gut micro-biota and in synbiotic preparations.

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1. Introduction

Fructans are oligo and polysaccharides consisting of fructan units with the single D-glucosyl unit at the reducing end. Fructans with lower degree of polymerization (DP_n) values ($DP_n < 10$) are oligofructose or fructooligosaccharides (FOS) and high ($DP_n > 10$ and < 60) are inulin (Roberfroid, 2005). Fructans are known to be prebiotics, resistant to digestion and absorption in the human small intestine. They undergo selective fermentation in the colon by beneficial bacteria such as *Lactobacillus* and *Bifidobacteria* to produce short chain fatty acids (SCFA) and also inhibit pathogens like *Escherichia coli* and *Clostridium* spp. (Zhang, Huang, Zeng, Wu, & Peng, 2013). Bifidobacteria and lactobacilli are among the group of gut microbiota considered to be the only microorganisms able to beneficially influence host health (Bielecka, Biedrzycka, Majkowska, Juskiewicz, & Wroblewska, 2002). Consequently, fructans can alter the colonic microbiota of the host toward a healthier composition. The degree of polymerization (DP) is an important property of fructans that affects their functionality (Rubel, Pérez, Genovese, & Manrique, 2014).

Data on fructan distribution and composition in foods is limited. Food sources of fructan include asparagus, sugar beet, garlic, chicory, onion, Jerusalem artichoke, wheat, honey, banana, barley, tomato and rye (Campbell et al., 1997; Sangeetha, Ramesh, & Prapulla, 2005). Banana is indicated as a good source of FOS (Campbell et al., 1997; Homme, Peschet, Puigserver, & Biagini, 2001). Garlic contains a mixture of FOS and inulin ranging from <1000 Da to ~6800 Da molecular mass and constitutes approximately 65% dry weight of garlic (~84% of its carbohydrate content) (Lawson & Wang, 1995). There is considerable research work on inulin from foods, particularly garlic and Jerusalem artichoke. Our earlier studies on fructan distribution in seven banana cultivars in South India demonstrated that *Nendran* variety had the highest content. It is also the variety used traditionally as weaning food (Shalini & Antony, 2015). Banana and garlic are consumed daily in all parts of India and per capita consumption of banana is about 10.5 kg/person/year (Mustaffa, 2012); onion and garlic about 7.83 kg/person/year (Gopal, Mahajan, Gawande, Sankar, & Khar, 2013).

India produces 29,724.6 million tonnes of bananas from an area of 802.8 ha. It is the largest producer of banana not only in Asia but also in the world and contributes 37.2% to global production followed, by China (6.60%) and Philippines (6.14%). Tamilnadu contributed 6736.4 million tonnes from 130.4 ha with total productivity of 5.17 million tonnes per hectare followed by Maharashtra, Gujarat and Andhra Pradesh (Indian Horticulture Database 2014). India produces 1252 million tonnes of garlic from an area

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of 231ha (Indian Horticulture Database 2014).

Fructan composition studies in banana and garlic have been carried out by few authors (Campbell et al., 1997; Hogarth, Hunter, Jacobs, Garleb, & Wolf, 2000; Muir et al., 2007; Van Loo, Coussement, De Leenheer, Hoebregs, & Smits, 1995). Fructan variations in Brazilian banana cultivars have been studied by Agopian, Purgatto, Cordenunsi, & Lajolo, 2009. Garlic fructans used as carbon source by *in vitro* batch fermentation inoculated with human fecal bacteria were investigated for its anaerobe counts, bifidobacteria and fecal microflora (Zhang et al., 2013). They proved that garlic fructans selectively stimulated the growth of beneficial *Bifidobacteria* from human fecal microflora. No such prebiotic studies are available till date in banana.

In order to confirm selectivity of a prebiotic it is necessary to study the preferential utilization of these substrates by the probiotic microbes using both *in vitro* and *in vivo* methods (Al-Sheraji et al., 2013). Knowledge of the synergistic interaction of pre and probiotics can suggest the potential synbiotic combinations to promote health or for use as therapeutics for specific conditions. The prebiotic effect of commercial FOS on LAB strains have been studied by few researchers (Durieux, Fougnies, Jacobs, & Simon, 2001; Kaplan & Hutkins, 2000; Rossi et al., 2005). Therefore, the aim of this work was to investigate the prebiotic activity of fructans from selected food sources on four LAB strains.

2. Materials and methods

2.1. Food selection

Preliminary screening for fructans in 48 commonly consumed fruits, vegetables and millets of South India identified *Nendran* banana (*Musa acuminata* x *balbisiana* - AAB Group) and two varieties of garlic – country garlic (*Allium oleraceum*) and hill garlic (*Allium ampeloprasum*) as the best sources, and hence used for this study. Each variety was purchased from ten different locations in and around Chennai city and a composite sample was used for all experiments.

2.2. Chemical compounds

1-kestose (GF2) and nystose (GF3) used for quantification assays and pepsin were purchased from Sigma-Aldrich, St. Louis, USA. A commercial FOS mixture (DP:2–10, 33% 1-kestose, 50% nystose, 13% 1-fructofuranosyl nystose, 2% sucrose, and 2% glucose) and inulin mixture (DP: 10–60, 99.5% DP $\geq$ 3, 0.5% 1-Kestose, $<$ 15% nystose), were obtained from Brenntag India Private Limited, India. The bacteriological growth media supplements like Proteose peptone, Beef extract, Yeast extract, Dextrose, Polysorbate, Ammonium citrate, Sodium acetate, Magnesium sulphate, Manganese sulphate, Dipotassium phosphate to prepare Man-Rogosa-Sharpe (MRS) glucose-free medium were obtained from Merck, India. Readymade MRS was also purchased from Hi-Media, Mumbai, India. All other reagents were analytical or HPLC grade unless otherwise specified.

2.3. Bacterial strains

Lactobacillus casei ATCC11578, *Lactobacillus acidophilus* ATCC4356 and *Escherichia coli* ATCC CRM-8739 were purchased in lyophilized form and maintained at −80 °C for long-term storage. All these strains are considered as probiotics as previously reported in different studies (Jain, McNaught, Anderson, MacFie, & Mitchell, 2004; Kaplan & Hutkins, 2000; Reid, 2008). Potential probiotic strains (based on *in vitro* screening) *Lactobacillus plantarum* and *Bacillus amyloliquifaciens* isolated from fermented *koozh* (Ilango & Antony, 2014) were also used.

2.4. Fructan characterization

The total fructan content of the samples was analysed after extraction using the megazyme Fructan HK Assay (AOAC, 2003). Fructan composition in terms of FOS (O'Donoghue et al., 2004) was obtained by HPLC and inulin (Ashwell, 1957) by colorometric assay.

2.4.1. Total fructans

2.4.1.1. Extraction. The method for extracting fructans fully described in the Megazyme Fructan HK Assay Procedure was followed. Homogenized sample (0.1–0.5 g) was weighed into a dry Pyrex beaker (100 mL capacity) and 80 mL of hot distilled water (pH 6) at 80 °C was added. The beaker was heated on a magnetic stirrer at 80 °C for 15 min until the sample was completely dispersed. The solution was cooled to room temperature (25 ± 2° C) and then quantitatively transferred to a 100 mL volumetric flask, and the volume was adjusted to 100 mL with distilled water.

Samples were further treated by filtering the solution though a Whatman No. 1 filter circle followed by immediate analysis. If the solution was still turbid, it was filtered again though a Whatman GF/A glass fiber filter paper. If analysis could not be undertaken immediately, then filtered samples can be stored at −20 °C. Samples were reheated to 80 °C and allowed to cool to room temperature (25 ± 2° C) before analysis. This extraction was carried out in triplicates for all samples. The extracted fructan was freeze dried for prebiotic efficacy studies.

2.4.1.2. Measurement. For total fructan content, two samples (A and B) were treated as follows: Sample A was treated with purified fructanase, which hydrolyzed fructan to fructose and glucose, while sample B was treated with blank. The concentration of glucose plus fructose was measured with a hexokinase/phosphoglucose isomerase (PGI)/glucose 6-phosphate dehydrogenase system. The fructan content was then measured by the difference between sample A and B and expressed on the basis of fresh weight (mg/100 g edible portion) and dry weight (g/100 g edible portion).

2.4.2. FOS

2.4.2.1. Extraction. The sample (2 g) was extracted based on the method described by O'Donoghue et al. (2004) with modifications. Distilled water (4.5 mL) was added to the sample and held for 10 min at 75 °C to extract the sugars. To the slurry, 7.5 mL methanol was added to give a final 62.5% (v/v) MeOH solution and extracted for 15 min at 55 °C. The slurry was then passed through a 0.2 μm Millex-GV syringe driven filter. Extracts were stored at −20 °C until further use.

2.4.2.2. Measurement. A liquid chromatography system (Shimadzu Corporation, Japan) supplied with an evaporative light-scattering detector (ELSD) was used for the analysis of sugars. Separation of sugars and FOS were achieved on a Shodex Asahipak NH2P- 50 4E column. The column oven temperature was maintained at 30 °C. The solvent gradient consisted of a linear increase/decrease in the amount of water in acetonitrile (% H_2O): 0–14 m, 20–45%; 14–18 m, 45–20%; 18–25 m, 20%. The flow rate was 0.75 mL m^{-1} and injections of 10 μL were made. Nitrogen was used as ELSD nebulizer gas (1.6 bar), the tube temperature was 90 °C and nebulizer temperature was set at 50 °C. The standard sugars used were glucose, fructose, sucrose, 1-kestose and nystose.

2.4.3. Inulin

For inulin analysis, the sample was extracted in 80% ethanol for 80 °C and 6 h to remove free sugars. The residue was filtered and extracted again in 80% ethanol for 10 min. The pooled extracts were combined and volume was made-up to 50 mL and analysed for

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