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Microbial ecology of sorghum sourdoughs: Effect of substrate supply and phenolic compounds on composition of fermentation microbiota

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ABSTRACT

The choice of the cereal substrate determines sourdough microbiota, however, the substrate-associated ecological factors for this phenomenon have not been elucidated. This study investigated the competitiveness of *Lactobacillus sanfranciscensis* LTH 2590, a wheat sourdough isolate, and four isolates from sorghum sourdoughs (ting), *Lactobacillus casei* FUA3166, *Lactobacillus harbinensis* FUA3199, *Lactobacillus parabuchneri* FUA3169, and *Lactobacillus coryniformis* FUA3307, in sorghum sourdoughs, sorghum sourdoughs supplemented with maltose, or wheat sourdoughs. Fermentations were characterised by determination of cell counts, pH, and quantification of metabolites. Maltose was the main carbon source in wheat sourdoughs whereas glucose was the major carbon source in sorghum. *L. coryniformis* and *L. parabuchneri* produced 1,3- and 1,2-propanediol from glycerol and lactate, respectively, metabolites that were previously not observed in sourdough. To determine the competitiveness of strains, wheat and sorghum slurries were inoculated with equal cells counts of *L. sanfranciscensis*, *L. parabuchneri*, and *L. casei* fermented at 28 °C or 34 °C and propagated by back-slopping every 24 h. Lactobacilli in sourdough were quantified by plating and species-specific quantitative PCR (qPCR). Generally, sorghum and wheat sourdoughs inoculated with isolates from ting gave no appreciable differences in the metabolites produced during the fermentation process. *L. sanfranciscensis* grew in wheat but not in sorghum sourdoughs, or sorghum sourdoughs supplemented with 2% maltose, 1% tryptone, 0.1% L-cysteine and 2% sucrose. Furthermore, *L.sanfranciscensis* decreased progressively during propagation of sorghum sourdoughs but ting isolates were overgrown by *L. sanfranciscensis* after three propagations in wheat sourdoughs independent of the incubation temperature. The anti-microbial activity of four different types of sorghum extracts was tested against *L. sanfranciscensis*, *L. parabuchneri*, and *L. casei* to correlate the resistance to phenolic compounds to growth in wheat or sorghum sourdoughs. *L. sanfranciscensis* was inhibited by phenolic extracts from sorghum flours whereas ting isolates were resistant. In conclusion, microbiota of sorghum sourdough differ from wheat and rye because sorghum contains active concentrations of antimicrobial phenolic compounds, and offers glucose as major carbon source.

1. Introduction

Sourdough has been traditionally used in baking applications to acidify and leaven bread dough (Hammes and Gänzle, 1998). Lactic fermented cereals are also used for further processing into beverages, crackers, gruels and porridges (Hammes et al., 2005; Vogel et al., 1999). The composition of sourdough microbiota is influenced by the fermentation process, particularly time, temperature, and dough yield (De Vuyst and Vancanneyt, 2007; Meroth et al., 2003; Vogel et al., 1999). Additionally, endogenous factors such as flour carbohydrates, enzymes, and microbial interaction impact microbial growth and metabolic activity (Gobbetti, 1998; Hammes and Gänzle, 1998; Meroth et al., 2003; Van der Meulen et al., 2007). Microbiota of sourdoughs propagated in wheat or rye flours do not exhibit characteristic differences (De Vuyst and Vancanneyt, 2007). However, the use of other cereal flours or pseudocereals selects for fermentation microbiota that differ from wheat and rye sourdoughs (Hammes et al., 2005; Moroni et al., 2011; Vogelmann et al., 2009).

Traditional fermentations of millet, sorghum, maize, pseudocereals, and pulses are carried out in Africa and Asia (Nout, 2009). One example is ting, a Botswana traditionally fermented sorghum product produced from sorghum flour, water and lactic fermentation (Sekwati-Monang and Gänzle, 2011). Microbiota of back-slopped sorghum sourdoughs only partially overlap with the microbiota of wheat and rye sourdoughs. *L. reuteri*, *L. plantarum*, and *L. fermentum* were isolated from sorghum as well as wheat and rye sourdoughs. *L. casei*, *L. coryniformis*, *L. parabuchneri*, *L. harbinensis*, however, were among dominant ting microbiota but are

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not frequently found in wheat- or rye sourdoughs. *L. sanfranciscensis*, a key organism in traditionally produced wheat and rye sourdoughs, was particularly absent in ting, kisra, or model sorghum sourdoughs performed at the laboratory scale (De Vuyst and Vancanneyt, 2007; Hamad et al., 1992; Sekwati-Monang and Gänzle, 2011; Vogelmann et al., 2009). Moreover, ting isolates exhibited usual metabolic activities, including lactate conversion to 1,2-propanediol, glycerol conversion to 1,3-propanediol (Sekwati-Monang and Gänzle, 2011), and metabolism of phenolic acids (Svensson et al., 2010).

In analogy to the selection of starter cultures for use in wheat and rye sourdough fermentations, the selection of cultures for sorghum sourdough fermentation requires strains that are highly adapted to the cereal substrate. Moreover, novel metabolites from sorghum-adapted fermentation microbiota allow novel applications of sourdough starter cultures (Gänzle et al., 2009). However, the substrate-associated ecological factors selecting for specific microbiota remain unknown. It was therefore the aim of this investigation to identify factors that determine the composition of fermentation microbiota in sorghum sourdoughs. Growth and metabolism of the ting isolates *L. casei* FUA3166, *L. harbinensis* FUA3199, *L. parabuchneri* FUA3169, and *L. coryniformis* FUA3307 in wheat and sorghum sourdoughs was compared to growth and metabolism of *L. sanfranciscensis*.

2. Materials and methods

2.1. Bacterial strains and growth conditions

L. sanfranciscensis LTH2590, a wheat sourdough isolate (Böcker et al., 1995), and four isolates from sorghum sourdoughs (ting) produced in Botswana, *L. casei* FUA3166, *L. harbinensis* FUA3199, *L. parabuchneri* FUA3169, and *L. coryniformis* FUA3307 (Sekwati-Monang and Gänzle, 2011), were cultivated in modified MRS medium (mMRS) (Stolz et al., 1996). Strains were grown overnight at 30 °C without agitation unless otherwise specified. To obtain defined inoculum for sourdough fermentations, cells from 10 mL overnight culture were harvested by centrifugation at 4000×*g* for 5 min at 15 °C, washed twice with sterile phosphate (PBS) buffer, and re-suspended in 10 mL of sterile tap water.

2.2. Model sourdough fermentations

White sorghum flour and whole wheat flour were obtained from a local supermarket. Pure sorghum cultivars (PAN 3860, Town and Segaolane) were obtained from the National Food Technology Research Centre, Kanye, Botswana, and ground in an Ultra Centrifugal Mill ZM200 (Retsch, Burlington, Canada) to a particle size of 0.5 mm or less. 10 g of flour was mixed with tap water and cell suspensions in tap water of 1 of the 5 lactobacilli to achieve an initial cell count of approximately 10^7 cfu/g, and incubated at 34 °C (ting isolates) or 28 °C (*L. sanfranciscensis*). Acid aseptic doughs were used as controls; acid aseptic doughs were acidified to a pH of 4.0 with a mixture of lactic and acetic acids (4:1) (v/v). Samples were taken every 24 h for characterisation of cell counts, pH, and the quantification of metabolites by HPLC as described below. Growth of *L. sanfranciscensis* was additionally monitored in sorghum sourdoughs supplemented with 2% maltose, 2% sucrose, 0.1% L-cysteine, or 1% tryptone. Fermentations were carried out in triplicate independent experiments.

To determine the competitiveness of strains, wheat and sorghum sourdoughs were inoculated with a strain cocktail consisting of *L. sanfranciscensis*, *L. parabuchneri*, and *L. casei*. Inocula were prepared by growing the three strains individually in mMRS broth at 34 °C or 28 °C for 18–24 h. Cell pellets were harvested by centrifugation at 4000×*g* for 5 min at 15 °C, washed twice with sterile phosphate (PBS) buffer, and re-suspended in 10 mL of sterile tap water. Sourdoughs were inoculated with the three strains to achieve approximately equal cell counts of 10^7 cfu/g, and fermented at 28 °C or 34 °C for 24 h. After 24 h of incubation, sourdoughs were propagated by using ripe sourdough to inoculate a new batch of sourdough with a 5% inoculum (1 g ripe sourdough, 10 g flour, 10 g sterile tap water). The propagation of sourdoughs was continued over four fermentation cycles corresponding to 4 days. Fermentations were carried out in triplicate independent experiments.

2.3. Determination of bacterial counts and quantification of substrates and metabolites

The pH of sourdoughs was measured with a glass electrode after dilution of sourdough samples with MilliQ water. Cell counts were enumerated after plating of serial 10-fold dilutions of sourdough samples on mMRS agar. In samples from sourdoughs inoculated with strain cocktails, colonies representing *L. sanfranciscensis* were readily differentiated from colonies representing *L. parabuchneri* or *L. casei* on the basis of the colony morphology. Differential cell counts for *L. sanfranciscensis* and (*L. parabuchneri* + *L. casei*) are reported. Sugars, organic acids and glycerol were quantified by HPLC using Aminex HPX-87 column, 300 mm × 7.8 mm (BioRad, Torrance, California, USA). For HPLC analyses, sourdoughs were diluted with 5 volumes of milliQ water and centrifuged to remove solids. Proteins were precipitated by addition of 50 μL of 70% perchloric acid to 1 mL sample, overnight incubation at 4 °C, and centrifugation to remove solids. Samples were eluted with 5 mmol/L H_2SO_4 at a flow rate of 0.4 mL/min and 80 °C. The injection volume was 10 μL and quantification was based on refractive index detection. Lactate was additionally quantified by UV detection at 210 nm to avoid interference with glycerol. Concentration of maltose, glucose, lactate, acetate, ethanol, glycerol, 1,2-propanediol and 1,3-propanediol was determined using external standards.

2.4. DNA extraction from bacterial cultures and sourdough

For extraction of DNA from bacterial cultures, cells of *L. casei*, *L. parabuchneri* and *L. sanfranciscensis* were harvested from overnight cultures. DNA was extracted using DNeasy Blood and Tissue Kit (Qiagen Inc., Toronto, Ontario, Canada).

For extraction of total DNA from sourdough samples, 10 g of sourdough was mixed with 90 mL of sterile saline-tryptone (8.5 g NaCl and 1 g tryptone per L). An aliquot of 50 mL was centrifuged at 4 °C for 5 min at 500×*g* to remove solids. Cells were harvested by centrifugation 15 min at 5000×*g* and cell pellets were stored at −20 °C until use. Frozen cell pellets were thawed, washed three times with 1 mL of sterile phosphate- buffered saline (8.0 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4 per L, pH 7.4), and DNA was extracted using DNeasy Blood and Tissue Kit (Qiagen). DNA was purified by adding an equal volume of phenol to the DNA solution, mixing, and recovery of the aqueous phase after centrifugation at 2000×*g*, 5 min. The aqueous phase was mixed with an equal amount of 24:1 (v/v) chloroform-isoamyl alcohol, and DNA was precipitated by adding 0.1 volume of 3 M sodium acetate (pH 5) and 2 volumes of ethanol, and incubated at −20 °C overnight. The precipitated DNA was recovered by centrifugation, dried at 50 °C for 5 min, and resuspended in 100 μL of sterile water. Quantity and quality of DNA were checked on a Nanodrop spectrophotometer system ND-1000, version 3.3.0 (Thermo Fisher Scientific Inc., Wilmington, DE, USA).

2.5. PCR and quantitative PCR analyses

PCR quantification of lactobacilli was based on primers specific for *L. sanfranciscensis*, *L. parabuchneri*, or the *L. casei*-group (Table 1). The oligonucleotide primers were purchased from Invitrogen (Burlington, ON, Canada). PCR was performed with a Gene Amp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) in a total volume 50 μL containing 5× buffer (10 μL), 1.5 μL of each deoxynucleotide triphosphate, 1 μL of each primer, 0.25 μL of GoTaq DNA polymerase (all