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# Community dynamics and metabolite target analysis of spontaneous, backslopped barley sourdough fermentations under laboratory and bakery conditions

Henning Harth, Simon Van Kerrebroeck, Luc De Vuyst *

*Vrije Universiteit Brussel, Faculty of Sciences and Bioengineering Sciences, Research Group of Industrial Microbiology and Food Biotechnology (IMDO), Pleinlaan 2, B-1050 Brussels, Belgium*

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ABSTRACT

Barley flour is not commonly used for baking because of its negative effects on bread dough rheology and loaf volume. However, barley sourdoughs are promising ingredients to produce improved barley-based breads. Spontaneous barley sourdough fermentations were performed through backslopping (every 24 h, 10 days) under laboratory (fermentors, controlled temperature of 30 °C, high dough yield of 400) and bakery conditions (open vessels, ambient temperature of 17–22 °C, low dough yield of 200), making use of the same batch of flour. They differed in pH evolution, microbial community dynamics, and lactic acid bacteria (LAB) species composition. After ten backsloppings, the barley sourdoughs were characterized by the presence of the LAB species *Lactobacillus fermentum*, *Lactobacillus plantarum*, and *Lactobacillus brevis* in the case of the laboratory productions (fast pH decrease, pH < 4.0 after two backslopping steps), and of *Leuconostoc citreum*, *Leuconostoc mesenteroides*, *Weissella confusa* and *Weissella cibaria* in the case of the bakery productions (slow pH decrease, pH 4.0 after eight backslopping steps). In both sourdough productions, *Saccharomyces cerevisiae* was the sole yeast species. Breads made with wheat flour supplemented with 20% (on flour basis) barley sourdough displayed a firmer texture, a smaller volume, and an acceptable flavour compared with all wheat-based reference breads. Hence, representative strains of the LAB species mentioned above, adapted to the environmental conditions they will be confronted with, may be selected as starter cultures for the production of stable barley sourdoughs and flavourful breads.


## 1. Introduction

Sourdough, a fermented flour-water mixture, represents a complex microbial ecosystem of yeasts and/or lactic acid bacteria (LAB) that is used worldwide in baked goods (De Vuyst and Neysens, 2005; De Vuyst et al., 2009, 2014; Minervini et al., 2014). In the case of a spontaneous sourdough fermentation process, these microorganisms originate from the flour itself, other dough ingredients, or the environment (Minervini et al., 2010; Scheirlinck et al., 2007, 2009; Siragusa et al., 2009). Upon backslopping, strains of certain species that are well adapted to the prevailing conditions such as the available carbohydrates and amino acids, a low pH and high acidity, and process parameters such as temperature, dough yield, and backslopping time dominate (De Vuyst et al., 2014; Minervini et al., 2014; Vogelmann and Hertel, 2011; Vrancken et al., 2011). Traditional, continuously backslopped or type I sourdoughs are carried out in artisan bakeries at room temperature and low dough yield, and harbour mostly heterofermentative LAB species (Ercolini et al., 2013; Lattanzi et al., 2013; Minervini et al., 2012b; Scheirlinck et al., 2008; Vrancken et al., 2010). Those sourdough processes have been mimicked in laboratory fermentors to study their species diversity and community dynamics (Minervini et al., 2012b; Van der Meulen et al., 2007; Vrancken et al., 2010, 2011; Weckx et al., 2010a, 2010b, 2011). All these sourdough fermentations have revealed the presence of *Lactobacillus fermentum*, *Lactobacillus plantarum*, and/or *Lactobacillus sanfranciscensis* as prevailing LAB species, whether or not in association with certain yeast species, such as *Candida humilis*, *Saccharomyces cerevisiae*, and *Wickerhamomyces anomalus*. *Lactobacillus sanfranciscensis* is typical for wheat, rye, and spelt sourdough fermentation processes that are characterized by low incubation temperature and appropriate backslopping procedures (De Vuyst et al., 2014; Scheirlinck et al., 2008). Alternatively, sourdough fermentations can be initiated by the addition of a sourdough starter culture that is competitive enough toward the background microbiota. Industrial, type II sourdoughs are characterized by higher fermentation temperatures (>30 °C), longer fermentation times (usually one step or no refreshment), and higher water contents than type I sourdoughs, and are run by either homofermentative (e.g., *Lactobacillus amylovorus*) or strictly heterofermentative (e.g., *Lb. fermentum*), heat- and acid-tolerant LAB species (Bessmeltseva et al., 2014; Meroth et al., 2003b; Ravyts and De Vuyst, 2011). A third variant of sourdough fermentation processes includes backslopping of starter culture-initiated fermentations (De Vuyst et al., 2014; Minervini et al., 2010; Siragusa et al., 2009).

* Corresponding author.
*E-mail address:* ldvuyst@vub.ac.be (L. De Vuyst).

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During sourdough fermentation, *Lb. fermentum* and *Lb. sanfranciscensis* degrade maltose into a mixture of lactic acid, acetic acid and/or ethanol, and carbon dioxide via the phosphogluconate pathway (Gänzle and Gobbetti, 2013; Gänzle et al., 2007; Gobbetti et al., 2005). Yeasts are mainly responsible for leavening and flavour production through glucose fermentation and amino acid conversions, respectively (De Vuyst and Neysens, 2005; De Vuyst et al., 2014; Guerzoni et al., 2013). All these microbial activities lower the pH of the cereal matrix and influence the nutritional and organoleptic properties of the sourdoughs and bakery products produced thereof (Arendt et al., 2007; Gänzle and Gobbetti, 2013; Poutanen et al., 2009). Furthermore, the competitive strength of sourdough-typical LAB species is based on the ability to use alternative external electron acceptors present in the sourdough matrix, such as fructose that is then reduced into mannitol; the expression of several stress responses, for instance arginine deiminase pathway activity that results in the excretion of citrulline and ornithine and the production of ammonia and extra ATP; and the feature to produce antimicrobial compounds (De Vuyst et al., 2009, 2014; Gänzle and Gobbetti, 2013; Gänzle et al., 2007; Gobbetti et al., 2005). Sourdough fermentation by LAB and yeasts results in a wide range of volatile organic compounds that, together with Maillard reactions and Strecker degradation of several compounds during the baking process, contribute to the bread flavour (Hansen and Schieberle, 2005; Pico et al., 2015; Rehman et al., 2006).

Most research on spontaneous sourdough fermentations has been performed on wheat and rye sourdoughs (Ercolini et al., 2013; Scheirlinck et al., 2007, 2008; Van der Meulen et al., 2007; Vrancken et al., 2010, 2011; Weckx et al., 2010a, 2010b, 2011). Research on sourdoughs from barley (*Hordeum vulgare* L.) flour is restricted to only a few studies, focusing on the potential of barley sourdoughs to improve the quality of the flour and the dough rheology of barley flour-based breads or on fermentations with LAB starter cultures to initiate barley fermentation (Coda et al., 2012; Hole et al., 2012; Liljeberg et al., 1995; Marklinder and Johansson, 1995; Marklinder et al., 1996; Rieder et al., 2012; Rizzello et al., 2012; Vogelmann et al., 2009; Zannini et al., 2009). Barley is an annual (summer barley) or biennial (winter barley) grass (belonging to the family *Poaceae*). It was one of the first domesticated cereals and it is now cultivated widely. As wheat and rye, barley contains gluten (little); as oat, it contains β-glucans. Barley bread is considered traditional English bread that was common up to the mid-nineteenth century, and was already known along the silk route according to archaeological findings in the Subeixi cemetery (500–300 BCE) in Xianjiang, China (Rubel, 2011; Shevchenko et al., 2014). However, barley is usually used for the production of malt, the raw material for beer and whiskey making (Bokulich and Bamforth, 2013).

The present systematic study aimed to determine the bacterial and yeast species diversity, community dynamics and metabolite kinetics of spontaneous barley sourdough fermentations performed through backslopping under laboratory and bakery conditions with the same batch of flour.

## 2. Materials and methods

### 2.1. Flour

The barley flour used throughout this study was provided by a local flour mill (West-Flanders, Belgium). It contained (m/m) 13.0% moisture, 2.0% ash, 75.0% carbohydrates, and 8.5% proteins.

### 2.2. Sourdough productions

Spontaneous barley sourdough fermentations were carried out through backslopping, both in the laboratory and in a small industrial bakery, in duplicate.

#### 2.2.1. Laboratory sourdough productions

Type II sourdoughs (8 kg) were produced in 15-litre laboratory fermentors (Biostat® C; Sartorius, Melsungen, Germany), as outlined previously (Van der Meulen et al., 2007). Therefore, the fermentors were sterilized in situ (121 °C, 2.1 bar, 20 min) in the presence of water. To start the backslopping procedure, 2.0 kg of barley flour was mixed with 6.0 L of sterile water, resulting in a dough with a dough yield (DY) of 400 [DY = (dough mass / flour mass) × 100]. The dough was incubated at 30 °C and kept homogeneous by continuous stirring (300 rpm). After 24 h of incubation, 800 g of ripe sourdough was collected into a sterile bottle to inoculate a fresh flour-water mixture (1.8 kg of flour and 5.4 L of water) in a second sterile fermentor on day 2. This dough was again incubated under the same conditions as described above. The backslopping procedure was carried out during a period of 10 days. The laboratory sourdough productions are further referred to as BF1 and BF2.

#### 2.2.2. Bakery sourdough productions

The backslopping procedure of the bakery sourdoughs (6.0 kg) was carried out in open vessels in a bakery facility. The fermentation was started by mixing 3.0 kg of flour with 3.0 L of water in one plastic vessel with a resulting DY of 200. This dough was incubated at ambient temperature without mixing; the temperature after refreshment averaged 22 °C and decreased to an average of 17 °C after 24 h of incubation due to the cooler temperature at night. After 24 h of incubation, 600 g of ripe sourdough was collected to inoculate a fresh flour-water mixture in a second vessel. This dough was again incubated under the same conditions as described above. The backslopping procedure was carried out during a period of 10 days. The bakery sourdough productions are further referred to as BF1′ and BF2′.

### 2.3. Sampling procedure

Before each refreshment step (every 24 h), samples of 50 mL were taken from the ripe sourdoughs to measure pH, to determine the total titratable acidity (TTA), and to perform platings; the latter were only performed at the start and before backslopping steps 1, 2, 4, 7, and 10. Further, approximately 50 mL of sourdough were centrifuged at 8507 ×*g* for 20 min at 4 °C to remove solids and the supernatants were stored at −20 °C for metabolite target analysis. Additionally, fresh sourdough samples (50 mL) were stored at −20 °C to perform culture-independent microbiological analyses.

### 2.4. Determination of pH and TTA

The pH was measured with an InoLab 720 pH meter (WTW, Weilheim, Germany). The TTA value was determined by suspending 10 g of sourdough into 90 mL of ultra-pure water. The TTA value was expressed as the amount (in mL) of 0.1 M NaOH needed to titrate the sample until a final pH of 8.5. Analyses of pH and TTA were performed in triplicate; averages are reported.

### 2.5. Culture-dependent community dynamics and identifications

To determine the counts of LAB and yeasts, 5 g of sourdough were mixed with 45 mL of saline (0.85% NaCl, m/v) and homogenized in a stomacher bag (Stomacher 400; Seward, Worthington, United Kingdom) for 10 min, as described previously (Van der Meulen et al., 2007). A tenfold dilution series of this suspension was made and 100 μL of each dilution was plated on modified de Man-Rogosa-Sharpe-5 (mMRS-5) agar medium (Meroth et al., 2003b), supplemented with cycloheximide (final concentration of 0.1 g/L; Sigma-Aldrich, Saint Louis, Missouri, USA), and yeast-glucose-chloramphenicol (YGC) agar medium (Meroth et al., 2003a), chloramphenicol (Sigma-Aldrich) being present in a final concentration of 0.1 g/L. The plates were incubated at 30 °C for 48 h. These platings