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Sourdough microbial community dynamics: An analysis during French organic bread-making processes

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ABSTRACT

Natural sourdoughs are commonly used in bread-making processes, especially for organic bread. Despite its role in bread flavor and dough rise, the stability of the sourdough microbial community during and between bread-making processes is debated. We investigated the dynamics of lactic acid bacteria (LAB) and yeast communities in traditional organic sourdoughs of five French bakeries during the bread-making process and several months apart using classical and molecular microbiology techniques. Sourdoughs were sampled at four steps of the bread-making process with repetition. The analysis of microbial density over 68 sourdough/dough samples revealed that both LAB and yeast counts changed along the bread-making process and between bread-making runs. The species composition was less variable. A total of six LAB and nine yeast species was identified from 520 and 1675 isolates, respectively. The dominant LAB species was *Lactobacillus sanfranciscensis*, found for all bakeries and each bread-making run. The dominant yeast species changed only once between bread-making processes but differed between bakeries. They mostly belonged to the *Kazachstania* clade. Overall, this study highlights the change of population density within the bread-making process and between bread-making runs and the relative stability of the sourdough species community during bread-making process.

1. Introduction

Sourdough is used as an alternative to baker's yeast to leave bread dough, although some bakeries use both (De Vuyst and Neysens, 2005). In France, the regulations define sourdough as "dough made from wheat or rye (…) with added water and salt (optional), containing a naturally acidifying microbiota made up primarily of lactic bacteria and yeasts" (Article 4 of Decree no. 93-1074 of September 13th 1993). In addition, the decree specifies that sourdough bread "has a potential maximum pH of 4.3 and an acetic acid content of at least 900 ppm". Many studies have now demonstrated the nutritional, sensory, texture and shelf-life advantages of using sourdough composed of lactic acid bacteria (LAB) and yeasts (Galle and Arendt, 2013; Gänzle, 2014; Poutanen et al., 2009).

Among LAB, the genera *Pediococcus*, *Leuconostoc*, *Weissella*, and especially *Lactobacillus*, have frequently been identified (De Vuyst et al., 2009; Hammes et al., 2005; Huys et al., 2013). Among yeasts, the species *Saccharomyces cerevisiae* and *Candida humilis* are those most commonly found (Iacumin et al., 2009; Minervini et al., 2012a; Vernocchi et al., 2004). Some stable and specific associations between LAB and yeasts, due to particular nutritional, trophic, and metabolic interactions, have been described (Corsetti and Settanni, 2007; De Vuyst and Neysens, 2005; De Vuyst and Vancanneyt, 2007; Gänzle et al., 2007; Gobbetti et al., 2005).

Recently, a number of studies have considered the influence of different factors on the microbial species composition of sourdough. For instance, the microbial community has been found to change according to the wheat species (Minervini et al., 2012a), the cereals used (Vogelmann et al., 2009), the temperature and the

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back-slopping time (Vrancken et al., 2011a), the location of the propagation (artisan bakery or laboratory) (Minervini et al., 2012b), and technological factors (Vogelmann and Hertel, 2011).

Despite the well-known microbial composition of sourdoughs in Italy (Lattanzi et al., 2013; Minervini et al., 2012a,b), Belgium (Scheirlinck et al., 2007, 2008), and Germany (Meroth et al., 2003a,b), few studies have investigated both LAB and yeast sourdough diversity in France (Huys et al., 2013 and references therein). In fact, previous works on French sourdoughs have considered only LAB diversity (Ferchichi et al., 2007; Robert et al., 2009; Valcheva et al., 2005; Vera et al., 2011).

Furthermore, to our knowledge, no previous study has investigated the dynamics of the microbial community during the bread-making process except for sweet baked products (Venturi et al., 2012). In addition, only few data are available on the long-term stability of sourdough ecosystems in bakeries (Gänzle and Vogel, 2003; Rosenquist and Hansen, 2000; Scheirlinck et al., 2008). The comparison of the microbiota in one conventional and one organic sourdough suggests that the genetic diversity is less stable in the organic sourdough compared to the conventional one (Rosenquist and Hansen, 2000). However, the stability of the microbiota was never investigated further in organic sourdoughs.

The aims of this study were (i) to analyze the stability of LAB and yeast counts in French organic sourdoughs along the bread-making process and between bread-making runs, (ii) to study the relationships between microbial counts and dough biochemical properties, and (iii) to characterize the LAB and yeast species composition throughout the bread-making processes and their long-term stability.

2. Materials and methods

2.1. Sourdough and bread collection

A total of five bakeries located in distinct regions of France were selected (Supplementary material S1), based on their choice to promote biodiversity conservation and to limit their ecological footprint through the use of natural sourdough and organic flour. A semi-structured interview to describe their bread-making practices was carried out among the five bakeries. The ingredients and technological parameters involved are summarized in Supplementary material S1. In all the bakeries, the chief sourdough was obtained by taking a piece of final leavened dough or dough after kneading at each bread-making run. Bakers' practices differed from one bakery to another (Supplementary material S1). Although temperatures were relatively the same in the bakeries, the number of back-sloppings before the final leavened dough step varied between bakeries, from one (in Bakeries 4 and 5) to three back-sloppings (in Bakery 2). Some other specificities might be noticed: a firm sourdough recipe in Bakery 5, a variation of the back-slopping period (short for Bakeries 1 and 2 (about 6 h)) and the percentage of sourdough added in final dough (higher for Bakeries 1 and 4 (>30%)).

Samples were taken at four steps during the bread-making process: chief sourdough (CS), final leavened dough (LD), dough after kneading (AK) and bread before baking (BB) (Supplementary material S2). Two independent samples were taken at each bread-making process step except for Bakery 1 and for the first bread-making run sampling for Bakeries 2 and 3. For each bakery, the sampling was repeated during two different bread-making runs, seven to ten months apart (Supplementary material S1). All samples were kept at 4 °C for three to seven hours before analysis. Then, LAB and yeast were enumerated for all collected samples. Finally, LAB and yeast species were identified for all samples of the first collection and for the final leavened dough samples of the second analyzed bread-making run.

2.2. LAB and yeast enumeration and isolation

Sourdough samples (10 g) were homogenized by adding 90 ml of sterile tryptone salt (TS) solution [0.85% (wt/vol) NaCl; 0.1% tryptone (wt/vol)] and mixing for 2 min using a Stomacher (AES Laboratories, France). A 10-fold dilution series from 10^{-2} to 10^{-6} was made and plated in triplicate using either a spiral plate method (Easyspiral, Intersciences, Saint Nom, France) or a spreading plate method on MRS5 agar (Meroth et al., 2003b) and on Yeast Extract (YE) media (yeast extract 1%, glucose 2%, agar 1.7%) to determine LAB and yeast counts, respectively. LAB were counted after incubation for 48 h at 30 °C under anaerobic conditions (Anaerocult A, Merck, Darmstadt, Germany). Yeasts were enumerated after incubation for 48 h to 72 h at room temperature under aerobic conditions.

For each sourdough sample, 30 LAB colonies and 40 yeast colonies were randomly picked from the plates and isolated on MRS5 agar and YE agar, respectively. After catalase and Gram tests, a total of 520 Gram-positive and catalase-negative LAB isolates were selected and stored at −80 °C in MRS5 medium containing glycerol (20%). A total of 1675 yeast isolates were stored at −80 °C in YE medium containing glycerol (17.4%).

The strains used as references for LAB and yeast species identification are listed in Supplementary material S3. The yeast type strains used in this study are preserved at CIRM-Levures (http://www6.inra.fr/cirm/Levures).

2.3. Determination of pH, titratable acidity and organic acids

Determination of pH, titratable acidity and organic acid content was carried out for each sourdough and dough sample as well as for bread. Ten grams of sourdough, dough or bread were mixed with 90 ml of TS solution in a Stomacher for 2 min. Then, 10 ml of the mixture was homogenized and the pH and total titratable acidity (TTA) were measured with an automatic titrator (pH-Matic 23, Crison). For D- and L-lactic and acetic acid measurements, 10 ml of the homogenized mixture was centrifuged at 7, 000 g for 10 min at room temperature and the supernatant was used for measurements using the Enzytec™ Kit as described in the instruction manual, and by measuring absorbance at 340 nm (Genesys 10). Acid amounts were expressed as g/kg of sourdough, dough or bread.

2.4. DNA extraction

The direct DNA extractions from sourdough or dough samples (30 g) were performed as described previously (Jaffrès et al., 2009).

The chromosomal DNA of bacterial strains was extracted from the pellet of bacteria grown in 5 ml MRS5. Then, DNA purification was carried out using a Qiagen DNeasy Blood and Tissue Kit, as described in the Qiagen instruction manual (Qiagen, SA, Courtaboeuf, France).

For DNA extraction from yeasts, strains were grown at 30 °C in 1 ml of YE (1% YE, 2% glucose) shaken at 200 rpm. After digestion of the pellet for one hour at 37 °C using 10 units of zymolyase (Euromedex, Souffelweyersheim, France) in 0.5 ml of sorbitol 1 M, Na2EDTA 0.1 M, pH 7.5 buffer, a classic method of DNA extraction was used in which the cell membrane was broken with a detergent (SDS), deproteinization was carried out with potassium acetate and finally DNA was purified on a Whatman unifilter (Whatman, Florham Park, NJ). For 28 isolates coming from Bakery 2, DNA glass bead extraction was adapted from Burke et al. (2000).