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House microbiotas as sources of lactic acid bacteria and yeasts in traditional Italian sourdoughs

Fabio Minervini, Anna Lattanzi, Maria De Angelis*, Giuseppe Celano, Marco Gobbetti

Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy

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ABSTRACT

This study aimed at understanding the extent of contamination by lactic acid bacteria (LAB) and yeasts from the house microbiotas during sourdough back-slopping. Besides sourdoughs, wall, air, storage box, dough mixer and flour of four bakeries were analyzed. Based on plate counts, LAB and yeasts dominated the house microbiota. Based on high throughput sequencing of the 16S rRNA genes, flour harbored the highest number of *Firmicutes*, but only few of them adapted to storage box, dough mixer and sourdough. *Lactobacillus sanfranciscensis* showed the highest abundance in dough mixer and sourdoughs. *Lactobacillus plantarum* persisted only in storage box, dough mixer and sourdough of two bakeries. *Weissella cibaria* also showed higher adaptability in sourdough than in bakery equipment, suggesting that flour is the main origin of this species. Based on 18S rRNA data, *Saccharomyces cerevisiae* was the dominant yeast in house and sourdough microbiotas, excepted one bakery dominated by *Kazachstania exigua*.

The results of this study suggest that the dominant species of sourdough LAB and yeasts dominated also the house microbiota.

1. Introduction

Sourdough, the natural starter widely used to manufacture leavened baked goods, originates from a mixture of flour and water that is spontaneously fermented by indigenous microorganisms. After several refreshments (5–7 days of propagation), a panel of lactic acid bacteria (LAB) and yeasts constitute the dominant sourdough microbiota. Yeasts and, especially, LAB are responsible for the sensory, rheology, nutritional, functional and shelf life properties of sourdough baked goods (Arendt et al., 2011; Gobbetti et al., 2005, 2014). Due to such relevance, sourdough is widely studied with more than 900 published items only in the last ten years.

The microbial composition of mature sourdoughs was previously characterized and reviewed (Huys et al., 2013), which revealed more than sixty LAB species belonging to *Leuconostoc*, *Pediococcus*, *Enterococcus*, *Weissella*, and, especially, *Lactobacillus* genera (De Vuyst et al., 2014). The diversity of lactic acid bacterium is larger than that of the yeast microbiota, since only six yeast species (*Saccharomyces cerevisiae*, *Kazachstania exigua*, *Candida humilis*, *Pichia kudriavzevii*, *Torulaspora delbrueckii*, and *Wickerhamomyces anomalus*) are mainly identified in sourdoughs (Huys et al., 2013). A single sourdough can harbor simple (few species) to very complex microbial consortia (Minervini et al., 2014). Some authors argued that the microbiota of artisan traditional sourdough is stable or with little variation over time (Scheirlinck et al., 2008; Vogel et al., 2011), some others demonstrated changes during propagation at laboratory and artisan bakery levels (Minervini et al., 2012b; Vogelmann and Hertel, 2011). In some cases, the stability of LAB and yeast strains, inoculated in dough to produce sourdoughs which were successively propagated at laboratory level, was shown to be strain-dependent (Vogelmann and Hertel, 2011). Selected strains of *Lactobacillus sanfranciscensis* and *Lactobacillus plantarum* were lost during daily back-slopping performed at laboratory level while others persisted (Scheirlinck et al., 2008, 2009; Siragusa et al., 2009; Minervini et al., 2010). *Lactobacillus helveticus* dominated in the lyophilized starte and industrial semi-fluid sourdough samples (Viiard et al., 2013). Indeed, the stability of the sourdough microbiota at artisan, large bakery and laboratory levels depends on a number of factors, including flour microbial composition, microbial interactions, composition of flour in terms of carbohydrates and free amino acids (FAA), endogenous enzymatic activities (e.g., amylase), specific technology parameters (e.g.,

* Corresponding author. Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, via Amendola 165/a, 70126 Bari, Italy.
E-mail address: maria.deangelis@uniba.it (M. De Angelis).

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leavening and storage temperature, pH and redox potential, dough hydration and yield, number of sourdough refreshment steps, fermentation time between refreshments, and the use of starters and/or baker's yeast), and bakery environment (Corsetti and Settanni, 2007; Gänzle et al., 2007; Gänzle and Vogel, 2003; Gobbetti et al., 2005; Hammes et al., 1996; Viiard et al., 2013). Obligately heterofermentative LAB were mainly correlated with flours (e.g., *Triticum durum* flour), showing high levels of maltose, glucose, fructose, and FAA (Minervini et al., 2012a). Recently, it was shown that four type I sourdoughs propagated for several years in firm condition (dough yield of 160) shifted the composition of LAB and yeasts under liquid (dough yield of 280) state of fermentation (Di Cagno et al., 2014). However, also using the same type of flour (e.g., *T. durum*) and technology, some sourdoughs change the dominant microbiota over a few years of operation, such as in the case of Pane di Altamura with Protected Designation of Origin (PDO) that showed *L. plantarum* in the year 2004 (Ricciardi et al., 2005) and *Wesseilla cibaria* (Minervini et al., 2012a) in the year 2011. Consequently, a contamination from the artisan and large bakery environment and/or presence of endophytic/epiphytic LAB and yeasts in the cereal plant can be hypothesized. Lactic acid bacterium and yeast species composition differs when comparing sourdoughs propagated at artisanal or laboratory level, because the level of contamination is supposed to be lower in the laboratory than in the artisan bakery (Minervini et al., 2012b; Vrancken et al., 2010).

A better knowledge of the microbial composition of bakery environment (house microbiota) may undoubtedly help to understand the origin of LAB and yeasts that can contaminate the sourdough during back-slopping. This study was aimed at evaluating, through plate count and culture-independent approaches, if the bakery environment is an effective source of LAB and yeasts to contaminate the sourdough during propagation. Four bakeries using sourdoughs, whose LAB and yeasts were previously characterized during artisan bakery or laboratory propagation (Minervini et al., 2012b) were selected for this study.

2. Materials and methods

2.1. Bakeries and workflow of traditional sourdoughs

Four artisan bakeries, which routinely used sourdough and are located in the South of Italy, were considered in the study: AM.B (Altamura, Bari), MT.A (Matera), MT.D (Matera), and VZ (Valenzano, Bari) (Minervini et al., 2012b). Sourdoughs were propagated by one step (daily back-slopping), without the use of starter cultures or baker's yeast. Amount of each ingredient, concentration of fermentable carbohydrates, total titratable acidity and temperature of incubation of each sourdough are showed in Table 1. Plans of each bakery and a key to all main elements are shown in Fig. S1. All bakeries adopt a similar workflow for the daily back-slopping of their sourdough: (i) one aliquot of sourdough (S, Fig. S1), obtained the day before, is taken from a dedicated storage box (SB, Fig. S1) and put into the bucket of a dough mixer (DM, Fig. S1). Sourdoughs are back-slopped in a dough mixer that is not used for kneading the bread dough. Dough mixers and storage boxes are daily cleaned up using tap water and coarse sponge. Once a week they are wiped with common vinegar (5% acetic acid).

2.2. Sampling of wheat flours, bakery environment and sourdoughs

Twenty grams of each wheat flour used in the four bakeries (AM.B, MT.A, MT.D, VZ) were sampled, transferred to laboratory and subjected to plate count and culture-independent analyses. Walls of the room wherein the sourdoughs were back-slopped, storage boxes, and clean buckets of dough mixer of the four bakeries were surface swabbed. In details, sterile rayon tip swabs (Nuova Aptaca Srl, Canelli, Asti, Italy) were moistened with sterile saline (NaCl, 9 g/L) solution and streaked across a 100 cm^2 square area of the target surface (Lahou and Uyttendaele, 2014). Swabs were inserted into tubes containing 3.5 mL of Amies gel transport medium without charcoal, in order to protect microorganisms from adverse conditions until plate count analysis (MacFaddin, 1985). Adjacent 100 cm^2-wide surfaces were sampled with sterile rayon tip swabs moistened with RNAlater®. Swabs were inserted into tubes containing 2.5 mL of RNAlater®, transferred to laboratory in dry ice and stored at −80 °C for culture-independent analysis. Air was sampled in each bakery by liquid impingement, using AGI-30 samplers (Ace Glass Co., Vineland, NJ) filled with 20 mL of either buffered peptone water, 0.01% of Tween, and 0.005% of anti-foam (for plate count analysis) or RNAlater® (for culture-independent analysis). The samplers were put in the room wherein the sourdoughs were back-slopped and were operated at a flow rate of 12.5 mL/min for 15 min (Adell et al., 2014). Sourdoughs used by the four bakeries (AM.B, MT.A, MT.D, VZ) were sampled at the end of fermentation. All samples were refrigerated in a cool box during the transport. Ten grams of sourdough were used, within two hours since sampled, for plate count analysis. Another aliquot (10 g), destined to culture-independent analysis, was stored in 10 mL of RNAlater® diluted (1:1) with water and kept at −20 °C until extraction of RNA. For each bakery, three replicate samples of flour, air, surfaces (wall, storage box, dough mixer), and sourdough were taken.

2.3. Enumeration of bacteria and yeasts

Cell densities of presumptive LAB, enterococci, staphylococci, enterobacteria, and yeasts were enumerated using the agar media (Oxoid Ltd., Basingstoke, UK) reported in Table 1S. Before inoculating the media, flours and sourdoughs were homogenized with 90 mL of sterile peptone water as previously described (Ercolini et al., 2013; Minervini et al., 2012a). Enumeration of microbial groups contaminating the surfaces of the bakery environment

Table 1
Ingredients, temperature of incubation, concentration of fermentable carbohydrates and total titratable acidity of the traditional sourdoughs object of study.

Sourdoughs	Type of flour	Amount (g per kg of dough) of:				Carbohydrates[a] (%, w/w)	TTA[b]	T[c] (°C)
		Flour	Sourdough	Water	NaCl			
AM.B	*Triticum durum*	549	109.5	330.5	11.0	0.9 ± 0.05	9.2 ± 0.5	25
MT.A	*Triticum durum*	622	62.0	311.0	5.0	0.9 ± 0.01	10.5 ± 0.6	25
MT.D	*Triticum durum*	625	62.5	312.5	0	0.8 ± 0.04	7.5 ± 0.2	25
VZ	*Triticum aestivum*	462	288.0	250.0	0	0.3 ± 0.02	10.8 ± 0.3	28

[a] Sum of fructose, glucose, maltose and sucrose.
[b] Total titratable acidity (mL of 0.1 N NaOH).
[c] Temperature of incubation.