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Drivers for the establishment and composition of the sourdough lactic acid bacteria biota

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ABSTRACT

The drivers for the establishment and composition of the sourdough microbiota, with particular emphasis on lactic acid bacteria, are reviewed and discussed. More than 60 different species of lactobacilli were identified from sourdoughs, showing the main overlapping between sourdough and human intestine ecosystems. The microbial kinetics during sourdough preparation was described by several studies using various methodological approaches, including culture-dependent and -independent (e.g., high throughput sequencing), and metabolite and meta-transcriptome analyses. Although the abundant microbial diversity harbored by flours, a succession of dominating and sub-dominating populations of lactic acid bacteria suddenly occurred during sourdough propagation, leading to the progressive assembly of the bacterial community. The contribution of all the potential sources (house microbiota, flour, types of flours and additional ingredients) for contaminating lactic acid bacteria was compared with the aim to find overlapping or specific routes that affect the sourdough microbiota. Once established and mature, pros and cons regarding the stability of the sourdough lactic acid bacteria biota were also reviewed, showing contradictory results, which were mainly dependent on the species/strains.

Probably, the future research efforts should be dedicated to decrease the sources/drivers of noticeable variation rather than to full standardization of the process for sourdough preparation and use.

1. Introduction

Sourdough is one of the most ancient examples of natural starter, where contaminating lactic acid bacteria and yeasts coexist. Indeed, the first historical track is dated 77 AC by Pliny the Elder (1972). Contrarily to other food and beverage fermentations, the studies describing the sourdough ecosystem date back almost fifty years ago. Spicher and the Spanish group coordinated by Benedito de Barber were among the first researchers who consistently studied the sourdough's world (e.g., Barber et al., 1989, 1991; Spicher, 1987). The first landmark description of *Lactobacillus sanfranciscensis* was published in 1971 (Kline and Sugihara, 1971; Sugihara et al., 1971). Later and continuously, an abundant literature has followed: 1122 published items dealing with sourdough were retrieved from the main literature databases in February 2016.

Undoubtedly, the knowledge about this natural starter has increased. A consensus on the capacity of sourdough to positively influence the sensory, nutritional, texture, and shelf-life features of baked goods was achieved (De Vuyst et al., 2009; Gobbetti et al., 2014). The main metabolic and functional traits of sourdough yeasts and, especially, lactic acid bacteria were described (for reviews see De Vuyst et al., 2009, 2014; Gobbetti et al., 2014; Minervini et al., 2014). Efforts to set up technology parameters (e.g., dough yield, percentage of sourdough inoculum, salt, pH, redox potential, time and temperature of fermentation, number and length of back slopping) and to establish their effects on the sourdough performance were also largely done (De Vuyst et al., 2009; Gänzle et al., 2007; Ganzle and Vogel, 2003; Gobbetti et al., 2005; Hammes et al., 1996; Minervini et al., 2014). As the use of sourdough at industrial and, especially, artisanal levels increased, the intensive research work took shape. About 30 to 50% of the breads manufactured in European countries require the use of sourdough. In Italy, ca. 200 different types of traditional/typical sourdough breads are manufactured, especially by small or medium-size specialized bakeries (INSR, 2000; Minervini et al., 2012a). Crackers, pizza, various sweet baked goods, and gluten-free products are also made using sourdough fermentation (De Vuyst et al., 2009; Gobbetti, 1998). With the exception of some specific recipe (e.g. Panettone), that requires different technological parameters (e.g., very low temperature), traditionally type I sourdough is made from a firm dough, fermented at 30–37 °C and continuously back slopped. Despite these evident progresses, the recent literature has markedly focused on the drivers that affect the establishment and, especially, the composition of the sourdough biota. A more comprehensive interpretation of such factors is pivotal to standardize the sourdough performance and to allow the propagation and use of this natural starter more manageable and safe. Lactic acid

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bacteria, the microbial group showing both the highest influence on the sourdough performance and the highest sensitivity to ecological determinants, were subjected to an in depth study. Notwithstanding, species belonging to *Leuconostoc*, *Lactococcus*, *Enterococcus*, *Pediococcus* or *Weissella* genera, which had also been isolated from sourdoughs (De Vuyst and Neysens, 2005; Ehrmann and Vogel, 2005; Hammes et al., 2005), the interest on most of the autochthonous sourdough species/strains was mainly confined to the *Lactobacillus* genus.

This review aims at describing and discussing the main recent and relevant data on diversity and, especially, factors (house microbiota, flour, flour varieties and ingredients) that drive the establishment and composition of the sourdough lactic acid bacteria biota. Once established, pros and cons regarding the stability of the sourdough lactic acid bacteria biota were also reviewed.

2. Diversity of lactobacilli

The diversity of the sourdough microbiota depends on a number of ecological (see below) and technology determinants (De Vuyst et al., 2009; Gänzle et al., 2007; Ganzle and Vogel, 2003; Gobbetti et al., 2005; Hammes et al., 1996; Minervini et al., 2014). Furthermore, the metabolic adaptability to sourdough stressing conditions, the nutritional interactions among microorganisms (e.g., between lactobacilli and yeasts), and the intrinsic robustness or weakness of microorganisms have an influence on the microbial diversity of sourdough (Minervini et al., 2014). Sourdoughs may harbor simple (few species) to rather complex (more than six species) microbial consortia (Minervini et al., 2014) but, given the above numerous factors, the differences in taxonomy and metabolism shown by sourdough lactic acid bacteria are not surprising (De Vuyst and Neysens, 2005; De Vuyst et al., 2002).

Table 1 lists, not exhaustively, the species of lactobacilli that were identified from sourdoughs made from various flours and of different geographical origins. Differences between culture-dependent and -independent methods of analysis in some cases make difficult the data comparison. Nevertheless, more than 60 different species of lactobacilli were associated to sourdough during time, which is certainly representative of the very large diversity.

Selecting the species most frequently isolated from sourdoughs, and searching for other ecosystems wherein such species were also found (Fig. 1), the major overlapping appears between sourdoughs and human intestine and feces. *Lactobacillus reuteri* inhabits gastro-intestinal tract of vertebrates and some sourdoughs. As recently suggested by comparative genomic analyses, vertebrate-symbiont strains of *L. reuteri* may have adapted to sourdough ecosystem, through positive selection exerted on several genes involved in energy conversion and carbohydrate metabolism (Zheng et al., 2015). Overall, the cross-contamination by lactobacilli of intestinal origin was postulated by several authors (De Angelis et al., 2006; Du Toit et al., 2003; Ehrmann and Vogel, 2005). On other hand, *Lactobacillus plantarum* is the most largely isolated species from other habitats. The ecological flexibility of *L. plantarum* might be reflected by its large genome size (Molenaar et al., 2005). Features of this huge environmental adaptation and potential of this bacterium were shown when the kinetics of growth, especially the steady state, of *L. plantarum* strains were compared using as substrate different vegetable juices and wheat flour hydrolysate used as the control. Juices, in particular cherry juice, induced specific metabolic traits. Although fermentable carbohydrates are available, decarboxylation of malic acid provides energy advantages due to the increased intracellular pH and the synthesis of reducing power. Conversion of branched chain amino acids into their corresponding 2-ketoacids leads to gain of ATP. Decarboxylation of histidine into histamine provides energy through the generation of proton motive force (Filannino et al., 2014).

3. Microbial kinetics during sourdough preparation

Although the manual procedure for preparing type I sourdough (e.g. mixing flour and water) might be extremely simple, its maintenance still represents a challenge that is mastered by only a minority of the artisanal bakeries, and has yet to be achieved at the industrial scale. Traditional protocol requires the mixing of water and flour, the leaving of the dough at an appropriate temperature for a consistent number of hours, and then a number of back-slopping steps, using a part of the previously fermented dough. But, what happens during back-slopping?

To the best of our knowledge, Onno and Roussel (1994) were the first who attempted to describe the bacterial evolution during sourdough preparation. This was done in term of balance between decreasing Gram-negative towards increasing Gram-positive biota. A number of relevant papers succeeded to this first description The establishment of the sourdough microbiota using wheat, spelt or rye flours occurred through a three-phase evolution, lasting 10 days, during which sourdough typical and less typical species alternated. All mature sourdoughs were dominated by hetero-fermentative lactobacilli (e.g., *L. plantarum* and *Lactobacillus fermentum*) (Van der Meulen et al., 2007; Weckx et al., 2010a, 2010b). The codominance between *Lactobacillus brevis*, *Lactobacillus rossiae*, *L. sanfranciscensis*, *Weissella cibaria* and *L. plantarum* was also shown in several traditional Sicilian (Southern Italy) sourdoughs (Ventimiglia et al., 2015). Meta-transcriptome hybridization data, based on a lactic acid bacteria functional gene microarray, confirmed the previous results for wheat and spelt sourdoughs, also showing the functional role for *Lactococcus lactis* in the early stage of sourdough preparation and the dominance of *Pediococcus pentosaceus* in some fermentations (Weckx et al., 2010b). Other studies also emphasized the succession of lactic acid bacteria species. During back slopping (20 or 30 °C) of semi-solid (dough yield of 200) rye sourdoughs, the bacterial dynamics was described by DGGE coupled with pyrosequencing of bar-coded 16S rRNA gene amplicon and identification of isolates through partial sequencing of 16S rRNA gene (Bessmeltseva et al., 2014). Also in this case hetero-fermentative lactobacilli dominated, but their proportion varied depending on the temperature. The microbial kinetics was studied during preparation of an organic gluten-free sourdough (Lhomme et al., 2014). *L. sanfranciscensis*, *L. plantarum*, and *Lactobacillus spicheri* dominated the first phases of propagation but the 2 last species suddenly decreased, being *L. sanfranciscensis* and *Lactobacillus sakei* the predominant species at the latest stages.

The bacterial dynamics turning flours into mature rye or soft or durum wheat sourdoughs was investigated through high throughput sequencing (Ercolini et al., 2013). Permutation analysis based on biochemical and microbial features, and diversity indices all together demonstrated the maturity of the sourdoughs after 5 to 7 days of propagation. Flours showed a very large diversity, being mainly contaminated by metabolically active genera (*Acinetobacter*, *Pantoea*, *Pseudomonas*, *Comamonas*, *Enterobacter*, *Erwinia* and *Sphingomonas*) belonging to the phylum Proteobacteria or Bacteroidetes (genus *Chryseobacterium*). Soon after 1 day of propagation (just adding water to flour and waiting for fermentation), this population was almost completely inhibited, except for the *Enterobacteriaceae*. Although members of the phylum Firmicutes were present at very low or intermediate relative abundances in the flours, they became dominant after 1 day of propagation. Firmicutes were almost exclusively represented by lactic acid bacteria. A succession of dominating and sub-dominating populations of *L. sakei* group, *Leuconostoc* spp., *Weissella* spp., *Lactobacillus* spp. (mainly *L. plantarum*) and *Lc. lactis* occurred during propagation, leading to the progressive assembly of the bacterial community (Ercolini et al., 2013).

4. Sources for contaminating sourdough lactobacilli

Overall, almost all the studies failed to link the species diversity of the sourdough biota with the geographical origin of the sourdough

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