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Review

Ecological parameters influencing microbial diversity and stability of traditional sourdough

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ABSTRACT

The quality of some leavened, sourdough baked goods is not always consistent, unless a well propagated sourdough starter culture is used for the dough fermentation. Among the different types of sourdough used, the traditional sourdough has attracted the interest of researchers, mainly because of its large microbial diversity, especially with respect to lactic acid bacteria. Variation in this diversity and the factors that cause it will impact on quality and is the subject of this review.

Sourdough microbial diversity is mainly caused by the following factors: (i) sourdough is obtained through spontaneous, multi-step fermentation; (ii) it is propagated using flour, whose nutrient content may vary according to the batch and to the crop, and which is naturally contaminated by microorganisms; and (iii) it is propagated under peculiar technological parameters, which vary depending on the historical and cultural background and type of baked good. In the population dynamics leading from flour to mature sourdough, lactic acid bacteria (several species of *Lactobacillus* sp., *Leuconostoc* sp., and *Weissella* sp.) and yeasts (mainly *Saccharomyces cerevisiae* and *Candida* sp.) outcompete other microbial groups contaminating flour, and interact with each other at different levels. Ecological parameters qualitatively and quantitatively affecting the dominant sourdough microbiota may be classified into specific technological parameters (e.g., percentage of sourdough used as inoculum, time and temperature of fermentation) and parameters that are not fully controlled by those who manage the propagation of sourdough (e.g., chemical, enzyme and microbial composition of flour).

Although some sourdoughs have been reported to harbour a persistent dominant microbiota, the stability of sourdough ecosystem during time is debated. Indeed, several factors may interfere with the persistence of species and strains associations that are typical of a given sourdough: metabolic adaptability to the stressing conditions of sourdough, nutritional and antagonistic interactions among microorganisms, intrinsic robustness of microorganisms, and existence of a stable house microbiota.

Further studies have to be performed in order to highlight hidden mechanisms underlying the microbial structure and stability of sourdough. The comprehension of such mechanisms would be helpful to assess the most appropriate conditions that allow keeping a given traditional sourdough as a stable microbial ecosystem, thus preserving, during time, the typical traits of the resulting product.

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1. Introduction

Sourdough is a mixture of flour (mainly wheat or rye) and water, fermented with lactic acid bacteria (LAB) and yeasts, which are responsible for its capacity to leaven a dough, while contemporarily and unavoidably acidifying it (De Vuyst and Neysens, 2005; Gobbetti, 1998; Vogel et al., 1999). In the modern bakery technology, sourdough represents an alternative to the use of baker's yeast (although bakers often use a combination of both leavening agents) to manufacture a variety of products such as bread, crackers, snacks, pizza and sweet baked goods, because it offers many advantages over baker's yeast: enhanced flavour (Hansen and Schieberle, 2005), prolonged shelf-life (Chavan and Chavan, 2011), improved dough structure (Arendt et al., 2007) and increased nutritional value (Gobbetti et al., 2013; Poutanen et al., 2009) of the leavened baked good. Although liquid (type II) and dried (type III) sourdoughs, produced at industrial level, are fairly widespread among bakers because they are easy to be used (Brandt, 2007), traditional (type I) sourdough tickles researchers' curiosity mainly for its large microbial diversity (De Vuyst et al., 2009). This feature of traditional sourdough is mainly caused by the use of a spontaneous multi-step fermentation, needed for obtaining a sourdough ("mature" sourdough) with a constant leavening and acidifying capacity (Hammes and Gänzle, 1998), and by the use of back-slopping as a tool (almost) daily applied for propagating sourdough (De Vuyst et al., 2009). For this reason, most of the studies dealing with microbial ecology of sourdough focused on traditional sourdough.

The microbial ecology of cereal fermentation has been reviewed by Hammes et al. (2005). Ecological determinants of sourdough microbiota were examined as part of the review by De Vuyst et al. (2009). Other reviews focused either on general aspects (Chavan and Chavan, 2011) or on features of sourdough other than microbial ecology (Arendt et al., 2011; Moroni et al., 2009; Yao et al., 2013). Since 2009, various studies have significantly advanced our knowledge about the microbial ecology of sourdough fermentations and have inspired the justification for this review. Therefore, the objectives of this review are: (i) to give an overview about how LAB and yeasts become the dominant microbial groups in traditional sourdough and how they interact with each other; (ii) to examine factors affecting microbial diversity of traditional sourdough in a systematical way; and (iii) to discuss about parameters influencing the microbial stability of traditional sourdough.

2. Production of sourdough

Traditional sourdough originates from multiple steps of fermentation. In the first step a dough, usually composed of just flour and water, is spontaneously fermented. Then, the fermented dough is used as inoculum for fermenting newly prepared dough, which, in turn, will be used as inoculum for a subsequent step of fermentation. Additional ingredients, such as grape juice/must, honey, hop, overripe fruit, salt, sugar, vinegar may be used in early fermentation steps, in order to include in the dough immediately available nutrients and pro-technological microorganisms. A protocol for the production of a mature "French style" sourdough is given in Fig. 1 (Onno and Roussel, 1994). The figure also shows the typical trend of cell density of different microbial groups during this process. Apart from the first fermentation, the operation named "back-slopping" (or "refreshment"), consisting in the inoculation of flour and water with an aliquot of previously fermented dough, is repeated before each fermentation step. Back-slopping is also applied later, for propagating mature sourdough over time (De Vuyst et al., 2009).

Dough is a nutrient-rich ecosystem. Complex carbohydrates (starch, above all) are present, but their partial hydrolysis to di-saccharides (maltose, above all) and mono-saccharides (fructose and glucose), by flour and microbial amylases, rapidly takes place. Nitrates, ammonia, and proteins constitute the nitrogen sources for microbial growth. During dough fermentation, proteins are hydrolysed to more easily usable nutrients (peptides and free amino acids, FAA) by flour and microbial proteinases. The values of water activity (a_w), ranging from 0.96 to 0.98, do not limit the growth of the majority of contaminant microorganisms. The pH is sub-acid, although, during dough incubation and as the number of back-slopping steps increases, it tends to become acid (values around 4.0) (Table 1 and Fig. 1). The redox potential gradually decreases during dough formation and incubation from positive to negative values (Hammes et al., 2005). Taking into account the above physic-chemical parameters, sourdough allows LAB and yeast to outgrow other microbial populations (Fig. 1). Specific influences of the sourdough ecosystem and its production on the microbial ecology of fermentation will be discussed in later sections.

3. Microbial dynamics from flour to mature sourdough

At the beginning of the first fermentation, the microbial population of dough reflects that of the flour, consisting of LAB, Gram-positive (e.g., *Bacillus* sp.) and Gram-negative (e.g., *Pseudomonas* sp.) aerobic bacteria, *Enterobacteriaceae*, yeasts and moulds (Fig. 1). Each microbial group is present at cell numbers generally not exceeding 5 log CFU/g (Onno and Roussel, 1994; Rocha and Malcata, 2012; Stolz, 1999). Through bacterial 16S rRNA pyrosequencing, it has been recently found that, before the first fermentation, several bacterial phyla (e.g., *Bacteroidetes*, *Cyanobacteria*, *Firmicutes*, and *Proteobacteria*) occur in the dough. However, the majority of these phyla either indicate the presence of a non-active population in the flours or are outcompeted by *Firmicutes* already after the first fermentation (Ercolini et al., 2013), which is consistent with already-known patterns in the microbial ecology of fermented foods (Humblot and Guyot, 2009; Jeong et al., 2013; Jung et al., 2013). Upon addition of water to flour, redox potential of the dough decreases (Hammes et al., 2005), favouring the growth of facultative anaerobes (*Enterobacteriaceae* and yeasts) and of LAB (Fig. 1), most of which are aerotollerant anaerobes. Because carbohydrate metabolism of LAB is highly adapted to mono- and di-saccharides (Gänzle and Gobbetti, 2013), lactic and acetic acids are produced leading to a decrease of pH of the dough. Such a decrease, usually becoming evident after the second fermentation step, may inhibit the growth of *Enterobacteriaceae*, while it is well tolerated by yeasts. Consequently, as the number of fermentation steps increases, LAB and yeasts become more and more adapted to the environmental conditions of sourdough (Fig. 1), until they dominate the mature sourdough (Hammes and Gänzle, 1998), at numbers ranging from 6 to 9 log CFU/g and from 5 to 8 log CFU/g, respectively (Lattanzi et al., 2013; Minervini et al., 2012a). Actually, time (intended as the consecutive fermentation steps) is the variable that mostly affects the structure of the sourdough microbiota (Rocha and Malcata, 2012; Weckx et al., 2010a). For instance, it has been recently found that a gradual succession between the active populations of *Proteobacteria* and *Firmicutes* occurs from the beginning of the first fermentation to the end of the second fermentation of rye-based

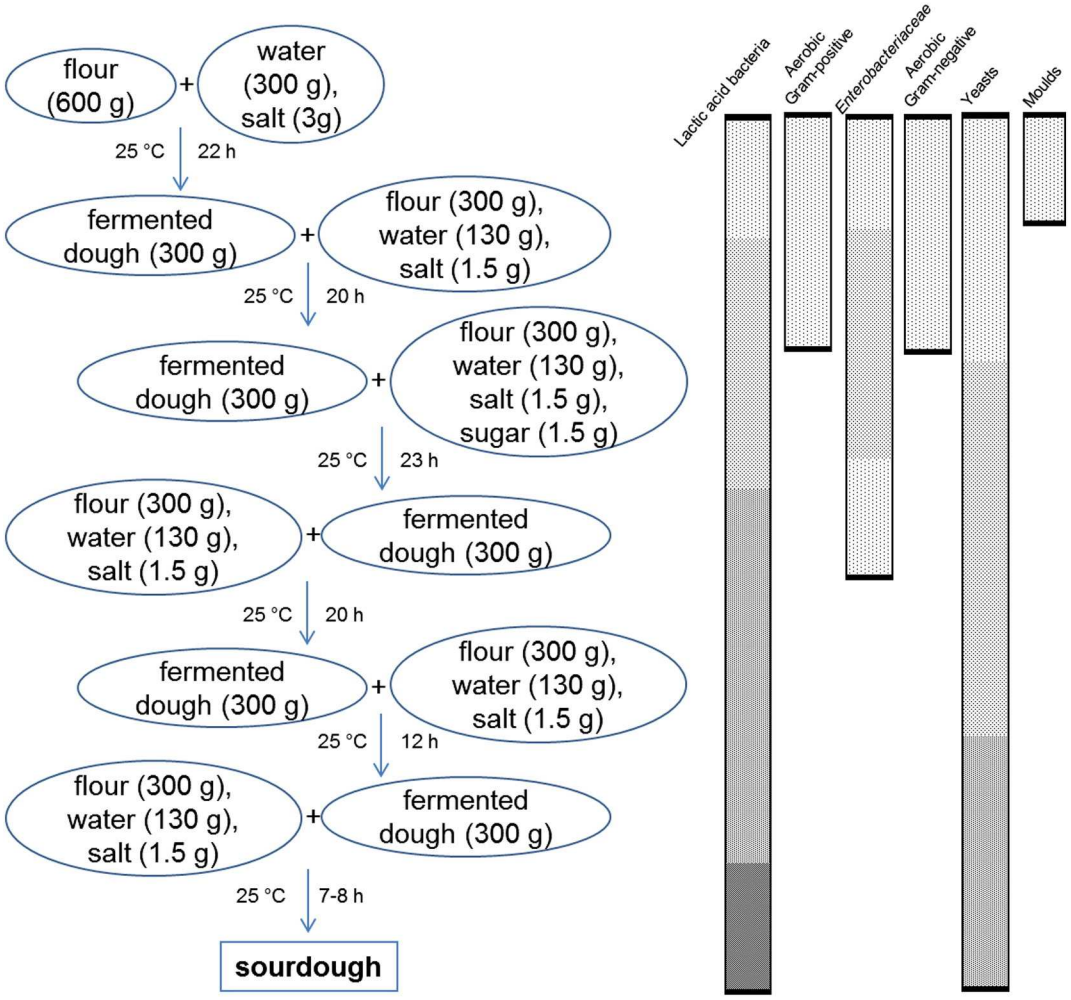

Fig. 1. Flow chart resuming the protocol of production of mature sourdough, according to the "French style" (adapted from Onno and Roussel, 1994), flanked by a graphical representation of the typical dynamic of different microbial groups occurring therein. For each microbial group, the number of dots in the bars is directly proportional to the cell density at a given stage of the protocol.

sourdough (Ercolini et al., 2013). A mature sourdough, i.e. a sourdough with constant cell densities of LAB and yeasts, acidification and leavening capacities, may be achieved in a number of days that varies (5–7 days) depending on the type of flour (Ercolini et al., 2013; Van der Meulen et al., 2007; Weckx et al., 2010a, 2010b).

Table 1
Ecological parameters and main relative effects on sourdough microbial ecosystem.

Parameter	Main effect(s)
Specific technological parameters	
Dough yield	Ratio lactic acid bacteria/yeasts; balance between homofermentative and heterofermentative lactic acid bacteria
% sourdough used as inoculum	Ratio lactic acid bacteria/yeasts; balance among different lactic acid bacterium genera
NaCl	Ratio lactic acid bacteria/yeasts
Redox potential	Balance between homofermentative and heterofermentative lactic acid bacteria
Fermentation time	Balance among differently stress-resistant lactic acid bacteria
Fermentation temperature	Ratio lactic acid bacteria/yeasts; balance between homofermentative and heterofermentative lactic acid bacteria
pH	Ratio lactic acid bacteria/yeasts; balance among different lactic acid bacterium genera
Number of back-slopping steps	Increased selective pressures; increased disturbance
Storage temperature	Cold stress as a cause of selective pressure
Not fully controllable parameters	
Flour	Introduction of contaminant microorganisms; slight modification of nutrient composition
House microbiota	Microbial stability of sourdough over time

Within LAB, a typical population dynamic, referred to as "the three-phase evolution", has been found, regardless of the type of flour. These three phases are: (i) dominance of LAB species belonging to the genera *Enterococcus*, *Lactococcus* and *Leuconostoc*; (ii) increasingly important presence of sourdough-specific LAB, such as species belonging to the genera *Lactobacillus*, *Pediococcus* and *Weissella*; and (iii) dominance of well-adapted sourdough strains, belonging to obligate heterofermentative species (e.g., *Lactobacillus sanfranciscensis*, *Lactobacillus fermentum*) and to *Lactobacillus plantarum* (Van der Meulen et al., 2007; Weckx et al., 2010a, 2010b), although strains of *Leuconostoc* sp. are sometimes encountered in the sourdough ecosystem (Corsetti et al., 2001; Minervini et al., 2012a, 2012b; Rocha and Malcata, 1999; Zotta et al., 2008). This succession of LAB is mainly driven by different tolerance to acidic conditions and to different adaptation mechanisms related to carbohydrate and nitrogen metabolism (Gänzle et al., 2007). Although different genera of LAB may inhabit the sourdough ecosystem, the most frequently encountered genus is *Lactobacillus* (De Vuyst e Neysens, 2005). Among this genus, and taking into account only identifications obtained through genotypic methods or polyphasic approach, the most common species isolated from sourdough are *Lactobacillus brevis*, *L. fermentum*, *Lactobacillus reuteri*, *Lactobacillus rossiae* and *L. sanfranciscensis* (obligate heterofermentative); *Lactobacillus alimentarius*, *Lactobacillus paralimentarius* and *L. plantarum* (facultative heterofermentative); *Lactobacillus amylovorus* and *Lactobacillus*

delbrueckii (obligate homofermentative) (for more details, refer to Huys et al., 2013).

Although a great variety of yeast species have been found in sourdoughs (Hammes et al., 2005), *Saccharomyces cerevisiae*, *Kazachstania exigua* (formerly *Saccharomyces exiguus*, anamorph *Candida holmii*) and *Candida humilis* (synonym *Candida milleri*) are those most frequently encountered, followed by *Pichia kudriavzevii* (formerly *Issatchenkia orientalis*, anamorph *Candida krusei*) (Garofalo et al., 2008; Huys et al., 2013; Iacumin et al., 2009; Vogelmann et al., 2009).

Apart from LAB and yeasts, sourdough may occasionally harbour acetic acid bacteria, such as *Acetobacter* sp. (Minervini et al., 2012b; Scheirlinck et al., 2008; Vogelmann et al., 2009), possibly affecting dough acidification. However the origin and role of these Gram-negative aerobic bacteria need to be elucidated.

4. Interactions between lactic acid bacteria and yeasts

In traditional sourdough, LAB and yeasts frequently interact mainly through metabolism of carbohydrates and of nitrogen sources, and through production of stimulatory or inhibitory compounds. The frequent association between *L. sanfranciscensis* and *C. humilis* and/or *K. exigua* is based on commensalism (De Vuyst et al., 2009). Indeed, *L. sanfranciscensis* preferentially uses maltose which, after being introduced in the cytoplasm, is hydrolysed by maltose phosphorylase to glucose-1-phosphate and glucose. This enables metabolization of glucose-1-phosphate without expenditure of ATP, while glucose is exported outside the cell and may be metabolized by maltose-negative yeasts (Neubauer et al., 1994). *L. sanfranciscensis*, as well as other obligate heterofermentative LAB, may gain extra ATP through the use of additional electron acceptors, such as fructose, oxygen, and extra pyruvate generated from citrate. Indeed, in the presence of additional electron acceptors, the intermediate acetyl-phosphate, instead of being reduced to ethanol, is converted into acetate, with concurrent production of extra ATP (Gobbetti, 1998). The generation of fructose from some flour oligosaccharides by specific enzymes of *C. humilis* causes an ecological advantage for the lactobacilli capable of using fructose as additional electron acceptor (Gobbetti et al., 1995; Stolz et al., 1995), and reduces the competition for carbohydrates between LAB and yeasts (Gobbetti and Corsetti, 1996). Competition for maltose and glucose may occur when obligate heterofermentative lactobacilli are associated with the maltose-positive *S. cerevisiae*. Indeed, this yeast species consume those flour carbohydrates at a higher rate than heterofermentative lactobacilli (Collar, 1996). Therefore, a decrease in the metabolism of heterofermentative lactobacilli is expected when associated with maltose-positive yeasts (De Vuyst and Neysens, 2005). However, traditional sourdough processes rarely lead to the depletion of fermentable carbohydrates. Indeed, maltose is continuously supplied by the activity of flour amylases and it never gets exhausted in few hours (3–8 h) of fermentation (De Vuyst and Neysens, 2005). The steady presence of maltose and other flour carbohydrates, along with the similar values of time of generation found for *L. sanfranciscensis*, *C. humilis* and S. *cerevisiae*, has been recently proposed as the cause of the stable and non-competitive association between these microbial species in traditional sourdoughs (Venturi et al., 2012).

In co-culture model systems, growth of *L. sanfranciscensis* and *L. plantarum* is stimulated by *K. exigua* and *S. cerevisiae*. This may be related to both the lack of competition for the nitrogen source and to the excretion of stimulatory compounds by yeasts (Gobbetti et al., 1994a). In the co-presence of organic and inorganic nitrogen sources, yeasts preferentially use the latter (e.g., ammonia), whereas LAB prefer to use FAA and, above all, small peptides. During growth or as a consequence of accelerated autolysis, yeast cells excrete essential and/or stimulatory amino acids for lactobacilli (Alexandre and Guilloux-Benatier, 2006; Gobbetti et al., 1994a; Velasco et al., 2004). A small peptide (Asp–Cys–Glu–Gly–Lys), identified in freshly prepared yeast extract, stimulates the growth of *L. sanfranciscensis* (Berg et al., 1981). On the other hand, lactobacilli contribute to proteolysis during sourdough fermentation, increasing the concentration of aliphatic, dicarboxylic and hydroxyl amino acid groups, most of which are used by yeasts (Gobbetti et al., 1994b).

Interactions between LAB and yeasts are often mediated by products of microbial metabolism. Obligate heterofermentative LAB synthesize acetic acid following heterolactic fermentation. In late stage of sourdough fermentation (pH $\approx$ 4.0), most of acetic acid is undissociated and therefore it may cross cytoplasmic membrane and enter the cell. Due to this, the growth of some yeasts (e.g., strains of *S. cerevisiae*) is inhibited. However, tolerance of other yeasts to acetic acid may influence microbial associations in sourdough (Suihko and Makinen, 1984). For instance, tolerance shown by *K. exigua* to acetic acid may be one of the causes of the stable association between this yeast and *L. sanfranciscensis* in the traditional sourdough used for making San Francisco bread (Kline and Sugihara, 1971). On the other hand, the antagonism of yeasts against LAB has not to be neglected (Viljoen, 2006), although, to our knowledge, it has not been reported for sourdough so far. This interaction is strain-dependent, at both the yeast and bacterium level and is related to production of inhibitory short chain fatty acids (e.g. hexanoic, octanoic, decanoic), sulphur dioxide, peptides, zymocidal proteins, and ethanol (Fleet, 2003).

Besides products of carbohydrate metabolism, microorganisms can release a wide array of secondary metabolites (Keller and Surette, 2006). The exposure of *S. cerevisiae* LBS and *L. sanfranciscensis* LSCE1 cells to oxidative, acid or osmotic sub-lethal stress gives rise to release of ethyl esters of some unsaturated long-chain fatty acids (Guerzoni et al., 2007). These molecules may be regarded as stress markers and have a possible physiological function in the cell–cell communication pathways (Black and Di Russo, 2006; Verstrepen et al., 2003). Furthermore, under oxidative stress, *L. sanfranciscensis* releases two 2(5H)-furanones (Guerzoni et al., 2007), which meet several criteria to be included into cell–cell communication molecules (Ndagijimana et al., 2006).

5. Ecological parameters in the sourdough ecosystem

In order to understand the combined effects exerted on the microbial growth by different ecological parameters, these are traditionally classified into endogenous (e.g., pH) and exogenous (e.g., temperature) parameters. However, this classification does not fit well to traditional sourdough, because complex interactions, resulting from manual operations and microbial activities, make it a peculiar microbial ecosystem. Thereby, it should be preferred to distinguish between specific technological parameters (e.g., percentage of sourdough, pH, temperature of fermentation, etc…) and parameters that are not fully (or at all) under the control of those who daily manage the propagation of sourdough, namely flour used in the propagation and role of the so-called “house” microbiota. All of these parameters and their combination are involved in the selection of the typical microbiota of a given sourdough. Furthermore, most of them also play a key-role in maintaining or disturbing the microbial structure of a given sourdough over time.

5.1. Specific technological parameters

Specific technological parameters and their relative main effects on sourdough microbiota are listed in Table 1 (De Vuyst et al., 2009; Hammes et al., 2005). The influence of the most studied parameters on the dominant sourdough microbiota is discussed below. Obviously, to consider one parameter at a time should not overlook the fact that microbial growth is influenced by multiple combinations of different parameters. Indeed, it is the continuous use of the same technological parameters that ultimately leads to the selection of microbial strains that are best adapted to the applied process conditions (Scheirlinck et al., 2009).

Dough yield (DY) is expressed by the ratio between dough weight and flour weight, multiplied by 100. Dough weight results from the sum of flour, water, starter inoculum and other ingredients, such as salt. For instance, the values of DY in the first and in the second fermentation steps illustrated in Fig. 1 are:

(First fermentation) DY = [(600 g + 300 g + 3 g)/600 g] * 100 = 150.5;

(Second fermentation) DY = [(300 g + 300 g + 130 g + 1.5 g)/(300 g + 200 g)] * 100 = 146.3.

Because water is, along with flour, the principal ingredient of the dough, DY is mainly related to the amount of water used in dough formulation: the higher is the amount of water, the higher is the value of DY. In most of cases, traditional sourdoughs are firm doughs, characterized by a value of DY of ca. 150–160. However, some traditional sourdoughs, such as those produced according to the "American style" (Kulp, 2003), may reach DY values of up to 225, corresponding to a_w value of ca. 0.98 (Hammes et al., 2005). Such values of a_w are not limiting for either LAB or yeasts. Yet, when a relatively high DY is combined with long (24–48 h) fermentation time, LAB are favoured over yeasts (Decock and Cappelle, 2005). Furthermore, relatively high values of DY, combined with higher temperatures (ca. 35–37 °C), favour the growth of homofermentative LAB (Decock and Cappelle, 2005; Onno and Roussel, 1994) (Table 1). Firmer sourdoughs (DY of ca. 160) are easily colonized by yeasts (minimal a_w for most of yeasts is 0.88), but represent a more selective environment for LAB, allowing the dominance of halotolerant strains (Hammes et al., 2005). Sometimes NaCl is used (1–3% on the total dough weight) during back-slopping (Fig. 1). While a low level of NaCl (up to 0.7%) stimulates growth of LAB, higher levels (1.6–3.2%) decrease their growth to a much greater extent than yeasts. Indeed, with increasing level of NaCl, the cell density ratio LAB/yeasts changed from 20:1 to 1:1 (Simonson et al., 2003) (Table 1).

Redox potential is mainly influenced by the level of oxygen incorporated during dough kneading. The presence of oxygen may represent an ecological advantage for those lactobacilli that are able to use it as external electron acceptor. Indeed, through the activity of enzymes such as NADH oxidase, NADH peroxidase, phosphotransacetylase, pyruvate dehydrogenase, and pyruvate oxidase, heterofermentative lactobacilli may generate additional energy by the activity of acetate kinase, which allows the recycling of NAD^+ without the need of ethanol formation (De Vuyst and Neysens, 2005) (Table 1). Accordingly, increased acidification was found when a sourdough started with *L. sanfranciscensis* CB1 had been insufflated with air. Furthermore, cell viability of the starter was not affected during 24 h of fermentation (De Angelis and Gobbetti, 1999).

The percentage of sourdough used as inoculum during daily back-slopping usually ranges from 10 to 40% of the total dough weight. As this parameter increases, the initial pH of the dough decreases, because of intrinsic acidity of sourdough. In this way, the percentage of sourdough influences growth rates of LAB (Brandt et al., 2004). Percentage of sourdough lower than 2% favoured growth of *L. sanfranciscensis* over that of *C. humilis*. On the opposite, the use of high (approaching to 50%) percentages of sourdough inhibited the growth of lactobacilli, but not that of yeasts, probably because of the low initial pH of the dough and of inhibitory concentrations of undissociated organic acids (Brandt et al., 2004) (Table 1).

Traditional sourdoughs have a pH range (3.5–4.3) that usually meets the growth requirements of the dominant sourdough microorganisms (De Vuyst and Neysens, 2005). Overall, within LAB, lactobacilli dominate this ecosystem, also because of their adaptation to low pH. However, genera of LAB present in the cereal kernels and flour, such as *Enterococcus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* and *Weissella* (Corsetti et al., 2007a), may dominate sourdoughs characterized by higher pH (Corsetti et al., 2007b; Rocha and Malcata, 1999; Zotta et al., 2008). Overall, low values of initial pH favour yeasts over LAB. When sourdough fermentation began under sub-acid conditions (initial pH ranging from 5.6 to 5.8), *L. sanfranciscensis* showed a higher multiplication factor than *C. humilis*. The opposite occurred at an initial pH value of 5.0 or lower, with lactobacilli being completely inhibited at pH 4.1 (Brandt et al., 2004) (Table 1).

The temperature of fermentation affects the microbial composition of sourdough. Overall, this parameter is inversely correlated to time of fermentation. Sourdough LAB have a growth temperature optimum of 30–40 °C, higher than that of yeasts (25–27 °C) (Brummer and Lorenz, 1991; Gänzle et al., 1998; Spicher and Stephan, 1999). This could be the rational for the so-called "baker's rule", according to which (relatively) low temperatures (20 to 26 °C) during sourdough fermentations are better for yeast growth than higher temperatures (Spicher and Stephan, 1999) (Table 1). Vrancken et al. (2011) highlighted a modulation of the microbiota as a function of time and temperature of back-slopping, going from almost exclusively *L. fermentum* at 30 °C and 37 °C, with back-slopping every 24 h, to a codominance of *L. fermentum* and *L. plantarum* at 30 °C and 48-h back-slopping, to the complete absence of these species at 23 °C. The latter fermentations were dominated exclusively by *Leuconostoc citreum*. Furthermore, stable numbers of yeasts and LAB were obtained in all the fermentation conditions, except for the fermentation at 37 °C and 24 h. Under these conditions, yeasts were almost completely absent, possibly reflecting an increased competitiveness of LAB compared to the yeasts or a growth limitation of the yeasts caused by the high temperature (Vrancken et al., 2011).

When considering the number of back-slopping steps, as it increases, the environmental conditions become more and more selective, resulting in the dominance of a very low number of species, such as *L. sanfranciscensis* (Lattanzi et al., 2013). Conversely, at each back-slopping new variables (e.g., batch of flour) may be introduced, causing modifications of the sourdough ecosystem in a relatively short time (Ottogalli et al., 1996) (Table 1).

5.2. Not fully controllable parameters

Flour used for propagating traditional sourdough may strongly influence microbial diversity, because it provides sourdough microorganisms with nutrients and, being non-sterile, is a vehicle of contaminant microorganisms, which, through daily and continuous back-slopping, could have the chance to become dominant (De Vuyst et al., 2009). Cereal flours have a highly heterogeneous nutrient composition, which offers the possibility for the simultaneous occupation of specific ecological niches by different microbial species and strains (De Vuyst et al., 2009). The capacity of some sourdough lactobacilli (e.g., *L. plantarum*) to ferment all wheat flour carbohydrates (pentoses included) may reduce the metabolic competition with yeasts (Corsetti et al., 2001), although catabolite repression by glucose could also occur (Stolz et al., 1993). *L. paralimentarius* was isolated from Apulian (Southern Italy) sourdoughs with a relatively high frequency and this could be related to the capacity, by all the isolates allotted to that species, to ferment the four main flour soluble carbohydrates (Corsetti et al., 2001). A comparative study of LAB and yeasts dominating 19 sourdoughs used for manufacturing traditional/typical Italian breads, and of nutrient (soluble carbohydrates and FAA) composition of the different flours used for propagation, highlighted that the type of flour (*Triticum durum* or *Triticum aestivum*), as well as the concentration of nutrients, may have a key-role for selecting the population of LAB (Minervini et al., 2012a). The main distinguishing features of *T. durum* flour were the higher concentrations of maltose, glucose, fructose and FAA. Compared to the *T. aestivum*-based sourdoughs, the *T. durum*-based sourdoughs were characterized by the sole or main presence of obligate heterofermentative LAB (mainly *L. sanfranciscensis*, *Leuconostoc* sp., *Weissella cibaria* and *L. brevis*), the lower number of facultative heterofermentative LAB, and the lower cell density of yeasts

(Minervini et al., 2012a). Obligate heterofermentative LAB were dominant in only three (Pane Casereccio di Reggio Calabria, Pane Casereccio del Molise and Bozza Pratese) out of nine *T. aestivum*-based sourdoughs. Because these three sourdoughs were based on *T. aestivum* flours characterized by the highest concentration of fermentable carbohydrates and total FAA, it was hypothesized that obligate heterofermentative LAB are less competitive than the two other metabolic groups in sourdough based on flours containing such nutrients at (relatively) low concentrations (Minervini et al., 2012a). Despite this, the role of the type of flour in the process leading to the formation of the microbial structure of mature sourdough deserves further studies. Indeed, it has been recently found that the number of shared Operational Taxonomic Units between sourdoughs based on different flours (rye, durum and soft wheat) increases as the process goes on (Ercolini et al., 2013). This means that initial differences of microbial community among doughs based on different flours tend to disappear as the number of back-slopping steps increases. This results in the definition of a core microbiota (consisting of LAB) which is shared between mature sourdoughs, regardless of the type of flour (Ercolini et al., 2013).

Although a given sourdough is commonly propagated over time using the same type of flour, seasonality characterizing crops affects nutrient composition of flour (Table 1). Because the capability to adapt to a specific substrate is highly strain-specific, even small changes of substrate quality will have effects on the microbiota (Vogelmann et al., 2009), with the exception of intrinsically robust microorganisms (Minervini et al., 2010; Siragusa et al., 2009).

Spontaneous sourdough fermentations carried out at laboratory level under semi-aseptic conditions indicate that flour used for propagation is one of the possible sources of LAB (Van der Meulen et al., 2007) and yeasts (Vrancken et al., 2010). In these conditions, if a given microbial species is not detected in the flour, it will not be in the sourdough. On the other hand, under the practical conditions of sourdough propagation (at the bakery), the influence of the "house" microbiota seems to be of outmost importance (Scheirlinck et al., 2009). "House" microbiota is the term used to indicate those microorganisms contaminating the bakery setting and equipment. This parameter is not fully controllable by those who manage sourdough propagation, unless a thorough sanitization plan is daily scheduled (Table 1). Because house microbiota may affect microbial stability of traditional sourdough (Scheirlinck et al., 2009), this parameter will be discussed later.

6. Factors influencing microbial stability of traditional sourdough

Some traditional sourdoughs are characterized by a microbial composition that remains stable over years (Scheirlinck et al., 2009; Venturi et al., 2012) and, sometimes, over decades (Böcker et al., 1990; Gänzle and Vogel, 2003). On the contrary, some studies highlighted a relative instability of the sourdough ecosystem, even in a relatively short time (Minervini et al., 2010, 2012b; Siragusa et al., 2009). Overall in traditional sourdough a stable microbiota is only achieved when the multiplication factors (expressed by the ratio between cell density at the end of fermentation and cell density at the beginning of fermentation) of the different microorganisms are very similar (Brandt et al., 2004). The use of specific technological parameters having constant values during time is undoubtedly a prerequisite for guaranteeing the microbial stability of a given sourdough (Vogelmann and Hertel, 2011). Yet, besides that, microbial stability is affected by the following factors: (i) metabolic adaptability to the stressing conditions of sourdoughs; (ii) nutritional interactions among microorganisms; (iii) intrinsic robustness of microorganisms; (iv) antagonistic interactions among microorganisms; and (v) existence of a stable house microbiota.

6.1. Metabolic adaptability to the stressing conditions of sourdough

During propagation and, above all, storage of sourdough, LAB are exposed to sub-optimal or critical values of temperatures and pH. In addition, when sourdough is stored, LAB may face with nutritional limitation (Gänzle and Gobbetti, 2013). The response to stress may be specific, i.e. following exposure to a given stress, LAB over-synthesize certain proteins which are not over-synthesized following exposure to a different stress. On the contrary, it may happen that different stresses (e.g., low temperature, NaCl) induce the over-synthesis of proteins, some of which are over-synthesized irrespective of the kind of stress (Hörmann et al., 2006).

To overcome deleterious effects caused by low temperature, bacteria have to develop a transient adaptive cold–shock response (Graumann and Marahiel, 1998). Sourdough LAB may continue to grow at a reduced rate after a temperature decrease of ca. 20 °C below their optimum (De Angelis and Gobbetti, 2004). *L. sanfranciscensis*, *L. plantarum*, *L. brevis*, *Lactobacillus hilgardii*, *L. alimentarius* and *Lactobacillus fructivorans* grew in wheat flour hydrolysate at 15 °C by increasing the lag phase (from ca. 2 to 5 h) and the generation time (from ca. 10 to 18 h). An array of 14–18 (depending on the species) cold-shock proteins (CSPs) were over-synthesized by *L. plantarum* DB200, *L. brevis* H12, *L. plantarum* 20B and *L. sanfranciscensis* CB1 upon cold adaptation (15 °C, 2 h) and subsequent freezing. The higher level of synthesis of CSPs was related to the cell recovery after freezing, which was higher than for cells directly (i.e. with no adaptation) subjected to freezing (De Angelis and Gobbetti, 2004).

Survival under acidic conditions is positively affected by the adaptation to low pH, a mechanism known as acid-tolerance response (Foster and Hall, 1991). After growth at a constant pH of 6.4, the survival of mid-exponential phase cells of *L. sanfranciscensis* CB1 decreased dramatically when suddenly subjected to pH 3.2–3.4, as set by lactic acid or by a mixture of lactic and acetic acids. Higher survival could be obtained upon adaptation to pH 5.0 for 1 h, prior to exposure to low pH, and was attributed to 15 acid-shock proteins over-synthesized by *L. sanfranciscensis* CB1 (De Angelis et al., 2001). Specific metabolic pathways of utilisation of glutamine and arginine may help LAB to overcome acidic stress. Strains of *L. sanfranciscensis* and of *L. reuteri* are able to convert glutamine into glutamate through glutaminase, generating ammonia that limits the decrease of intracellular pH (Vermeulen et al., 2007). Several lactobacilli possess all the enzymes of the arginine deiminase pathway (De Angelis et al., 2002; Rollan et al., 2003; Thiele et al., 2002; Vrancken et al., 2009). In this pathway arginine is converted into ornithine and ammonia, with the consumption of two protons, which allows microbial cells to spare ATP required for proton extrusion (Konings, 2002). Both glutamate and ornithine contribute to the flavour of the product (De Vuyst et al., 2009).

Compared to cold and acid stress adaptation, nutrient limitation faced by microorganisms has received scarce attention. Carbon catabolite repression could cause nutrient limitation to some microorganisms in the sourdough ecosystem. Indeed, glucose excreted by some lactobacilli (e.g., *L. sanfranciscensis*), following utilisation of maltose, may induce catabolite repression in other LAB and yeasts. When glucose is exhausted, these microorganisms could start to metabolize other carbohydrates (e.g., maltose), provided that these latter have not been totally consumed by the abovementioned lactobacilli. Stress from nutrient limitation causes phenotypic responses encompassing the use of external electron acceptors, the preferential and/or simultaneous use of unconventional energy sources (e.g., amino acids, ribose and deoxyribose), and the interaction with exogenous and flour enzymes (Gänzle and Gobbetti, 2013).

6.2. Nutritional interactions among microorganisms

Overall, it is obvious that the absence of competition for carbohydrates, as well as for other nutrients, may be a key-factor in the microbial stability of traditional sourdough. Differences in the use of flour soluble carbohydrates may result in a non-competitive association of different species of LAB, such as in the case of the stable association between *L. sanfranciscensis* and *L. plantarum*. The former species

preferentially utilizes maltose and is generally unable to ferment fructose, whereas the latter species preferentially ferments glucose and fructose with maltose metabolism being subject to carbon catabolite repression (Corsetti et al., 2001; Gobbetti, 1998).

As discussed previously, stable, non-competitive associations exist between the maltose-negative, acid-tolerant *K. exigua* or *C. humilis* and the maltose-positive *L. sanfranciscensis* in traditional sourdoughs (Gobbetti, 1998; Hammes et al., 1996). The association between *L. sanfranciscensis* and *C. humilis* has been recently confirmed as stable, regardless of technological parameters applied during propagation and of the presence of potentially competing microorganisms (*L. fermentum*, *Lactobacillus helveticus*, *Lactobacillus pontis*, *L. reuteri*, *Lactobacillus johnsonii*, *S. cerevisiae*, *I. orientalis*) deliberately added (Vogelmann and Hertel, 2011).

6.3. Intrinsic robustness of microorganisms

The frequent predominance of *L. sanfranciscensis* as the key-lactic acid bacterium of traditional sourdough is possibly the result of the selective pressures that arise through the environmental conditions pertinent to the typical technological parameters (relatively low fermentation temperature, continuous back-slopping) applied during propagation of sourdough (Corsetti et al., 2001; Foschino et al., 1999; Kline and Sugihara, 1971). Recently, the genome analysis of *L. sanfranciscensis* TMW 1.1304 showed that, although the genome size is the smallest within the lactobacilli (ca. 1.3 Mbp), ribosomal RNA operons are present at the highest density among all known genome of free-living bacteria. This feature could allow this bacterium to respond quickly to favourable conditions of sourdough, and to initiate suddenly fermentative metabolism and fast growth (Vogel et al., 2011). However, the capacity of this species to persist in sourdough seems to be strain-dependent. The structure and stability of the dominant bacterial population were investigated during 10 day-long continuous propagation (at laboratory level, under semi-aseptic conditions) of nine different sourdoughs singly started with as many strains of *L. sanfranciscensis* (Siragusa et al., 2009). Only three starters dominated throughout ten days of continuous back-slopping, whereas the other strains progressively decreased to less than 3 log CFU/g (Fig. 2a). *Weissella confusa*, *L. sanfranciscensis*, *L. plantarum*, *L. rossiae*, *L. brevis*, *Lactococcus lactis* ssp. *lactis* and *Pediococcus pentosaceus* were identified as the dominant bacterial species in the flour used for back-slopping. At the end of propagation, one strain of *L. sanfranciscensis*, belonging to the contaminant microbiota of the flour, was found in all the sourdoughs. Persistent starters were found in association with other biotypes of *L. sanfranciscensis* and with *W. confusa* or *L. plantarum*. The three persistent *L. sanfranciscensis* strains were further used as single starters for the production of as many sourdoughs, which were propagated, under the same conditions as above, by using a different flour, whose lactic acid bacterium population in part differed from the previous one. Also in this case all the three starters persisted during propagation (Siragusa et al., 2009). A subsequent study was set up with a similar approach, aiming to investigate the robustness of seven sourdough strains of *L. plantarum*. In this case, five out of seven strains maintained elevated cell numbers (ca. 9 log CFU/g) throughout ten days of daily propagation. The other two strains progressively decreased to less than 5 log CFU/g (Fig. 2b) (Minervini et al., 2010).

Overall, these two studies showed that, during daily propagation, intrinsic robustness of strains seems to play a key-role in the establishment of the peculiar microbiota of a given sourdough (Minervini et al., 2010; Siragusa et al., 2009). The ability of some LAB and yeasts to adapt to many different substrates underlies their intrinsic robustness (Vogelmann et al., 2009). In particular, robustness of *L. plantarum* strains is attributed to several factors: ability to use the main flour carbohydrates, as well as low concentration carbohydrates (e.g., pentoses) and alternative energy sources (sugar nucleotides, amino acids, etc…), adaptation to environmental stresses, rapid acidification, synthesis of

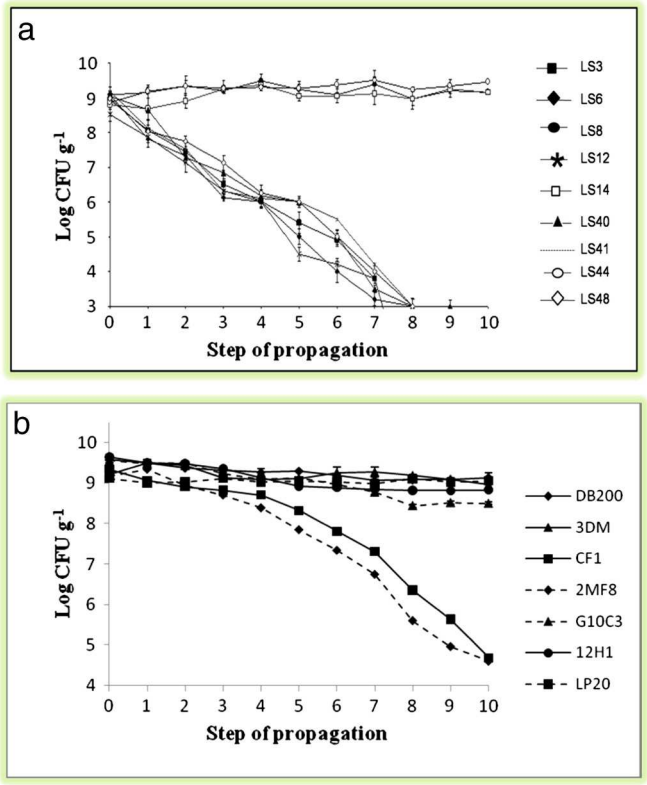

Fig. 2. Persistence of *Lactobacillus sanfranciscensis* and *Lactobacillus plantarum* strains in sourdough after continuous 10 day-long propagation. In (a) the LS numbers refer to the different strains of *L. sanfranciscensis*. In (b) the alpha-numerical codes refer to the different strains of *L. plantarum* (adapted from Siragusa et al., 2009 and from Minervini et al., 2010).

diacetyl and hydrogen peroxide (showing antimicrobial activity), and especially synthesis of bacteriocins (Gobbetti et al., 2005), such as plantaricins. Plantaricins were active also during sourdough fermentation (Settanni et al., 2005) and they inhibited growth of other *L. plantarum* strains (Todorov et al., 1999). Some strains of *L. plantarum* synthesize plantaricin A (plnA), which acts as a pheromone in the mechanism of inter-species cell–cell communication (Di Cagno et al., 2010). Under conditions that mimicked the sourdough fermentation, the level of plnA depended on the microbial partner and plnA acted as inhibitory compound, especially towards some strains of *L. sanfranciscensis* (Di Cagno et al., 2010).

6.4. Antagonistic interactions among microorganisms

Several sourdough LAB may synthesize antimicrobial compounds, like bacteriocins, bacteriocin-like inhibitory substances (BLIS) and antibiotics (Corsetti et al., 1996; Olsen et al., 1995). Bacteriocins are small peptides or proteins, ribosomically synthesized, endowed with bactericidal or bacteriostatic activity especially towards bacteria not strictly correlated, from a phylogenetic point of view, to the producing bacterium (De Vuyst and Vandamme, 1994). BLIS are antimicrobial molecules, sharing some characteristics with bacteriocins, but not purified at homogeneity (Tagg, 1991). The first screening studies about antimicrobial activity of sourdough LAB trace back to the early '90. Bavaricin A, synthesized by *Lactobacillus sakei*, was characterized (Larsen et al., 1993). Subsequently, BLIS C57, synthesized by *L. sanfranciscensis* C57 (Corsetti et al., 1996) and plantaricin ST31, synthesized by *L. plantarum* ST31 (Todorov et al., 1999) were described. Production of BLIS has been also reported for several *L. plantarum*, *Lactobacillus pentosus* and *L. rossiae* strains (Corsetti et al., 2004). Several studies report about LAB, such as *L. amylovorus* DCE 471 (Messens and De Vuyst, 2002) and *Lc. lactis* M30 (Hartnett et al., 2002), isolated from sourdough, that

showed interesting bacteriocinogenic properties during sourdough fermentation (Gänzle and Gobbetti, 2013).

Reutericyclin is a low molecular weight antibiotic, synthesized by some strains of *L. reuteri*, which is active against a broad range of Gram-positive bacteria (including some LAB), in concentrations of less than 1 mg/l (Gänzle et al., 2000). The reutericyclin-producing *L. reuteri* LTH2584 was isolated in 1988 from SER, an in house rye sourdough prepared for the production of a commercially available baking aid (Böcker et al., 1995). Relevant cell counts of *L. reuteri* were observed over a 10 year-long monitoring of the SER sourdough microbiota (Gänzle and Vogel, 2003). Synthesis of reutericyclin in dough inhibited the growth of a reutericyclin-sensitive indicator strain of *L. sanfranciscensis* (Gänzle and Vogel, 2003). Several other reutericyclin-producing strains of *L. reuteri*, isolated in high cell counts from SER sourdough, showed a two- to four-fold higher tolerance to reutericyclin than *L. sanfranciscensis* strains and most other lactobacilli (Gänzle et al., 2000). These findings highlight that production of reutericyclin provides a competitive advantage to the producer strains, thus contributing, along with other factors (e.g., adaptation to available substrates, minimal pH and temperature required for growth), to the stable persistence of *L. reuteri* in the SER sourdough over a period of 10 years (Gänzle and Vogel, 2003).

6.5. Existence of a stable house microbiota

Previous introduction of flour into the bakery environment helps to build up a house microbiota that may serve as an important inoculum for subsequent sourdough fermentations (De Vuyst et al., 2009). In order to highlight the influence of the environment of propagation on the diversity of the lactic acid bacterium and yeast microbiotas, seven traditional sourdoughs were daily back-slopped for 80 days at an artisan bakery or in the laboratory, using the same batch of flour and applying the same technological parameters (Minervini et al., 2012b). While cell density of LAB and related biochemical features (pH, total titratable acidity and concentration of organic acids) were not affected by the environment of propagation, the number of yeasts and the concentration of ethanol markedly decreased from artisan bakery to laboratory propagation. Denaturing Gradient Gel Electrophoresis showed that the DNA band corresponding to *S. cerevisiae* was no more detectable in four out of seven sourdoughs propagated in the laboratory. Twelve species of LAB were variously identified through a culture-dependent approach. All sourdoughs harboured a certain number of species and strains, which were dominant throughout time and, in several cases, varied depending on the environment of propagation (Tables 2–4). Overall, *L. plantarum*, *L. sakei* and *W. cibaria* dominated some sourdoughs propagated at artisan bakeries, whereas *Ln. citreum* seemed to be more persistent under laboratory conditions. Other LAB (*Lactobacillus curvatus*, *Lc. lactis* and *P. pentosaceus*) were only temporarily revealed, along with stable species, and largely differed between artisan bakery and laboratory levels. On the other hand, some strains (e.g., *L. sanfranciscensis* s1 in sourdough MT.A used for Pane di Matera PGI and *L. plantarum* s15 in sourdough AM.B used for Pane di Altamura PDO) showed intrinsic robustness, because they were among the dominant LAB regardless of the environment of propagation (Tables 2 and 4). Permutation analysis based on bacterial diversity, assessed through culture-dependent and -independent methods, showed that in five out of seven cases, sourdoughs propagated at artisan bakery and those propagated in the laboratory diverged. This may be explained probably by incomplete control of relevant factors and by the influence of house microbiota, whose level of contamination is supposed to be much higher in the bakery than in the laboratory (Minervini et al., 2012b).

Regarding yeasts, differences among artisan- and laboratory-propagated sourdoughs confirmed the importance of the environment of propagation as primary source of *S. cerevisiae* (Vrancken et al., 2010). This could be related to the frequent use of baker's yeast, which may become a common contaminant of the environment of propagation (De Vuyst and Neysens, 2005). However, the bakery environment may also affect bacterial diversity and stability of traditional sourdough. In order to test this hypothesis, flours, sourdoughs and their environment of propagation were sampled from two artisan Belgian bakeries (D01 and D10) (Scheirlinck et al., 2009). The sourdoughs produced at bakery D10 were mainly dominated by *L. sanfranciscensis*, which was detected in the air of the storage and work rooms, as well as on benches and dough mixer. This finding demonstrates that this species circulates throughout the environment of propagation. Conversely, sourdough samples produced in bakery D01 were characterized by a higher bacterial diversity (*L. paralimentarius*, *L. plantarum* and *Lactobacillus spicheri*). Both *L. plantarum* and *L. spicheri* were detected on the hands of the baker, but of these two species only *L. plantarum* was also detected in the flour and in the air of the bakery. These results indicate the importance of air as a potential carrier of LAB in food-processing environments (Scheirlinck et al., 2009). Furthermore, *L. plantarum*, *L. spicheri* and *L. sanfranciscensis* isolates coming from sourdough and bakery environment samples were genetically indistinguishable. This suggested that the sourdoughs and their corresponding environments of propagation were dominated by a single strain of these species. Despite the use of different flour batches and possible variations in flour characteristics during subsequent propagation of the sourdoughs analysed, those strains appeared to persist in the doughs over at least 3 years of sampling. This persistence may be the result of the continuous use of the same fermentation parameters and of significant contamination from the environment of propagation (Scheirlinck et al., 2009). Overall, the results of this study suggest that bakery environment, because of its usually high level of microbial

Table 2
Species and strains of lactic acid bacteria isolated from traditional sourdough MT.A propagated at artisan bakery and laboratory levels for 1, 20, 40, 60, and 80 days. The dot indicates the presence of strains. Data were obtained from Minervini et al. (2012b).

	Bakery-day 1	Bakery-day 20	Bakery-day 40	Bakery-day 60	Bakery-day 80	Laboratory-day 20	Laboratory-day 40	Laboratory-day 60	Laboratory-day 80
Lactobacillus parabrevis s1							●		
Lactobacillus plantarum s1	●	●							
Lactobacillus plantarum s2	●		●						
Lactobacillus plantarum s3	●	●	●	●	●				
Lactobacillus plantarum s4			●						
Lactobacillus plantarum s5							●		
Lactobacillus sakei s1	●								
Lactobacillus sanfranciscensis s1	●	●	●	●	●	●	●	●	●
Lactobacillus sanfranciscensis s2	●	●							
Lactobacillus sanfranciscensis s3	●	●							
Leuconostoc citreum s1	●								
Pediococcus pentosaceus s1			●						

Table 3
Species and strains of lactic acid bacteria isolated from traditional sourdough MT.C propagated at artisan bakery and laboratory levels for 1, 20, 40, 60, and 80 days. The dot indicates the presence of strains. Data were obtained from Minervini et al. (2012b).

	Bakery-day 1	Bakery-day 20	Bakery-day 40	Bakery-day 60	Bakery-day 80	Laboratory-day 20	Laboratory-day 40	Laboratory-day 60	Laboratory-day 80
Lactobacillus brevis s1							●		
Lactobacillus curvatus s1						●			
Lactobacillus plantarum s7	●	●		●					
Lactobacillus plantarum s8	●	●	●						
Lactobacillus plantarum s9	●	●	●	●	●				
Lactobacillus sanfranciscensis s4		●							
Leuconostoc citreum s4	●					●	●	●	●

Table 4
Species and strains of lactic acid bacteria isolated from traditional sourdough AM.B propagated at artisan bakery and laboratory levels for 1, 20, 40, 60, and 80 days. The dot indicates the presence of strains. Data were obtained from Minervini et al. (2012b).

	Bakery-day 1	Bakery-day 20	Bakery-day 40	Bakery-day 60	Bakery-day 80	Laboratory-day 20	Laboratory-day 40	Laboratory-day 60	Laboratory-day 80
Lactobacillus casei s9		●							
Lactobacillus plantarum s15	●	●	●	●	●	●	●	●	●
Lactobacillus plantarum s16							●		
Lactobacillus sanfranciscensis s9	●								
Lactobacillus sanfranciscensis s10	●								
Lactobacillus sanfranciscensis s11	●	●	●						
Lactobacillus sanfranciscensis s12					●				
Lactococcus lactis ssp. *lactis* s3				●					

contamination, may be the source not only of yeasts, but also of LAB that, by virtue of their intrinsic capacities, may or may not dominate traditional sourdough.

7. Conclusions

The microbial ecology of traditional sourdough is currently a matter worthy of further investigation. This is mainly due to the complexity of microbiota, the influence of flour and environment of propagation. Sodium chloride and quality of water could affect the sourdough microbial community, and this could be one of the targets for future research. Overall, traditional sourdoughs are characterized by relative microbial stability, provided that their propagation is carried out applying constant technological parameters and in their usual environment. However, differences in the composition of flour nutrients, as well as possible bacteriophage infections (Foschino et al., 2005), may result in disturbance of the microbial community. Such modifications can be limited at the level of strains, i.e. a given sourdough stably harbours one or more microbial species, but different strains alternate (Minervini et al., 2012b). The reasons for this succession are not very clear and are probably hidden in the myriad of sourdough ecological niches, whose dynamics are difficult to understand (De Vuyst et al., 2009). Modifications of the typical sourdough microbiota may occur even at the level of species. Indeed, it has to be taken into account that when sourdough is propagated, not only dominant but also sub-dominant microorganisms (e.g., *Enterococcus* sp., *Pediococcus* sp.) are perpetuated. Therefore, any disturbance against dominant LAB may allow subdominant to become prevailing (Corsetti et al., 2007b).

Succession of strains and species during sourdough propagation could affect the technological performances of sourdough and this, in turn, would influence the characteristics of the product. Furthermore, during final fermentation, contribution of the sourdough microbiota to the product quality is presumably affected by the use of ingredients, such as flour, water, (eventually) baker's yeast, and (in the case of sweet products such as Panettone) sucrose, eggs, and butter. For instance, if baker's yeast is used, the contribution of the sourdough yeasts to the leavening of the dough will probably be restricted. Therefore, further studies about that matter are warranted.

One of the main issues of the bakers is that the variations of the sourdough performances cannot be eliminated but, at most, can be attenuated. Although this variability may be regarded as a trait guaranteeing the artisanal, irreproducible quality of sourdough-based leavened baked goods, bakery industry obviously considers that as a weakness. Therefore, further studies have to be performed in order to highlight hidden mechanisms underlying the microbial structure and stability of sourdough. The comprehension of such mechanisms would be helpful to assess the most appropriate conditions that allow preserving the typical traits of traditional sourdough over time.

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