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Review

The sourdough microflora: biodiversity and metabolic interactions

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The production of sourdough bread can be traced back to ancient times. Sourdough is a mixture of flour and water that is fermented with lactic acid bacteria (LAB). Sourdough is an intermediate product and contains metabolically active yeast and LAB strains. The LAB that develop in the dough may originate from selected natural contaminants in the flour or from a starter culture containing one or more known species of LAB. Sourdough can be produced in bakeries or obtained from commercial suppliers. The microbial ecology of the sourdough fermentation is determined by ecological factors. Microbiological studies have revealed that more than 50 species of LAB, mostly species of the genus *Lactobacillus*, and more than 20 species of yeasts, especially species of the genera *Saccharomyces* and *Candida*, occur in this ecological niche. The sourdough microflora is composed of stable associations of lactobacilli and yeasts, in particular due to metabolic interactions. As shown for certain industrial sourdough processes, such microbial associations may endure for years, although the fermentation process runs under non-aseptic conditions. A reproducible and controlled composition and activity of the sourdough microflora is indispensable to achieve a constant quality of sourdough bread.

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Introduction

The production of sourdough bread can be traced back to ancient times (Spicher, 1999a). Whereas bread is a staple food in many European diets, sourdough bread production contributes to cultural and geographical identity too. Artisan bread production, that often employs sourdough processes or the use of pre-ferments, provides a wide, regional variety of breads and specialty bakery products. In fact, many wheat breads and cakes are original to the Mediterranean countries, the San Francisco bay, and Southern America, whereas numerous bakery preparations made with rye, wheat, barley, or mixed flours are typical for Germany, Central and Eastern Europe, and Scandinavia (Stephan & Neumann, 1999a,b). In Italy sourdough is used in more than 30% of bakery products, which include numerous different types of sourdough breads (Ottogalli, Galli, & Foschino, 1996). Most of these products originate from very old traditions and differ in the type of flour, other ingredients, type of sourdough, technology, and shelf-life.

In northern Italy, sweet leavened baked products, obtained from sourdoughs, are typical, and are traditionally made for religious festivities. Panettone cake in Milan and Pandoro in Verona are manufactured for Christmas, while Colomba is a Milanese cake for Easter (Ottogalli *et al.*, 1996). Other local products include Bisciola in Valtellina, Lagaccio biscuit in Genoa, and Focaccia Dolce in the Venetian region, which is called Veneziana in Lombardy. Also, traditional pizzas, *i.e.* flat leavened breads, and snacks for breakfast or coffee time such as Cornetto, Pandorino and Brioche, and other small cakes for infants are typical Italian bakery products. Due to the superior sensory quality and the prolonged shelf-life of the resulting baked goods, sourdough processes have retained their importance in modern baking technology. Moreover, their production and consumption contributes to the gastronomy of many countries nowadays.

Sourdough is a mixture of flour and water that is fermented with lactic acid bacteria (LAB), mainly heterofermentative strains, elaborating lactic acid and acetic acid in the mixture, and hence resulting in a sour taste of the end product. Sourdough plays an important role in the

preparation of bread dough to favour technological properties (for instance improved dough machinability), nutritional properties (for instance through phytate hydrolysis), organoleptic properties (for instance bread volume, crumb texture, and a unique flavour), and keeping properties (shelf-life) (Hammes & Gänzle, 1998; Salovaara, 1988).

Sourdough can be freshly produced in bakeries or can be obtained from commercial suppliers (living, liquid sourdough or dried, non-fermenting sourdough). Examples of commercial sourdoughs are the San Francisco sour for wheat bread production, a process that has been carried out in the San Francisco bay area for over 130 years (Kline & Sugihara, 1971) and the Böcker–Reinzucht–Sauer (BRS) for rye bread production (Böcker, Vogel, & Hammes, 1990). However, many rye bread bakeries in Europe still use traditionally fermented sourdoughs, which have been kept metabolically active for decades by addition of fresh flour and water at regular time intervals. In Italy, a great number of bakeries produce bread products by such traditional means (*e.g.* Panettone), which require long time for fermentation and result in products with typical sensorial characteristics and longer shelf-lifes. Finally, 'home' baking of sour rye bread is still practised in many countries.

Dough acidification is a prerequisite for rye baking to inhibit the flour α-amylase. Maltose catabolism and the use of alternative electron acceptors (*e.g.* fructose) play an important role in this process (Stolz, Böcker, Hammes, & Vogel, 1995; Stolz, Böcker, Vogel, & Hammes, 1995; Stolz, Hammes, & Vogel, 1996). Acidificiation also activates cereal phytases, making more nutrients available (Fretzdorff & Brümmer, 1992). Further, sourdough fermentation promotes a solubilisation of rye pentosans at the dough stage and thus enhances water binding of the dough, since gluten are lacking in rye (Martinez-Anaya & Devesa, 2000). Other metabolic activities of sourdough LAB, which are of importance for bread quality, are their proteolytic activity (Di Cagno *et al*., 2002; Gobbetti, Simonetti et al., 1994; Gobbetti, Smacchi, & Corsetti, 1996; Gobbetti, Smacchi, Fox, Stepaniak, & Corsetti, 1996), the formation of volatile aromatic compounds and aroma precursors (Gobbetti, Simonetti et al., 1995; Röcken, Rick, & Reinkemeier, 1992; Thiele, Gänzle, & Vogel, 2002), arginine metabolism enhancing the roasty flavour of bread (De Angelis *et al*., 2002), and the production of antibacterial compounds, antifungal substances, antiropiness activities, and exopolysaccharides in dough, which potentially affects bread texture, staling and/or shelf-life (Corsetti, Gobbetti, Balestrieri, Russi, & Rossi, 1998; Corsetti, Gobbetti, Rossi, & Damiani, 1998; Corsetti, Gobbetti, & Smacchi, 1996; Gobbetti, 1998; Hammes & Gänzle, 1998; Katina, Sauri, Alakomi, & Mattila-Sandholm, 2002; Korakli, Pavlovic, Gänzle, & Vogel, 2003; Korakli, Rossmann, Gänzle, & Vogel, 2001; Lavermicocca et al., 2000; Lavermicocca, Valerio, & Visconti, 2003; Pepe, Blaiotta, Moschetti, Greco, & Villani, 2003).

Sourdough is an intermediate product for dough and bread preparation and contains metabolically active microorganisms. Due to their artisan and region-dependent handling, sourdoughs are an immense source of diverse LAB and yeast species and strains. The LAB developing in the dough may originate from selected natural contaminants in the flour or from a starter culture containing one or more known species of LAB. Cell densities exceeding 10^8 colony forming units (CFU)/g of dough are usual in the sour ferments. As a general rule, LAB are the predominant microorganisms and in many cases yeasts are present in significant numbers (Vogel, Knorr, Müller, Steudel, Gänzle, & Ehrmann, 1999; Vogel, Müller, Stolz, & Ehrmann, 1996).

The microbial ecology of the sourdough fermentation is dependent on both endogenous and exogenous factors (Hammes & Gänzle, 1998; Hammes, Stolz, & Gänzle, 1996; Vogel *et al*., 1996). Endogenous factors are determined by the chemical and microbiological composition of the dough, exogenous factors mainly by temperature and redox potential. In practice, strong effects are exerted by process parameters such as dough yield (water activity), addition of salt, amount and composition of the starter, number of propagation steps, and fermentation time. The impact of these parameters during continuous propagation of sourdough causes the selection of the characteristic LAB and yeast microflora, and meanwhile preventing the growth of other microorganisms originating from contamination of the raw materials or the bakery environment. As shown for certain industrial sourdough processes, such microbial associations may endure for years, only because of the selective pressure exerted by the environmental conditions, although the fermentation process still runs under non-aseptic conditions (see below).

Microbiological studies have revealed that many species, mostly of the genus *Lactobacillus*, and several yeast species, especially species of the genera *Saccharomyces* and *Candida*, occur in this ecological niche. The biodiversity of sourdough LAB can be regarded as restricted or diverse. In the case of a restricted biodiversity, one has to know that a limited but increasing number of LAB species are unique for sourdough. Besides, opportunistic strains may occur in sourdough, which might play a role in the sourdough fermentation or occur as contaminants. In the case of a wide biodiversity, one may recognise different microbial consortia and particular microbial interactions in diverse sourdough ecosystems. This dual view on the biodiversity of sourdough will be discussed in this paper in the light of its microbiological composition and metabolic interactions.

Sourdough microorganisms

LAB and yeasts are often associated in sourdough. The LAB:yeast ratio in sourdoughs is generally 100:1 (Ottogalli *et al*., 1996). Whereas in the majority of fermented foods homofermentative LAB play an important role, heterofermentative LAB are dominating in sourdough, especially

when traditionally prepared (Corsetti *et al*., 2003; Corsetti, Lavermicocca, Morea, Baruzzi, Tosti, & Gobbetti, 2001; De Vuyst *et al*., 2002; Kline & Sugihara, 1971; Meroth, Walter, Hertel, Brandt, & Hammes, 2003; Rocha & Malcata, 1999). Indeed, acetic acid, an important end product of heterofermentation, plays a major role in the flavour of sourdough. Further, *Lactobacillus* strains are more frequent than *Leuconostoc*, *Weissella*, and *Pediococcus* species; lactococci, enterococci, and streptococci are rarely found. The dominance of (obligate) heterofermentative lactobacilli in sourdoughs can be explained mainly by their competitiveness in and adaptation to this particular environment (see below).

Cereal microflora

The microflora of raw cereals is composed of bacteria, yeasts and fungi (10^4–10^7 CFU/g), while flour contains 2×10^4–6×10^6 CFU/g (Stolz, 1999). The bacteria are mainly mesophilic, and are also found in spontaneously fermented sourdoughs. They include Gram-negative aerobes (*e.g*. *Pseudomonas*) and facultative anaerobes (*Enterobacteriaceae*), as well as Gram-positive LAB: homofermentative rods (*L. casei*, *L. coryniformis*, *L. curvatus*, *L. plantarum*, and *L. salivarius*), heterofermentative rods (*L. brevis* and *L. fermentum*), homofermentative cocci (*E. faecalis*, *L. lactis*, *P. acidilactici*, *P. parvulus*, and *P. pentosaceus*), and heterofermentative cocci (*Leuconostoc* and *Weissella*). Also, undesirable *Staphylococcus aureus* and *Bacillus cereus*, as well as other bacteria, may be present. The following yeasts have been detected, either in the cereals (few up to 9×10^4 CFU/g) or flours (up to 2×10^3 CFU/g): *Candida*, *Cryptococcus*, *Pichia*, *Rhodotorula*, *Torulaspora*, *Trichosporon*, *Saccharomyces*, and *Sporobolomyces*. It should be emphasised that *S. cerevisiae* is not found in the raw materials; its occurrence in sourdough may be explained by the application of baker's yeast in most daily bakery practice (Corsetti *et al*., 2001; Galli, Franzetti, & Fortina, 1987). Among fungi (ca. 3×10^4 CFU/g), *Alternaria*, *Cladosporium*, *Drechslera*, *Fusarium*, *Helminthosporium*, and *Ulocladium* (from the field), and *Aspergillus* and *Penicillium* (from the storage), are found.

Spontaneous sourdough fermentation

Sourdough is rich in fermentable carbohydrates and possesses an initial pH of 5.0–6.2, which is rather low. It therefore allows a spontaneous development of characteristic LAB, derived from the cereals or flours, and depending on the flour preparation and sourdough production technology applied. Traditional sourdough processes, however, do usually not rely on the fortuitous flora, but on the use of mother doughs that are continuously maintained over long periods of time according to a defined and typical cycle of preparation, and that may extent to several decades or even longer; the mother dough represents the natural microbial inoculum for the subsequent doughs. During spontaneous fermentation, the LAB fastly dominate the Gram-negative enterobacteria; both lactobacilli (homofermentative *L. casei*, *L. delbrueckii*, *L. farciminis*, *L. plantarum*, and heterofermentative *L. brevis*, *L. buchneri*, and *L. fermentum*) and pediococci (*P. acidilactici*, *P. pentosaceus*) develop, among which homofermentative lactobacilli are dominating, without a significant difference between wheat and rye (Stolz, 1999). *Leuconostocs* and *Weissella* may play a role during the first phase of the fermentation. They can be important for growth association with lactobacilli. Pediococci usually exist at the end of the fermentation processes of material from plant origin. The following yeast species develop during spontaneous rye sourdough fermentation: *S. turbidans*, *S. marchalianus*, *T. albida*, *S. exiguus*, *S. cerevisiae*, and *Saturnispora saitoi* (Stolz, 1999).

Sourdough fermentation through backslopping

When backslopping is applied, one can find the microflora of spontaneous sourdough fermentations (where homofermentative lactobacilli dominate), but mainly heterofermentative lactobacilli are found. The so-called sourdough lactobacilli *Lactobacillus sanfranciscensis* (Kline & Sugihara, 1971), *L. pontis* (Vogel *et al*., 1994), *L. panis* (Wiese, Strohmar, Rainey, & Diekmann, 1996), *L. paralimentarius* (Cai, Okada, Mori, Benno, & Nakase, 1999), *L. frumenti* (Müller, Ehrmann, & Vogel, 2000a), and *L. mindensis* (Ehrmann, Müller, & Vogel, 2003) are considered typical to sourdough environments, in particular, in batters with an extended fermentation period and/or higher temperatures, because their competitive metabolism has adapted to this environment (see below). Whilst species as *L. brevis* and *L. plantarum* may be considered as ubiquitous, *Lactococcus* is perhaps deliberately used, and lactobacilli of the *L. acidophilus* cluster and even *L. reuteri* are most probably of intestinal origin. About 50 different species of sourdough LAB have been reported until now. Many researchers still report on the existence of non-identifiable and perhaps new sourdough LAB species and/or strains (De Vuyst *et al*., 2002; Rosenquist & Hansen, 2000).

Several factors account for the dominance of sourdough lactobacilli during traditional dough preparation. First, their carbohydrate metabolism is highly adapted to the main energy sources in dough, maltose and fructose. Utilisation of maltose via maltose phosphorylase and the pentose phosphate shunt with fructose as co-substrate results in a higher energy yield than homofermentative maltose degradation (Stolz, Böcker, Hammes, & Vogel, 1995; Stolz, Böcker, Vogel, & Hammes, 1995; Stolz *et al*., 1996). Second, the growth requirements of *L. sanfranciscensis* with respect to temperature and pH match the conditions encountered during sourdough fermentation (Gänzle, Ehrmann, & Hammes, 1998). Third, sourdough lactobacilli possess several stress response mechanisms to overcome acid, high/low temperatures, high osmolarity/dehydration, oxidation, and

starvation (De Angelis, Bini, Pallini, Cocconcelli, & Gobbetti, 2001). These three factors increase the competitiveness and adaptation of these strains to this peculiar environment. Fourth, the production of antimicrobial compounds, both organic acids (lactate, acetate, and others) and proteinaceous compounds (for instance, bacteriocins), improves their competitiveness and may contribute to their stable persistence in sourdough fermentations (Gänzle & Vogel, 2002; Gobbetti, 1998; Hammes & Gänzle, 1998; Messens & De Vuyst, 2002).

Species of sourdough lactobacilli exhibit unique technological properties related to the flavour, texture, staling, and shelf-life of sourdough bread (Gobbetti, 1998; Hammes & Gänzle, 1998). For example, strains of the species *L. sanfransiscensis* and *L. pontis* are shown to improve the taste and flavour of bread (Gobbetti, Corsetti, & Rossi, 1996; Hansen & Hansen, 1996; Thiele *et al*., 2002). In general, heterofermentative metabolism, by means of the fermentation quotient (*i.e.* the molar ratio between lactic acid and acetic acid), mainly influences the flavour of various leavened baked products. Further, *L. sanfranciscensis* and *L. plantarum* produce a wide range of volatile compounds (Damiani *et al*., 1996; Hansen & Hansen, 1996). In general, heterofermentative LAB mainly produce ethylacetate with some alcohols and aldehydes, and homofermentative LAB produce diacetyl and other carbonyls, while iso-alcohols are produced by the yeast.

Yeasts

More than 20 species of yeasts are found in sourdoughs (Rossi, 1996; Stolz, 1999). *S. cerevisiae* is frequently present (or added) due to the use of baker's yeast (Barber & Báguena, 1988, 1989; Barber, Báguena, Martinez-Anaya, & Torner, 1983; Boraam, Faid, Larpent, & Breton, 1993; Coppola, Pepe, Masi, & Sepe, 1996; Corsetti *et al*., 2001; Galli, Franzetti, & Fortina, 1988; Gobbetti, Corsetti, Rossi, La Rosa, & De Vincenzi, 1994; Paramithiotis *et al*., 2000; Rocha & Malcata, 1999; Rosenquist & Hansen, 2000; Salovaara & Savolainen, 1984; Spicher, 1987; Spicher, Schröder, & Schöllhammer, 1979; Strohmar & Diekmann, 1992; Succi *et al*., 2003). In particular, *S. exiguus* (*T. holmii* or *C. holmii* or *S. minor*) (Foschino, Terraneo, Mora, & Galli, 1999; Galli *et al*., 1988; Galli & Ottogalli, 1973; Gobbetti, Corsetti, Rossi, La Rosa, & De Vincenzi, 1994; Infantes & Tourneur, 1991; Spicher & Schröder, 1978; Sugihara, Kline, & Miller, 1971), *C. humilis* (*C. milleri)* (Boraam *et al*., 1993; Gullo, Romano, Pulvirenti, & Giudici, 2003; Spicher, 1987), and *I. orientalis* (*C. krusei*) (Coppola *et al*., 1996; Gobbetti, Corsetti, Rossi, La Rosa, & De Vincenzi, 1994; Spicher & Schröder, 1978; Succi *et al*., 2003) are yeasts associated with LAB in sourdoughs. However, a much greater variety of additional yeast species have been isolated from sourdoughs (Hammes *et al*., 2004; Rossi, 1996). The great variability in the number and type of species found depends on several factors including the degree of dough hydration, the type of cereal used, the leavening temperature, and the sourdough maintenance temperature (Gobbetti, Corsetti, Rossi, La Rosa, & De Vincenzi, 1994). For instance, in Italian sourdough that is generally used for the production of durum wheat bran flour bread more than 95% of the yeast belong to the species *C. humilis*, whose dominance is stable in time (Gullo *et al*., 2003).

Classification of sourdough production processes

Sourdoughs have been classified into three types, based on the kind of technology applied for their production, as used in artisan and industrial processes (Böcker, Stolz, & Hammes, 1995):

- type I sourdoughs or traditional sourdoughs;
- type II sourdoughs or accelerated sourdoughs;
- type III sourdoughs or dried sourdoughs.

Each type of sourdough is characterized by a specific sourdough LAB microflora (Table 1).

Type I sourdoughs

Type I sourdoughs are produced with traditional techniques and are characterized by continuous, daily refreshments to keep the microorganisms in an active state, as indicated by a high metabolic activity, above all with regard to leavening, *i.e.* gas production. The process is performed at ambient temperature (20–30 °C) and the pH is about 4.0. Examples of baked goods so obtained are San Francisco sourdough French bread, Panettone and other brioches, Pugliese, Toscanon and Altamura bread, and three-stage sourdough rye bread. Traditional, type I sourdoughs encompass pure culture, pasty sourdough starter preparations from different origin (type Ia), spontaneously developed, mixed culture sourdoughs made from wheat and rye or mixtures thereof and prepared through multiple stage fermentation processes (type Ib), and sourdoughs made in tropical regions fermented at high temperatures (type Ic) (Stolz, 1999).

Pure culture sourdoughs (type Ia) are derived from natural sourdough fermentations. These sourdoughs are composed of a well-adapted microflora, which is typical for the sourdough. They maintain a stable composition, have a high souring activity, and are resistant against microbial contamination. An example of a type Ia sourdough is a starter preparation containing *L. sanfranciscensis* for the production of San Francisco French bread.

L. sanfranciscensis (previously named *L. sanfrancisco* or *L. brevis* subsp. *lindneri*) was first reported in the San Francisco sourdough French bread process to be responsible for acid production (Kline & Sugihara, 1971). It is an obligate heterofermentative LAB species that can produce large amounts of lactic acid and acetic acid from maltose. It is hence responsible for the souring activity in this sourdough bread, and it helps in dough leavening by gas production (Gobbetti & Corsetti, 1997; Gobbetti, Corsetti,

Table 1. Classification of sourdoughs and the corresponding characteristic microflora

Type Ia	Type Ib	Type Ic	Type II	Type III
Obligate heterofermentative	Obligate heterofermentative	Obligate heterofermentative	Obligate heterofermentative	Obligate heterofermentative
L. sanfranciscensis	*Lactobacillus* spp. [a] *L. brevis* *L. buchneri* *L. fermentum* *L. fructivorans* *L. pontis* *L. reuteri* *L. sanfranciscensis* *W. cibaria*	*Lactobacillus* spp. [b] *L. fermentum* *L. reuteri*	*L. brevis* *L. fermentum* *L. frumenti* *L. pontis* *L. panis* *L. reuteri* *L. sanfranciscensis* *W. confusa*	*L. brevis*
	Facultative heterofermentative *L. alimentarius* *L. casei* *L. paralimentarius* *L. plantarum*			Facultative heterofermentative *L. plantarum* *P. pentosaceus*
	Obligate homofermentative *L. acidophilus* *L. delbrueckii* *L. farciminis* *L. mindensis*	Obligate homofermentative *L. amylovorus*	Obligate homofermentative *L. acidophilus* *L. delbrueckii* *L. amylovorus* (rye) *L. farciminis* *L. johnsonii*	
Yeasts *Candida humilis* (*T. holmii*, *C. milleri*) *S. exiguus*	Yeasts *Candida humilis*	Yeasts *Issatchenkia orientalis* (*Candida krusei*)	Yeasts No yeasts *S. cerevisiae* may be added	

[a] Phylogenetically related to *L. brevis*.
[b] Phylogenetically related to *L. pontis*.

& Rossi, 1996). Maltose-negative, asporogenous strains of the yeast *S. exiguus* are mainly responsible for the leavening function in this particular acidic environment (Sugihara *et al*., 1971).

Traditionally, fermentation times of between 3 and 48 h are used to manufacture wheat and rye sourdoughs (Stephan & Neumann, 1999a,b). For instance, a traditional rye sourdough production process (type Ib) consists of three fermentation steps, including fresh sour, basic sour and full sour. The mother dough or starter, when fully developed, serves as the inoculum for each batch of bread dough. The continuity of the microflora is ensured by consecutive re-inoculation of a new batch from a previous batch, the so-called indirect fermentation with refreshments (back-slopping). The microflora plays an important role in the acidification and leavening of the dough as well as in aroma formation.

The major part of the microflora of type Ib sourdough preparations consists of obligate heterofermentative strains of *L. sanfranciscensis*, selected only by the environmental conditions induced by the sourdough fermentation technology applied. Depending on the fermentation conditions, other species such as obligate heterofermentative *L. brevis* and related *Lactobacillus* spp., *L. buchneri*, *L. fermentum*, *L. fructivorans*, *L. pontis*, *L. reuteri*, and *W. cibaria*, facultative heterofermentative *L. alimentarius*, *L. casei*, *L. paralimentarius*, and *L. plantarum*, and obligate homofermentative *L. acidophilus*, *L. delbrueckii*, *L. farciminis*, and *L. mindensis* occur in relevant cell counts (Hammes & Gänzle, 1998; Vogel *et al*., 1999). The most prominent metabolic activity of these microorganisms in sourdough is the production of acid and carbon dioxide. Gas production is required for leavening of the dough unless baker's yeast is added. If yeast is present naturally, *C. humilis* is often associated with *L. sanfranciscensis* and *L. pontis*. The stable coexistence of these microorganisms in the same substrate is explained in part by their identical growth rates, in turn determined by temperature and pH (Gänzle *et al*., 1998).

Type Ic sourdoughs are, for instance, African sorghum sourdoughs that are produced at higher temperatures (> 35 °C). They contain the obligate heterofermentative *L. fermentum*, *Lactobacillus* spp. related to *L. pontis*, and *L. reuteri* species, as well as the obligate homofermentative *L. amylovorus* (Hamad, Böcker, Vogel, & Hammes, 1992; Hamad, Dieng, Ehrmann, & Vogel, 1997). The yeast most often associated with this type of sourdough is *I. orientalis*.

Type II sourdoughs

The industrialisation of the baking process for rye bread, and the industrial demand for faster, more efficient,

controllable, large-scale sourdough fermentation processes resulted in the development of type II sourdoughs, which are semi-fluid silo preparations. Those bakery pre-products serve mainly as dough acidifiers. Several modified, accelerated sourdough fermentation processes exist. Sourdough processes with continuous propagation and long-term one-step fermentations are common now; they guarantee more production reliability and flexibility. A recent trend of industrial bakeries exists in the instalment of continuous sourdough fermentation plants (Stolz & Böcker, 1996).

Typical type II processes last for 2–5 days and are often carried out at increased fermentation temperature (usually $>30\,^{\circ}C$) to speed up the process (Böcker *et al*., 1995; Hammes & Gänzle, 1998). Those sourdoughs exhibit a high acid content at a pH of <3.5 after 24 h of fermentation. The microorganisms are commonly in the late stationary phase and therefore exhibit restricted metabolic activity only. The high dough yields of these preparations permit pumping of the dough. They are frequently used in local bakeries. As those sourdoughs are stored fresh until use (up to one week), they can be produced in large quantities. In industry, they are applied for the production of dried sourdough products as well.

Under the conditions of type II sourdoughs, *L. sanfranciscensis* is not competitive enough to dominate the fermentation. The completely different process parameters of type II sourdough fermentations result in a different microbial ecosystem with respect to composition and population dynamics. The obligate homofermentative *L. acidophilus*, *L. delbrueckii*, *L. amylovorus* (rye), *L. farciminis*, and *L. johnsonii*, and obligate heterofermentative *L. brevis*, *L. fermentum*, *L. frumenti*, *L. pontis*, *L. panis*, *L. reuteri*, as well as *Weissella* (*W. confusa*) species are found (Müller, Wolfrum, Stolz, Ehrmann, & Vogel, 2001; Vogel *et al*., 1999).

Type III sourdoughs

Type III sourdoughs are dried doughs in powder form, which are initiated by defined starter cultures. They are used as acidifier supplements and aroma carriers during breadmaking. They mostly contain LAB that are resistant to drying and are able to survive in that form, *e.g*. heterofermentative *L. brevis*, and facultative heterofermentative *P. pentosaceus* and *L. plantarum* strains. The drying process (spray-drying or drum-drying) also leads to an increased shelf-life of the sourdough and turns it into a stock product until further use. Dried sourdoughs are convenient, simple in use, and result in standardized end products. They can be distinguished in colour, aroma, and acid content (Stolz & Böcker, 1996).

Type 0 doughs and the use of starter cultures

In contrast to type I preparations, doughs of types II and III require the addition of baker's yeast (*S. cerevisiae*) for leavening. During continuous propagation (type I) the temperature is lower and the rate of re-inoculation often exceeds 30%, resulting in a lower start pH that promotes yeast growth (*S. exiguus*, *C. humilis*, *I. orientalis*, *S. cerevisiae*). In most cases, yeast preparations contain LAB, which contribute to acidification and aroma development in pre-doughs often used for the production of soda crackers (USA), baguettes (France) and Ciabatta (Italy) (Fields, Hoseney, & Varriano-Marston, 1982). These sourdoughs are sometimes referred to as type 0 doughs.

As a consequence of the use of commercially available bulk starter cultures for dairy and meat fermentations, a new trend is the use of commercial starter cultures in sourdough fermentations. Indeed, they usually lead to standardized end products, although their selection has not always been done in a rational way; on the other hand, they do not allow flexibility in applications, and due to loss of genetic material through repeated use their application often results in decreased biodiversity (Leroy & De Vuyst, 2004). Commercial starters for sourdough fermentation should at least acidify the dough quite reasonably and hence be isolated from a cereal environment to be adapted to the process, survive the drying process (to produce powder forms which are easy to handle), and contribute to the aroma of the end product. Unfortunately, most strains in use are not well-adapted to the cereal environment, cannot compete with the endogenous microflora, and require a frequent inoculation. As recent developments, single- and multiple-strain starter cultures have been introduced into practice, such as dried preparations containing *L. delbrueckii* and *L. brevis* or *L. plantarum*. Freeze-dried preparations containing *L. sanfranciscensis* have been developed as well. Finally, cultures containing *L. plantarum*, *L. brevis*, and *L. fructivorans* or *L. brevis*, *L. pontis*, and *S. cerevisiae* are available (Hammes & Gänzle, 1998).

Microbial consortia

The distribution of the taxa of LAB is highly variable from one sourdough ecosystem to another (Table 2). In general, growth rate and yield of microorganisms are governed by a multitude of ecological factors (Spicher, 1999b). In sourdoughs, these factors are temperature, pH, redox potential, ionic strength, dough yield, and microbial products, such as lactate, acetate, carbon dioxide, and ethanol, as well as factors resulting from substrates present in the cereal fraction and from endogenous and microbial enzymatic reactions.

In addition, it should be noticed that in all of the microbiological surveys carried out, reference is usually made to single isolations performed only once per fermentation. Moreover, not always modern taxonomic identification techniques and polyphasic approaches, as is usually the case today, have been carried out for their characterisation (Corsetti *et al*., 2003; De Vuyst *et al*., 2002). In addition, only few data on the influence of time on the composition of the sourdoughs have been reported (Gullo *et al*., 2003; Meroth *et al*., 2003; Müller *et al*., 2001). Finally, it should be emphasised that the LAB isolated from

Table 2. Biodiversity of sourdough lactic acid bacteria in sourdoughs of different origin

Country	Product/method of isolation and identification	Lactic acid bacteria	Reference
Belgium	Wheat/rye sourdoughs polyphasic approach	*L. brevis*, *Lactobacillus* spp. [a], *L. plantarum*, *L. sanfranciscensis*, *L. paralimentarius*, *P. pentosaceus*, *L. helveticus*	Unpublished results
Denmark	Rye sourdough phenotypical	*L. reuteri*, *L. panis*, *L. amylovorus*	Rosenquist and Hansen (2000)
Finland	Sour rye dough phenotypical	*L. acidophilus*, *L. plantarum*, *L. casei*	Salovaara and Katunpää (1984)
France	Wheat bread phenotypical	*L. plantarum*, *L. casei*, *L. delbrueckii* subsp. *delbrueckii*, *L. acidophilus*, *L. brevis*, *Leuc. mesenteroides* subsp. *mesenteroides*, *Leuc. mesenteroides* subsp. *dextranicum*, *P. pentosaceus*, *L. curvatus*	Infantes and Tourneur (1991)
Germany	Wheat sourdough phenotypical	*L. delbrueckii*, *L. plantarum*, *L. casei*, *L. fermentum*, *L. buchneri*, *L. brevis*	Spicher (1959)
	Rye bread phenotypical	*L. acidophilus*, *L. farciminis*, *L. alimentarius*, *L. casei*, *L. plantarum*, *L. brevis*, *L. sanfranciscensis*, *L. fructivorans*, *L. fermentum*, *L. buchneri*	Spicher and Schröder (1978) and Spicher et al. (1979)
	Rye sourdough phenotypical	*L. acidophilus*, *L. casei*, *L. plantarum*, *L. farciminis*, *L. alimentarius*, *L. brevis*, *L. buchneri*, *L. fermentum*, *L. fructivorans*, *L. sanfranciscensis*, *Pediococcus* spp.	Spicher (1984)
	Wheat sourdoughs (Panettone, wheat bread) phenotypical	*L. plantarum*, *L. casei*, *L. farciminis*, *L. homohiochii*, *L. brevis*, *L. hilgardii* (spontaneous); *L. sanfranciscensis*, *L. brevis*, *L. hilgardii*, *W. viridescens* (masa madre)	Spicher (1987)
	Rye sourdough RAPD-PCR	*L. amylovorus*, *L. pontis*, *L. frumenti*, *L. reuteri*	Müller et al. (2001)
	Rye bran PCR-DGGE	*L. sanfranciscensis*, *L. mindensis* (type I rye sourdough); *L. crispatus*, *L. pontis*, *L. panis*, *L. fermentum, L. frumenti* (type II rye sourdough); *L. johnsonii*, *L. reuteri* (type II rye bran sourdough)	Meroth et al. (2003)
Greece	Wheat sourdoughs polyphasic approach	*L. sanfranciscensis*, *L. brevis*, *Lactobacillus* spp. [a], *L. paralimentarius*, *W. cibaria*	De Vuyst et al. (2002)
Italy	Panettone phenotypical	*L. brevis, L. plantarum*	Galli and Ottogalli (1973)
	Panettone, Brioche phenotypical	*L. sanfranciscensis*, *L. fermentum*, *L. plantarum*, *Leuc. mesenteroides*, *Pediococcus* spp.	Galli et al. (1988)
	Umbrian wheat sourdoughs phenotypical	*L. sanfranciscensis*, *L. plantarum*, *L. farciminis*	Gobbetti, Corsetti, Rossi, La Rosa, and De Vincenzi (1994)
	Pizza (Naples) phenotypical	*L. sakei*, *L. plantarum*, *Leuc. gelidum*, *Leuc. mesenteroides*	Coppola et al. (1996)
	Verona sourdoughs RAPD-PCR	*L. sanfranciscensis*	Zapparoli et al. (1996, 1998)
	Lombardian mother sponges species-specific PCR	*L. sanfranciscensis*	Foschino et al. (1999)
	Apulian wheat sourdoughs 16S rDNA sequencing 16S/23S rRNA spacer region PCR	*L. sanfranciscensis*, *L. alimentarius*, *L. brevis*, *Leuc. citreum*, *L. plantarum*, *L. lactis* subsp. *lactis*, *L. fermentum*, *L. acidophilus*, *W. confusa*, *L. delbrueckii* subsp. *bulgaricus*	Corsetti et al. (2001)
Iran	Sangak phenotypical	*Leuc. mesenteroides, L. plantarum, L. brevis, P. cerevisiae*	Azar et al. (1977)
Mexico	Pozol (maize) 16S rDNA sequencing	*L. lactis, S. suis, L. plantarum, L. casei, L. alimentarius, L. delbrueckii*	Escalante et al. (2001)
Morocco	Sourdough ferments traditional starter sponges phenotypical	*L. plantarum, L. brevis, L. buchneri, L. casei, Leuc. mesenteroides, Pediococcus* sp.	Boraam et al. (1993)
	Soft wheat flour phenotypical	*L. plantarum, L. delbrueckii, L. buchneri, L. casei, L. sanfranciscensis, Leuc. mesenteroides, P. pentosaceus*	Faid et al. (1994)
Portugal	Broa phenotypical	*Leuconostoc* spp., *L. brevis*, *L. curvatus*, *L. delbrueckii*, *L. lactis* subsp. *lactis*, *E. casseliflavus*, *E. durans*, *E. faecium*, *S. constellantus*, *S. equinus*	Rocha and Malcata (1999)
Russia	Rye sourdough phenotypical	*L. plantarum*, *L. brevis*, *L. fermentum*	Kazanskaya et al. (1983)
Spain	Wheat sourdough phenotypical	*L. brevis*, *L. plantarum*	Barber et al. (1983)

(*continued on next page*)

Table 2 (*continued*)

Country	Product/method of isolation and identification	Lactic acid bacteria	Reference
	Wheat sourdough phenotypical	*L. brevis*, *L. plantarum, L. cellobiosus, Leuc. mesenteroides*	Barber and Báguena (1988, 1989)
Sudan	Kisra (sorghum sourdough)	*L. fermentum*, *L. reuteri*, *L. amylovorus*	Hamad et al. (1992)
	Kisra RAPD	*E. faecalis*, *L. lactis, L. fermentum, L. reuteri, L. vaginalis, L. helveticus*	Hamad et al. (1997)
Sweden	Rye/wheat phenotypical	*L. fermentum, L. delbrueckii, L. acidophilus, L. plantarum, L. rhamnosus*, *L. farciminis*, *L. fermentum*, *L. sanfranciscensis*, *L. brevis, W. viridescens*	Spicher and Lönner (1985)
	Rye sourdough phenotypical	*Lactobacillus* sp., *P. pentosaceus*	Lönner, Welander, Molin, Dostálek, and Blickstad (1986)
USA	San Francisco sourdough French bread phenotypical	*L. sanfranciscensis*	Kline and Sugihara (1971)

[a] Phylogenetically related to *L. brevis*.

many sourdoughs are difficult to cultivate in common laboratory media. This may be because these bacteria have been selected during repeated sourdough propagation, resulting in a flora with specialised nutrient requirements and growth conditions (Böcker *et al*., 1990; Kline & Sugihara, 1971; Rosenquist & Hansen, 2000; Salovaara & Katunpää, 1984). As an example, Böcker *et al*. (1990) observed that their *L. sanfranciscensis* isolates required fresh baker's yeast and wheat bran to grow in a basal medium.

LAB consortia can be broad, limited, and/or unique. The most often isolated LAB from German type I rye sourdoughs are strains of *L. sanfranciscensis* followed by *L. brevis* (Böcker *et al*., 1995, 1990; Spicher, 1959, 1984, 1987; Spicher & Schröder, 1978; Spicher *et al*., 1979). San Francisco sourdough and Panettone are characterized by a homogeneous microflora, consisting of *L. sanfranciscensis* and *S. exiguus* only (Foschino *et al*., 1999; Kline & Sugihara, 1971; Sugihara *et al*., 1971). Further, *L. sanfranciscensis* is very abundant in type I wheat sourdoughs (Table 2). As mentioned above, this species is characteristic for and dominates type I sourdough fermentations, because it is probably selected only by the environmental conditions induced by the sourdough fermentation technology applied (Böcker *et al*., 1995; Corsetti *et al*., 2001; Foschino *et al*., 2001, 1999; Kline & Sugihara, 1971; Meroth *et al*., 2003). The physiological and genetic characteristics of a number of *L. sanfranciscensis* strains could not distinguish isolates from sourdough samples collected in different geographical regions of Italy (Foschino *et al*., 2001). Most of the *L. sanfranciscensis* isolates only ferment glucose and maltose, and do not ferment fructose (Corsetti *et al*., 2001); the latter energy source is also fermented by *L. brevis* (De Vuyst *et al*., 2002).

L. brevis and *L. plantarum* seem to be abundant in type Ib sourdoughs (Table 2). As *L. plantarum* is facultative heterofermentative, it cannot make the dough rise like other lactobacilli such as *L. sanfranciscensis* and *L. brevis* (obligate heterofermentative). However, several studies report on the association of *L. sanfranciscensis* and *L. plantarum* (Gobbetti, 1998), for instance, in Italian wheat sourdoughs (Table 2).

Many sourdoughs contain associations of hetero- and homofermentative LAB strains (Table 2). As mentioned above, homofermentative LAB dominate in spontaneous fermentation processes; heterofermentative sourdough lactobacilli drive sourdough fermentation processes with backslopping. However, this does not exclude the presence of homofermentative LAB in the latter sourdoughs. The eventually established LAB consortia commonly reflect the media resources (carbohydrates, amino acids, vitamins) and environmental conditions (temperature, pH, redox potential). Further, their microbiological composition is strongly influenced by the process parameters, the use of a starter, and/or the use of baker's yeast. As an example, the microorganisms associated with French natural sourdoughs are those commonly found in cereal products, namely *L. plantarum*, *L. casei*, *L. delbrueckii*, *L. acidophilus*, *L. brevis*, *Leuc. mesenteroides*, and *P. pentosaceus* (Infantes & Tourneur, 1991). Several Apulian sourdoughs made from rye or the wheat flour species *Triticum aestivum* do not contain such complex associations (Corsetti *et al*., 2001). Sourdoughs from Foggia and Lecce seem to typically contain associations between *L. sanfranciscensis* and *Leuc. citreum*, and between *L. sanfranciscensis* and *L. alimentarius*, respectively. The facultative heterofermentative species *L. alimentarius* is typical for Apulian (Lecce) wheat sourdoughs that use durum wheat flour (*T. durum*). This may indicate that *L. plantarum* is substituted for *L. alimentarius* in its association with *L. sanfranciscensis*, probably because of a stronger association of *L. alimentarius* with *L. sanfranciscensis*, in particular upon prolonged fermentation (Corsetti *et al*., 2001). A common feature of *L. alimentarius* isolates is the capacity to ferment all four soluble carbohydrates contained in wheat flour, *i.e.* maltose, sucrose, glucose, and fructose. Finally, *L. sanfranciscensis*,

L. alimentarius and *L. plantarum* have also the capacity to use pentoses (xylose and/or arabinose). The capacity to ferment a large range of wheat flour carbohydrates may reduce the metabolic competition with yeasts and may have important technological repercussions during sourdough fermentation (see below).

Greek wheat sourdough LAB isolates belong to the species *L. sanfranciscensis*, *L. brevis*, *L. paralimentarius*, and *W. cibaria*, a unique consortium (De Vuyst *et al*., 2002). It may be assumed that isolates earlier assigned to the species *W. confusa* (Table 2) have been misidentified (De Vuyst *et al*., 2002). They probably belong to the very recently described species *W. cibaria*, a species that is both genomically and phenotypically highly similar to *W. confusa* (Björkroth *et al*., 2002). A misidentification may also have occurred for sourdough isolates previously assigned to the species *L. alimentarius* (Table 2), which probably belong to the species *L. paralimentarius* (De Vuyst *et al*., 2002). Notice that *L. alimentarius*, *L. paralimentarius*, *L. farciminis*, *L. kimchii*, and *L. mindensis* are close relatives (De Vuyst *et al*., 2002; Ehrmann *et al*., 2003); this is also the case for *L. frumenti*, *L. pontis*, *L. panis*, *L. oris*, *L. vaginalis*, and *L. reuteri* (Müller *et al*., 2000a; Müller, Ehrmann, & Vogel, 2000b; Wiese *et al*., 1996).

Finally, strains of some species are rarely isolated from sourdoughs. For instance, *L. fermentum* is dominating in Swedish sourdoughs (Spicher & Lönner, 1985), and in Russian sourdoughs it occurs together with *L. brevis* and *L. plantarum* (Kazanskaya, Afanasyeva, & Patt, 1983). In the case of German type II rye sourdoughs where *L. fermentum* was dominating, it has been shown that the *L. fermentum* detected originated from the baker's yeast used (Meroth *et al*., 2003). *L. curvatus* is seldomly isolated from sourdough (Infantes & Tourneur, 1991), as is also the case for *L. crispatus* (Meroth *et al*., 2003). Also, *L. helveticus* and *L. delbrueckii* subsp. *bulgaricus* occur only occasionally (Faid, Boraam, Zyani, & Larpent, 1994; Hamad *et al*., 1997). However, LAB species such as *L. acidophilus*, only occasionally found in some sourdoughs (Infantes & Tourneur, 1991; Salovaara & Katunpää, 1984; Spicher, 1984), are found in more than 50% of the Umbrian sourdoughs, may be indicating a selection due to typical and regional conditions of sourdough production (Gobbetti, Corsetti, Rossi, La Rosa, & De Vincenzi, 1994).

Microbial interactions

The sourdough microflora is usually composed of stable associations of lactobacilli and yeasts, because of their growth requirements with respect to temperature, pH, and organic acids, as well as metabolic interactions between these microorganisms (see below). However, in some sourdoughs, LAB and yeasts compete for the available substrates, resulting in heterogeneous populations that reflect the media resources and environmental conditions (see above). This in turn may change the mother dough completely in a short time in the case of continuous propagation and backslopping (Ottogalli *et al*., 1996). Weak microbial associations include, for instance, LAB present as contaminants in pre-doughs. Further, when baker's yeast is added, the microflora changes, and, consequently, the organoleptic properties of the final product are influenced. Similarly, the use of selected starters influences the fermentation and the properties of the final product as compared with spontaneous sourdoughs.

The importance of antagonistic and synergistic interactions between lactobacilli and yeasts are based on the metabolism of carbohydrates and amino acids and the production of carbon dioxide (Gobbetti & Corsetti, 1997; Gobbetti, Corsetti, & Rossi, 1994a,b). Typical mutual associations involve *L. sanfranciscensis* and either *S. exiguus* or *C. humilis* (Ottogalli *et al*., 1996). As mentioned above, these yeasts usually share type I sourdough environments with the lactobacilli. Maltose is the preferred energy source for *L. sanfranciscensis* and is not utilized by either *S. exiguus* or *C. humilis* (maltose-negative yeasts; use sucrose, glucose, and fructose). Maltose is continuously delivered by flour amylases. In the abundance of maltose and under stress conditions, several strains of the species *L. sanfranciscensis* hydrolyse maltose through constitutive, intracellular maltose phosphorylase activity (without the expenditure of ATP), and accumulate glucose in the medium in a molar ratio of about 1:1 (lack of hexokinase activity) (Stolz *et al*., 1996). This glucose affects the ecological system as it may be metabolised by its producers, by other LAB, and by the yeasts. However, it may initiate glucose repression in competitors for maltose, while the maltose phosphorylase reaction is not repressed by glucose (Hammes *et al*., 1996). The glucose may then be utilised by the maltose-negative yeasts. Due to the faster consumption of maltose, and especially glucose, by *S. cerevisiae*, a decrease in the metabolism of *L. sanfranciscensis* is expected when associated with maltose-positive yeasts. However, the disappearance of *S. cerevisiae* from the microbial population of sourdough during consecutive fermentations is related to the repression of the genes involved in maltose fermentation, so that maltose cannot be utilized, and to the rapid depletion of sucrose.

The lack of competition between *L. sanfranciscensis* and *S. exiguus* for maltose is fundamental for the stability of this association. The sourdough yeasts do not affect the cell yield of *L. sanfranciscensis*, because pH is the limiting factor for growth of the lactobacilli (*e.g. L. sanfranciscensis* does not grow below pH 3.8). The maltose, amino acid, and peptide concentrations are not depleted during wheat or rye sourdough fermentations. The cell yield of the maltose-negative yeasts is lower in the presence of lactobacilli, both in wheat and rye doughs, because their growth is inhibited by the accumulation of metabolic end products. However, the glucose concentration in rye flours and whole-wheat flours remains high enough to support yeast growth throughout the fermentation. Fermentations that employ

white wheat flours as the raw material are characterized by low concentrations of glucose, and small amounts of lactic acid are produced because of the low buffering capacity. In these doughs, depletion of glucose and fructose may occur and limit the growth of the yeasts. The sourdough lactobacilli may also generate additional energy by the activity of acetate kinase in the presence of electron acceptors, which allows the recycling of NAD^+ without the need of ethanol formation, and in parallel the synthesis of a higher level of acetic acid. Electron acceptors used by *L. sanfranciscensis* include fructose and citrate/malate/fumarate, which are reduced to mannitol and lactate/citrate, respectively (Stolz, Böcker, Hammes, & Vogel, 1995; Stolz, Böcker, Vogel, & Hammes, 1995). Furthermore, NAD^+ can be recycled in the NADH oxidase reaction (oxygen as electron acceptor). Fructose is present in the flour in gluco-fructosans, which are degraded by the maltose-negative *C. humilis*. Yeast invertase has been shown to be responsible for the liberation of fructose from fructo-oligosaccharides in dough (Brandt & Hammes, 2001). Hence, maltose–fructose and maltose–citrate co-metabolisms of *L. sanfranciscensis* reduce the competition for carbohydrates between LAB and yeasts (Gobbetti & Corsetti, 1996; Gobbetti, Corsetti, & Rossi, 1995). The practical relevance of these interactions is the change in the fermentation quotient affecting the baking and sensorial properties of sourdough bread.

Finally, different microbial interactions and technologies affect the synthesis of volatile compounds that contribute to the flavour and aroma of sourdough products. The development of bacteria that ferment all soluble flour carbohydrates will affect the metabolic competition and acidification processes mentioned above; for instance, *Leuconostoc* and *Weissella* do ferment maltose, glucose, fructose, and sucrose, while some strains of *L. lactis* subsp. *lactis* do not ferment maltose neither sucrose. Also, while yeasts greatly contribute to the leavening and, with heterofermentative LAB, to the sensory quality, facultative heterofermentative and homofermentative LAB will dominate the acidification and, as the production of lactic acid by these strains is much lower, the effect on FQ is uncertain and difficult to control (Martinez-Anaya, Llin, Macias, & Collar, 1994; Röcken *et al*., 1992). While sourdoughs started with an association of *L. sanfranciscensis* and other homo- or heterofermentative LAB and/or *S. exiguus* are characterized by a balanced aroma profile, sourdoughs produced with an association of *L. sanfranciscensis* and *S. cerevisiae* contain higher concentrations of the yeast fermentation products and a lower amount of the bacterial compounds (Meignen *et al*., 2001). Associations of *L. sanfranciscensis*, *L. plantarum*, and *S. cerevisiae* guarantee an equilibrated aroma profile in wheat sourdough breads (Hansen & Hansen, 1996). On the other hand, acetic acid is lost during freeze-drying of the sourdough. By providing fructose, this may be compensated by the high levels of acetate produced by fructose-positive strains such as *L. brevis* (Meignen *et al*., 2001). Also, the addition of pentosans may result in higher acetate contents (Gobbetti *et al*., 1999). Further, a sourdough fermentation with *L. plantarum* with the addition of pentosan extracts and pentosanases, which liberate arabinose from the pentosans, increases the acidification rate, titratable acidity, and acetic acid content in comparison with a traditional sourdough (Gobbetti *et al*., 2000).

Stable sourdoughs

A reproducible and controlled composition and activity of the sourdough microflora is indispensable to achieve a constant quality of sourdough bread. In bakery practice, as mentioned above, sourdough is usually sustained by repeated inoculation. It is believed that some sourdoughs are maintained over several centuries, *e.g.* the continuous use of Böcker–Reinzucht–Sauerteig (BRS) sourdough over seven decades has been documented (Böcker *et al*., 1990). Despite annual changes in raw materials, seasonal changes in temperature, as well as ample opportunity for contamination from either the raw materials or the bakery environment, the sourdough microflora often is remarkably stable. Monitoring of the microflora of two industrial Danish sourdoughs over a period of 7 months revealed only minor shifts in the composition of the lactobacilli (Rosenquist & Hansen, 2000). In BRS, a sourdough starter propagated according to traditional procedures, the composition remained stable on strain level over a period of at least two decades as experimentally revealed (Böcker *et al*., 1990; Gänzle *et al*., 1998; Spicher & Schröder, 1978).

As mentioned above, mainly four factors account for the dominance of lactobacilli in sourdough: their highly adapted carbohydrate metabolism, their growth requirements for temperature and pH that match the conditions encountered during sourdough fermentation, their possible stress responses, and their excretion of antimicrobial compounds that may contribute to a stable persistence. For instance, the microflora of SER sourdough, an in house rye sourdough prepared for the production of a commercially available baking aid (Böcker *et al*., 1995), has been monitored over a period of 10 years. Over these 10 years of continuous propagation, considerable shifts are observed concerning the composition of the dough microflora. However, relevant cell counts of *L. reuteri* are found at each isolation time. All isolates exhibit similar physiological properties and molecular typing reveals closely related patterns (Gänzle & Vogel, 2002). Two isolates obtained in 1994 and 1998 are identical and produce reutericyclin, a low-molecular-mass antibiotic active against a broad range of Gram-positive bacteria in concentrations of less than 1 mg/l, including those LAB relevant in sourdough fermentations (Gänzle, Höltzel, Walter, Jung, & Hammes, 2000). The reutericyclin concentration in dough fermented with *L. reuteri* was 5 mg/kg. Reutericyclin produced *in situ* by *L. reuteri* is active in dough against reutericyclin-sensitive *L. sanfranciscensis*, and hence provides a competitive advantage to the producer strain, and contributes to the stable persistence of *L. reuteri*

in the industrial sourdough. Similarly, the production of antibacterial and antimould substances by *L. sanfransiscensis* may be related to its predominance and may contribute to the stability of sourdough products also protecting insensitive yeasts (Gobbetti, 1998).

Similarly, *L. amylovorus*, *L. brevis*, *L. fermentum*, *L. frumenti*, *L. panis*, *L. pontis*, and *L. reuteri* seem to remain dominant for a long time during continuous propagation of type II sourdoughs supporting their important role during these fermentations (Hammes & Gänzle, 1998; Meroth *et al*., 2003; Müller *et al*., 2000a,b). Moreover, they are enriched during continuous propagation of these doughs (Meroth *et al*., 2003). Their persistence is ascribed to a competitive metabolism and adaptation (see above). However, the process temperature is an important ecological factor strongly affecting the competitiveness of lactobacilli in sourdough fermentations. For instance, during continuous propagation of type II sourdoughs at higher temperature (40 °C instead of 30 °C), *L. frumenti* and *L. panis* are dominating instead of *L. pontis* and *L. reuteri* (Meroth *et al*., 2003).

Finally, the use of competitive strains might help to develop new, stable, controlled sourdough starter cultures for type II sourdough fermentation processes (Messens & De Vuyst, 2002). For instance, it has been shown that *L. amylovorus* strain DCE 471 is a fast acidifier, optimally grows under the temperature and pH conditions prevailing during type II wheat and rye sourdough fermentations, and produces a bacteriocin, amylovorin L (De Vuyst *et al*., 2004; De Vuyst, Callewaert, & Pot, 1996; Messens, Neysens, Vansieleghem, Vanderhoeven, & De Vuyst, 2002) that suppresses the background microflora, conditions that all improve its competitiveness (Messens *et al*., 2002; Neysens, Messens, & De Vuyst, 2003; Neysens, Messens, Gevers, Swings, & De Vuyst, 2003). Furthermore, since the strain was isolated from corn steep liquor, it is adapted to a cereal environment and may help in elaborating maltose from the starch through its amylase activity; in addition, it is able to ferment maltose and fructose simultaneously. Its competitiveness in wheat and rye sourdoughs have been demonstrated recently (De Vuyst *et al*., 2004).

Conclusion

In-depth studies of the biodiversity of the microflora of traditional sourdough products are interesting from an academic and industrial point of view. Recently, several new species have been identified and many others will soon be discovered. Further, investigations of the population dynamics of traditional sourdoughs and commercial sourdough starters revealed basic mechanisms of their propagation and stability. Modern, molecular approaches such as denaturing gradient gel electrophoresis and microarray analysis will further help to confirm and to know the real flora of sourdough ecosystems. Finally, an increased knowledge of the sourdough microflora is useful to better control both artisan and industrial fermentation processes, and to protect typical local productions, in particular in view of rural development, to guarantee an 'Appellation d'Origine Protégée' (AOP) status, and for the development of high quality products (quality labels).

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