The sourdough microflora: Interactions of lactic acid bacteria and yeasts

M. Gobbetti

Institute of Dairy Microbiology, Agriculture Faculty of Perugia, S. Costanzo, 06126 Perugia, Italy (tel. & fax: +39-75-32387)

Sourdough bread is a traditional product with great potential. This can only be achieved if the interactions between the lactic acid bacteria and yeasts that populate the sourdough are understood. The trophic and non-trophic interactions between sourdough lactic acid bacteria and yeasts are reviewed with particular emphasis on the metabolism of the carbohydrates and nitrogen compounds, the production of CO₂ and other volatile compounds, and antimicrobial activity.

Sourdough LAB and yeasts

Lactobacilli, obligately homofermentative and facultatively or obligately heterofermentative, are the typical sourdough LAB. Lactobacillus sanfranciscensis [2] (synonym Lb. brevis subsp. lindneri), Lb. plantarum and Lb. brevis are the most frequently isolated lactobacilli (Table 1). Some strains, initially classified as Lb. brevis, were recently allotted to the new species Lb. pontis [10]. Leuconostoc sp. and Enterococcus sp. are occasionally found or used in sourdough processes.

Several species of yeasts are found in sourdoughs; Saccharomyces cerevisiae is frequently present or is added (Table 1). The amount of S. cerevisiae may be overestimated due to the lack of reliable systems for identifying and classifying yeasts from this habitat [11]. In particular S. exiguus (imperfect state Torulopsis holmii or Candida holmii, physiologically similar to C. milleri), and C. krusei, Pichia norvegensis and Hansenula anomala are yeasts associated with LAB in sourdoughs. The LAB:yeast ratio in sourdoughs is generally 100:1 [9].

Like other fermented foods produced by mixed microflora, the organoleptic, health and nutritional properties of baked sourdough goods depend on the cooperative activity of LAB and yeasts.

Metabolism of carbohydrates

Rye and especially wheat flours contain low amounts of soluble carbohydrates. In wheat flour, the total concentration of maltose, sucrose, glucose and fructose varies from 1.55 to 1.85% depending on the balance between starch hydrolysis, by the flour and microbial enzymes and microbial consumption [12].

The utilization of soluble carbohydrates by LAB and, consequently, their energy yield, and lactic and acetic acid production are greatly influenced by the associated and the responsible metabolite(s) to be identified. Knowledge, exploitation and improvement of the stability of associated sourdough LAB and yeasts is necessary in order to avoid losing the variety of regional specialities and to meet consumer and industry demands.

This review focuses on the trophic and non-trophic interactions between sourdough LAB and yeasts based on the metabolism of carbohydrates and nitrogen compounds, the production of CO₂ and other volatile compounds, and on the antimicrobial activity.
yeasts and vary according to the type of sugars. The results obtained are contradictory because of the complexity of the ecosystem. In a continuous sourdough fermentation the association between \textit{Lb. sanfranciscensis} and \textit{S. cerevisiae} was optimal for producing acetic acid, while yeast extract did not produce the same effect [13]. \textit{Torulopsis holmii} was found to improve dough acidification by \textit{Lb. sanfranciscensis} and \textit{S. cerevisiae} enhanced acid production by \textit{Lb. sanfranciscensis} and \textit{Lb. plantarum} [14, 15].

The lack of competition between \textit{Lb. sanfranciscensis} and \textit{S. exiguus} for maltose is fundamental for the stability of this association in San Francisco French bread [5]. \textit{S. exiguus} preferentially uses glucose or sucrose and has a high tolerance for the acetic acid produced by the heterolactic metabolism [16]. The lack of competition for the main carbon source seems to be one of the prerequisites for the stability of LAB/yeast associations in food fermentations (e.g. the yeast flora in fermented milks such as kefir, leben and koumiss is mainly non-lactose-fermenting).

However, most reports show that LAB multiply and produce lactic and acetic acids more slowly in mixtures with yeasts than in pure culture [1, 17]. The trophic relationships that occur between the prevailing sourdough LAB (\textit{Lb. sanfranciscensis} and \textit{Lb. plantarum}) and yeast (\textit{S. cerevisiae}) have been studied in co-culture model systems [18]. Bacterial growth and production of lactic and acetic acids decreased due to the faster consumption of maltose and, especially, of glucose by \textit{S. cerevisiae} when associated with \textit{Lb. sanfranciscensis} in a synthetic medium containing these carbon sources. Sourdough yeasts and LAB have different kinetics for carbohydrate uptake. Most of the yeasts take up hexose and maltose by high affinity transport systems, while the disaccharide uptake of LAB (\textit{Lb. brevis}) is strictly dependent on the external concentration and is less effective [1]. Indeed, the stimulation of bacterial growth in the co-cultures by yeast has only been observed when wheat flour extract supplied with large amounts of the various soluble carbohydrates is used. The imbalance between yeast consumption and starch hydrolysis by flour enzymes leads to the rapid depletion of soluble carbohydrates during wheat sourdough fermentation which, in turn, decreases LAB acidification due to microbial competition [19, 20]. This situation is less pronounced in rye dough fermentation due to the greater flour enzyme activity, which increases the availability of soluble carbohydrates [21]. In wheat doughs fermented by yeasts and LAB, the concentration of maltose may remain between 2 and 5 g/kg [22]. It has been shown that maltose may accumulate because it is not metabolized by some yeasts until the available glucose and fructose supplies are depleted [23]. The maltose transport system is inducible in sourdough yeasts and glucose and fructose repress the permease. [12]

The addition of selected carbon sources to the wheat dough has been proposed in order to enhance the production of lactic and acetic acids by LAB associated with \textit{S. cerevisiae} [24]. Co-fermentations are another metabolic route, which enable sourdough LAB to use non-fermentable substrates, thus increasing their adaptability. A co-fermentation of fructose and maltose or glucose has been observed in a fructose-negative strain of \textit{Lb. sanfranciscensis} [25, 26]. Through its reduction to mannitol, fructose acts as an additional electron acceptor and increases ATP and acetic acid by the acetate kinase reaction. A co-metabolism of citrate and maltose or glucose was also observed by the same strain of \textit{Lb. sanfranciscensis} [27]. The same metabolism was observed in fruit juices: the competition between \textit{S. cerevisiae} and \textit{Lb. plantarum} for glucose promoted the citrate co-metabolism by the lactic acid bacterium [28].

\textit{Lb. sanfranciscensis} hydrolyses maltose and accumulates glucose in the medium in a molar ratio of about 1 maltose to 1 glucose [18, 29]. The glucose 1-phosphate produced by maltose phosphorylase is further metabolized, whereas glucose is not used but it is excreted in order to avoid excessive intracellular concentrations. The maltose uptake and glucose excretion in \textit{Lb. sanfranciscensis} were analysed by Neubauer et al. [30]. Maltose transport occurs by a secondary transport system (maltose-H\textsuperscript{+} symport) and is driven by the proton motive force (PMF). Excretion of glucose takes place only if hexokinase activity is not induced. Once the maltose is depleted, the consumption of the excreted

### Table 1. Examples of lactic acid bacteria and yeasts isolated in sourdoughs

<table>
<thead>
<tr>
<th>Lactic acid bacteria</th>
<th>Yeasts</th>
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glucose begins. The glucose excreted during sourdough fermentation may be used by maltose-negative yeasts such as S. exiguus or may prevent competitors from using maltose by glucose repression, thereby giving an ecological advantage to Lb. sanfranciscensis. The frequent disappearance of S. cerevisiae from the microbial population of sourdough during consecutive fermentations is also related to the repression of genes involved in maltose fermentation [31]. In addition, some strains of S. cerevisiae are sensitive to the acetic acid produced by LAB, especially at the normal sourdough pH (4.0–4.5) which favours the undissociated lipophilic and membrane-diffusible form of the organic acid. At times, it is necessary to use large amounts of baker’s yeast to compensate for the poor survival of wild-type yeasts in consecutive sourdough fermentations [16]. In Lb. sanfranciscensis strains, the use of maltose is very effective and is not subject to glucose repression. Lb. sanfranciscensis, Lb. pontis, Lb. reuteri, and Lb. fermentum [10] are unique among the Lactobacillaceae in that they phosphorylate maltose and maltose phosphorylase may be considered to be a key enzyme for the lactobacilli growth during sourdough fermentation.

When Lb. plantarum is associated with yeasts (S. cerevisiae or S. exiguus) in the presence of sucrose as carbon source [18], cell yield and lactic acid production increase. The hydrolysis of sucrose by yeasts liberates glucose and fructose which are then more rapidly depleted than the sucrose by LAB [18, 32]. Yeasts hydrolyse sucrose about 200 times faster than the released hexoses are fermented [12], causing the rapid disappearance of sucrose during sourdough fermentations [33].

Metabolism of nitrogen compounds

Amino acids accumulate during wheat sourdough fermentation [34]. Peptides and amino acids play an essential role as important flavour precursors of baked sourdough products [35] and interfere in the physical properties of the dough [1, 36]. Proteolysis by flour enzymes [37], spontaneous microflora and, especially, by various LAB strains [36, 37] has been shown. Amino acids are also derived from the cell mass of microorganisms [38, 39] and, to a lesser extent, from other metabolisms [40]. Mixing ingredients (flour, water, salt and microbial mass) induces lytic degradation of the microbial cells when incorporated into the dough. The microbial biomass releases varying amounts of specific amino acids: S. cerevisiae mainly releases γ-aminobutyric acid, proline, valine, isoleucine, glycine and alanine and peptides in sourdoughs [1], while LAB mainly release glycine and alanine. In co-culture model systems [41] with an adequate supply of carbon source and vitamins, the stimulation of the Lb. sanfranciscensis and Lb. plantarum growth by S. cerevisiae and S. exiguus has been related to both the lack of competition for the nitrogen source (in a mixture of NH4Cl and amino acids, yeasts preferentially use NH4+) and to the yeast excretion of specific amino acids and small peptides, either during growth [42, 43] or as a consequence of an accelerated autolysis [44]. It seems that glucose uptake induces an outflow of amino acids from the yeast cells, by affecting a change in the permeability of the plasma membrane. Studies on the interactions between LAB and yeasts in grape must have shown a stimulated bacterial growth caused by vitamins, amino acids and peptides released by yeasts as a consequence of an accelerated lysis caused by bacterial enzymes [45]. The liberation of amino acids by yeasts made the growth of Lb. sanfranciscensis possible even in a medium initially deficient in essential amino acids (valine and isoleucine) [41]. An experiment conducted by Challinor and Rose in 1954 [46] showed the growth of Lactobacillus spp. in media lacking some essential amino acids when it was cultured with S. cerevisiae. Berg et al [47] identified a growth stimulant factor for Lb. sanfranciscensis as a small peptide (Asp-Cys-Glu-Gly-Lys) contained in freshly prepared yeast extractives, and commercial yeast, liver or protein hydrolysates are inadequate substrates [48]. The preference of bacteria for peptide-bound amino acids rather than free amino acids is relevant; direct transport of peptides into the cell prior to hydrolysis reduces the metabolic energy used for amino acid uptake.

Yeast generally lead to a depletion of amino acids [37, 49]. However, it has been shown that an initially high cellular concentration (10^7 cfu/g) of a non-proteolytic yeast in the dough, resulted, during fermentation, in a greater amount of free amino acids which satisfied the requirements of LAB [39]. The efficient utilization of specific amino acids and/or peptides generated by yeasts provides a competitive advantage and contributes to the stability of LAB in sourdough.

The use of Lb. sanfranciscensis and Lb. plantarum during sourdough fermentation caused a considerable increase in the total concentration of free amino acids. When compared with the unstarted sourdough, LAB proteolysis increases the concentration of aliphatic, dicarboxylic and hydroxy amino acid groups which, for the most part, are stimulatory for bacterial growth and are used by yeasts [39]. The peptide hydrolase and proteinase systems of Lb. sanfranciscensis have been characterized and their subcellular localization [50]. Compared with the activities of other sourdough LAB, Lb. sanfranciscensis strains show the highest amionopeptidase, dipeptidase, tripeptidase and iminopeptidase activities. A 58 kDa cell-envelope serine-proteinase from Lb. sanfranciscensis CB1 has been purified and characterized [51]. When compared with the activity of the cell-envelope proteinase from Lb. delbrueckii subsp. bulgarius B397 and with the PIII proteinase from Lactococcus lactis subsp. lactis SK11, it is characterized by lower activity on αs1- and β-caseins and by a higher
capacity to produce peptides from the gliadin. A higher substrate specificity for vegetable proteins than for caseins and a great adaptability to the sourdough environment have been assumed.

Production of CO₂ and other volatile compounds

Even though yeast cell concentrations and the type of yeast are the major parameters determining gas production rates [52], LAB have an influence on yeast leavening and CO₂ production (Fig. 1) [53]. In addition, LAB alone may make a major contribution to leavening in rye sourdough [31, 54]. Analyses conducted by using a rheofermentometer showed that the production of CO₂ by other yeasts (e.g. *S. exigua*) is not comparable to the high gassing power of *S. cerevisiae*. Compared with *S. cerevisiae* alone, the associative growth of *S. cerevisiae* and *Lb. sanfranciscensis* decreased to one third the time necessary to reach the maximum production of CO₂ by the yeast. An increase in total CO₂ was also observed when associated with *S. exigua*. When *Lb. plantarum* was associated with *S. cerevisiae*, it caused an increase in CO₂ produced and improved the capacity of the dough to retain the gas. Some LAB (e.g. *Lb. plantarum* and *Lb. brevis*, or their combinations) were also shown to increase the volume of sourdough breads started with *S. cerevisiae* [55]. The lactic acid, mainly produced by facultatively heterofermentative LAB such as *Lb. plantarum*, is responsible for a more elastic gluten structure. The addition (6 g/kg) of fructose to the sourdough produced by *S. cerevisiae* and *Lb. sanfranciscensis* increased acetic acid production and the LAB cell number, decreased ethanol production, slightly affected CO₂ production and doubled the gas production rate (Fig. 1).

The flavour of leavened baked goods is influenced by the raw materials [56], sourdough fermentation [57, 58], proofing, baking and by the type of starters [59]. Even though the greatest amount of aroma substances is formed during baking [3], sourdough fermentation is essential for achieving an acceptable flavour, since chem-

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**Fig. 1.** Rheofermentometer gas release curves of sourdoughs produced with: (A) *S. cerevisiae* 141; (B) *Lb. brevis* subsp. *lindneri* CB1; (C) *S. cerevisiae* 141 and *Lb. brevis* subsp. *lindneri* CB1; (D) *S. cerevisiae* 141 and *Lb. plantarum* DC400 and (E) *S. cerevisiae* 141 and *Lb. brevis* subsp. *lindneri* CB1 in the presence of fructose (6 g/kg). The area delimited by the upper line of the gas release curve corresponds to the total volume of CO₂ (ml) produced; the area delimited by the lower line of the gas release curve corresponds to the total volume of CO₂ (ml) retained in the sourdough; *T₁* indicates the time (min) to reach the maximum curve height; *T₂* the time (min) when the porosity of the dough develops [53].
ically acidified breads failed in sensory quality [60]. The importance of the individual starters used and of the microbial interactions on the production of volatile compounds has been carefully considered [61]. A statistical discrimination based on the volatiles produced showed that each type of metabolism (hetero-, homo-lactic and alcoholic) which takes place in sourdough fermentation is defined by typical compounds (Fig. 2) [62]. The differentiation is mainly related to 2-methyl-1-propanol and 2,3-methyl-1-butanol (main products of the yeast fermentation), diacetyl (mainly produced with other carbonyls by homofermentative LAB), and ethylacetate (mainly produced with some alcohols and carbonyls by heterofermentative LAB). Loaves made with the addition of *Lb. plantarum* or *Lb. sanfranciscensis* had a higher content of 2,3-methyl-1-butanol; with the association of LAB and yeasts, the bread attained a higher flavour quality, which may be caused by a higher content of 2,3-methyl-1-butanol, 2-methyl-propanoic acid, 3-methyl-butyric acid and 2-phenyl-ethanol [63]. The microbial interactions also affect volatile synthesis (Table 2). While sourdoughs started with the association of *Lb. sanfranciscensis* and other homo- or hetero-fermentative LAB and/or *S. exiguus* are characterized by a balanced profile, the sourdoughs produced with the *Lb. sanfranciscensis*–*S. cerevisiae* mixture had higher concentrations of the yeast fermentation products (1-propanol, 2-methyl-1-propanol and 3-methyl-1-butanol) and fewer bacterial compounds [62, 64]. An activation of the yeast metabolism in the presence of the homofermentative LAB was demonstrated by Hansen *et al.* [59], but the same effect, probably due to the combination of bacterial acidification and proteolysis [39, 65], may be attributed to *Lb. sanfranciscensis*. Hansen and Hansen [61] used the association of *Lb. sanfranciscensis*, *Lb. plantarum* and *S. cerevisiae* to guarantee an equilibrated aroma in wheat sourdough breads.

**Self-protection of the sourdough**

Spicher and Mastik [66] first reported inhibition of the typical bacterial flora of flour by sourdough LAB cultures. Homofermentative LAB have greater inhibitory effects against coliforms than heterofermentative LAB [6]. Gänzle *et al.* [67] purified inhibition compound from *Lb. reuteri* LTH 2584. The inhibitory spectrum differed markedly from bacteriocins of meat-associated lactobacilli and included cereal-associated microorganisms as well as food pathogens or spoilage organisms such as *B. subtilis*. A screening among different strains of *Lb. sanfranciscensis* showed inhibition of *Bacillus subtilis* but not against sourdough yeasts [68]. A bacteriocin-like inhibitory substance (BLIS C57) which is heat-stable (100°C for 20 min), insensitive to lipase and α-amylase, of a protein nature, with an inhibitory spec-

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**Table 2. Quantity of volatiles in sourdoughs produced by associated starters. The amount of volatiles is expressed as relative peak area = (peak area of compound/total area)×100. Adapted from [62]**

<table>
<thead>
<tr>
<th>Starters</th>
<th>Alcohols</th>
<th>Carbonyls</th>
<th>Ethylacetate</th>
<th>Others*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lb. plantarum</em> DC400-1b. lacticus CC1</td>
<td>4.8 a (16.7–11.7) b</td>
<td>34.5 a (68.4–27.3) b</td>
<td>56.7 a (10.1–58.1) b</td>
<td>4.0 (4.8–4.9)</td>
</tr>
<tr>
<td><em>Lb. sanfrancisco</em> CB1-1b. fructivorans DD1</td>
<td>12.0 a (23.6–10.0) c</td>
<td>24.9 a (15.9–9.5) c</td>
<td>59.8 ab (56.0–76.0) c</td>
<td>3.3 (6.5–4.5)</td>
</tr>
<tr>
<td><em>S. cerevisiae</em> 141-S. exiguus M14</td>
<td>49.4 d (39.1–32.7) e</td>
<td>18.1 c (23.1–16.0) d</td>
<td>36.9 d (25.8–47.6) ab</td>
<td>1.6 (6.0–3.7)</td>
</tr>
<tr>
<td><em>Lb. sanfrancisco</em> CB1-1b. plantarum DC400</td>
<td>12.1 af</td>
<td>30.8 c</td>
<td>52.4 ab</td>
<td>4.7 af</td>
</tr>
<tr>
<td><em>Lb. sanfrancisco</em> CB1-S. cerevisiae 141</td>
<td>81.1 e</td>
<td>5.4 e</td>
<td>12.6 e</td>
<td>0.9 af</td>
</tr>
<tr>
<td><em>Lb. sanfrancisco</em> CB1-S. exiguus M14</td>
<td>75.6 e</td>
<td>11.4 e</td>
<td>11.9 e</td>
<td>1.1 af</td>
</tr>
<tr>
<td><em>Lb. plantarum</em> DC400-S. cerevisiae 141</td>
<td>71.3 e</td>
<td>16.7 ef</td>
<td>11.9 e</td>
<td>0.1 af</td>
</tr>
<tr>
<td><em>Lb. plantarum</em> DC400-S. exiguus M14</td>
<td>18.1 f</td>
<td>40.3 d</td>
<td>40.7 d</td>
<td>0.9 af</td>
</tr>
</tbody>
</table>

The number in parentheses indicates the quantity of volatiles produced by the individual strains. Values in the same column with different letters differ significantly (P < 0.05). Letters on the right of the parentheses refer to the sum of values in parenthesis.

*The values indicate in the column are not significantly different (P < 0.05).*

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**Fig. 2.** Distribution of hetero- (Δ), homofermentative lactic acid bacteria (□) and yeast strains (○) in the plain formed by the two functions of the linear discriminant analysis based on the determination of 30 volatile compounds during sourdough fermentation with individual strains. Solid symbols mean the centroid of the group [62].

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trum centred about LAB, with a bactericidal or bacteriolytic mode of action and with a chromosomally-located encoding gene was isolated from *Lactobacillus sanfranciscensis* C57. Except for the *Lb. fructivorans* strains, all the other strains from sourdough are inhibited by BLIS C57. *Listeria monocytogenes* is also sensitive. A mixture of acetic, caproic, formic, propionic, butyric and n-valeric acids, acting in a synergistic way, was responsible for the antimould activity of *Lb. sanfranciscensis* CB1 [69]. It inhibited moulds related to bread spoilage such as *Fusarium*, *Penicillium*, *Aspergillus*, and *Monilia*, and caproic acid played a key role in inhibiting fungi growth (Fig. 3). Inhibition of some strains of *Candida* sp. but not *Saccharomyces* sp. has been shown. The production of antibacterial and antimould substances by LAB and, especially, by *Lb. sanfranciscensis* may be related to its predominance and may contribute to the stability of sourdough products also protecting insensitive yeasts. Like other natural systems (e.g. kehr grains) in which associations of LAB and yeasts are used in food fermentation, sourdough appears to be self-protecting and self-regulating. Many of these microbial systems, when correctly used, maintain a remarkably constant composition.

References


Fig. 3. Inhibition of *Fusarium graminearum* 623 by different mixtures of organic acids (total concentration 16 mM) produced by *Lactobacillus sanfrancisco* CB1. Wells contained the mixture of acetic, caproic, formic, propionic, butyric, and n-valeric acids (well 1); the mixture without acetic (well 2), caproic (well 3), formic (well 4), butyric (well 5) and propionic (well 6) acids, respectively [69].


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