Lactobacillus sanfrancisco a key sourdough lactic acid bacterium: a review

M. Gobbetti* and A. Corsetti

Sourdough may be considered a traditional product with a great future. Studies on the sourdough microflora have recently been improved. In this paper, research on Lactobacillus sanfrancisco, a key sourdough lactic acid bacterium, is reviewed. The physiology, trophic relationships with sourdough related organisms and the genetics are considered in order to explain and improve the biotechnological performances of Lb. sanfrancisco.

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Introduction

Lactobacillus sanfrancisco, named after the city from where the sourdough microorganism was first isolated, is an obligatory heterofermentative Lactobacillus with phylogenetic relationship to the Lactobacillus casei-Pediococcus group (Hammes and Vogel 1995). It was first isolated by Kline and Sugihara (1971) and subsequently revised by Weiss and Schillinger (1984) for inclusion in Approved List of Bacterial Names.

Characteristics of Lb. sanfrancisco are a 36–38 mol% G+C of the DNA (Kandler and Weiss 1986), a Lys-Ala type murein without teichoic acid in the cell wall (Weiss and Schillinger 1984) and one to three large mesosomes at the site of the membrane-wall interface (Nelson et al. 1971). Different strains harbour varying numbers and sizes of plasmids (Lønner et al. 1990, Gobbetti et al. 1996a). DNA–DNA hybridization showed that Lb. sanfrancisco is synonymous with Lactobacillus brevis subsp. lindneri (Spicher and Schroder 1978).

Lb. sanfrancisco growth is very fastidious: it requires fresh yeast extractives, unsaturated fatty acids (mainly oleic acid) (Sriranganathan et al. 1973) and it preferentially ferments maltose rather than glucose. Occasionally, strains that ferment sucrose, ribose, gluconate, galactose, raffinose or fructose have been identified (Gobbetti et al. 1994a, Onno and Roussel 1994, Vogel et al. 1994, Hammes and Vogel 1995).

Lb. sanfrancisco has been widely isolated from rye and wheat sourdoughs of several bread-producing areas and from sourdoughs used to make Panettone (Table 1). Traditional productions by various stages of continuous propagation and sourdoughs produced by commercial starter cultures are effective in reducing the growth of fortuitous bacteria and permit a predominance of Lb. sanfrancisco which, with very few exceptions (Martínez-Anaya et al. 1990), is considered to be a key lactic acid bacteria (LAB) in the biotechnology of baked sourdough products.
**Lactobacillus sanfrancisco and its interactions with sourdough yeasts**

The sourdough ecosystem may be defined as a strict microbial consortium and an understanding of the interactions that occur between *Lb. sanfrancisco* and yeasts is fundamental for evaluating the real role of this bacterium. The association between *Lb. sanfrancisco* and *Saccharomyces exiguus* is typical in the production of San Francisco french bread (Sugihara et al. 1970) and Panettone (Gobbetti et al. 1994a), and the association between *Lb. sanfrancisco* and *Saccharomyces cerevisiae* has been studied in sourdoughs from different countries (Spicher et al. 1982, Wlodarczyk 1985), in gluten-free baked goods (Wlodarczyk et al. 1993) and has been found to be optimal in a continuously operating sourdough fermentation system (Vollmar and Meuser 1992).

The trophic relationships that occur between *Lb. sanfrancisco* and the prevailing sourdough yeasts (*S. cerevisiae* and *S. exiguus*) have been studied in both co-cultural model systems and during sourdough fermentation. Stimulation of *Lb. sanfrancisco* growth has been related to an increased availability of specific amino acids and peptides excreted by yeasts. In alcoholic beverages, it has been shown that *S. cerevisiae* may release these nitrogen compounds either during growth (Thorne 1957, Suomalainen and Oura 1971) or as a consequence of an accelerated autolysis (Lyons and Rose 1977). The liberation of amino acids by yeasts has made possible the growth of *Lb. sanfrancisco* even in a medium initially deficient of essential amino acids (valine and isoleucine) (Gobbetti et al. 1994b). Berg et al. (1981) have identified a growth stimulant factor for *Lb. sanfrancisco* in a small peptide (Asp±Cys±Glu±Gly±Lys) contained in the freshly-prepared yeast extracts and commercial yeast, liver or protein hydrolysates are inadequate substitutes (Sugihara and Kline-Wlodarczyk 1985), in gluten-free baked goods (Wlodarczyk et al. 1993) and has been found to be optimal in a continuously operating sourdough fermentation system (Vollmar and Meuser 1992).

The consumption of soluble carbohydrate and the energy yield of *Lb. sanfrancisco* are greatly influenced by the associated yeasts.

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**Table 1.** Source of isolation of *Lactobacillus sanfrancisco* (syn. *Lb. brevis* subsp. *lindneri*).

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<thead>
<tr>
<th>References</th>
<th>Origin</th>
<th>Source of isolation</th>
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<tr>
<td>Spicher 1959</td>
<td>Germany</td>
<td>Sour rye starters</td>
</tr>
<tr>
<td>Kline and Sugihara 1971</td>
<td>California (USA)</td>
<td>Wheat sourdough</td>
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<tr>
<td>Spicher and Schröder 1978</td>
<td>Germany</td>
<td>Pure culture sourdough starters</td>
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<tr>
<td>Wlodarczyk 1985</td>
<td>Poland</td>
<td>Bread starters</td>
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<tr>
<td>Nout and Creemers-Molenaar 1986</td>
<td>Netherlands</td>
<td>Wheatmeal sourdough starters</td>
</tr>
<tr>
<td>Spicher 1987</td>
<td>Italy, Switzerland, Germany</td>
<td>Panettone and wheat bread sourdough</td>
</tr>
<tr>
<td>Galli et al. 1988</td>
<td>Switzerland, Germany</td>
<td>Italian</td>
</tr>
<tr>
<td>Hansen et al. 1989a</td>
<td>Sweden</td>
<td>Panettone, brioches, wheat and rye bread sourdough</td>
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<td>Lönnner et al. 1990</td>
<td>Denmark</td>
<td>Rye sourdoughs</td>
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<td>Lonner et al. 1990</td>
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<td>Hammes 1990</td>
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<td>Commercial starter culture</td>
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<td>Okada et al. 1992</td>
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<td>Purely cultured sourdough</td>
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<td>Hochstrasser et al. 1993</td>
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<td>Rye sourdough sponge</td>
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<td>Wheat sourdough</td>
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<td>Gobbetti et al. 1994a</td>
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<td>Faid et al. 1994</td>
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<tr>
<td>Ganzle et al. 1995</td>
<td>Germany</td>
<td>Sourdough</td>
</tr>
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and vary according to the type of sugars. The lack of competition between Lb. sanfrancisco and S. exiguus for maltose is fundamental for the stability of this association (Sugihara et al. 1970). S. exiguus preferentially uses sucrose or glucose and has a high tolerance to the acetic acid produced by heterofermentative metabolism (Suihko and Makinen 1984). Due to the faster consumption of maltose, and especially glucose, by S. cerevisiae, a decrease in the bacterial metabolism has been observed when associated with Lb. sanfrancisco in a synthetic medium (Gobbetti et al. 1994c). A stimulation of the bacterial growth in the co-cultures has been only observed using wheat flour extract supplied with 2-5 g l⁻¹ of various soluble carbohydrates. An imbalance between yeast consumption and starch hydrolysis by the flour enzymes leads to a rapid depletion of some soluble carbohydrates during sourdough fermentation (Gobbetti et al. 1994d). However, the maltose concentration may frequently remain between 2-5 g kg⁻¹ in wheat doughs fermented by yeasts and LAB (Martinez-Anaya et al. 1993). Barber et al. (1991) have shown an accumulation of maltose because its metabolism by some yeasts may not begin until the available glucose and fructose are depleted. Nevertheless, the microbial competition for maltose and glucose represents a critical point in wheat flours with very low concentrations of soluble carbohydrates. The addition of selected carbon sources to the dough has been proposed in order to enhance the production of lactic and acetic acids by Lb. sanfrancisco (Corsetti et al. 1994).

Lb. sanfrancisco hydrolyzes maltose and accumulates glucose in the phosphate buffer (Fig. 1) in a molar ratio of about 1:1 (Stolz et al. 1993, Gobbetti et al. 1994c). The glucose-1-phosphate produced by maltose phosphorylase is further metabolized, whereas glucose is not utilized but excreted outside the cell in order to avoid excessive intracellular concentrations. Excretion of glucose takes place in concomitance with maltose availability, and after maltose has been depleted the consumption of the excreted glucose begins. The glucose excreted during sourdough fermentation may be utilized by maltose-negative yeasts or better may prevent competitors from utilizing abundant maltose by glucose repression thereby giving an ecological advantage to Lb. sanfrancisco. The disappearance of S. cerevisiae from the microbial population of sourdough during consecutive fermentations has been related to the repression of genes involved in maltose fermentation (Nout and Creemers-Molenaar 1986). In Lb. sanfrancisco strains, maltose utilization is very effective and is not subject to glucose repression. The maltose uptake and glucose excretion in Lb. sanfrancisco has been analyzed by Neubauer et al. (1994). Maltose transport occurs by a secondary transport system (maltose-H⁺ symport) and is driven by the proton motive force (PMF). The maltose carrier protein(s) is constitutively expressed, and is highly dependent on pH with an optimum at 5.6-5.2 which matches with the pH of sourdough. The glucose efflux in maltose-grown cells is catalyzed by a transport system which is induced during growth on maltose, and which can mediate homologous glucose-glucose exchange but not a maltose-glucose exchange, thus indicating a maltose-inducible glucose uniport system which is responsible for the excretion of the excess glucose. Lb. sanfrancisco, Lactobacillus reuteri, and Lactobacillus fermentum (Vogel et al. 1994) are unique among the Lactobacillaceae in that they phosphorylize maltose, and maltose phosphorylase may be considered to be a key enzyme for the predominance of lactobacilli during sourdough fermentation.

Although sucrose fermentation strains of Lb. sanfrancisco have been isolated, the invertase activity of yeasts liberates fermentable glucose and enables a partial growth of sucrose- and fructose-negative strains, which leads to a metabolic mutualism in sourdough fermentation (Gobbetti et al. 1994c).

Lb. sanfrancisco has a positive influence on yeast leavening and CO₂ production (Gobbetti et al. 1995a). Even though the inoculum size of the baker's yeast is the major parameter in determining the gas production rates (Akdogan and Ozilgen 1992), analyses conducted by using a rheofermentometer have shown that the associative growth of S. cerevisiae and Lb. sanfrancisco...
decreased at one third the time necessary to reach the maximum production of CO₂ by the yeast. An increase in the total CO₂ has also been observed when associated with S. exigus M14.

**Co-metabolisms in *Lb. sanfrancisco***

Co-fermentations enable micro-organisms to use substrates that are otherwise non-fermentable, and increase the microbial adaptability to difficult ecosystems. Under the influence of several ecological factors the homo- and heterofermentative LAB have a great aptitude for producing metabolites other than lactic acid (Ceslovszky et al. 1992) and for co-fermentations (Romano et al. 1987, Cunha and Foster 1992) which lead to an increase of the energy yield.

A fructose-negative strain of *Lb. sanfrancisco* has been shown to co-ferment fructose when it is in the presence of maltose or glucose (Gobbetti et al. 1995b). About two moles of fructose are consumed for each mole of maltose. In comparison with the growth with maltose alone, the main variations which occur in co-fermentation are: an increase in the cell yield correlated with the additional amount of ATP generated by the metabolism of fructose, increases in production of lactic acid and especially of acetic acid, synthesis of mannitol and a decrease in ethanol amount. *Lb. sanfrancisco* uses maltose as an energy source and fructose as an additional electron acceptor, thereby obtaining an optimal growth rate (Axelsson 1993). The action of fructose as electron acceptor occurs via its reduction to mannitol accompanied by a decrease in the level of ethanol, which is normally produced from the degradation of maltose (Fig. 1). This enables *Lb. sanfrancisco* to produce ATP through the acetate kinase reaction, and in parallel, to synthesize a higher level of acetic acid. A stoichiometric acetate production (two moles of fructose depleted per mole of acetate formed) by *Lb. sanfrancisco* has been first reported by Stolz et al. (1995a). As shown for other co-fermentations (Dills et al. 1980, Romano et al. 1987), maltose crosses the membrane by PMF and generates ATP by the hexose monophosphate pathway. The ATP produced energizes the transport of fructose which may enter the cell through facilitated diffusion (uniport) by the same or modified maltose permease (Dills et al. 1980). As a consequence of the maltose-fructose mediated co-fermentation, the addition of fructose to the wheat flour induces the utilization of fructose by *Lb. sanfrancisco* CB1 during fermentation. Compared with the sourdough without fructose added, a greater amount of acetic acid is produced, as well as a lower fermentation quotient, which drops into the optimal range of 1.5–4.0 (Spicher 1983). By providing fructose, Rocken et al. (1992) increased the acetic acid production of *Lactobacillus brevis* (fructose-positive strain) to the high levels that could compensate the subsequent loss of acetic acid during the freeze drying of the sourdough. Similar changes in the sugar metabolism of obligate heterofermentative lactobacilli may also be induced by aeration (Ng 1972, Condon 1987). Indeed, the switch from one branch to the other may be governed by the presence of oxygen or other suitable electron acceptors, such as fructose, citrate and malate (Kandler 1983, Condon 1987, Stolz et al. 1993). The influence of the electron acceptors on the metabolism of *Lb. sanfrancisco* has been shown in depth by Stolz et al. (1995a) (Fig. 1). However, while the improvement in the acetic acid concentration by controlled aeration may be a suitable method in well-equipped laboratories, it cannot be done in normal bakeries.

A co-metabolism of maltose or glucose and citrate has also been shown in *Lb. sanfrancisco* citrate-negative strains (Gobbetti and Corsetti 1996). The production of succinic acid by the partial dissimilation of citrate through the succinic pathway (Radler 1975, Lutgens and Gottschalk 1980) and a biphasic impedance curve have been typical of this co-metabolism. During growth of *Lb. sanfrancisco* in a medium containing both maltose and glucose or other electron acceptors, the changes in the redox potential were consistent with the formation of acetate or ethanol in addition to lactate and with a biphasic growth not related to the sequential utilization of the various carbon sources (Stolz et al. 1993, 1995a). Since pyruvate is also an
Figure 1. Metabolic key reactions of Lactobacillus sanfrancisco: metabolism of maltose and fate of potential electron acceptors upon maltose fermentation. The enzymes involved are given in the following list: (1) maltosephosphorylase; (2) phosphoglucomutase; (3) hexokinase; (4–8) enzymes of the phosphogluconate pathway; (8) phosphoketolase; (9–14) enzymes of the Embden–Meyerhof glycolytic pathway; (15) phosphotransacetylase; (16) alcohol dehydrogenase; (17) acetate kinase; (18) mannitol dehydrogenase; (19) malolactic enzyme; (20) citrate lyase; (21) oxaloacetate-decarboxylase; (22) lactate dehydrogenase; (23) NADH–H₂O₂ oxidase; (24) NADH-peroxidase; (25) glucose phosphate isomerase; (26) erythritol dehydrogenase and erythrose-4-P-phosphotransferase. Reproduced by permission of Stolz et al. (1995b).
intermediate of the citrate metabolism of LAB (Cogan 1987), in cells co-metabolizing citrate, pyruvate coming from two pathways is used to reoxidize NADH/NADPH (Fig. 1). This leads the cell to save acetaldehyde from reduction to ethanol and the precursor acetylphosphate is diverted to acetic acid, the production of acetic acid being greater than can be accounted for in terms of maltose fermentation. A sourdough started with Lb. sanfrancisco CB1 and with an addition of citrate to the dough was characterized by an appropriate quotient of fermentation.

Proteolytic activity of Lb. sanfrancisco

Accumulation of amino acids during sourdough fermentation has been determined (Collar et al. 1991). Although some authors have shown a proteolysis by flour enzymes (Kratchovil and Holas 1984, 1988, Spicher and Nierle 1988), the increase of the amino acid content has been related to the spontaneous microflora, and especially, to the presence of various LAB strains (Spicher and Nierle 1984, 1988, Collar and Martinez 1993, Mascaros et al. 1994). To a lesser extent, amino acids may also be derived from other metabolic routes (Okuara and Harada 1971) and from the cell mass of micro-organisms (Rothenbuehler et al. 1982, Gobbetti et al. 1994e). The sourdough microflora requirements (Spicher and Nierle 1988, Collar et al. 1991), the environmental factors (Collar and Martinez 1993, Mascaros et al. 1994) and yeasts which, as a balance between release and consumption, lead to a depletion (Collar and Martinez 1993) are the main parameters which influence the kinetics of amino acids. Peptides and amino acids also play an essential role as important flavour precursors of the baked sourdough products (Spicher 1983).

The use of Lb. sanfrancisco in sourdough fermentation has been related to a considerable increase of the total concentration of free amino acids (Spicher and Nierle 1984, Gobbetti et al. 1994e). When compared with the unstarted sourdough, the sourdough fermented by Lb. sanfrancisco showed increases in the concentration of aliphatic, dicarboxylic and hydroxy amino acid groups which, for the most part, are stimulatory for the bacterial growth (Spicher and Nierle 1984, Gobbetti et al. 1994e). The synthesis of D-alanine and D-glutamic acid isomers has also been observed by Lb. sanfrancisco and other sourdough starters. Cellular excretion of D-forms or enzymatic isomerization of L-amino acids may explain the biotic generation (Gobbetti et al. 1994e).

The peptide hydrolase and proteinase systems of Lb. sanfrancisco have been characterized and their subcellular distribution localized (Gobbetti et al. 1996b). Compared with the activities of other sourdough LAB, Lb. sanfrancisco strains show the highest aminopeptidase, dipeptidase, tripeptidase and iminopeptidase activities. Most peptidases are contained in the cytoplasm except for dipeptidase which is located in the membrane, and di- and tri-peptidase have a higher overall specific activity than the other peptidases. Because wheat flour contains considerable amounts of low molecular weight peptide (Quaglia 1984), the di- and tri-peptidases may be more important than other proteolytic activities for the predominance of LAB in the sourdough. Although iminopeptidase activity is limited when compared with those from strong proteolytic bacteria, it may represent a pre-requisite due to the high content of proline residues in the gluten (Quaglia 1984). Endopeptidase and proteinase activities have been shown to be pronounced and distributed in the cytoplasm and cell wall–envelope of Lb. sanfrancisco, respectively.

A cell-envelope 58 kDa proteinase, a cytoplasmic 67 kDa dipeptidase and 75 kDa aminopeptidase from Lb. sanfrancisco CB1 have been purified to homogeneity and characterized (Table 2) (Gobbetti et al. 1996c). The proteinase is a serine enzyme most active at pH 7.0 and 40°C which, when compared with the activity of the cell-envelope proteinase from Lactobacillus delbrueckii subsp. bulgaricus B397 and with the P11 proteinase from Lactococcus lactis subsp. lactis SK11, 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. Both dipeptidase and aminopeptidase have optimum activity at pH 7-5 and 35–30°C, respectively. The dipeptidase is a metalloenzyme which shows high affinity for peptides containing hydrophobic amino acids but has no activity on tri- or larger peptides showing several features in common with another dipeptidase isolated from Lb. delbrueckii subsp. bulgaricus B14 (Wohlrab and Bockelmann 1992). After glutamic acid and proline, the hydrophobic amino acids are the major amino acid residues contained in the gluten of wheat flour (Carnovale and Miuccio 1981). Aminopeptidase is also inhibited by metal-chelating agents, shows a broad N-terminal hydrolytic activity including di- and tri-peptides and, according to the classification proposed by Tan et al. (1993), has been defined as an aminopeptidase type N. All the characterized enzymes from Lb. sanfrancisco CB1 maintain relatively high activity at the pH (4.0–5.0) and temperature (30–35°C) of sourdough fermentation.

### Acidification rates and production of volatile compounds by Lb. sanfrancisco

Heterolactic metabolism by means of fermentation quotient mainly influences the flavour of the various leavened baked products (Spicher 1983, Lonner and Preve-Åkesson 1989). Acetic acid is assumed to act as a flavour enhancer and, together with lactic acid, is a catalyst during the Maillard type-reaction (Seibel and Brummer 1991). Minor acids (propionic, isobutyric, butyric, α-methyl n-butyric, isovaleric, and valeric acids) generated by the activity of microbial enzymes also contributed to the titratable acidity and flavour (Galal et al. 1978). Lonner and Preve-Åkesson (1988) studying the properties of LAB in sourdough have found that Lb. sanfrancisco is the heterofermentative bacteria with the best properties.

A characterization of sourdough LAB based on nine parameters of acidification rates was done (Gobbetti et al. 1995c, d). Lb. sanfrancisco strains isolated from sourdoughs are characterized by the highest variability among the heterofermentative species and differ markedly from the collection cultures. They are characterized by a rather long latency phase, high maximum-acidification rate and by tolerance to acidity. These pre-requisites lead to an elevated production of lactic and acetic acids (3.48–3.70 g kg⁻¹ and 0.38–0.44 g kg⁻¹, respectively). Acetic acid in heterofermentative cultures accounts for about 16–29% by weight of the total lactic acid (Lund et al. 1989) but this percentage varies according to the firmness of the dough and the fermentation temperature (Salovaara and Valjakka 1987, Onno and Roussel 1994). The screening of Lb. sanfrancisco cultures showed strains which differed completely due to their fastest acidification. The total lactic acid produced by Lb. sanfrancisco approaches those of a weak homofermentative strain such as Lactobacillus alimentarius (Gobbetti et al. 1995d).

The flavour of leavened baked goods is influenced by the raw materials (McWilliams and McKey 1969, Hansen and Hansen 1994a), sourdough fermentation (Hansen et al. 1989a, Lund et al. 1989), proofing, baking

### Table 2. Characteristics of the purified proteinase, dipeptidase and aminopeptidase from Lactobacillus sanfrancisco CB1

<table>
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<th>Dipeptidase</th>
<th>Aminopeptidase</th>
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<tr>
<td>Molecular mass</td>
<td>58 kDa</td>
<td>67 kDa</td>
<td>75 kDa</td>
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<tr>
<td>pH optimum</td>
<td>7.0</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Relative activity</td>
<td>higher than 70%</td>
<td>higher than 70%</td>
<td>higher than 70%</td>
</tr>
<tr>
<td>pH 4.0–5.0</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Temperature optimum</td>
<td>40°C</td>
<td>35°C</td>
<td>30°C</td>
</tr>
<tr>
<td>Substrate specificity</td>
<td>Gliadin</td>
<td>Leu-Leu</td>
<td>Leu-pNA</td>
</tr>
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and by the type of starters (Hansen et al. 1989b). The volatile flavour compounds of the wheat bread crust have been identified by Schieberle and Grosch (1985) which also determined the differences between the bread crumb and crust and the variations of these potent odorants during storage of wheat bread (Schieberle and Grosch 1992, 1994). Even though the greatest amounts of aroma substances are formed during baking (Spicher 1983), sourdough fermentation is essential for achieving an acceptable flavour because chemically acidified breads failed in sensory quality (Rothe and Ruttillof 1983). The importance of the individual starters used and of the microbial interactions on the production of volatile compounds has been carefully considered (Hansen and Hansen 1994b).

Sourdoughs fermented with the heterofermentative lactobacilli had a higher titratable acidity, higher acetic acid content, lower yeast count and lower content of yeast fermentation products than those fermented with homofermentative lactobacilli (Hansen et al. 1989a). While ethanol and ethylacetate were produced in the highest amounts in sourdoughs fermented with Lb. sanfrancisco, ethyl-n-propanoate, butyl-acetate and n-pentyl acetate were only produced in the sourdoughs started with yeasts (Hansen and Hansen 1994b). Lb. sanfrancisco strains show a wide and homogeneous profile of volatiles which differ greatly from those of the other heterolactic species (Damiani et al. 1996). It may be defined as unique among the sourdough LAB and irreplaceable in sourdough production. Ethylacetate (less than with the other species), alcohols (ethanol, 1-propanol, 2-methyl-1-pentanol, 1-heptanol and 1-octanol), aldehydes (3-methyl-1-butanal, heptanal, trans-2-heptanal, octanal and nonanal) and acetic acid are the main compounds produced by Lb. sanfrancisco. Some of these compounds, and in particular 3-methyl-1-butanal and nonanal (Schieberle and Grosch 1994), have been considered as potent odorants in sourdough rye and wheat breads (Hansen et al. 1989b).

The microbial interactions also affect the volatile synthesis. While sourdoughs started with the association of Lb. sanfrancisco and other homo- or heterofermentative LAB and/or S. exigus are characterized by a balanced profile, the sourdoughs produced with the association Lb. sanfrancisco–S. cerevisiae 141 contained higher concentrations of the yeast fermentation products (1-propanol, 2-methyl-1-propanol and 3-methyl-1-butanol) and a lower amount of the bacterial compounds (Gobbetti et al. 1995e, Damiani et al. 1996). An activation of the yeast metabolism in the presence of the homofermentative LAB was demonstrated by Hansen et al. (1989b), but the same effect, probably due to the combination of bacterial acidification and proteolysis (Levesque 1991, Gobbetti et al. 1994e), may be attributed to Lb. sanfrancisco. Hansen and Hansen (1994b) used the association of Lb. sanfrancisco, Lactobacillus plantarum and S. cerevisiae to guarantee an equilibrated aroma in wheat sourdough breads.

**Antimicrobial activity and genetics of Lb. sanfrancisco**

Spicher and Mastik (1988) first reported inhibition of the typical bacterial flora of flour by sourdough LAB cultures. Homofermentative LAB have greater inhibitory effects than heterofermentative against coliforms (Barber and Baguena 1989). A mixed culture pre-ferment of lactic and propionic acid bacteria has been used in breadmaking in order to produce propionic acid for its antimicrobial properties (Javanainen and Linko 1993). Larsen et al. (1993) purified and characterized the bavarian A from Lactobacillus bavaricus M1401 isolated from sourdoughs and Ganzle et al. (1995) recently purified a bacteriocin from Lb. reuteri LTH 2584 which differed from the bactericins of meat-associated lactobacilli, such as sakacin P, or antibiotics, such as nisin. A screening among different strains of Lb. sanfrancisco has shown inhibition of Bacillus subtilis but not against sourdough yeasts and moulds (Corsetti et al. 1996). Two main groups of Lb. sanfrancisco strains were differentiated for their inhibitory spectrum. According to the classification proposed by Jack et al. (1995), a bacteriocin-like inhibitory substance (BLIS C57) which is heat-stable (100°C for 20 min), insensitive to
Lactobacillus sanfrancisco a key sourdough lactic acid bacterium

Lipase and α-amylase, of a protein nature, with an inhibitory spectrum centered about LAB, with a bactericidal or bacteriolytic mode of action and with a chromosomally located encoding gene has been isolated from Lb. sanfrancisco C57. With the exception of the Lactobacillus fructivorans strains, all the other strains from sourdough, as well as most of the dairy LAB strains, are inhibited by BLIS C57. Listeria monocytogenes is also sensitive. A narrower spectrum of inhibition has generally been observed from bacteriocins produced by lactobacilli (Klaenhammer 1993). The production of anti-bacterial substances by Lb. sanfrancisco may be related to its predominance and may contribute to the stability of sourdough products.

Basic knowledge about the genetics of sourdough LAB must increase because to date very few studies have been conducted. Lonner et al. (1990) have examined the plasmid contents of LAB isolated from different types of sourdoughs, including Lb. sanfrancisco, and compared them with the plasmid contents of culture collection strains. No plasmids or one plasmid of varying size (ca. 4·2–38 kbp) have been identified in the Lb. sanfrancisco strains from the collection, while strains isolated from sourdoughs have a high number of plasmids. Wild-type strains, other than the inoculated strains, gradually become dominant during sourdough fermentation. They possess a high number of plasmids which are assumed to be related to the carbohydrate fermentations. On the contrary, a ca. 17·0 kbp cryptic plasmid unrelated to maltose fermentation, bacteriocin production or to the proteolytic activity has constantly been identified (Gobbetti et al. 1995b, 1996b, Corsetti et al. 1996) in Lb. sanfrancisco strains.

A rapid method for reliable and simultaneous identification of different LAB in fermented food, including Lb. sanfrancisco, has been developed by Ehrmann et al. (1994). Sixteen-S and 23-S rRNA-targeted, species-specific oligonucleotides have been applied as capture probes in a non-radioactive reverse dot-blot hybridization.

pC194Amy, a construct containing an amylase encoding gene, has been introduced in Lb. sanfrancisco CB1 by electroporation and the Amy gene was expressed (Gobbetti et al. 1996a). Lb. sanfrancisco CB1 was also transformed with pGKV210, pNZ12 and pMG36e by electroporation. Amylase activity was extracellular and retained for at least 140 generations. The transformant degraded starch despite the presence of maltose, regardless of its concentration and had similar or slightly greater growth than the wild type on maltose. The expression of the α-amylase activity in Lb. sanfrancisco could potentiate the fermentation ability of this strain, reduce the competition between LAB and yeasts for the soluble carbohydrates of the flour and have a positive role in reducing staling of baked sourdough products.

Conclusions

Lb. sanfrancisco is physiologically well-adapted to the sourdough system (utilization of specific amino acids and peptides, presence of maltose phosphorylase, pronounced proteolytic activity and synthesis of antimicrobial compounds), physiologically related to the sourdough yeasts (very strict trophic relationships with yeasts are based on fundamental metabolisms) and biotechnologically indispensable for producing typical baked sourdough products (the acidification rate and the volatile compound productions differed greatly from the other sourdough LAB). However, its performances must still be improved by selection of strain or, better, of balanced starter associations, by conditioning the sourdough ecosystem (e.g. use of additional electron acceptors such as fructose or citrate) and probably, in the future, by using engineered strains.

Acknowledgements

The research was supported by the National Research Council of Italy, Special Project RAISA, Sub-project 4, Paper No. 3090.

References

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