Chapter 7
Physiology and Biochemistry of Lactic Acid Bacteria

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7.1 Introduction

In the past decades, studies on the physiology and biochemistry of sourdough lactic acid bacteria provided insight into the microbial ecology of sourdough as well as the effect of the metabolic activity of lactic acid bacteria on flavor, texture, shelf-life, and nutritional properties of leavened baked goods. Lactic acid bacteria are the dominant microorganisms of sourdough. Their metabolic versatility favors adaptation to the various processing conditions and the metabolic interactions with autochthonous yeasts determine mechanisms of proto-cooperation during sourdough fermentation [1–3]. *Lactobacillus* species are most frequently found in sourdough fermentations although species belonging to the genera *Pediococcus*, *Enterococcus*, *Lactococcus*, *Weissella* and *Leuconostoc* were also identified ([4–6], see Chap. 5). A large number of *Lactobacillus* species were first identified from sourdoughs or fermentation processes of cereals [5]. This chapter gives an overview of the general growth and stress parameters, carbohydrate and amino acid metabolism, synthesis of exopolysaccharides and antimicrobial compounds, and the conversion of phenolic compounds and lipids of lactic acid bacteria during sourdough fermentation.

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7.2 General Growth and Stress Parameters

The large diversity of lactic acid bacteria associated with sourdough fermentation (Chap. 5), is matched by a comparable diversity of general growth and stress parameters. Different processes of sourdough fermentation select for organisms with different growth parameters. Generally, type I sourdoughs, which are characterized by frequent back-slopping to achieve leavening without addition of baker's yeast, select for organisms growing rapidly in cereal substrates. Under these conditions, *Lactobacillus sanfranciscensis* is often found as the predominant organism. Type II sourdoughs, which are characterized by long fermentation times and high fermentation temperatures, select for acid-tolerant organisms and *L. pontis*, *L. fermentum*, *L. reuteri* and related organisms are frequently found [6–8].

This effect of fermentation parameters on sourdough microbiota is reflected by the response of the growth of sourdough lactic acid bacteria to pH, temperature, and NaCl concentration. Mathematical models for the growth of sourdough lactic acid bacteria were developed for *L. sanfranciscensis* [9], *L. pontis* [10], *L. amylovorus* [10, 11] and *L. plantarum* [12]. The optimum temperature for growth is species specific; mesophilic organisms grow optimally between 30 and 35°C, while thermophilic organisms do not grow at ambient temperature (25°C) and grow optimally between 40 and 45°C [9–11, 13]. Traditional sourdough fermentations in Europe are typically carried out in the temperature range of 25–35°C (Chap. 4) and are thus dominated by mesophilic lactic acid bacteria. Many industrial processes and cereal fermentations in tropical climates are conducted at higher temperatures and accordingly select for thermophilic lactic acid bacteria [7, 8].

The optimum pH of sourdough lactic acid bacteria is typically between 5.0 and 6.0 [9–11], matching the pH of sourdough after inoculation with 5–20% of a previous batch of sourdough. Remarkably, the pH of wheat flour, about 6.2–6.5, is close to the maximum pH permitting growth of *L. sanfranciscensis,* pH 6.7 [9]. The minimum pH of growth of *L. sanfranciscensis* and *L. pontis* was determined as pH 3.9 and pH 3.5, respectively. Growth and lactic acid production by *L. plantarum* continues until a pH of 3.1 is reached [12]. The higher pH tolerance of *L. pontis* corresponds to its competitiveness in type II sourdoughs, which select for acid-tolerant lactic acid bacteria. The acid tolerance of *L. pontis* and related organisms adapted to type II sourdoughs is dependent on conversion of arginine and glutamate, which consume intracellular protons, and on the formation of exopolysaccharides. These metabolic pathways are discussed in more detail below.

Lactic acid bacteria tolerate concentrations of undissociated acetic and lactic acids that exceed by far those concentrations that are typically encountered in sourdough [9–11]. Growth of *L. sanfranciscensis* and *L. pontis* is observed at lactate concentrations of up to 300 and 500 mmol/L, respectively [9, 10]; both organisms also tolerate high concentrations of acetic acid. This high tolerance for organic acids contrasts with the response of yeasts, which are inhibited by undissociated organic acids but not by low pH. Moreover, because the pH but not the organic acid concentration limits the growth of lactic acid bacteria in sourdough, the selection of cereal

substrates with a high buffering capacity, for example whole wheat flour or bran, allows the production of sourdough or sourdough products with a high concentration of organic acids and a corresponding high total titrable acidity.

The response of sourdough lactic acid bacteria to high salt concentrations is also species specific. Generally, obligate heterofermentative lactobacilli are more sensitive to NaCl in comparison to other lactobacilli. For example, the heterofermentative *L. sanfranciscensis* and *L. pontis* are inhibited by 4% NaCl whereas *L. plantarum* and *L. amylovorus* tolerate up to 6% NaCl [9, 10, 12].

7.3 Metabolism of Carbohydrates

Lactic acid bacteria belong to three metabolic categories: (1) obligately homofermentative organisms, which ferment hexoses through the EMP (Embden-Meyerhof-Parnas) pathway to lactate as the major end product of carbohydrate metabolism (Fig. 7.1). Pentoses are not fermented. Examples of obligately homofermentative lactobacilli occurring in sourdough include *L. delbrueckii*, *L. acidophilus*, *L. farciminis*, *L. amylovorus*, and *L. mindensis*. (2) Obligately heterofermentative organisms, which ferment hexoses and pentoses through the 6-PG/PK (6-phosphogluconate/phosphoketolase) pathway and synthesize equimolecular amounts of lactate and ethanol or acetate. CO_2 is additionally produced from hexoses (Fig. 7.2). Key examples of obligately heterofermentative lactobacilli in sourdough are *L. sanfranciscensis*, *L. rossiae*, *L. brevis*, *L. pontis*, and *L. fermentum*. (3) Facultatively heterofermentative organisms, which ferment hexoses through the EMP pathway, and pentoses and gluconate through the 6-PG/PK pathway. Examples of facultatively homofermentative lactobacilli occurring in sourdough include *L. plantarum*, *L. alimentarius*, *L. paralimentarius*, and *L. curvatus* [13].

Homofermentative metabolism of hexoses under anaerobic conditions yields 2 mol ATP per mol hexose. In contrast, heterofermentative metabolism of hexoses under anaerobic conditions yields only 1 mol ATP per mol hexose unless co-substrates are present (Fig. 7.1). Contrarily to other fermented foods where obligately homofermentative species have the major role, obligately heterofermentative lactic acid bacteria are dominant in sourdough fermentations [6]. Several factors determine the dominance of heterofermentative strains: (1) the metabolism of maltose via maltose phosphorylase activity, simultaneous fermentation of hexoses and pentoses through the 6-PG/PK pathway, and the use of fructose and other substrates as an external acceptor of electrons; (2) the optimal pH and temperature which often coincides with the values of sourdough fermentation; (3) the capacity of showing alternative phenotype responses and to markedly adapt under various environmental stresses; and (4) the synthesis of a large spectrum of antimicrobial compounds [6]. The variable sources of fermentable carbohydrates in sourdough determine phenotypic responses that include the use of external acceptors of electrons, the preferential and/or simultaneous use of nonconventional energy sources, and the interaction with exogenous or endogenous enzymes from the flour.

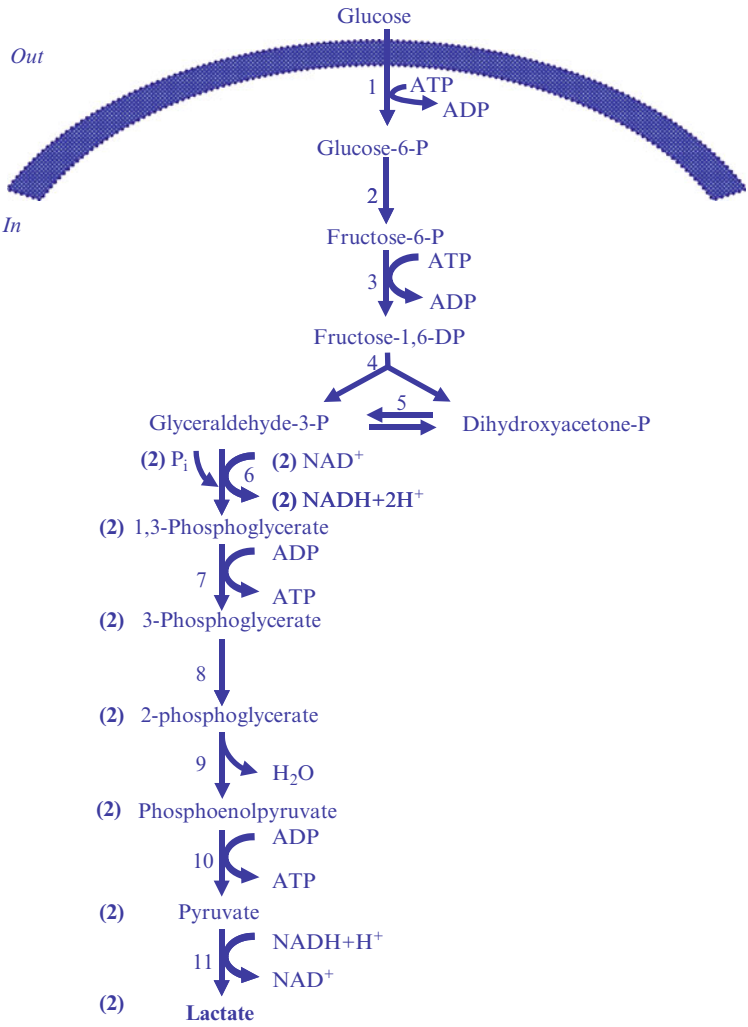


Fig. 7.1 Embden-Meyerhof-Parnas (EMP) pathway; homolactic fermentation. The final products of glucose metabolism are in *bold*. (2) indicates the formation of two moles of each compound. *1* Glucokinase, *2* glucose-6-phosphate isomerase, *3* phosphofructokinase, *4* fructose 1,6-bisphosphate aldolase, *5* triosephosphate isomerase, *6* glyceraldehyde 3-phosphate dehydrogenase, *7* 3-phosphoglycerate kinase, *8* phosphoglycerate mutase, *9* enolase, *10* pyruvate kinase, *11* lactate dehydrogenase

7.3.1 Use of External Acceptors of Electrons

Because the energy yield of heterofermentative metabolism of hexoses is low, the competitiveness of obligate heterofermentative lactobacilli depends on the use of external electron acceptors. The practical relevance of the use of external acceptors of electrons is represented by the substantial modification of the

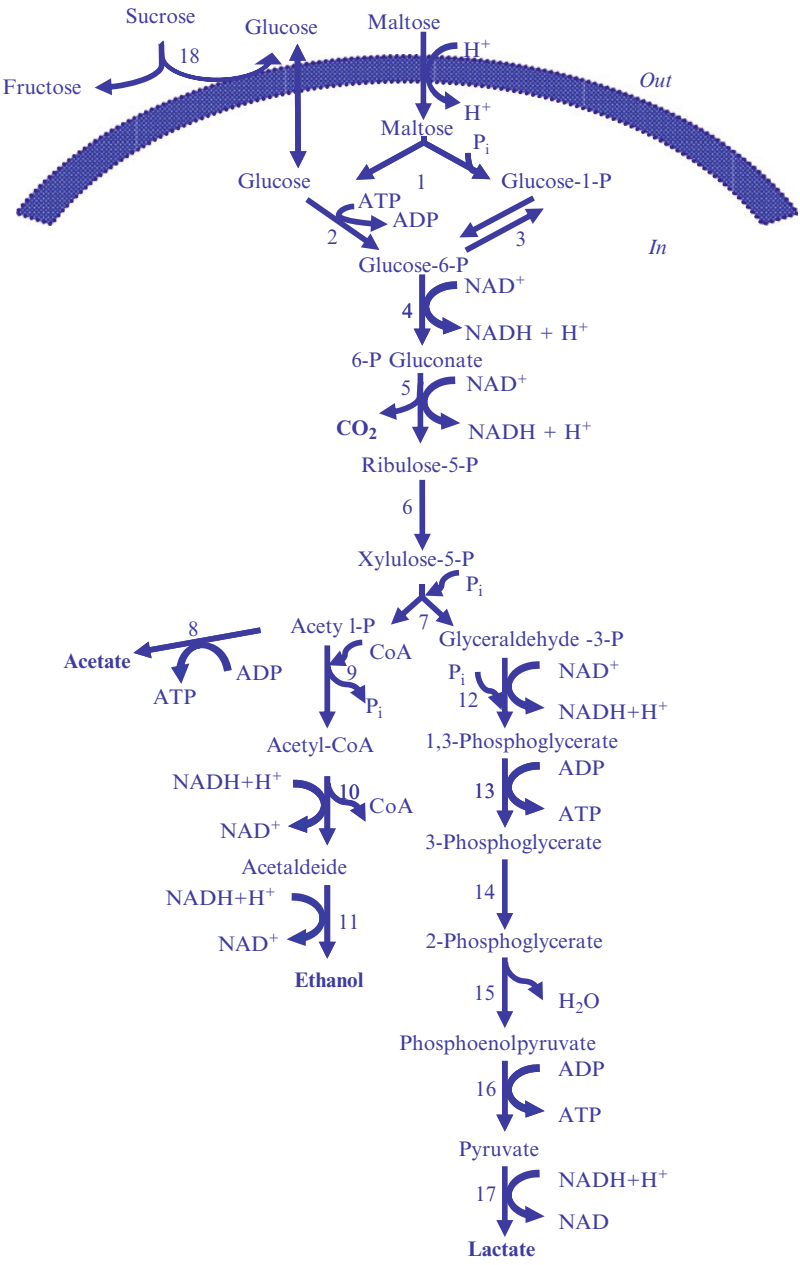


Fig. 7.2 6-Phosphogluconate/phosphoketolase pathway (6-PG/PK); heterolactic fermentation. The final products of glucose metabolism are in *bold* (Adapted from [3]). *1* Maltose phosphorylase, *2* hexokinase, *3* phosphoglucomutase, *4* glucose-6-phosphate dehydrogenase, *5* 6-phosphogluconate decarboxylase, *6* epimerase, *7* phosphoketolase, *8* acetate kinase, *9* phosphotransacetylase, *10* aldehyde dehydrogenase, *11* alcohol dehydrogenase, *12* glyceraldehyde 3-phosphate dehydrogenase, *13* 3-phosphoglycerate kinase, *14* phosphoglycerate mutase, *15* enolase, *16* pyruvate kinase, *17* lactate dehydrogenase, *18* levansucrase

Fig. 7.3 Examples of some reactions that allow NADH + H^+ co-factor reoxidation (Adapted from [3]). *1* Mannitol dehydrogenase, *2* alcohol dehydrogenase, *3* glutathione dehydrogenase, *4* NADH oxidase. *GSSG*, oxidized glutathione; *GSH* reduced glutathione; *R=O*, aldehyde (e.g., hexanal); *R-OH*, corresponding alcohol (e.g., hexanol)

fermentation quotient (see Chap. 4) which positively influences the sensory and shelf-life characteristics of sourdough baked goods [2]. Additional ATP is synthesized when acetyl-phosphate is employed for acetate synthesis through acetate kinase (Fig. 7.2). The synthesis of acetate and ATP as alternative metabolites from acetyl-phosphate requires the availability of co-substrates to oxidize NADH that were generated in the upper branch of the 6-PG/PK pathway. When external acceptors of electrons are available, the recycling of NADH is achieved without the need to synthesize ethanol from acetyl-phosphate. Most heterofermentative lactic acid bacteria are capable of fructose reduction to mannitol to achieve co-factor regeneration (Fig. 7.3). Fructose is quantitatively converted to mannitol by most heterofermentative lactic acid bacteria under acidic conditions [3, 14]. When maltose-negative and maltose-positive sourdough lactic acid bacteria are associated, fructose conversion may have a further role [2]. Most strains of *Weissella* spp. differ from other heterofermentative lactic acid bacteria because they do not convert fructose to mannitol with concomitant acetate formation [15]. The activity of the mannitol dehydrogenase of *L. sanfranciscensis* LTH2581 is optimal at 35°C and pH 5.8–8.0 [16]. Once synthesized, mannitol could be further used as an energy source by strains of *L. plantarum*. This occurs under anaerobiosis and in the presence of ketoacids (e.g., pyruvate) as electron acceptors [17].

Oxygen is also used as an external electron acceptor and is reduced to H_2O with H_2O_2 as an intermediate ([2, 18]; Fig. 7.3). Aerobiosis also induced the expression of a 12.5-kDa superoxide dismutase (SOD), probably Mn^{2+} dependent [18]. Overall, lactic acid bacteria possess various enzymes that are involved in the detoxification of oxygen radicals. NADH-peroxidases and the system involved in the transport of L-cysteine are specifically used by sourdough lactobacilli to detoxify H_2O_2 [19]. The latency phase of growth and cell yield of *L. sanfranciscensis* CB1 are positively influenced by traces of oxygen and Mn^{2+} [18]. When aldehydes are available in the environment, *L. sanfranciscensis* showed R-specific activity by NADH-dependent alcohol dehydrogenase [20, 21] (Fig. 7.3). For instance, the reduction of hexanal to hexanol activates the acetate kinase pathway and the synthesis of acetic acid. Also the reduction of oxidized glutathione (GSSG) into reduced glutathione (GSH), via glutathione dehydrogenase, protects against oxidative stress, and allows the synthesis of acetic acid [22] (Fig. 7.3).

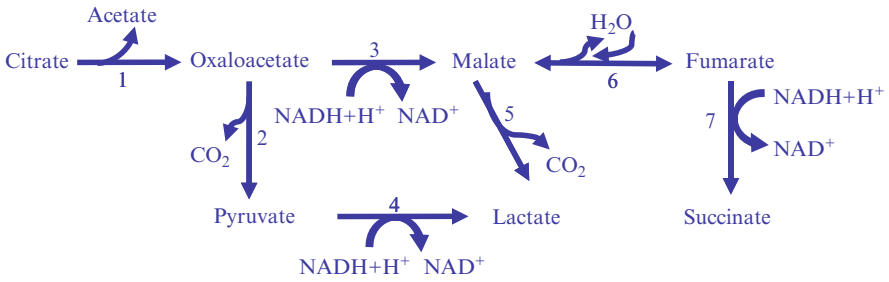


Fig. 7.4 Citric acid metabolism (Adapted from [3]). *1* Citrate lyase, *2* oxaloacetate decarboxylase, *3* malate dehydrogenase, *4* lactate dehydrogenase, *5* malolactic enzyme, *6* fumarase. *7* succinate dehydrogenase

7.3.2 Metabolism of Organic Acids

Sourdough lactic acid bacteria exhibit strain-dependent metabolism of citrate, malate, and fumarate; a few species are also capable of anaerobic lactate metabolism. Citrate, malate, and lactate conversion consumes intracellular protons and thus increases the acid tolerance of lactic acid bacteria. The initial steps of citrate metabolism by lactic acid bacteria are transport by citrate permease and the reaction catalyzed via citrate lyase (Fig. 7.4). Two alternative destinations are possible for oxaloacetate: the first allows the synthesis of succinic acid; the second proceeds via decarboxylation into pyruvate [23]. *Lactococcus lactis* converts part of the pyruvate into α-acetolactate. This reaction takes place when external acceptors of electrons (e.g., citrate) are available, resulting in a surplus of pyruvate with respect to the amount needed to regenerate NADH through lactate dehydrogenase. α-Acetolactate is reduced to acetoin or nonenzymatically converted to diacetyl, an important flavor compound of the bread crumb [24]. Diacetyl formation is not observed in obligate heterofermentative lactobacilli and conversion of citrate to succinate is the most common route for other sourdough lactic acid bacteria. Nevertheless, the synthesis of diacetyl was found in sourdoughs fermented with *L. plantarum*, *L. farciminis*, *L. alimentarius* and *L. acidophilus* [25]. *Lactobacillus sanfranciscensis* uses the route of the pyruvate to convert citrate into lactate and acetate, also including the co-fermentation with maltose [26, 27]. The regeneration of the co-factor during the reaction catalyzed by the lactate dehydrogenase allows the additional synthesis of acetate. Lactate formation from citrate does not cause a decrease of the value of pH, and, therefore, the citrate metabolism of *L. sanfranciscensis* during sourdough fermentation is not limited by the low pH [3]. The use of citrate during sourdough fermentation thus favors an increase of lactate and acetate concentrations. Malate and fumarate are also converted to lactate by *L. sanfranciscensis* [3, 26] (Fig. 7.4). In sourdough, the synthesis of pyruvate and lactate may also result from the catabolism of nonconventional substrates (e.g., amino acids). For instance, serine could be deaminated into ammonium and pyruvate. This latter may be, in turn, reduced to lactate. Pyruvate may be synthesized directly (e.g., alanine) or indirectly (e.g., aspartic acid) through the reaction of transamination [17].

Lactate conversion has been described for *L. parabuchneri*, an isolate from ting, a lactic-fermented sorghum sourdough [28]. Lactate conversion to propanediol proceeds through NADH-dependent reduction of lactate to lactaldehyde and 1,2 propanediol. The reduction of two NADH to NAD^+ allows the concomitant formation of acetate from lactate [29]. Lactate conversion to 1,2-propanediol occurs mainly in the stationary phase of growth and the consumption of lactate improves the stationary phase survival of *L. parabuchneri* [30].

The practical relevance of the use of nonconventional energy sources is more complex and less diffuse within the population of sourdough lactic acid bacteria. In some cases, the addition (e.g., citrate) is needed for conditioning of the microbial metabolism. In other cases (e.g., deamination or transamination of amino acids), it is possible to note a large phenotype variability depending on the biotypes.

7.3.3 Preferential and/or Simultaneous Use of Energy Sources

Lactic acid bacteria use various sources of energy according to hierarchical features that are determined through mechanisms of global control, mainly regulated at the transcription level [31]. When bacteria are subjected to a mixture of energy sources, they preferentially use the substrate that ensures the highest cell yield. When growing on glucose-containing mixtures, *L. amylovorus* DCE 471 always consumed glucose most rapidly, which seemed to steer growth during the early phase. Maltose consumption started only when low levels of glucose were reached [32]. The presence of glucose repressed the fermentation of fructose, maltose, and sucrose of *L. paralimentarius* and *Weissella cibaria* [33]. However, maltose, sucrose and fructose metabolism is not repressed by glucose in the sourdough-adapted species *L. reuteri* and *L. sanfranciscensis*. Levansucrase, the only enzyme responsible for sucrose metabolism in *L. sanfranciscensis* and a major contributor to sucrose metabolism in *L. reuteri* is constitutively expressed or regulated independent of the carbohydrate supply [35, 39]. In *L. reuteri*, sucrose phosphorylase is induced by sucrose or raffinose but not repressed by glucose [40]. The efficient and preferential metabolism of maltose and sucrose by several obligate heterofermentative lactobacilli from sourdough likely reflects adaptation to cereal substrates where sucrose and raffinose are the major carbon sources in the resting grain while maltose is the major carbon source that is liberated by cereal enzymes during fermentation.

Metabolism via maltose phosphorylase or sucrose phosphorylase activity allows a higher energy yield because the chemical energy of the glycosidic bond is employed for ATP synthesis [34, 35]. Both enzymes are frequently found in heterofermentative lactic acid bacteria from sourdough. Expression of maltose phosphorylase in *L. sanfranciscensis* and *L. reuteri* is constitutive and not repressed by glucose, in contrast, hexokinase activity is observed only if glucose is available. During growth on maltose, *L. sanfranciscensis* phosphorylyses maltose and accumulates glucose in the environment, according to the molar ratio of ca. 1:1 (Fig. 7.2) [34, 36]. Glucose accumulated by *L. sanfranciscensis* is available for maltose-negative yeasts and lactic acid bacteria. Nevertheless, the release of glucose from the cell takes place only

when the activity of the enzyme hexokinase is not induced [37]. The accumulation of glucose was not found for some strains of *L. sanfranciscensis* that were cultivated in the presence of maltose and fructose. Therefore, it was hypothesized that once liberated the glucose is used through the activity of the enzyme hexokinase, which is in turn induced by the presence of fructose [38].

Some strains of *L. sanfranciscensis* have the capacity to express β-glucosidase activity, but this activity is repressed by glucose [41]. Lactobacilli from sourdough also show simultaneous rather than consecutive fermentation of pentoses and hexoses. Compared to growth on maltose as the only energy source, *L. alimentarius* 15 F, *L. brevis* 10A, *L. fermentum* 1 F e *L. plantarum* 20B showed the highest growth and acidification rates, and the highest cell yield when cultivated in the presence of xylose, ribose, and arabinose [42]. Other sourdough lactic acid bacteria showed the highest performance in terms of growth and synthesis of acetic acid when cultivated in the presence of a mixture of pentose carbohydrates [43]. The presence of pentoses induces the synthesis of the phosphoketolase enzyme in facultatively heterofermentative lactic acid bacteria (e.g., *L. plantarum*) and allows the second half of the 6-PG/PK pathway to proceed (Fig. 7.2). The selection of lactobacilli with the capacity to ferment pentose carbohydrates is thus a suitable alternative to sucrose addition to increase acetate formation.

7.4 Proteolysis and Catabolism of Free Amino Acids

7.4.1 Proteolysis

Lactic acid bacteria are characterized by multiple amino acid auxotrophies. Lactic acid bacteria depend on substrate-derived proteases or on the activity of their proteolytic system to satisfy the nitrogen metabolism [44]. The proteolytic systems of lactic acid bacteria includes serine cell-envelope-associated proteinase (CEP – PrtP) which is associated to the cell wall, oligopeptide and amino acid transporters, and a large number of intracellular peptidases [45] (Fig. 7.5). Contrary to lactic acid bacteria in dairy fermentations, *L. sanfranciscensis* ATCC 27651^T and most other sourdough lactobacilli do not possess a cell-envelope-associated proteinase and depend on cereal-associated proteases [46, 47]. The comparison between sourdoughs and chemically acidified doughs showed that the degradation of the native proteins is mainly due to the activity of cereal endogenous proteinases [46, 48, 49]. The acidification by sourdough lactic acid bacteria favors the activation of aspartate-proteinases from cereals, which have an optimal pH of activity that ranged between 3.0 and 4.5 [50]. Nevertheless, selected strains of sourdough lactobacilli showed the capacity to hydrolyze albumins, globulins, and gliadins during fermentation [51–53]. Oligopeptides (4–40 amino acids) are transported inside the bacterial cell and hydrolyzed through a complex system of peptidases. An overview of the intracellular peptidases of *L. sanfranciscensis* is shown in Fig. 7.6. Several intracellular peptidases of *L. sanfranciscensis* CB1 were biochemically characterized: a 65-kDa metal-dipeptidase (PepV), with

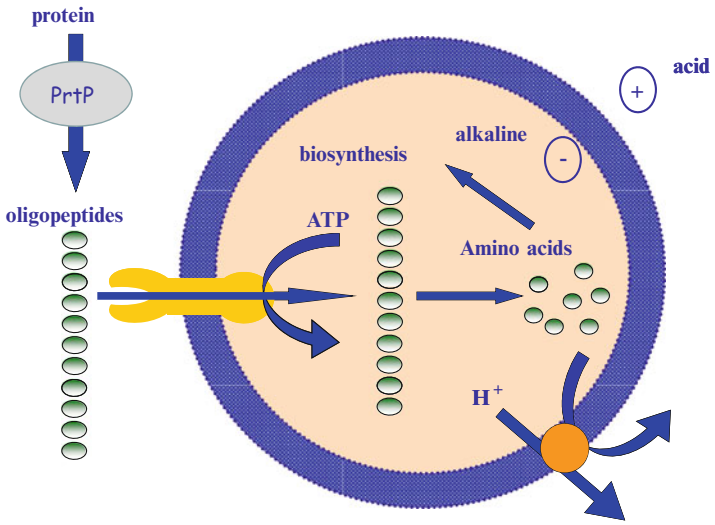


Fig. 7.5 Proteolytic system of lactic acid bacteria (Adapted from [44]). *PrtP*, Cell-envelope-associated proteinase (CEP)

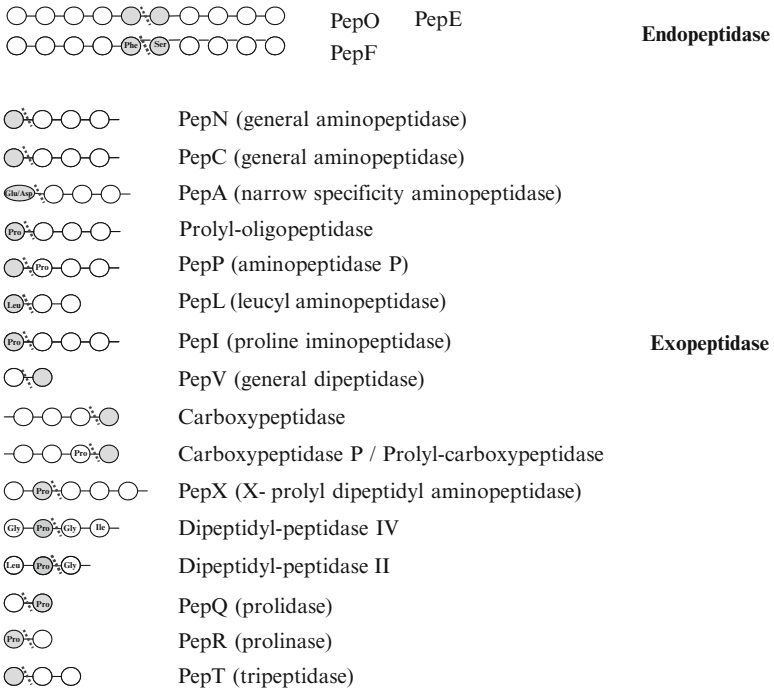


Fig. 7.6 Peptidase system of lactic acid bacteria (e.g. *Lactobacillus sanfranciscensis*) and substrate specificity (Adapted from [50])

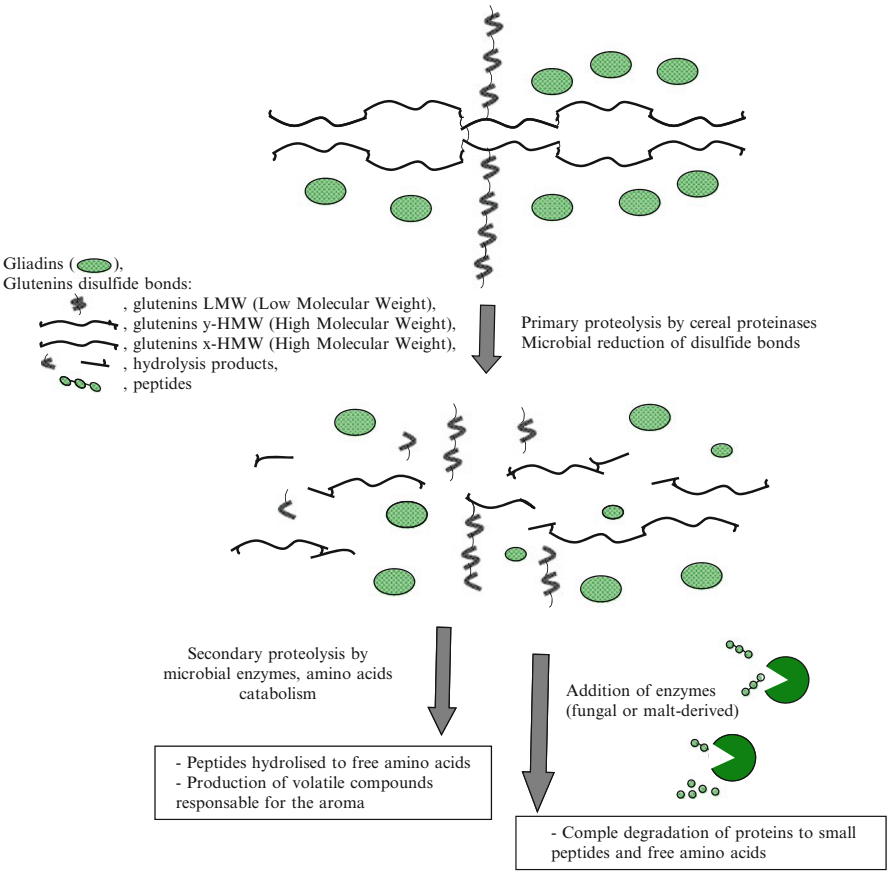


Fig. 7.7 Proteolytic scheme that occurs during sourdough fermentation. Figure shows substrates, enzymes and the activities of primary and secondary proteolysis (Adapted from [50])

elevated specificity towards dipeptides containing hydrophobic amino acids; a 75-kDa general aminopeptidase (PepN); and an X-prolyl-dipeptidyl aminopeptidase (PepX), which exclusively hydrolyzes substrates with the N-terminal sequence X-Pro [51, 54]. The capacity to hydrolyze oligopeptides adjacent to proline residues was shown in selected sourdough lactobacilli. However, the proteolytic system of lactic acid bacteria does not cleave peptides with the sequence motif XPP [55]. Overall, sourdough fermentation increases amino acid concentrations compared to doughs that are chemically acidified [48, 52]. Proteolysis during sourdough fermentation proceeds in two stages (Fig. 7.7): (1) primary proteolysis that releases oligopeptides, and is mainly operated by cereal endogenous proteinases that are activated at low pH and by accumulation of thiols; and (2) secondary proteolysis that releases free amino acids and small-sized peptides, and is exclusively operated through peptidase activity of lactic acid bacteria.

The degradation of the cereal proteins has a fundamental importance for the rheology and sensory features of leavened baked goods. High molecular weight glutenins (HMWG) and gliadins, following the decreasing order of solubility of fractions γ, α, and ω, are hydrolyzed into alcohol-soluble oligopeptides [47]. The partial hydrolysis of glutenins during sourdough fermentation results in the depolymerization and solubilization of the glutenin macropolymer (GMP), thus affecting the viscoelastic properties of the dough. The degree of polymerization of GMP is also influenced by reducing agents. The reduced glutathione (GSH) is the most important reducing agent in wheat dough. GSH may be subjected to an exchange reaction with the thiol groups of the gluten proteins, thus reducing the intermolecular disulfide bond and favoring the decrease of the molecular mass of the GMP [50]. Sourdough heterofermentative lactic acid bacteria possess glutathione reductase activity (see Sect. 3.1), which reduces the extracellular oxidized GSSG to GSH. The continuous recycling of GSSG into GSH keeps the level of SH groups in the dough elevated and increases the number of SH groups in the gluten proteins [56].

The proteolytic system of sourdough lactic acid bacteria contributes to the accumulation of bioactive peptides in sourdough. Fermentation of rye malt sourdoughs with *L. reuteri* resulted in the accumulation of angiotensin-converting-enzyme inhibitory peptides [55]; the formation of peptides with antioxidant activity was also demonstrated [57]. Sourdough fermentation in combination with fungal enzymes was also demonstrated to decrease the concentration of gluten to levels of less 10 ppm, which are tolerated by celiac patients [52, 58]. This hydrolyzed wheat flour was used for the manufacture of baked goods and administered to celiac patients for 60 days. As shown by hematology, immunology and histological analyses, the consumption of 10 g of equivalent gluten per day was absolutely safe for celiac patients [59, 60]. Nine peptidases were partially purified from the pooled cytoplasm extract of the above-selected sourdough lactobacilli and used to hydrolyze the 33-mer epitope, the most common immunogenic peptide generated during digestion of *Triticum* species. At least three peptidases, general aminopeptidase type N (PepN), X-prolyl dipeptidyl aminopeptidase (PepX), and endopeptidase (PepO) were necessary to detoxify the 33-mer without generation of related immunogenic peptides. After 14 h of incubation, the combination of at least six different peptidases totally hydrolyzed the 33-mer into free amino [61]. Peptidase activities of sourdough lactobacilli show considerable diversity at the strain level [55, 62] and the expression of genes coding for peptidases and peptide transport is under control of the concentration of peptides that are present during sourdough fermentation [46].

7.4.2 Amino Acid Metabolism

Peptides and free amino acids represent the substrates for microbial conversion, or are transformed during baking to volatile flavor compounds (Table 7.1). Catabolism of free amino acids by sourdough lactic acid bacteria not only has sensory implications but also increases acid resistance and the microbial energy yield under

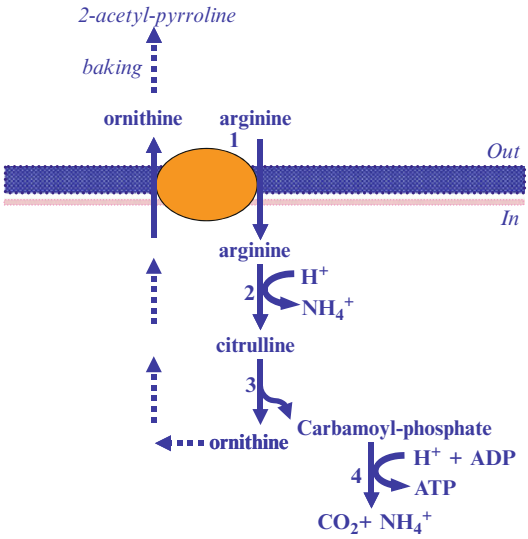
Table 7.1 Examples of amino acid precursors and derived carbonylic compounds, which are generated during sourdough fermentation and/or during baking

Amino acid precursor	Derived carbonylic compound
Leucine	3-Methylbutanol
Isoleucine	2-Methylbutanol
Valine	2-Methylpropional
Alanine	Acetaldehyde
Methionine	Methional
Phenylalanine	Phenylacetaldehyde
Threonine	2-Hydroxypropional

starvation conditions [63, 64]. Major pathways for amino acid conversion by lactic acid bacteria include decarboxylation reactions, transamination, and metabolism by lyases (for review, see [65]). These pathways lead to the synthesis of ketoacids, aldehydes, acids and alcohols, which are important flavor compounds of baked goods (Table 7.1) [66]. Major pathways that are relevant in sourdough fermentation are outlined in more detail below.

The catabolism of arginine (Arginine Deiminase, ADI) was studied in depth on sourdough lactic acid bacteria (Fig. 7.8). Three enzymes are involved, arginine deaminase (ADI), ornithine carbamoyl transferase (OTC), and carbamate kinase (CK). A fourth protein, located at the cell membrane which acts as transporter, allows the antiporter exchange between arginine and ornithine [67]. The activity of ADI, OTC, and CK enzymes is a species-specific property of several obligately heterofermentative species, including *L. rossiae*, *L. reuteri*, *L. brevis*, *L. hilgardii*, *L. fermentum*, *L. pontis*, and *L. fructivorans* [68]. Generally, arginine is quantitatively converted to ornithine by ADI-positive lactic acid bacteria during sourdough fermentation [48]. The activity of ADI, OTC, and CK of *L. rossiae* CB1 is well adapted to the acidity (pH 3.5–4.5) and temperature (30–37°C) conditions of sourdough fermentation [69]. The ADI pathway in *L. fermentum* IMDO 130101 is upregulated in response to temperature and salt stress conditions [70]. During sourdough fermentation, the expression of the ADI pathway favors: (1) microbial growth and survival, which determines a constant composition of the microbial population in the sourdough; (2) the enhanced tolerance of lactic acid bacteria to acidity by contributing to homeostasis of the intracellular pH; and (3) an increased synthesis of ornithine which is converted, during baking, into 2-acetyl-1-pyrroline, the compound responsible for the typical flavor of the bread crust.

Glutamine is the most abundant amino acid of wheat proteins; *L. sanfranciscensis* and other sourdough lactobacilli convert glutamine into glutamate (Fig. 7.9). Glutamine conversion to glutamate improves the adaptation of lactobacilli to sourdough acidity due to consumption of protons and liberation of ammonia which increases the extracellular pH. The synthesis of glutamate also has a positive effect on the sensory properties of leavened baked goods [71]. Glutamate is alternatively decarboxylated to γ-aminobutyrate (GABA), or converted to α-ketoglutarate (αKG) by glutamate dehydrogenase (EC 1.4.1.3). Heterofermentative lactobacilli preferably



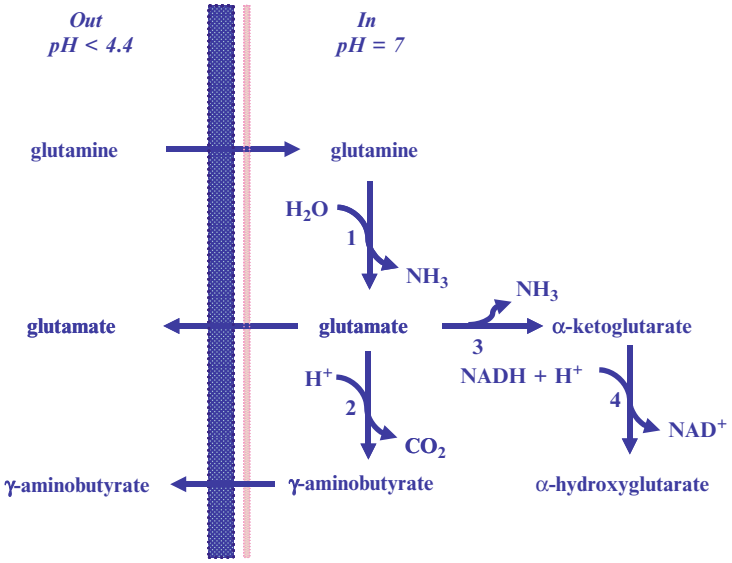


Fig. 7.8 Arginine deiminase catabolism (ADI) in sourdough lactic acid bacteria (Adapted from [3]). *1* Arginine-ornithine antiporter, *2* arginine deaminase, *3* ornithine transcarbamylase, *4* carbamate kinase

Fig. 7.9 Glutamine and glutamate metabolism of sourdough lactic acid bacteria. *1* Glutaminase, *2* glutamate decarboxylase, *3* glutamate dehydrogenase, *4* alcohol dehydrogenase

employ αKG as an electron acceptor to regenerate NADH [20]. αKG also acts as the preferred amino acceptor in transamination reactions with leucine, phenylalanine, and other amino acids [72]. After peptide transport, the transamination reactions are the second limiting factor for the conversion of amino acids by lactic acid bacteria [73]. Consequently, the addition of α-ketoglutarate into the food matrix or the selection of glutamate dehydrogenase positive strains considerably increases the

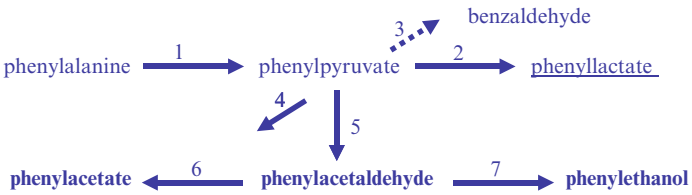


Fig. 7.10 Phenylalanine catabolism in *Lactobacillus plantarum* and *Lactobacillus sanfranciscensis*. The most abundant metabolic products are *underlined*, those responsible for the aroma compounds are in *bold* (Adapted from [3]). *1* Transaminase, *2* dehydrogenase, *3* chemical oxidation, *4* multienzyme complex, *5* decarboxylase, *6* and *7*, dehydrogenase

conversion of amino acids. Glutamate dehydrogenase activity is variable depending on the biotype of lactic acid bacteria [73]. This enzyme is also dependent on NADH + H^+, which links the catabolism of glutamate of *L. sanfranciscensis* DSM20451 to the regeneration of NADH during the central metabolism of carbohydrates (Fig. 7.9) [74]. Glutamate decarboxylase (GAD) activity was also found in sourdough lactic acid bacteria [75, 76]. GAD converts L-glutamate to GABA, through a single-step α-decarboxylation. GABA, a four-carbon nonprotein amino acid, has several functional activities. Sourdough fermentations with *L. plantarum*, and *Lc. lactis* or *L. reuteri* allowed the synthesis of GABA in concentrations of up to 9 g/kg, comparable to those found in functional preparations [75, 76]. Glutamate decarboxylase-mediated acid resistance was shown to contribute to the persistence of *L. reuteri* in type II sourdough fermentations [64].

The catabolism of phenylalanine by *L. sanfranciscensis* and *L. plantarum* is shown in Fig. 7.10. Leucine, isoleucine, and valine undergo a similar mechanism. Alternative pathways for leucine metabolism were proposed but remain to be validated by enzymatic and genetic analyses [77]. Phenyl-lactate or phenyl-acetate are the main end products of the catabolism of phenylalanine. For *L. plantarum* TMW1.468, a glutamate-dehydrogenase negative strain, the synthesis of phenyl-lactate is increased through addition of αKG but not with glutamate and citrate added. Conversely, for *L. sanfranciscensis* DSM20451, a glutamate-dehydrogenase positive strain, the synthesis of phenyl-lactate is increased through addition of αKG, glutamate and peptides containing glutamate. However, αKG and glutamate favor the conversion of phenylalanine only when fructose or citrate are also present, probably owing to the co-factor dependence of glutamate dehydrogenase [74]. During sourdough fermentation, *L. plantarum* TMW1.468 and *L. sanfranciscensis* DSM20451 synthesize ca. 0.35–1.1 mM of phenyl-lactate; the concentration of 1.5 mM inhibits the growth of *L. sanfranciscensis* DSM20451 during growth in mMRS at pH 5.3 [3].

Cystathionine lyase activities are also diffuse within sourdough lactic acid bacteria (Fig. 7.11) [78]. A homotetrameric 160-kDa cystathionine γ-lyase was purified and characterized from *L. reuteri* DSM 20016 [79]. The enzyme is active towards a large number of amino acids and amino acid derivatives, including methionine, and allows the synthesis of ammonia, α-ketobutyrate, and low-molecular-weight volatile sulfur compounds.

Fig. 7.11 Cystathionine metabolism (Adapted from [169]). *1* Cystathionine-γ-lyase, *2* cystathionine-β-lyase, *3* cystathionine-β-synthetases

7.5 Synthesis of Exopolysaccharides

The formation of exopolysaccharide by lactic acid bacteria during sourdough fermentation improves culture survival and stress resistance, impacts dough rheology and bread texture, and allows production of bread with specific nutritional functionality. EPS from lactic acid bacteria can be categorized on the basis of their composition or their biosynthesis. EPS composed of only one type of constituting monosaccharide are classified as homopolysaccharides (HoPS). Examples include levan, which is composed of fructose, or dextran, which is composed of glucose. Heteropolysaccharides (HePS) are composed of two or more constituting monosaccharides. Lactic acid bacteria employ two alternative routes for EPS synthesis, intracellular synthesis from sugar nucleotides by glycosyltransferases, and extracellular synthesis from sucrose by glucansucrases or fructansucrases. EPS produced by glucansucrase or fructansucrase activity are invariably HoPS composed of glucose or fructose, respectively [80, 81]. EPS produced by intracellular glycosyltransferases predominantly are HePS with glucose, galactose, N-acetylglucosamine, N-acetylgalactosamine, rhamnose, or fucose as constituting monosaccharides [82–84]. Noticeable exceptions of HoPS produced by intracellular glycosyltransferases include a galactan from *Lc. lactis* [85] and β-glucan produced by *Pediococcus parvulus* and *Oenococcus oeni* [86].

Large-scale screening of strain collections of sourdough lactic acid bacteria revealed that very few isolates are capable of HePS formation [39, 87–89]; only one report documents formation of HePS in sourdough [90]. In contrast, HoPS formation is a frequent trait of sourdough lactic acid bacteria and any sourdough is likely to contain at least one HoPS-forming strain [39, 88]. Moreover, the in situ formation of HoPS during sourdough fermentation as well as the effect of HoPS on bread quality is well documented ([91, 92]), for a review see [93] and Chap. 8). Subsequent paragraphs will hence only briefly discuss HePS formation and focus on HoPS formation and applications.

7.5.1 EPS Biosynthesis and HoPS Structure

HePS formation by lactic acid bacteria is mediated by large gene clusters that are encoded on plasmids or the chromosome (for reviews, see [82–84, 94]). EPS gene clusters generally code for proteins regulating EPS biosynthesis (EpsA in *Streptococcus thermophilus* Sfi6), polymerization, export, and chain length determination (EpsB, EpsC, and EpsD in *S. thermophilus* Sfi6), and one or several

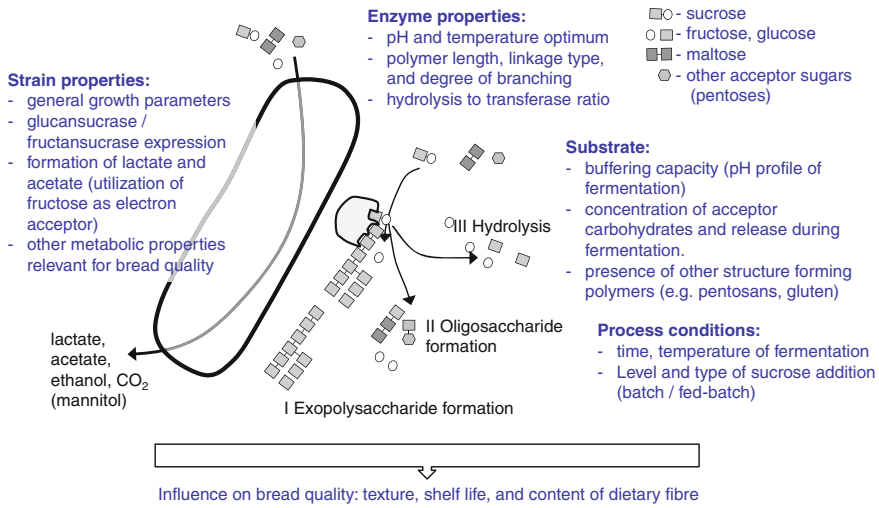


Fig. 7.12 Factors influencing yield of exopolysaccharides and the influence of exopolysaccharides on bread quality. Glucansucrases catalyze three alternative reactions: (I) polymerization of glucose or fructose to exopolysaccharides, (II) glycosyl transfer to acceptor carbohydrates to form oligosaccharides, (III) sucrose hydrolysis to glucose and fructose. EPS properties and yield are additionally dependent on the concentration of substrate-derived acceptor carbohydrates, and the ambient pH and temperature

phosphor-glycosyltransferases or glycosyltransferases (EpsE, EpsF, EpsG, and EpsI in *S. thermophilus* Sfi6). EPS is synthesized by glycosyltransferases with sugar nucleotides derived from glucose-6-phosphate as substrates. The repeating unit of the polysaccharide, consisting of two to eight monosaccharides, is assembled by sequential addition of monosaccharides to the membrane-bound lipid carrier undecadeprenyl pyrophosphate. The repeating unit is exported and polymerized extracellularly. HePS biosynthesis requires energy-rich sugar nucleotides as precursors, and the assembly and export of the repeating units competes with the peptidoglycan biosynthesis; therefore, HePS yields are relatively low, typically well below 1 g/L.

HoPS synthesis is mediated by extracellular cell-wall associated or soluble glucansucrases or fructansucrases [80, 81]. Both groups of enzymes are classified as retaining glycosyl hydrolases and use sucrose as substrate. Fructansucrases but not glucansucrases also employ raffinose, stachyose, or verbascose as substrate [40]. Catalysis by glucansucrases and fructansucrases proceeds through a covalently linked glucosyl-enzyme or fructosyl-enzyme intermediate, respectively, with subsequent transfer of the glycosyl moiety to a growing polymer chain, a suitable acceptor carbohydrate, or water (Fig. 7.12). Sucrose hydrolysis or oligosaccharide formation are thus alternative reactions to polysaccharide synthesis [80, 95–97]. The ratio of sucrose hydrolysis to oligo- or polysaccharide synthesis depends on the enzyme structure as well as the substrate concentrations [98, 99]. Because the catalytic mechanism retains the chemical energy of the glycosidic bond of the substrate, HoPS synthesis does not require energy-rich substrates or co-factors. Several recent reviews

provide an excellent overview on structure-function relationships of glucansucrases and fructansucrase [80, 81, 100].

HoPS produced from sucrose are composed only of fructose or glucose but nevertheless have a large diversity with respect to linkage type, or degree of branching, and molecular weight. Dextran is mainly composed of α–(1→6) linked glucose molecules with a varying degree of α–(1→3) or α–(1→2) linkages and α–(1→3,6) branching points. Dextran is produced by many strains of *Leuconostoc* spp. and *Weissella* spp. but dextran-forming *Lactobacillus* spp. were also described [80, 81, 88]. Mutan is mainly composed of α–(1→3) linked glucose moieties and is produced by *Streptococcus* spp. and *L. reuteri* [80, 81]. Glucans with alternating α–(1→6) and α–(1→3) or alternating α–(1→4) and α–(1→6) linkages are referred to as alternan and reuteran, respectively. Reuteran formation has to date exclusively been observed in strains of *L. reuteri* [81]. The current knowledge on structure-function relationships of glucansucrases allows the manipulation of the linkage type [101] as well as the molecular weight and degree of branching [102]. Information on structure-function relationships of glucansucrases has also been used for identification of wild-type glucansucrases producing polymers with desired properties [103]. The linkage type in the oligosaccharides formed by glucansucrases generally matches the linkage types found in the corresponding polysaccharide [81, 97]. For example, *L. reuteri* 121 produces α–(1→4) and α–(1→6) linked reuteran with a relative molecular weight of 8×10^7; in the presence of glucose or maltose as acceptor carbohydrates, maltose and isomaltose or isomaltotriose and panose are produced. Fructans produced by lactic acid bacteria include the predominantly β–(2→1) linked inulin and the predominantly α–(2→6) linked levan; both polymers contain β–(2→1→6) branching points [80, 81, 98, 104]. A majority of fructan-producing sourdough isolates form levan. Remarkably, levansucrases form predominantly inulin-type fructo-oligosaccharides and hetero-oligosaccharides from sucrose [95, 98].

7.5.2 *Ecological Function of HoPS Production and HoPS Formation in Dough*

The ecological function of glucansucrases and fructansucrases for cereal-associated lactic acid bacteria was related to biofilm formation, sucrose and raffinose utilization, and stress resistance. In oral streptococci, glucansucrases and fructansucrases are primarily responsible for sucrose-dependent biofilm formation on the tooth enamel, and are considered major virulence factors of these organisms [105]. Analogous to the biofilm formation by oral streptococci, colonization and biofilm formation by *L. reuteri* in nonsecretory epithelia of the proximal intestinal tract of animals was found to be dependent on HoPS formation. The reuteransucrase of *L. reuteri* TMW1.106 was a major contributor to formation of the extracellular biofilm matrix, the levansucrase in the same organism functions as a glucan-binding protein. Both enzymes are required for colonization of reconstituted lactobacilli-free mice by *L. reuteri* TMW 1.106 [106].

Glucansucrases and fructansucrases use one of the two constituting monosaccharides of sucrose for poly- or oligosaccharide synthesis, the other monosaccharide, fructose and glucose, respectively, accumulates in the fermentation substrate. Levansucrases also release the α-galactosides melibiose, manninotriose, and manninotetraose from raffinose, stachyose, and verbascose, respectively [40]. In *L. sanfranciscensis* LTH2590, levansucrase is the only enzyme capable of sucrose and raffinose hydrolysis [40, 98]. In *L. reuteri* and *Lc. mesenteroides*, levansucrase, glucansucrase and sucrose phosphorylase constitute alternative pathways for sucrose (and raffinose) metabolism. Metabolism by extracellular levansucrases is the preferred metabolic route for raffinose, stachyose, and verbascose, presumably because the resulting α-galactosides have a smaller degree of polymerization, which facilitates transport across the cytoplasmic membrane [40]. Glucansucrase activity invariably accumulates fructose and thus supports acetate formation by heterofermentative lactic acid bacteria [98]. Because high quantities of acetate have detrimental effects on the structure of wheat bread [107], dextran-producing *Weissella* spp. that do not convert fructose to mannitol with concomitant acetate formation exhibited superior performance in baking applications when compared to *L. reuteri* or *L. sanfranciscensis* [91, 92, 108].

A contribution of HoPS formation to stress resistance of lactic acid bacteria has particularly been demonstrated for *L. reuteri*. Here, HoPS or fructo-oligosaccharides increase survival at acid conditions during the stationary phase [103], and improves survival of freeze-dried *L. reuteri* during storage [109]. The protective effects of levan and fructo-oligosaccharides are partially attributable to their specific interaction with phospholipids of biological membranes [109, 110]. However, protective effects are not limited to levan and fructo-oligosaccharides; dextran and β-glucan also improved acid resistance and stationary phase survival [86, 111].

Lactic acid bacteria producing HoPS from sucrose in laboratory medium generally also produce HoPS during growth in sourdough [39]. HoPS concentrations typically range from 1 to 8 g/kg dough [39, 91, 92, 98, 112]; more than 10 g/kg were reported in optimized, pH-controlled fermentations [113]. Glucansucrases and fructansucrases are extracellular enzymes, thus, their activity is governed by ambient conditions rather than intracellular pH and substrate concentrations (Fig. 7.12). The optimum pH of levansucrases and glucansucrases of sourdough lactobacilli ranges from 4.5 to 5.5, matching the pH profile of sourdough fermentations [98, 99]. HoPS yields in sourdough were maximized by pH-controlled fermentations at a pH of 4.7 [113]. Because sucrose acts both as glycosyl-donor and glycosyl-acceptor in HoPS formation, the sucrose concentration has a decisive influence on the ratio of sucrose hydrolysis, oligosaccharide formation, and polysaccharide formation. Below 10 g/kg sucrose, sucrose hydrolysis is the predominant reaction [98, 114]. Increasing sucrose concentrations initially increase the formation of levan over hydrolysis. After a further increase of the sucrose concentration, sucrose increasingly acts as a glycosylacceptor and oligosaccharide formation is the predominant reaction catalyzed by glucansucrases or fructansucrases [98, 114, 115]. In sourdough fermentation with 100 g/kg sucrose, *L. sanfranciscensis* LTH2590 produced 11 g/kg fructo-oligosaccharides but only 5 g/kg levan [98]. High reuteran yields from *L. reuteri* TMW1.106 were achieved in pH static fed-batch fermentations

that maintained sucrose concentrations at 10% throughout fermentation [113]. The addition of acceptor carbohydrates other than sucrose also influences HoPS yields in sourdough fermentations. Maltose is a stronger acceptor for glucansucrases compared to glucose [97, 115]. Correspondingly, dextran yields in wheat sourdoughs, characterized by high maltose concentrations throughout fermentation, were substantially lower compared to yields by the same strains in sorghum fermentations, which are characterized by low maltose and high glucose concentrations [15]. Maltose, xylose, and arabinose also act as alternative acceptor carbohydrates for levansucrase activity [95, 96].

Exopolysaccharide-producing sourdough starter cultures have found industrial application to improve the textural properties of bread [116]. In addition to the function of HoPS as hydrocolloids in baking applications, specific HoPS were found to prevent pathogen adhesion to eukaryotic cells [117] and to exhibit prebiotic activity [92, 118]. HoPS-producing lactic acid bacteria thus enable the formulation of baked goods with specific health properties.

7.6 Antimicrobial Compounds from Sourdough Lactic Acid Bacteria

Leavened baked goods can become contaminated by spores of the genus *Bacillus*, which survive baking, yeasts, mainly belonging to the genera *Pichia* and *Zygosaccharomyces*, which colonize the surface and negatively affect the sensory properties, and, especially, by moulds, mainly belonging to the genera *Penicillium*, *Aspergillus* and *Cladosporium* which alter the color and the sensory properties, and, in some cases, synthesize mycotoxins. More than 40 species of fungi were described as contaminants of baked goods. Although chemical preservatives (e.g., sorbate and propionate, ethanol) are routinely used for preventing the contamination of leavened baked goods, sourdough lactic acid bacteria show a number of natural bio-preservative features that are complementary to chemical preservatives, or can even substitute their use. Antimicrobial compounds from sourdough lactic acid bacteria were additionally shown to influence sourdough microbiota, and to contribute to the stability of individual strains.

The inhibitory activity of sourdough lactic acid bacteria is generally attributable to rapid consumption of oxygen and fermentable carbohydrates, and the formation of lactate with concomitant reduction of the pH. Additional metabolites with specific antimicrobial activity include diacetyl, hydrogen peroxide, acetate and other short-chain fatty acids, and reuterin. Acetate formation by heterofermentative lactobacilli in sourdough is readily adjusted by addition of sucrose or pentoses [39], and contributes to shelf-life extension of bread (see below). The odor threshold of diacetyl, butyrate, and caproate is substantially lower than the concentrations required for antimicrobial activity; these compounds can thus not be accumulated in bread without adverse effects on the sensory bread quality. Although individual sourdough lactic acid bacteria are capable of reuterin synthesis, reuterin formation has not been achieved in cereal fermentations [119].

7.6.1 Antifungal Compounds from Sourdough Lactic Acid Bacteria

Antifungal compounds synthesized by sourdough lactic acid bacteria include diacetyl, hydrogen peroxide, acetate, propionate, caproate, 3-hydroxy fatty acids, phenyllactate, cyclic dipeptides, reuterin, and fungicidal peptides [120]. Numerous reports describe a substantial increase of the mould-free shelf life of bread owing to antifungal activities of sourdough lactic acid bacteria [121, 122]. However, metabolites of lactic acid bacteria responsible for the antifungal effect remain in most cases unknown. Acetate inhibits fungal growth only at concentrations that significantly impair the sensory and textural quality of bread [30, 107, 123]. Phenyllactate and 4-hydroxyphenyllactate were initially characterized as antifungal metabolites from *L. plantarum* ITM21B [124]. Lactobacilli capable of producing phenyllactate and hydroxyphenyllactate delayed the growth of *Aspergillus niger* and *Penicillium roqueforti* for up to seven days of bread storage [125]. The concentration of phenyllactate accumulated by lactobacilli in sourdough fermentation, however, is about 100 fold lower than its minimal inhibitory concentration [74, 126] and the antifungal effect of the sourdough is thus not attributable to phenyllactate accumulation. Likewise, the levels of antifungal cyclic dipeptides in bread are 1,000 fold lower than their MIC, but above the threshold imparting a metallic and bitter taste [127]. Accordingly, the antifungal effect of sourdough lactic acid bacteria is attributed to a synergistic activity of several compounds. The antifungal effect of *L. reuteri*, *L. plantarum*, and *L. brevis* in the presence of Ca propionate (0.2%, w/w) [128] delayed fungal growth by 8 days compared to the bread started with baker's yeast alone. It was hypothesized that a synergistic activity between acetic acid and phenyllactate with chemical preservatives was responsible for this effect. The antifungal effect of *L. buchneri* and *L. diolivorans* against growth of four moulds on bread was attributed to a combination of acetate and propionate [30]. The preservative effect of *L. amylovorus* [121, 122] was attributed to the synergistic activity of more than ten antifungal compounds, including phenyllactate, phenolic acids, fatty acids, and cyclic dipeptides [129].

In addition to the antifungal metabolites of lactic acid bacteria, inhibitory peptides derived from the substrate may also contribute to the preservative effect. A water-extract from beans in combination with sourdough fermented with *L. brevis* AM7 contained three natural inhibitory compounds, two phaseolins (NCBI n. gi 130169, gi 403594) and one lectin (NCBI n. gi 130007) [130]. Antifungal peptides were also identified from the water-extract of sourdough. The combined activity of the above compounds determined a delay in fungal growth of up to 21 days, leading to a shelf life for the bread that was comparable to that found when using Ca propionate (0.3% w/w). A very extensive bread shelf life was also achieved by using sourdough lactic acid bacteria and amaranth flour or wheat germ [131, 132]. Sourdough fermented with the nonconventional yeast *Wickeramomices anomalus* LCF1695 and *L. plantarum* was shown to exhibit strong antifungal activity based on synergistic activities between the inhibitory peptides liberated by *L. plantarum*

and ethyl-acetate produced by *W. anomalus* [133]. Fungal contamination was delayed by 28 days under pilot plant bakery conditions. Although indubitable progress has been achieved in the making and storage of baked goods, fungal contamination remains one of the main problems in terms of long-term shelf life of bakery products. The search for novel methods of bio-conservation appears to be one of the most promising tools in this regard, even though a combination of various inhibitory compounds appears to be inevitable to markedly extend the storage of leavened baked goods.

7.6.2 Antibacterial Compounds from Sourdough Lactic Acid Bacteria

Antibacterial metabolites from lactic acid bacteria include reuterin and the organic acids described above. However, the emphasis of research related to antibacterial activities was placed on bacteriocins, ribosomally synthesized peptides with antibacterial activity against closely related organisms, and reutericyclin. The prevention of bread spoilage by rope-forming bacilli does not require the selection of specific protective cultures. Growth of endospores of *Bacillus* spp. is readily inhibited by modest acidification as is characteristic for sourdough bread [134–136]. Acetate and propionate are more effective against rope-forming bacilli than lactic acid [134]. Remarkably, the bacteriocins nisin and pedicoin, either included as additives, or generated in situ by bacteriocin-producing lactic acid bacteria, were ineffective [134].

Bacteriocin formation by sourdough lactic acid bacteria was described particularly for strains of *L. sakei*, *L. plantarum* and *L. amylovorus* (for a review, see [137, 138]). Bacteriocins appear not to be suitable for extending the shelf life of bread but formation in sourdough fermentation was shown to enhance the stability of sourdough microbiota. *Lactococcus lactis* M30, a lacticin 3147-producing strain [139], produced a bacteriocin during fermentation. The inhibitory activity persisted under the low values of pH of sourdough and after thermal treatments that corresponded to baking temperatures. Similarly, the bacteriocin-producing strain *L. amylovorus* DCE471 persisted for a long time during sourdough propagation [140]. The competitiveness of *L. pentosus* 2MF8, which synthesized a bacteriocin-like inhibitory substance [141] and *Lc. lactis* subsp. *lactis* M30, which synthesized lacticin 3147 [139] were studied during sourdough propagation. *Lactococcus lactis* subsp. *lactis* M30 showed a larger spectrum of inhibition compared to *L. pentosus* 2MF8, and did not inhibit the growth of *L. sanfranciscensis*. After 20 days of back-slopping, the persistence of *Lc. lactis* subsp. *lactis* M30 inhibited the indicator strain *L. plantarum* 20, without interference in the growth of *L. sanfranciscensis* CB1. The above-described features of bacteriocins, together with the demonstration of the in situ inhibitory activity, encourage the use of antimicrobial compounds to facilitate the persistence of the starter cultures and the conditioning of the microbial interactions that occur during sourdough fermentation.

Reutericyclin is a tetramic acid derivative with a broad spectrum of activity against Gram-positive bacteria, including rope-forming bacilli [142, 143]. To date, all reutericyclin-producing strains were isolated from an industrial rye sourdough. Reutericyclin is produced to active concentrations in sourdough and the persistence of *L. reuteri* strains during long-term propagation of a rye sourdough was attributed to the synthesis of reutericyclin (for a review, see [144]). The use of reutericyclin-producing *L. reuteri* as a sourdough starter also caused a delay in the growth of *Bacillus* sp. during bread storage [144].

7.7 Metabolism of Phenolic Compounds and Lipids

The major phenolic compound in wheat and rye is ferulic acid bound to cell wall polysaccharides [145]. Changes in the ferulic acid content during sourdough fermentation are predominantly the result of oxidation reactions and cereal enzymes [146, 147]. In contrast, cereal grains used in African cereal fermentations, particularly sorghum and millet, are rich in phenolic compounds, including phenolic acids, phenolic acid esters, desoxyanthocyanidins, and tannins [148, 149]. In these cereal grains, metabolism of phenolic compounds influences product properties, and the content of antimicrobial phenolic compounds influences the microbial ecology of sorghum sourdough fermentations [150]. A review on the metabolism of food phenolics by lactic acid bacteria, based predominantly on isolates from wine and vegetable fermentations, is provided by Rodriguez et al. [151].

Conversion of phenolic compounds is based on glycosyl hydrolases, for example β-glucosidase and α-rhamnosidase, which convert flavonoid glycosides to the corresponding aglycones [152, 153], esterase degrading methyl gallate, tannins, or phenolic acid esters [154], and decarboxylases and reductases with activity on phenolic acids [151]. The strain-specific metabolism of phenolic compounds is particularly well described for *L. plantarum* [151, 155]. Decarboxylation of hydroxyl cinnamic acids generates the corresponding vinyl derivatives [156]. Reductases hydrogenate the double bond of hydroxyl cinnamic acids or their decarboxylated vinyl derivatives [151, 156]. The spectrum of activities is strain specific; organisms capable of conversion of hydroxy cinnamic acids harbor reductase activity, decarboxylase activity, or both [150, 151, 156]. Analysis of phenolic compounds during sorghum sourdough fermentation revealed that strain-specific glycosyl hydrolase, decarboxylase, and phenolic acid reductase contributed to the conversion of phenolic compounds [151].

Phenolic compounds, including phenolic compounds from sorghum and millet, exhibit strong antimicrobial activity [148, 150]. Particularly the activity of hydroxy benzoic and hydroxy cinnamic acids is well characterized [157]. The resistance of lactic acid bacteria towards phenolic acids is highly strain specific and the strain-specific capability for phenolic acid conversion corresponds to resistance [157, 158]. Because the conversion of hydroxy cinnamic acids by reductase- or decarboxylase activities reduced the antimicrobial activity of caffeic

acid two to fivefold, metabolism was recently identified as a mechanism of detoxification [157]. To date, lactic acid bacteria capable of conversion of phenolic compounds were predominantly isolated from fermented beverages (wine, whisky, beer), or African sorghum fermentations. These organisms are likely a suitable source for starter cultures of phenolic-rich cereals and pseudocereals used in gluten-free baking [159].

Lipid metabolism in sourdoughs is poorly described and particularly lipase activity of sourdough lactic acid bacteria appears not to be relevant. However, sourdough fermentation influences lipid oxidation and the influence of lipid oxidation products on bread flavor. During flour storage and during dough mixing, chemical oxidation and cereal-derived lipoxygenase, respectively, convert free linoleic acid to lipid peroxides. Peroxides are chemically converted to lipid aldehydes, i.e., (E)-2-nonenal and (E,E)-2,4-decadienal, with a strong influence on the flavor of the bread crumb [21]. Heterofermentative lactobacilli reduce these aldehydes to the corresponding alcohols with a much lower flavor threshold through alcohol reductase activity. Homofermentative lactobacilli may increase lipid oxidation through formation of hydrogen peroxide [21]. Baker's yeast also reduced flavor-active aldehydes resulting from lipid oxidation, but slower and through different metabolic pathways [21]. Thiol accumulation by sourdough lactic acid bacteria [56] may additionally influence lipid oxidation. Cysteine and related thiol compounds reduce linoleic acid peroxides to the corresponding hydroxy fatty acids [160], and thus interrupts the reaction cascade leading to flavor-active aldehydes.

7.8 Cell-to-Cell Communication

Bacteria synthesize, release, sense, and respond to small signaling molecules that are defined as auto-inducers. The signaling molecules accumulate in the environment and lead to a series of physiological and biochemical responses when the quorum (threshold concentration) is reached. The term quorum sensing is derived from this consideration, and is mostly used to describe cell-to-cell communication [161]. Overall, the mechanisms of intraspecies communication include the use of acyl-homoserine lactones (AHL) and auto-inducing peptides (AIP) for Gram-negative and -positive bacteria, respectively [162, 163]. The interspecies communication is mainly based on signaling molecules such as furanone derivatives. The mechanisms involve the LuxS protein or AIP molecules and the three-component regulatory system (3CRS) [161]. Several studies considered the microbial dynamics during sourdough fermentation [164]. Within this complex food ecosystem, an understanding of the mechanisms of interspecies communication should give new insights into the physiological response of sourdough lactic acid bacteria and the interactions in an heterogeneous microbial community that govern growth and metabolism. The mechanism of cell communication was studied in *L. sanfranciscensis* CB1 co-cultivated with other sourdough lactic acid bacteria [165]. The highest number of dead and/or damaged cells of *L. sanfranciscensis* CB1 was found

when co-cultured with *L. plantarum* DC400 or *L. brevis* CR13. The co-cultivation with *L. rossiae* A7 did not interfere with survival compared to the mono-culture. The analysis of the proteome revealed the induction of several cytoplasm proteins of *L. sanfranciscensis* CB1, especially when associated with *L. plantarum* DC400. The majority of the induced proteins had a key role in the mechanisms of response to environmental stresses, leading to the hypothesis that co-cultivation with some species of lactic acid bacteria could be considered like an environmental stress which promoted cell communication. A central role for the LuxS protein was established, while also furanone derivatives were identified as presumptive signaling molecules. The synthesis of volatile compounds and the activity of peptidases also decreased when *L. sanfranciscensis* CB1 was cultivated together with *L. plantarum* DC400. The mechanisms of cell communication were also studied in *L. plantarum* DC400 [166]. The co-cultivation of this strain with other sourdough lactic acid bacteria did not interfere with survival compared to the mono-culture. Nevertheless, the analysis of the proteome revealed the induction of proteins involved in the response to environmental stresses and cell communication. *Lactobacillus plantarum* DC400 synthesized the pheromone plantaricin A (PlnA) at variable concentrations that depended on the associated microbial species [167]. The co-cultivation with *L. pentosus*, *L. brevis* and other sourdough lactic acid bacteria species did not modify the synthesis of PlnA compared to the mono-culture conditions and did not show effects on the viability and survival of the other species. On the contrary, the co-cultivation with *L. sanfranciscensis* increased the synthesis of PlnA and caused a decrease of the viability of this species. The same effect was found during growth of *L. sanfranciscensis* in the presence of the chemically synthesized PlnA. Under these environmental conditions, the analysis of the proteome revealed the over-expression of proteins that had a central role in the energy metabolism, catabolism and biosynthesis of proteins and amino acids, stress responses, homeostasis of the redox potential, and programmed cell death. Notwithstanding antimicrobial activities due to substrate competition and synthesis of inhibitory compounds (e.g., phenyl lactic acid), and the elevated capacity to adapt to changing environments, it could be hypothesized that the dominance of *L. plantarum* during sourdough fermentation [168] may relate to the synthesis of the pheromone/bacteriocin PlnA as stimulated by mechanisms of quorum sensing.

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