Update in bread fermentation by lactic acid bacteria

G. Rollán[*,1], C.L. Gerez[1], A. M. Dallagnol[1], M.I. Torino[1] and G. Font[1,2]

[1]Centro de Referencia para Lactobacilos (CERELA)-CONICET, San Miguel de Tucumán 4000, Argentina.
[2]Cátedra de Microbiología Superior, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, San Miguel de Tucumán 4000, Argentina.

*Corresponding author: e-mail: rollan@cerela.org.ar Phone: +54-381-4311720. Fax: 54-381-4005600

The lactic fermentation of cereals improves the food quality through the development of flavor, enhancement of the nutritional value and shelf life, and by removing toxic or antinutritional factors of food products. Lactic acid bacteria (LAB) strains are able to improve the shelf life of several food products. The efficiency of the LAB cultures determined in *in vitro* assays was confirmed in bread manufacture. Data obtained put in evidence the suitability of selected LAB strains to be used as natural food-grade biocontrol agents for reducing mould spoilage in bakery products. Also, the proteolytic activity of LAB might be used as a tool to reduce the allergenic fragments of wheat-baked goods. Several strains displayed activity on 31-43 and 62-75 α-gliadin-fragments while the 57–89 peptide, the most resistant to proteolysis, was only degraded by a combination of LAB strains with different peptidase profiles.

These results point out the advantages of using selected LAB strains as starter cultures for sourdough fermentation

Key-words: Lactic acid bacteria; Biopreservation; Gluten hydrolysis

1. Introduction

For several thousand years, bread has been one of the major constituents of the human diet, making the baking of yeast-based and sourdough breads one of the oldest biotechnological processes. Sourdough is an intermediate product between dough and traditional bread preparation, containing flour, water and metabolically active microorganisms, mainly lactic acid bacteria (LAB) and yeast. During the fermentation of the dough, the metabolic products of LAB improve the organoleptic and technological properties of bread as well as their shelf life, nutritional value [1, 2] and healthy aspect [3- 5].

The newest developments on the biochemistry and physiology of LAB in the sourdough ecosystem are considered here, with particular emphasis on anti-microbial compounds synthesis and decrease of certain allergen products derived from gluten which is present in wheat, barley and rye baked foods and is involved in celiac disease.

2. Biodiversity

Cereal flours are not microbiologically sterile, bacteria, yeasts and fungi being present at ~ 10^4–10^6 CFU/g. This microflora is amended during sourdough fermentation. A remarkable property of LAB is their versatility in respect not only to catabolic and anabolic pathways but also to the continuous changes of the environment [6]. Besides these adaptations, dominance of sourdough LAB depends on the technology used for sourdough production. Microbial interactions, type of flour, low and variable availability of nutrients, environmental stresses during processing and changes in the technology are some of the mayor factors which influence the biochemical and physiological responses of LAB and that determine the stability of the microbial communities in sourdough [7, 8]. The main genera of LAB isolated from sourdough are *Lactobacillus (Lact.), Leuconostoc, Pediococcus* and *Weissella*, and the majority of the strains belongs to the genus *Lactobacillus* [9]. De Vuyst and Vancanneyt [10] reported the most representative species groups: *Lact. alimentarius*, *Lact. buchneri*, *Lact. casei*, *Lact. delbrueckii*, *Lact. fructivorans*, *Lact. plantarum*, and *Lact. reuteri.* Moreover, from homemade sourdoughs of the mountain region of north-west Argentina, pediococcoci and heterofermentative lactobacilli are ubiquitous microbiota [11]. Furthermore, in this region a novel specie within the genus *Pediococcus* was demonstrated by polyphasic analysis, and it was named *Pediococcus argentinicus* sp. nov. [12].

Yeasts are associated with LAB in sourdough, generally at a ratio of 1:100 [13]. The usual yeasts species belong to the genera *Saccharomyces* (S. *exiguous*), *Candida* (C. *humilis*) and Issatchenkia *orientalis* (*Candida krusei*) [14, 15]. *S. cerevisiae* is not found in the raw materials; its presence in sourdough may be explained by the application of baker´s yeast in most daily bakery practice [16, 17]. In view of the fact that flour cannot be subjected to heat-sterilization, the incidence and number of certain types of microorganisms will strictly depend on a combination of available substrates and specific technological parameters [1, 10].

3. Types of sourdoughs

On the basis of applied technology, sourdoughs have been grouped into three types: type I (sourdough which is restarted using a part of the previous fermentation), type II (generally used as dough-souring supplements in semi-fluid preparations) and type III (are dried preparations) [18, 19]. Unlike type I sourdoughs, types II and III doughs require the addition of baker's yeast (*S. cerevisiae*) as leavening agent [20].

4. Impact of lactic acid bacteria in sourdough fermentation

The application of sourdough has a long tradition in the production of wheat and rye breads. Sourdough plays a crucial role in the development of the sensorial, nutritional, and safety quality of fermented products. The metabolic activity of LAB during sourdough fermentation may contribute to the improvement of cereal products in different ways, such as lengthening their shelf-life; hydrolyzing proline-rich allergenic fragments [21, 22, 4]; improving the texture and palatability of whole grain, fiber-rich, or gluten-free products; stabilizing / increasing levels of bioactive compounds and enhancing mineral bioavailability [23-25].

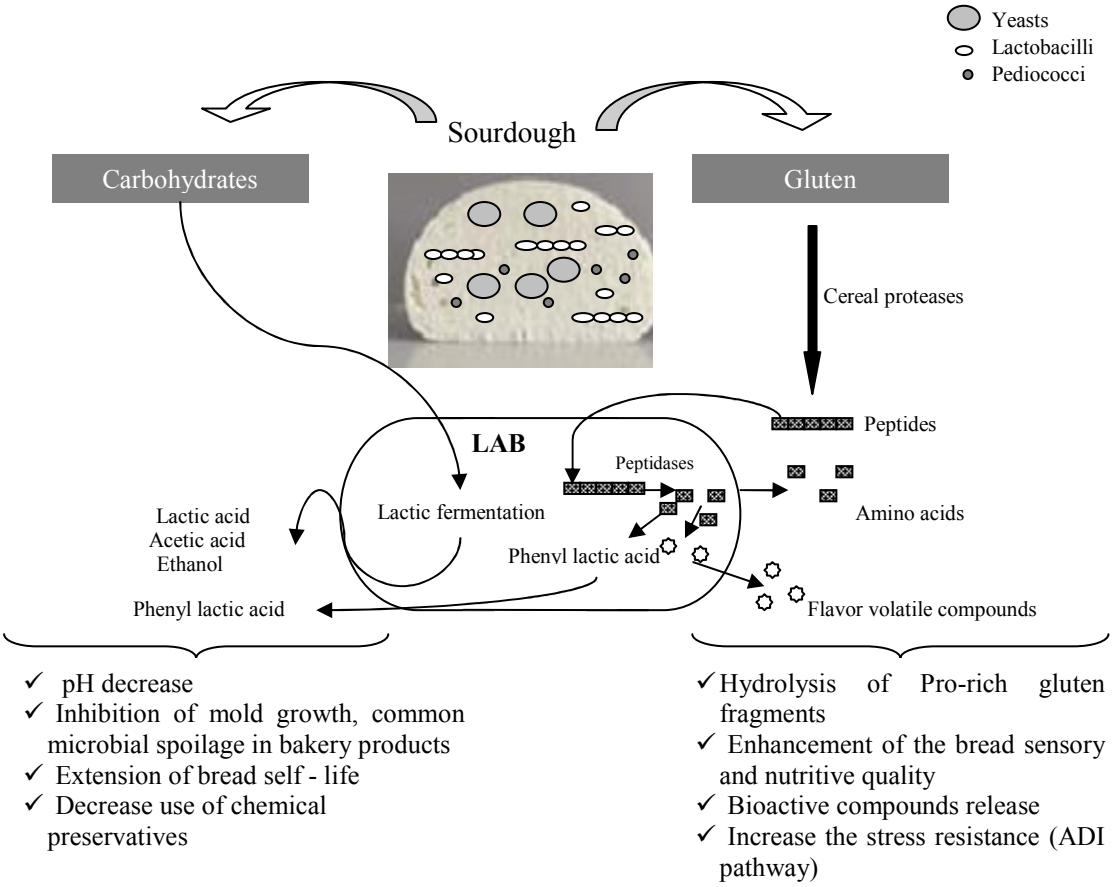

Fig. 1. LAB in sourdough fermentation

Because of the stress conditions that occur during the sourdoughs fermentation (i.e. energy starvation and acidic medium), a major problem for the deliberate use of microorganisms is their limited resistance to this harmful environment [26]. De Angelis et al [27] and Rollán et al [28] demonstrated that arginine catabolism through the arginine deiminase pathway (ADI) enhanced the cell growth (via the ATP production) and increase the tolerance to acid environmental stress (through ammonium production).The triggering factor for the ADI pathway in *Lact. reuteri* CRL 1098 would be the depletion of energy source rather than the pH attained by cultures at the stationary phase, whereas in *Lact. sanfranciscis* CB1, the presence of carbon source and low concentration of both arginine and oxygen are the conditions that promote the arginine catabolism throughout this pathway.

5. Antifungal lactic acid bacteria as biopreservation agent

Fungal growth is the most frequent cause of spoilage in bakery products mainly due to *Aspergillus*, *Fusarium*, and *Penicillium* genera. Statistics show that Argentinean small factories register losses in packaged bread as high as 20-40%, mainly due to the lack of good manufacture practices in addition to the warm climate in this country [29]. In addition to the great economic loses derived from the presence of mould, another concern is the potential mycotoxin production that may cause public health problems [30].

5.1 Fungi contamination control in bakery products

5.1.1 Conventional methods

The preservation of baked products includes suitable packaging techniques (such as modified atmospheres) [31] and the application of chemical conservatives. Currently, the protection of baked goods from fungal spoilage is mainly reached through the use of organic acids as inhibitors such as propionic, sorbic, acetic and benzoic acids and some of their salts [32]. Present trends in the bakery industry have included the desire for high-quality foods, which are minimally processed and do not contain chemical preservatives. For this reason, the level of additives have been reduced in the new EU regulations, allowing the concentrations of propionate, the most commonly used, up to 0.3% (wt/wt) for packaged sliced breads. However, fungal growth still occurs in these conditions, meaning that the food preservation is not guaranteed [33].

5.1.2 Novel strategies to fungal control

Consumer demands for more natural foods have stimulated the research on biological (i.e. vegetal and microbial) preservation systems. In this aspect, LAB are organisms of interest for biopreservation since they have been used for centuries in various fermented food, either by its natural presence in raw materials (spontaneous fermentation) or its addition as pure starter cultures. Recently, LAB have received scientific attention because of their antifungal potential since LAB strains from cereals with antifungal activity have been reported [34-36, 29]. However, the application of these antifungal LAB cultures in baked food is still limited despite of the advances on the characterization of antifungal metabolites (i.e. peptides, organic acids) regarding molecular weight, heat-resistance, spectrum of action and effectiveness.

Previously, the increased shelf-life of bakery products was attributed to the lactic and acetic acids produced by LAB during sourdough fermentation [37] (**Fig. 1**). Nowadays another bioactive compound produced during sourdough fermentation has also been recognized, such as the phenyllactic acid (derived from the phenylalanine metabolism) which is active against several fungal species isolated from bakery products, flour and cereals, including some mycotoxigenic species and bacterial contaminants [33, 29]. Dal Bello et al. [38] have showed that addition of *Lact. plantarum* strains inhibit the outgrowth of *Fusarium* spp. in wheat bread. The compounds responsible for the antifungal activity were characterized at the chemical level and were identified as lactic and phenyllactic acids; and two cyclic dipeptides cyclo (L-Leu–L-Pro) and cyclo (L-Phe–trans-4-OH-L-Pro). The combination of these antifungal strains using 20% sourdoughs into wheat bread formulations with 0.3 or 0.1 % calcium propionate (CP), showed strong synergistic effect, substantially increasing the shelf life of bread [39]. Also, Gerez et al [29] reported that the inclusion of three antifungal LAB allowed reducing the concentration of CP by 50% to attain a shelf-life similar to that of traditional bread containing 0.4% CP. This starter culture improves the fermentation quotient and the leaving volume of the dough. The LAB strains present in this starter have the ability to inhibit *Aspergillus, Fusarium,* and *Penicillium*, the main contaminants in bread. The most effective antifungal compounds were acetic and phenyllactic acids. Recently, Gerez et al [40] reported the use of a ready-to-use biopreservative starter for non-sliced packed bread using selected antifungal LAB (*Lact. plantarum* CRL 778) and low cost ingredients compatible with the food matrix. The combination of this starter with CP (0.4%) increased 2.6 times the shelf-life compared to breads prepared without LAB.

An interesting bio-strategy reported by Zhang et al [41], considers the production of propionate from lactate by *Lact. diolivorans* in co-fermentation with *Lact. buchneri* during sourdough fermentation. The application of this experimental sourdough (20%) in bread inhibited the growth of moulds for more than 12 days. Hence, the use of propionate-producing cultures could replace the addition of propionate as preservative. The CP can also be replace by a combination of antifungal LAB and a water-soluble extract of the vegetable *Phaseolus vulgaris* cv. Pinto [42]. Three proteins (Phaseolin alpha-type precursor, phaseolin and a lectin) were shown to be responsible of the antifungal activity of this extract.

The reduction of bread spoilage represents a demanding topic for the baker. Moreover, consumer demands for additive-free foods increases the need to seek for natural alternative preservation systems. The data obtained up to now put into evidence the suitability of selected LAB strains to be used as natural food-grade bio-control agents for reducing mould spoilage in bakery products and assuring their safety and quality.

6. Proteolysis during sourdough fermentation

The hydrolysis of the flour protein during dough fermentation is of crucial importance for bread quality. The sourdough fermentation results in an increase of amino acids concentrations, while in dough fermentation by yeast only, a decrease of these compounds was observed [43]. Recent studies elucidated the contributions of cereal and microbial enzymes to the proteolysis, peptide degradation and amino acid turnover during sourdough fermentation [44, 45]. The degradation of proteins observed in sourdough may be attributed to the proteolytic activity of LAB and active proteases of cereal flour under acidic conditions. Microbial acidification shifts the dough to pH 3.5–4.0, the optimum pH of the cereal proteinases which play an important role in the primary proteolysis of gluten. The released peptides are hydrolyzed to aminoacids (secondary proteolysis) by intracellular LAB peptidases. These metabolites may be further metabolized or accumulated in the dough (**Fig 1**).

The proteolytic system of LAB species commonly isolated from traditional sourdoughs, such as *Lact. sanfranciscensis, Lact. brevis and Lact. plantarum,* has been subjected to several studies [11, 21, 46, 47]. This system provides substrates (small peptides and free amino acids) for microbial conversion of amino acids to flavour precursor compounds [48, 49], as well as, compounds with interest from nutritional and technological point of views [50, 51]. Likewise, LAB might be used as a tool to hydrolyse pro-rich allergenic fragments in wheat-baked goods [3, 4, 52, 53].

6.1 Proteolysis impact in celiac disease

Cereal proteins are frequent causes of food allergies; celiac disease (CD) is one of the most common food intolerance problems that occur in 1 out of every 130-300 persons of the European and United States populations [54, 55] and 1 of each 100 persons from Argentine. CD is a chronic inflammatory disorder characterized by damage of the small intestinal mucosa caused by the gliadin fraction of wheat gluten and similar alcohol-soluble proteins (prolamins) of barley and rye in genetically susceptible subjects [56]. This disease, increasingly diagnosed throughout the world, can only be controlled by maintaining a strictly gluten-free diet. Rice, maize, sorghum, millet, buckwheat, amaranth, and quinoa are cereals and pseudocereals suitable for celiac patients, who often also suffer also from the lack of dietary fibre and non-efficient mineral absorption. Oat has slightly different prolamins (avenins) which has been recently approved as ingredient in gluten-free labeled products by EC (if the cross-contamination from wheat, barley and rye can be avoided and gluten content of the oat product remains <20 mg/kg). Patients with CD are not capable to consume some of the most common market products, namely breads, baked goods and other food products made with wheat flour [57].

Several fragments of the primary structure of α-gliadin are known to be allergenic, e.g., fragments 31–43, 62–75 and 57–89 (33-mer, the more significant in CD) have been identified. These fragments are difficult to hydrolyze because they contain high amounts of proline residues within their sequence. Since LAB have an active proteolytic system upon gluten [11], it has been proposed that some of the allergenic fragments could be detoxified by enhancing their hydrolysis during the food processing. The peptidase enzymes of LAB are able to reduce the pro-rich fragments of wheat-baked goods [4, 45, 58]. Nevertheless, the ability of LAB to hydrolyse α-gliadin fragments could not be correlated to an individual peptidase; it is unlikely that a single strain possesses the complete pattern of peptidases required for hydrolyzing all the latent peptides where Pro is implicated.

Different studies revealed the positive effect of the use of selected sourdough cultures to eliminate risks of contamination by gluten. De Angelis et al [59] reported that prolonged fermentation of dough by certain LAB would be a potential tool to decrease the risk of rye contamination in gluten-free products for celiac patients. Rizzello el al [45] indicated the use of a combination of sourdough LAB, selected for their peptidase systems, and active fungal proteases capable to hydrolyze wheat and rye flour proteins during prolonged liquid fermentation. The kinetics of the hydrolysis of the 33-mer by lactobacilli are highly efficient; through *in vitro* assays, the absence of toxicity of hydrolyzed wheat proteins were confirmed. Di Cagno et al [60] demonstrated that bread made with wheat flour hydrolyzed during processing showed to be non-toxic after its administration to CD patients during 60 days.

Germinated cereals or other proteases allow an extensive degradation of proteins in sourdoughs in fermentation protocols that may be used to develop new products for individuals with gluten intolerance [51]. However, the gluten exclusion results in very important problems for bakers, and at present many gluten-free products on the market are of low quality, exhibiting poor mouth feel and flavor. It has been reported that gluten-free breads have a tendency to show rapid staling and a weak aroma. These disadvantages could be overcome by the application of sourdough [24, 61].

Conclusions

The results presented in this review point out some advantages of using selected LAB strains as starter cultures for sourdough fermentation. The data collected so far suggest that these positive effects represent the new frontier for the production of natural high quality bread with less allergenic compounds and lengthen shelf-life.

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