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Lactic Acid Bacteria as Starter-Cultures for Cheese Processing: Past, Present and Future Developments

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1. Introduction

The identification of solutions to improve the life and health of consumers, providing safe and nutritious foods, is the major concern in Food Science. Toward that goal, preservation methods such as salting, drying, high/low temperature application, fermentation, and more recently, pulsed electric field, high pressure and radiation - alone or in combination – may be applied. The chosen method will depend on the type of raw materials, availability of the method, cost, effectiveness and degree of change it causes to the flavor and nutritional features of the food product. Fermentation, also called biopreservation, is a cheap, widely accessible method that meets today's increasing consumer's demand for minimally processed/preserved food products. Biopreservation with lactic acid bacteria (LAB) is indeed one of the oldest and highly efficient forms of non-thermal processing method. Cheese production is based on LAB ability to ferment sugars, especially glucose and galactose, so to produce lactic acid and aroma substances that give typical flavors and tastes to fermented products. LAB also release antimicrobial metabolites so called bacteriocins, which are considered safe and natural preservatives, with great potential to be used on their own, or synergistically with other methods in food preservation.

2. Lactic acid bacteria in dairy processing

Milk is a highly perishable food raw material, therefore, its transformation in cheese or other form of fermented dairy product, provides an ideal vehicle to preserve its valuable nutrients (Table 1), making them available throughout the year. It is known that while unprocessed milk can be stored for only a few hours at room temperatures, cheeses may reach a shelf-life up to 5 years (depending on variety).

Animal	Fat	Casein	Lactose	Albumin	Ash	Water
	%					
Cow	3.75	3.0	4.75	0.4	0.75	87.3
Goat	6.0	3.3	4.6	0.7	0.84	84.5
Ewe	9.0	4.6	4.7	1.1	1.0	79.6
Camel	3.0	3.5	5.5	1.7	1.5	84.8
Buffalo	6.0	3.8	4.5	0.7	0.75	85

Note: In cheese, these nutrients will appear at a concentration approximately ten times higher, while the water content decreases.

Table 1. Approximate composition of milk from various species of mammals

Fermentation with lactic acid bacteria (LAB) is a cheap and effective food preservation method that can be applied even in more rural/remote places, and leads to improvement in texture, flavor and nutritional value of many food products. LAB have a long and safe history of application and consumption namely in cheese processing (Aquilanti et al., 2006, Caplice & Fitzgerald, 1999, Giraffa et al., 2010, Ray, 1992; Wood, 1997; Wood & Holzapfel, 1995) thus being generally regarded as safe (GRAS). Increasing knowledge of LAB physiology, together with new developments in processing technology, is leading to their application beyond traditional starter culture application, namely in new food safety roles and direct health applications.

2.1. LAB as starter-cultures in cheese processing

Cheese-making is based on application of LAB in the form of defined or undefined starter cultures that are expected to cause a rapid acidification of milk through the production of lactic acid, with the consequent decrease in pH, thus affecting a number of aspects of the cheese manufacturing process and ultimately cheese composition and quality (Briggiler-Marco et al., 2007).

The earliest productions of cheeses were based on the spontaneous fermentation, resulting from the development of the microflora naturally present in the raw milk and its environment. The quality of the end product was a reflex of the microbial load and spectrum of the raw material. Spontaneous fermentation was later optimized through backslopping, i.e., inoculation of the raw material with a small quantity of whey from a previously performed successful fermentation, and the resulting product characteristics depended on the best-adapted strains dominance (Leroy & De Vuyest, 2004). Today, backslopping is still used to produce many artisanal raw-milk cheeses, namely those bearing the PDO (Protected Designation of Origin) status, which are considered to be an important source of LAB genetic diversity, as well as being crucial from an economic and even ecologic point of view, since production of said cheeses (usually processed on a small-scale) contributes to local employment and maintains people functioning as “guardians of local environment” in regions that otherwise would be deserted.

The starter-culture applied in this, so-called, natural fermentation, is usually a poorly-known microflora mix that although having a predominance of LAB, may also contain non-LAB microorganisms, and its microbial diversity and load is usually variable over time. In fact, studies directed to characterize traditional cheeses show that those made from raw milk harbor a diversity of LAB (Bernardeau et al., 2008) depending on geographical region, where a few may show particular interesting technological features that upon optimization may have industrial applications (Buckenhiiskes, 1993). For example, because wild strains need to withstand the competition of other microorganisms to survive in their hostile natural environment, they often produce antimicrobials substances called bacteriocins (Ayad et al., 2002), which are natural antibacterial proteins that can be incorporated directly into fermented foods as such (food-grade) or indirectly as starter culture (Bernardeau et al., 2008). Although nisin is today the only bacteriocin that reached commercial status, approved worldwide as a natural food preservative, many other bacteriocins may soon reach similar status. Recently, our work (to be published) with LAB isolates from traditional portuguese raw-milk cheeses, revealed several lactobacilli having antibacterial activity against pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella newport* and even *E.coli*. Future studies may allow us using these isolates or their metabolites, applied *in situ* or *ex situ* fashion, in applications where food safety is a concern.

Moreover, traditional cheeses also obtain their flavor intensity also from the non-starter lactic acid bacteria (NSLAB), which are not part of the normal starter flora but develop in the product, particularly during maturation, as a secondary flora (Beresford, et al., 2001). The isolation and optimization of wild-type strains from traditional products, to be used as starter cultures in cheese processing, is indeed a highly active field of research in Food Science today.

2.2. LAB food safety and cheese technology

Cheese is made in almost every country of the world and there are more than 2000 varieties, made from milk of several mammals, processed industrially or by traditional methods (Figure 1).

However, despite the large number of varieties, the basic steps required in any cheese processing are essentially the same, and slight variations in any of these steps may result in products of different general quality (Figure 2).

Milk treatment. In large-scale cheese processing, the milk is heat-treated, e.g. 73 °C for 15 seconds, to destroy pathogens and reduce microbial numbers, while in most traditional PDO raw-milk cheeses heat treatment is not applied. Also the milk may be standardized, i.e. the fat content may be increased or reduced, or the casein-to-fat ratio may be adjusted.

Starter-culture addition. The type of commercially available starter preparation to be used will be determined by the cheese recipe. As previously stated, large-scale processing relies on using defined, commercially available starters, while for traditional cheeses, a natural fermentation (whey from the previous lot) is often used.



Figure 1. (Top) - Brine salting of cheeses in a large-scale plant processing 20 tons of cheese a day. (Bottom) - Small-scale unit processing 50 Kg per day of a traditional PDO cheese

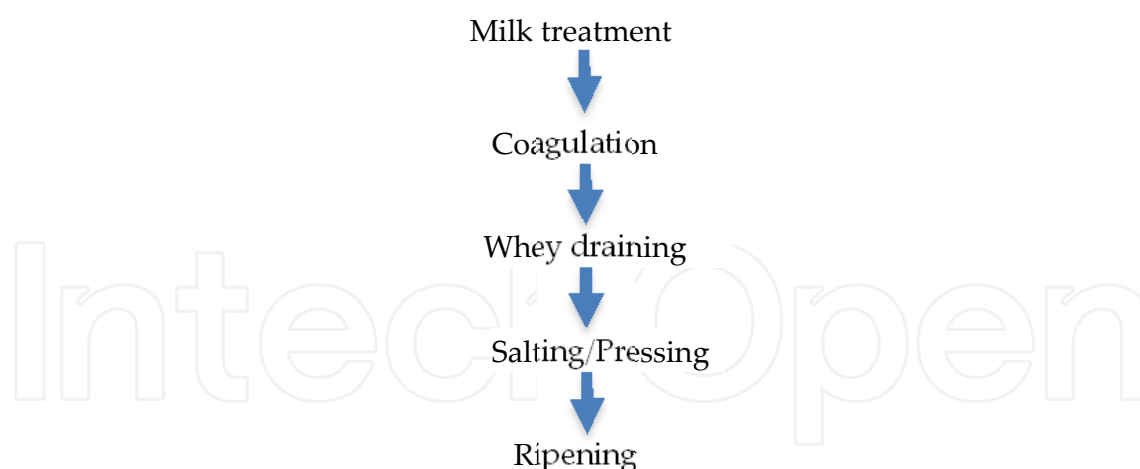


Figure 2. Common steps to most cheese making processes

Coagulation. During coagulation, modifications on the milk protein complex occur under defined conditions of temperature and by action of a coagulant agent, which changes the physical aspect of milk from liquid to a jelly-like mass. Various coagulants are available, e.g. lemon juice, plant rennet or more commonly a proteolytic enzyme such as chymosin (rennin) or – due to high demand from the cheese industry - proteolytic enzymes from the mould *Rhizomucor miehei* obtained via biotechnology. These enzymes have an acidic nature, meaning they have optimum activity in a slightly acidic environment. Therefore, the action of LAB in this phase is crucial as they are required to rapidly release enough lactic acid, to lower the milk pH from 6.7 to near 6.2, (thus creating an appropriate environment for optimum activity of rennin) and later to pH 4.5 as the processing proceeds, creating an inhospitable environment for many unwanted bacteria, thus increasing the end product safety.

Cutting the coagulum. The resulting coagulum may be cut with appropriate knives into curd particles of a defined size, e.g. 1–2 cm, or it may be transferred into containers or cheese moulds. The cutting or ladling of the coagulum is a very important step in the manufacture of some cheese varieties as it determines the rate of acid development and the body (firmness) and texture of the cheese.

Heating or cooking the curds. Heating (37–45 °C, depending on the type of cheese) the curds and whey affects the rate at which whey is expelled from the curd particles and the growth of the starter microorganisms. During heating, the curds and whey are often stirred to maintain the curd in the form of separate particles.

Whey removal. After heating and stirring, and when the curd particles have firmed and the correct acid development have taken place, the whey is removed allowing the curd particles to mat together.

Milling the curd. In cheeses such as Cheddar, when the curd has reached the desired texture, it is broken up into small pieces to enable it to be salted evenly. Milling the curd can be done either by hand or mechanically. Salting is usually done to enhance the taste of the curd and to increase its safety and shelf life.

Ripening. Finally, for most cheeses, the resulting mass is molded and put to ripening for periods that may vary from 15 days to one, two or more years. Ripening is a slow phase, crucial for the development of aroma and flavor, brought about by the action of the many enzymes released by LAB. During ripening the protein in cheese is broken down from casein to low molecular weight peptides and amino acids. Proteolysis is the major – and certainly the most complex of biochemical events that take place during ripening of most cheese varieties and LAB play an important role in it. This happens while the cheeses are stored in the curing cabinets and in some cases in caves, usually with temperature and humidity controlled (Figure 3).



Figure 3. Cheese ripening in cabinets with controlled temperature and humidity.

During coagulation, the initial step of casein hydrolysis is performed by chymosin (milk coagulant) and proteinases from starter lactic acid bacteria, starter moulds and other microorganisms. The further degradation of high molecular weight peptides produced at the initial step, is subsequently catalysed to low molecular weight peptides by endopeptidases from LAB during ripening (see Fig. 4 and 5).

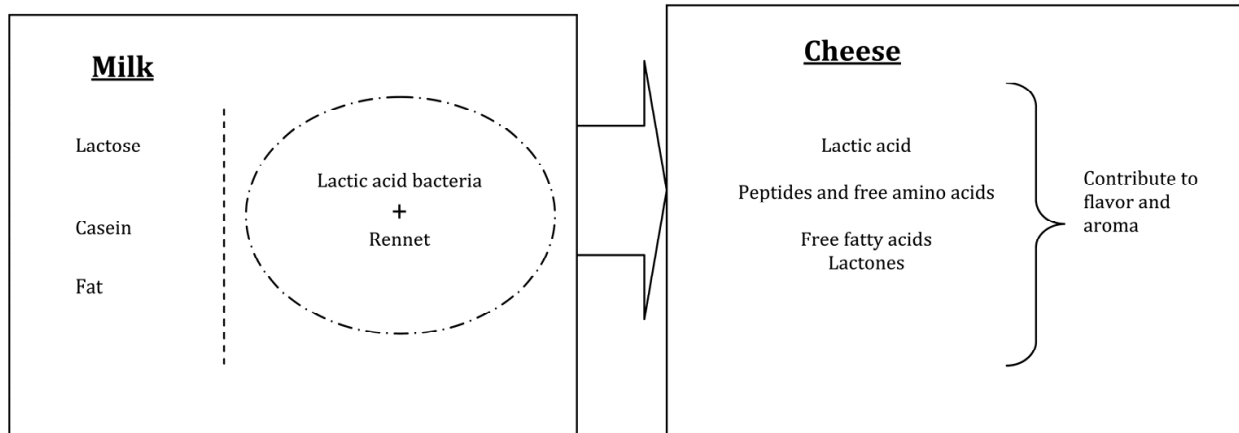


Figure 4. Simplified view of the biochemical changes that lead to texture and flavour changes in cheeses.

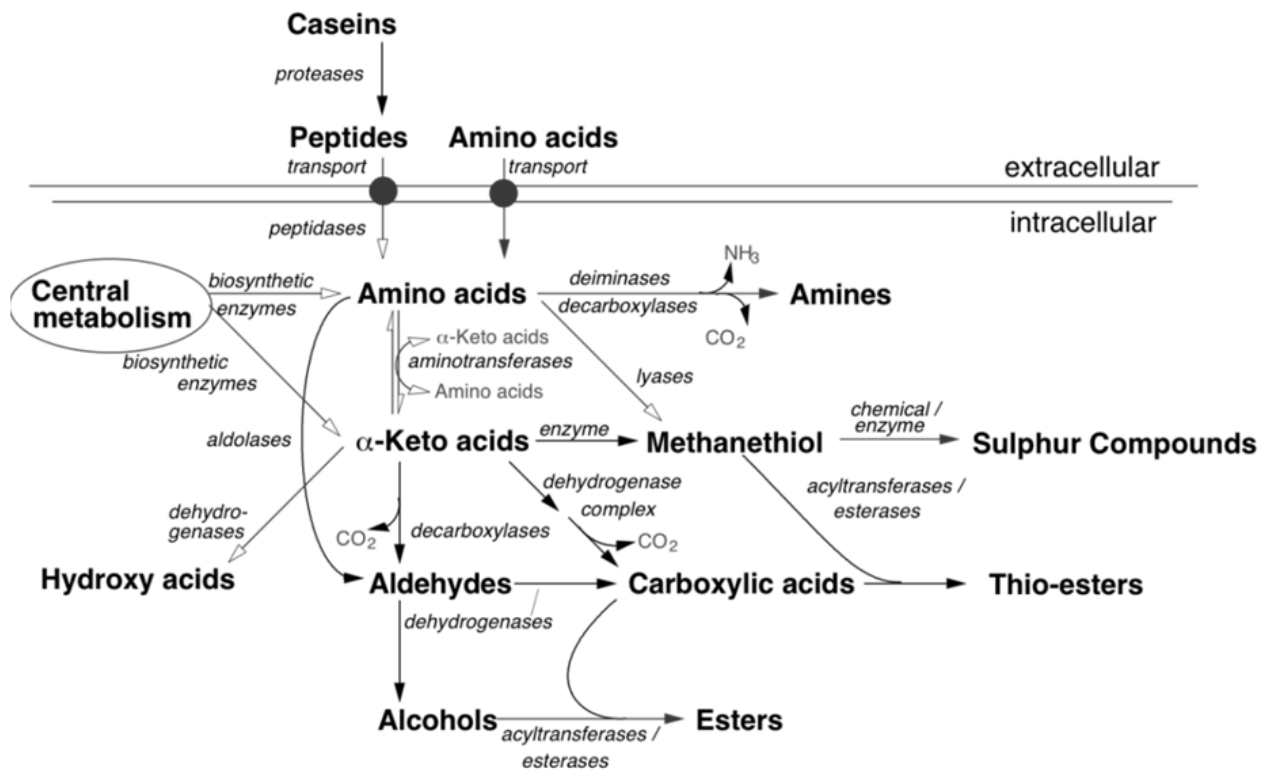


Figure 5. General pathways leading to intracellular metabolites, and their degradation routes to potential flavour compounds. More specifically, pathways from methionine to flavour compounds (methanethiol, thioesters, sulphur compounds) are shown (Adapted from Kranenburg et al., 2002).

Primary proteolysis in cheese is defined as changes in β -, γ -, α s-caseinpeptides, and other minor proteins that are detected by PAGE (Figure 6). Primary proteolysis leads to the

formation of large water-insoluble peptides and smaller water-soluble peptides (Fox, 1993, Mooney *et al.*, 1998). Secondary proteolysis products include those peptides, proteins and amino acids soluble in the aqueous phase of cheese and are extractable as the water-soluble nitrogen (WSN) fraction. The WSN fraction is a complex mixture of large, medium, and small peptides and amino acids. These components result from the action of milk clotting enzymes, milk proteases, starter LAB and contaminating microorganisms (Rank *et al.*, 1985).

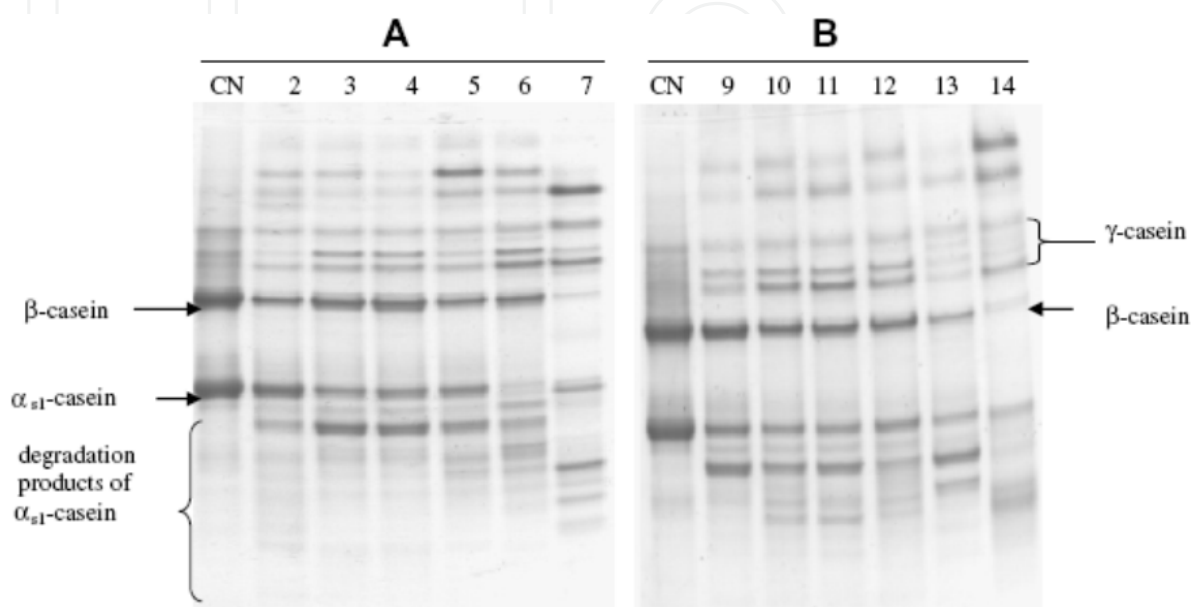


Figure 6. Evolution of proteolysis via urea-polyacrylamide gel electrophoresis in São Jorge cheeses from dairies A and B, by 1, 15, 30, 60, 90 or 130 days of ripening. Lanes 1, 8 and 15, Na- caseinate; lanes 2-6: cheese A; lanes 9-14: cheese B (Kongo *et al.*, 2012).

Typical cheese pH values measured at 3–7 days after manufacture are 4.9–5.5 in most firm and hard ripened varieties, and 4.4–4.8 in fresh lactic and most soft ripened varieties (Table 2 and Figures 7 and 8).

Operations	Swiss type		Gouda		Cheddar		Feta		Cottage	
	Time	pH	Time	pH	Time	pH	Time	pH	Time	pH
Add starter	0	6.60	0	6.60	0	6.60	0	6.60	0	6.60
Add rennet	15	6.60	35	6.55	30	6.55	75	6.50	60	6.50
Cut	45	6.55	70	6.50	75	6.50	115	6.4	300	4.80
Drain or dip into forms	150	6.35	100	6.45	195	6.3	130	NA	360	5.0
Milling	NA	NA	NA	NA	315	5.45	NA	NA	NA	NA
Pressing	165	6.35	130		390	5.40	NA	NA	NA	NA
Demoulding	16 h	5.30	8 h	5.40	10 h	5.20	24 h	4.6	NA	NA
Minimum pH	1 wk	5.20	1 wk	5.20	1 wk	5.10	1 wk	4.4	NA	NA
Retail	6 mo	5.6	6 mo	5.6	4 mo	5.3	6 wk	4.4	2–14 d	5.2

Table 2. Typical pH *vs* time profiles for several cheese varieties (time is in minutes unless otherwise noted).

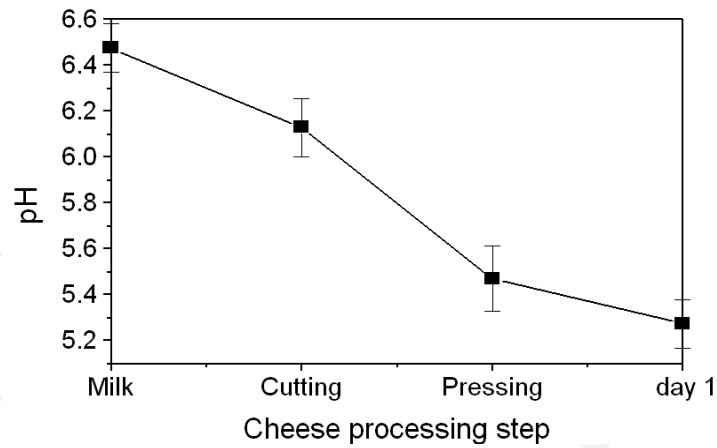


Figure 7. Evolution of pH (average ± standard deviation) in experimental cheese made with a starter culture of autochthonous São Jorge cheese LAB isolates.

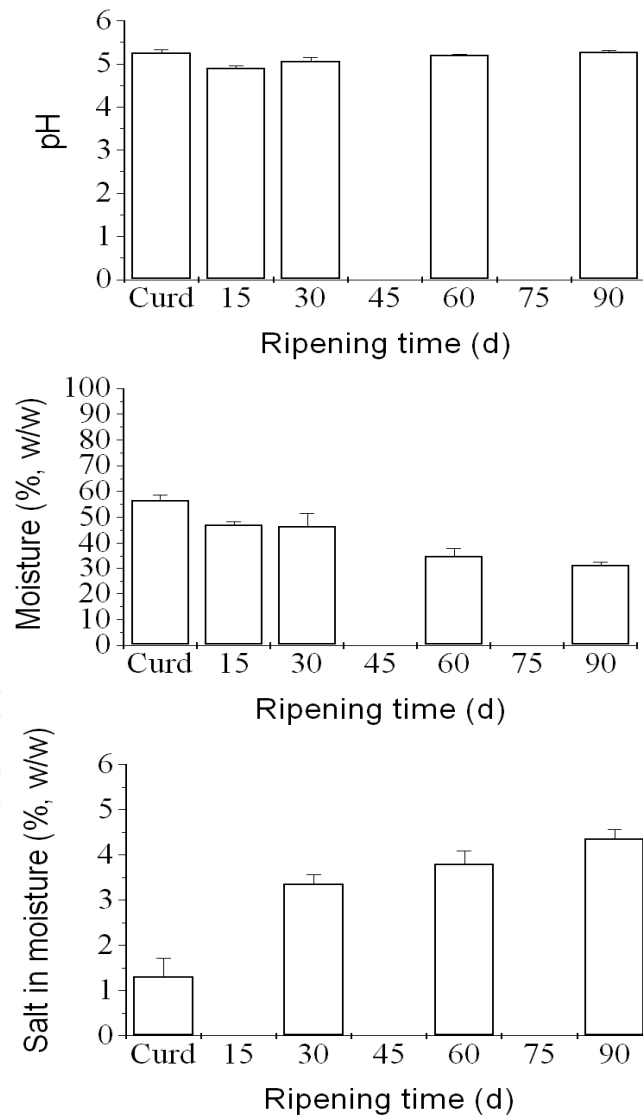


Figure 8. Evolution of physicochemical parameters (average ± standard deviation) throughout ripening of cheeses made with an experimental starter culture.

During processing, the pH history of the cheese is a good indicator of the actual product safety. For example a 'slow vat' allows more time at high pH for undesirable bacteria to grow, while during cheese ripening, unwanted bacteria may grow due to an acidity neutralization resulting from secondary microflora growth such as moulds. For most ripened varieties the combination of a low pH and ripening time, which leads to moisture decrease in the cheese, will in general cause a gradual decline of all groups of bacteria due to increasing inhospitable conditions inside the cheese.

The pH history of a cheese and the hygienic practices applied in its manufacture are thus key factors to guarantee safe products. Thus, the isolation of autochthonous LAB intend to be used for development of specific starter cultures with improved acid production and other antimicrobial activities may be an excellent way towards reaching the goals of simultaneously obtaining safe traditional cheeses, still bearing their unique flavors.

Nowadays, western consumers still enjoy artisan cheeses thanks to their outstanding gastronomic qualities; however, in most industrialized countries the large-scale cheese processing is the most important branch of the food industry. In such cases, there is a strong need to control the fermentation process towards maximum efficiency in terms of yields and standardization of the end product. This, and the need to fulfill the safety assurance of the final product, is usually achieved by, among other improvements, adding a high dosage of pure LAB selected starter cultures, commercially available (today's world starter culture market is more than US\$1 billion), as well as by heat treating the raw milk, most commonly by pasteurization.

3. Development of new starter cultures for cheese processing

Traditional raw-milk cheeses are highly valued for their flavors, while large-scale products are often perceived by the consumer as "boring" (Law, 2001) – a consequence of the elimination by pasteurization, of the flora that has a key role in flavor development; and this puts the food industry under pressure to look for alternative LAB cultures capable of improving products flavor (Leroy & De Vuyst, 2004).

Today, the increased understanding of the genomics and metabolomics of food microbes opens up new perspectives for starter-cultures improvements and through genetic engineering it is now possible to express their desirable properties or suppress undesirable features (Del- cour, De Vuyst, & Shortt, 1999; Law, 2001; Mogensen, 1993).

Originally, starter cultures for the cheese industry were maintained by daily propagation, and later, they became available as frozen concentrates and dried or lyophilised preparations, produced on an industrial scale, some of them allowing direct vat inoculation (Sandine, 1996). Because the original starter cultures were mixtures of several undefined microbes, the daily propagation, eventually led to shifts of the ecosystem resulting in the disappearance of certain strains. Because some important metabolic traits in LAB are plasmid-encoded, there was a risk that they would be lost during propagation (Weerkamp et al., 1996). Lactococci are generally used as starter cultures in the production of industrial

cheeses and cultured milk products. In traditional cheeses the natural starter cultures may harbor many different species and strains.

On the other hand, cheeses manufactured in a standard (large-scale) processing manner, are considered as safer because of the application of pasteurization and following the standard hygienic practices, including the HACCP. Traditional cheeses have their own specific processing methods, namely the common use of raw milk, however the hygienic procedures and HACCP approaches adapted to their specificities should be applied as well.

Species / subspecies	Main uses / Other comments
Lactococcus	Mesophilic starter used for many cheese types.
<i>Lc. lactis</i> subsp. <i>lactis</i>	Used in Gouda, Edam, sour cream and lactic butter. Mesophilic starter used for many cheese types.
<i>Lc. lactis</i> subsp. <i>lactis</i> biovar <i>diacetylactis</i>	
<i>Lc. lactis</i> subsp. <i>cremoris</i>	
Streptococcus	Thermophilic starter used for yogurt and many cheese types particularly hard and semi hard high-cook cheeses.
<i>Sc. thermophilus</i>	
Lactobacillus	
<i>Lb. acidophilus</i>	Probiotic adjunct culture used in cheese and yogurt.
<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i>	Thermophilic starter for yogurt. and many cheese types, particularly hard and semi hard high-cook cheeses.
<i>Lb. delbrueckii</i> subsp. <i>lactis</i>	Used in fermented milks and high-cook cheese.
<i>Lb. helveticus</i>	Thermophilic starter for fermented milks and many cheese types particularly hard and semi hard high-cook cheeses
<i>Lb. casei</i>	Cheese ripening adjunct culture.
<i>Lb. plantarum</i>	Cheese ripening adjunct culture.
<i>Lb. rhamnosus</i>	Cheese ripening adjunct culture.
Leuconostoc	Mesophilic culture used for Edam, Gouda, fresh cheese, lactic butter and sour cream.
<i>Ln. mesenteroides</i> subsp. <i>cremoris</i>	
Brevibacterium	Used in smear surface-ripened cheeses, Camembert, Stilton and Limburger and as a cheese ripening adjunct culture.
<i>Brev. linens</i>	
Propionibacterium	Used in Gruyère and Emmental cheeses.
<i>Prop. Acidipropionici</i>	Used in Gruyère and Emmental cheeses.
<i>Prop. freudenreichii</i> subsp. <i>shermanii</i>	

Table 3. Main bacteria associated with cheeses or other fermented products (From: Broome et al., 2003).

As previously stated, LAB are only a part of the complete microflora of raw milk (Kongo et al, 2007) and this, associated to other technological methods such as pressing, allows the production of a diversity of traditional cheeses (Parguel, 2011). This raw-milk microflora represents the contamination from the environment (air, utensils, the animal skin), and the load and its diversity will thus vary with local, season and livestock type, influenced by temperature.

These microbial mixes have an interdependent activity when together in their ecosystem and therefore their physiological properties may differ when the biodiversity is disrupted. In fact, it has been shown that certain microbial associations reveal a higher protecting effect against pathogens such as listeria, than when their association diversity is disrupted, (Montel 2010) see Figure 9.

Bacteriocinogenic probiotic bacteria could be beneficial when used as starter cultures in cheese, as they may prolong the shelf-life of the products, while simultaneously providing the consumer with a healthy advantage at a low cost (Gomes et al. 1998). The presence of bacteriocins in foods is, in general, seen as safe for consumers because bacteriocins are inactivated by pancreatic or gastric enzymes (Liu et al., 2011).

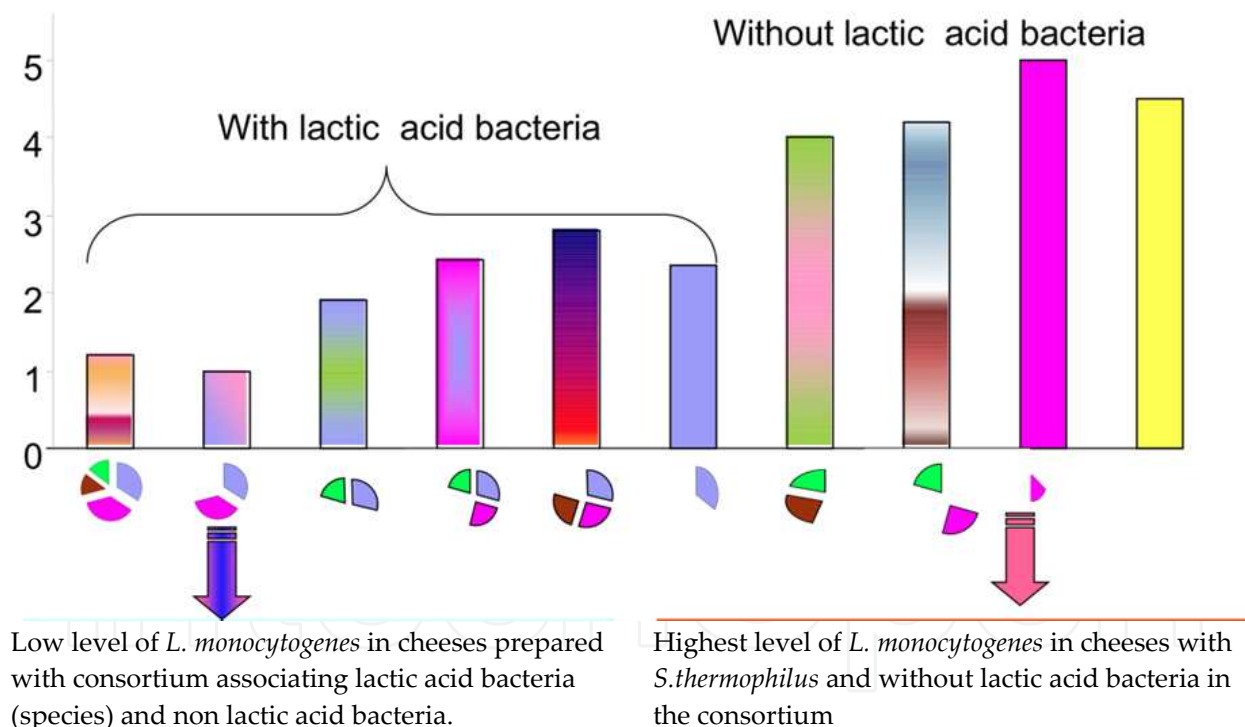


Figure 9. Level of *L. monocytogenes* in the core of Saint-Nectaire type cheese (28d) (Adapted from Montel & Samelis, (2010).

3.1. EPS-producing cultures and acceleration of cheese ripening

Many LAB produce exopolysaccharides (EPS), which may provide viscosifying, stabilizing, and water-binding effects in cheeses. The growing demand for all-natural, healthy food products, foods with low fat or sugar content and low levels of additives, as well as cost

factors has increased the interest of food industry to use LAB polysaccharides. Research has also shown that EPS+ LAB can enhance the functional properties of low fat cheese and that the excellent water-binding properties and moisture retention of EPS can improve the melting properties of low fat Mozzarella cheese. These properties show that EPS have wide technical potentials for development of novel and improved food products with enhanced texture, mouth-feel, taste perception and stability, representing potential sources for economic gains for the dairy industry.

EPS have also the potential to be used as surface carriers of bacteriocins or bacteriocin-producing LAB, and species such as *Leuconostoc mesenteroides*, *Streptococcus mutans* and several lactobacilli (*Lactobacillus brevis*, *Lactococcus lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *Lactobacillus casei*, *Lb. sake*, *Lb. rhamnosus*,) and thermophilic (*Lb. acidophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. helveticus* and *S. thermophilus*) are known to produce EPS. The isolation and characterization of EPS from new wild LAB species, which are ubiquitous in traditional cheeses, is a key strategy towards finding strains with optimized production of EPS.

Finally, cheese ripening is a lengthy and costly process. Therefore, attenuated starter cultures with high autolysis are being sought towards increasing the amount of endogenous peptides, thus accelerating the cheese ageing process as well as enhancing flavour and texture. These cultures may be obtained via application of several techniques such as pulsed electric field, heat treatment, freeze–thawing and lysozyme treatment (Briggs, 2003).

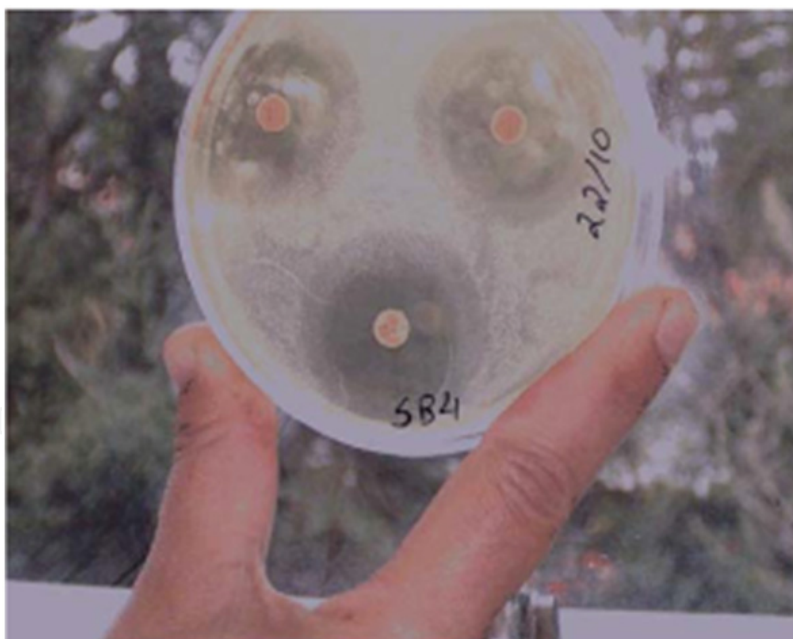


Figure 10. Antilisterial activity of LAB isolates from a traditional cheese.

Thus, the cheese industry is looking for new types of LAB starter-cultures bearing several properties: – cultures that increase microbial safety or offer one or more organoleptic, technological, nutritional (enzymes, or polyunsaturated fatty acids - PUFAs) or health advantages such as probiotic properties, starter cultures with increased resistance to bacteriophage, (recall that high product loss, especially in cheese manufacturing, is often

associated with bacteriophages (Parente and Cogan, 2004), cultures that produce EPS and cultures that accelerate cheese ripening.

3.2. Methods used to characterize LAB for starter cultures development

To characterize new LAB isolates, phenotypic methods relying on physiological or biochemical criteria have been widely applied (Montel, Talon, Fournaud, & Champomier, 1991, Kongo et al., 2007). These phenotypic profiling methods are very important - especially related to finding the technological features, such as the acidification, proteolytic and lipolytic activity, of a new isolate (see Tables 3 and 4, and Figure 11) and have the advantage of requiring less sophisticated equipment. In most of the cases however, these tests are insufficient for accurate species identification due to the great number of different LAB species with similar phenotypic characteristics (Temmerman et al. 2004).

Characteristics	<i>Lactobacillus</i>	<i>Enterococcus</i>	<i>Lactococcus</i>	<i>Leuconostoc</i>	<i>Pediococcus</i>	<i>Streptococcus</i>
CO ₂ from glucose	+/-	-	-	+	-	-
Growth at 10 °C	+/-	+	+	+	+/-	-
Growth at 45 °C	+/-	+	-	-	+/	+/-
Growth at 6.5% NaCl	+/-	+	-	+/-	+/	-
Growth at 18% NaCl	-	-	-	-	-	-
Growth at pH 4.4.	+/-	+	+/-	+/-	+	-
Growth at pH 9.6	-	+	-	-	-	-
Type of lactic acid	D, L, DL	L	L	D	L, DL	L

Table 4. Phenotypic characteristics for discrimination of common LAB for dairy processing (modified from Batt, 1995).

Enzyme	Genus					
	<i>Lactobacillus</i>			<i>Enterococcus</i>		
	Per cent of strains giving indicated reaction*			Per cent of strains giving indicated reaction*		
	-	+	++	-	+	++
Alkaline phosphatase	100			100		
Esterase	100			100		
Lipase	100				25	75
Leucine aminopeptidase	12	88				100
Cystine aminopeptidase	100				100	
α -Chymotrypsin	100				100	
Acid-phosphatase	88	24		15	75	
Phosphoamidase		100			100	
β -Galactosidase		100			50	50
β -Glucuronidase		100			25	75
α -Glucosidase	100			100		
β -Glucosaminidase	100			100		

*API-ZYM color: grade 0, negative (-); grades 1 through 3, positive (+); grades 4 and 5, plus positive (++)

Table 5. Enzyme profiling of 14 representative LAB isolates found in Sao Jorge traditional cheese (from Kongo et al., 2007).

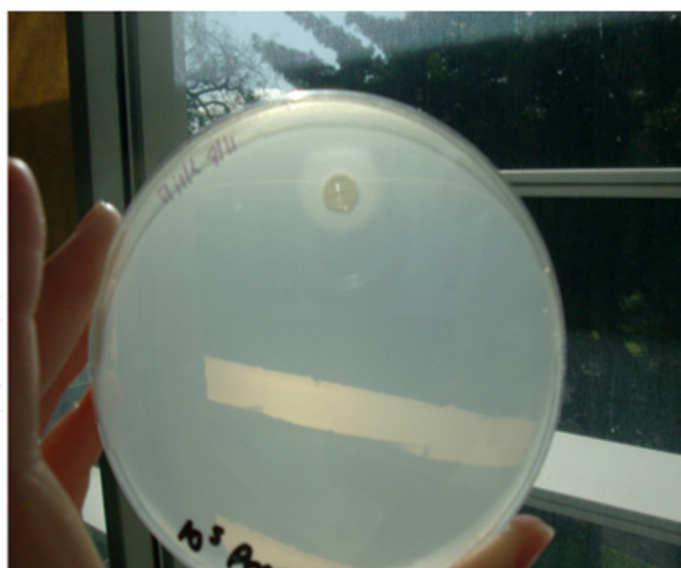


Figure 11. Proteolytic activity of a *Lactobacillus* ssp isolate on milk agar.

Molecular biology (genotypic) methods (Figure 12) on the other hand - largely DNA-based techniques - offer much greater discriminatory power, all the way to differentiation of individual strains (Aymerich et al., 2006, Cocolin et al., 2004, Furet et al., 2004, Prabhakar et al., 2011). Thus, a combination of both phenotypic and genotypic identification techniques (so called polyphasic approach) is preferred (Temmerman et al., 2004, Aquilanti et al., 2006).

Num/	Label/	DuPont ID/	DuPont ID	Label	RiboPrint(R) Pattern
1	Q1A	DUP-18182	<i>Lactobacillus</i>	<i>paracasei</i> ss. <i>paracasei</i>	
2	Q2A	DUP-14061	<i>Lactobacillus</i>	<i>plantarum</i>	
3	Q3A	DUP-13076	<i>Lactobacillus</i>	<i>paracasei</i> ss. <i>paracasei</i>	
4	Q1B	DUP-16545	<i>Enterococcus</i>	<i>faecalis</i>	
5	Q1C	DUP-13077	<i>Lactobacillus</i>	<i>paracasei</i> ss. <i>paracasei</i>	
6	Q2B	DUP-18014	<i>Lactobacillus</i>	<i>paracasei</i> ss. <i>paracasei</i>	
7	Q3B	DUP-18182	<i>Lactobacillus</i>	<i>paracasei</i> ss. <i>paracasei</i>	
8	Q3C	<None>	<None>	<None>	

Figure 12. Ribotyping as molecular biology technique for identification of LAB to type or strain level. (Kongo et al., 2007)

Finally, it should be mentioned that there are concerns today that commensal bacterial populations from food and the gastrointestinal tract (GIT) of humans and animals, such as LAB, could act as a reservoir for antibiotic resistance genes, and therefore, be transferred to possibly pathogenic bacterial species, complicating the treatment of a disease or infection and leading to the spread of antibiotic-resistant bacteria (Ammor et al., 2007). Thus, before using new isolates as starter cultures or as probiotics, the antibiotic resistance must be addressed.

The European Food Safety Agency (EFSA) proposed a system for a pre-market safety assessment of selected groups of microorganisms, leading to granting a “Qualified Presumption of Safety (QPS)”. Therefore, EFSA proposed that a safety assessment of a

defined taxonomic group, such as a genus or group of related species could be made based on establishing identity, body of knowledge, possible pathogenicity and end use (European Commission 2007). The 33 *Lactobacillus* species shown in Table 6 are the ones that in 2007 EFSA stated could be considered to have QPS-status. In addition to *Lactobacillus* species, also other LAB species have been granted QPS –status. They include three leuconostocs, (*Ln. citreum*, *Ln. lactis* and *Ln. mesenteroides*), three pediococci (*P. acidilactici*, *P. dextrinicus* and *P. pentosaceus*), *Lc. lactis* and *Streptococcus thermophilus*.

<i>Lb acidophilus</i>	<i>Lb farciminis</i>	<i>Lb paracasei</i>
<i>Lb amylolyticus</i>	<i>Lb fermentum</i>	<i>Lb paraplantarum</i>
<i>Lb amylovorus</i>	<i>Lb gallinarum</i>	<i>Lb pentosus</i>
<i>Lb alimentarius</i>	<i>Lb gasserii</i>	<i>Lb plantarum</i>
<i>Lb aviaries</i>	<i>Lb heveticus</i>	<i>Lb pontis</i>
<i>Lb brevis</i>	<i>Lb hilgardii</i>	<i>Lb reuteri</i>
<i>Lb buchneri</i>	<i>Lb johnsonii</i>	<i>Lb rhamnosus</i>
<i>Lb casei</i>	<i>Lb kefiranoferiens</i>	<i>Lb sakei</i>
<i>Lb crispatus</i>	<i>Lb kefirii</i>	<i>Lb salivarius</i>
<i>Lb curvatus</i>	<i>Lb mucosae</i>	<i>Lb sanfranciscensis</i>
<i>Lb delbrueckii</i>	<i>Lb panis</i>	<i>Lb zeae</i>

Table 6. *Lactobacillus* (Lb) species with QPS- status according to EFSA (from Korhonen, 2010).

Lactobacilli are generally susceptible to antibiotics inhibiting the synthesis of proteins, such as chloramphenicol, erythromycin, clindamycin and tetracycline, and more resistant to aminoglycosides (neomycin, kanamycin, streptomycin and gentamicin. While some species show a high level of resistance to glycopeptides (vancomycin and teicoplanin), susceptibility to bacitracin will vary greatly (Ammor et al, 2007; Coppola et al., 2005).

Antibiotic	Species							
	Lactobacillus obligate homofermentative	Lactobacillus heterofermentative	Lactobacillus plantarum	Enterococcus spp	Pediococcus pp	Leuconostoc	Lactococcus lactis	Streptococcus thermophilus
Ampicillin	4	4	4	8	4	4	4	4
Vancomycin	4	IR	IR	8	IR	IR	4	4
Gentamycin	8	8	64	512	4	4	8	8
Kanamycin	16	16	64	1024	4	8	8	8
Streptomycin	16	16	64	1024	4	8	16	16
Neomycin	16	16	32	1024	8	8	8	8
Erythromycin	4	4	4	4	4	4	4	4
Clindamycin	4	4	4	4	4	4	4	4
Tetracycline	8	8	32	16	4	4	4	4
Chloranphenicol	4	4	8	8	4	4	8	8
Trimethoprim	8	8	8	8	8	8	n.r	n.r

Key: IR, intrinsically resistant

Table 7. Microbiological break points ($\mu\text{g mL}^{-1}$) categorizing some LAB species as resistant (Adapted from Ammor et al., 2007)

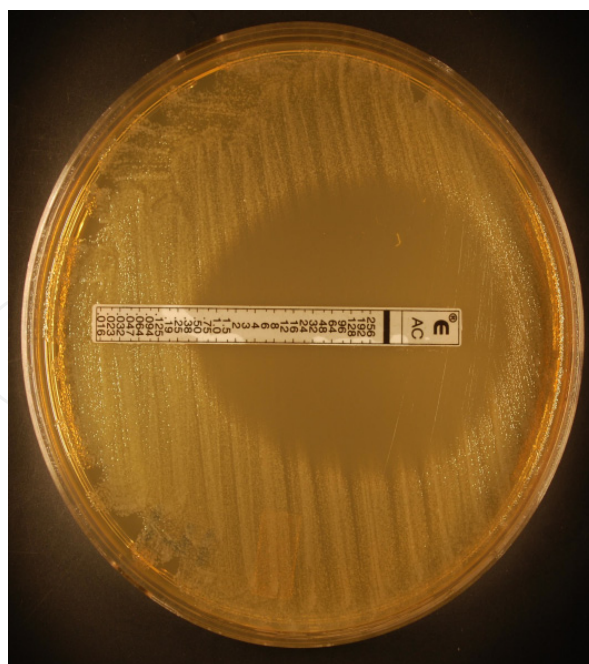


Figure 13. Result of a screening for antibiotic resistance of a *Lactobacillus paracasei* isolate

4. Concluding remarks

LAB are important in cheese processing because (i) they increase food safety through the release of lactic acid and bacteriocins, (ii) produce aromas and flavor and accelerate the maturation process of cheese via their proteolytic and lipolytic activities, bringing economic advantages to the industry, (iii) bring about desirable food textures via release of polysaccharides that increase the viscosity and firmness, and reduce susceptibility to syneresis, (iv) they may be used to deliver polyunsaturated fatty acids (PUFA) and vitamins, leading to dairy products with increased nutritional value, (v) specific probiotic strains contribute to liberation of health-enhancing bioactive peptides improving absorption in the intestinal tract, stimulating the immune system, exerting antihypertensive, antithrombotic effects, or functioning as carriers for minerals.

Novel insights arising from use of Bioinformatics, Systems Biology and Bioengineering approaches will offer perspectives for the application of a new generation of starter cultures for cheese-making, having enhanced functional features and offering several health, marketing, and technological advantages, contributing to the development of small and medium sized enterprises on the one hand, and product diversification of large companies on the other.

However, there are still many developments to be achieved towards fully realizing the many foreseen potential of LAB or their products. For example extraction and purification of bacteriocins is still difficult as they form micelles or clumps with the nitrogen sources already in the growth medium. On the other hand while genetic engineering may offer many solutions related to optimal use of LAB, they may not be easily allowed by food legislation.

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