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Review

Non-starter lactic acid bacteria used to improve cheese quality and provide health benefits

Luca Settanni*, Giancarlo Moschetti

SENFIMIZO Department, Section of Phytopathology and Agricultural Microbiology, University of Palermo, Viale delle Scienze 4, 90128 Palermo, Italy

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A B S T R A C T

Non-starter lactic acid bacteria (NSLAB) dominate cheese microbiota during ripening. They tolerate the hostile environment well and strongly influence the biochemistry of curd maturation, contributing to the development of the final characteristics of cheese. Several NSLAB are selected on the basis of their health benefits (enhancement of intestinal probiosis, production of bioactive peptides, generation of gamma-aminobutyric acid and inactivation of antigenotoxins) and are employed in cheese-making. This review describes the ecology of NSLAB, and focuses on their application as adjunct cultures, in order to drive the ripening process and promote health advantages. The scopes of future directions of research are summarised.

1. Introduction

Lactic acid bacteria (LAB) constitute a heterogeneous group of genera which share many physiological features. LAB owe their designation to their capacity to ferment sugars primarily into lactic acid via homo- or heterofermentative metabolism (Salminen and von Wright, 1998). LAB are characterized by being Gram-positive, catalase-negative, non-spore-forming, low G + C content and facultative anaerobic (Kandler and Weiss, 1986). LAB are also usually known to be non-motile organisms, with the exception of *Lactobacillus agilis* and the recently described species *Lactobacillus ghanensis* (Nielsen et al., 2007) and *Lactobacillus capillatus* (Chao et al., 2008). The heterogeneity of this bacterial group is well expressed in their morphological traits, since they may appear rod or coccal shaped, in single cells or couple, tetrads and short or long chains. Due to the limited biosynthetic abilities (Klaenhammer et al., 2005) and their high requirements in terms of carbon and nitrogen sources (Salminen and von Wright, 1998), the natural habitat of LAB is represented by nutritionally rich environments; they are generally associated with plant and animal raw materials, fermented food products, animal skin and mucous membranes.

Thanks to some of their metabolic properties, LAB are generally employed because they significantly contribute to the flavour, texture, nutritional value and microbial safety of fermented foods (Caplice and Fitzgerald, 1999; Settanni and Corsetti, 2008). For these reasons, LAB find numerous industrial applications; the most commonly selected genera belong to *Lactococcus* (milk), *Lactobacillus* (milk, meat, vegetables, cereals), *Leuconostoc* (vegetables, milk), *Pediococcus* (vegetables, meat), *Oenococcus* (wine), and *Streptococcus* (milk) (De Vuyst and Tsakalidou, 2008). LAB also provide competitiveness against pathogenic bacteria colonizing the gastrointestinal tracts (Corfield et al., 2001), thus, several food applications depend on their probiotic effects.

Among food fermentations, milk-based productions are common to many societies, and, nowadays, they are being produced even in South-eastern Asian countries that have not been traditional cheese and fermented milk consumers. Cheeses may be classified following different criteria that also include the type of microorganisms involved in the ripening process. Among the different schemes of cheese classification, one of the most recent (Mucchetti and Neviani, 2006) considers the distinction of the following raw materials and inocula: pasteurized milk and selected starters; pasteurized milk and natural starters; thermal treated

* Corresponding author. Tel.: +39 091 23896043; fax: +39 091 7028855.
E-mail address: luca.settanni@unipa.it (L. Settanni).

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milk and natural starters; raw milk and selected starters; raw milk and natural starters; raw milk without starters.

Cheese during ripening is a hostile environment, typically characterized by the presence of salt, low moisture, 4.9–5.3 pH value, low temperatures, and a deficiency of nutrients (Turner et al., 1986). The difficult conditions are inhibiting towards many microbial groups, except some LAB species which are able to tolerate the environment of cheese (Peterson and Marshall, 1990) and may exert several beneficial effects.

This paper reviews the current knowledge about LAB employed as non-starter cultures for their role in cheese ripening, with a major emphasis on their contribution to generate health benefits associated with cheese consumption.

2. LAB and their role in cheese-making

Milk is a nutritionally rich medium where all microbial groups, generally associated with food matrices, may be found: pathogenic, spoilage and useful microorganisms. Within the last group, LAB are commonly isolated at significant concentrations (Franciosi et al., 2009a). They are naturally present in milk as contaminants by the udder surface, milking equipment, stable environment and/or during transport and filling operations, storage surfaces, and dairy factory environment (Eneroth et al., 1998; Mc Phee and Griffiths, 2002). Several species belonging to *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* genera are often recognised from raw milk (Franciosi et al., 2009a; Wouters et al., 2002).

LAB may play different roles in cheese-making: some species participate in the fermentation process, whereas some others are implicated in the maturation of cheese. In the first case, LAB rapidly ferment lactose producing high concentrations of lactic acid and are designated as starter LAB (SLAB), while LAB responsible for the ripening process are indicated as non-starter LAB (NSLAB). The group of SLAB mainly includes *Lactococcus lactis* and *Leuconostoc* spp. among mesophilic species and *Streptococcus thermophilus*, *Lactobacillus delbrueckii* and *Lactobacillus helveticus* among thermophilic species (Fox et al., 2004). The group of NSLAB is particularly heterogeneous with lactobacilli being mostly represented: *Lactobacillus farciminis* among obligately homofermentative species, *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus plantarum*, *Lactobacillus pentosus*, *Lactobacillus curvatus* and *Lactobacillus rhamnosus* among facultatively heterofermentative species and *Lactobacillus fermentum*, *Lactobacillus buchneri*, *Lactobacillus parabuchneri* and *Lactobacillus brevis* among obligately heterofermentative species (Gobbetti et al., 2002; Coeuret et al., 2004; Švec et al., 2005). The non-*Lactobacillus* species of NSLAB commonly isolated during cheese ripening are *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Enterococcus durans*, *Enterococcus faecalis*, *Enterococcus faecium* and also leuconostocs with the same species that act as starter cultures (Chamba and Irlinger, 2004).

Evolution of SLAB and NSLAB in raw milk cheese follows a general dynamic (Fig. 1). SLAB are high in number (about 10^8–10^9 cfu/g) at the beginning of ripening and decrease regularly by two or more log cycles during ageing (Beuvier and Buchin, 2004; Franciosi et al., 2008). On the contrary, NSLAB are present at low concentrations after pressing which, however, may increase of about four – five orders of magnitude within a few months (Fox et al., 2004).

The presence of adventitious NSLAB introduces variability into the ripening process that cannot be easily controlled by the cheese-maker, thus, several productions may be subjected to fluctuations in the final characteristics (Franciosi et al., 2008): inter-factory differences (Antonsson et al., 2003; De Angelis et al., 2001), but also differences between cheeses produced at the same factory on different days and between cheeses from different vats of the same day were registered (Fitzsimons et al., 1999; Williams et al., 2002). For all these reasons, the dominance of wanted NSLAB strains, is crucial to minimize microbial variability during the ripening process.

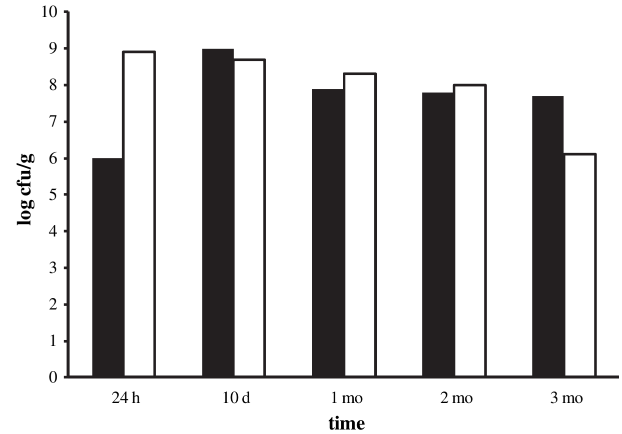

Fig. 1. Evolution of SLAB (empty columns) and NSLAB (full columns) during ripening of Puzzone di Moena cheese. Data from Franciosi et al. (2008).

NSLAB are traditionally selected as a means of determining the organoleptic characteristics of the final cheese (Beresford and Williams, 2004). However, other characteristics are being considered during the selection process, in particular their “health” properties.

3. Contribution of NSLAB to cheese ripening

After curd formation, enzymatic transformations targeting different constituents of curd take place. A cheese matrix may be left ageing for variable periods, stored under temperature and humidity controlled regimes. The enzymes that participate in the ripening phenomenon may be originated from milk, rennet and microorganisms. Milk enzymes, mainly plasmin and acid protease acting on β- and αS1-casein, respectively, find unfavourable pH, whereas rennet enzymes are inactivated during curd cooking and inhibited by salt. Hence, a basic role in the transformation of curd in cheese is played by microbial enzymes released by SLAB, NSLAB and/or other microorganisms naturally present in milk or added by the cheese-maker (McSweeney, 2004a; Upadhyay et al., 2004), as well as those released by microorganisms that contaminate cheese products during ripening through salt, equipment, and storing environment. For this reason, it is possible to modify or accelerate cheese ripening by increasing the level of these enzymes in cheese curd (Upadhyay et al., 2007).

Proteolysis in cheese is of paramount importance for its ecology and, especially, for the final texture and flavour. Besides residual lactose, present at very low concentrations, NSLAB utilize carbohydrates deriving from glycomacropeptides of caseins and glycoproteins deriving from fat globule membranes; heterofermentative NSLAB may ferment pentoses liberated from SLAB lysis. Also residual citrate is present at low levels and fats do not represent relevant nutritional sources. Thus, peptides and aminoacids, present at high concentrations, constitute the main nutritional compounds for NSLAB (Fox et al., 2004).

Cheese flavour is an outcome of the action of several enzymes. In general, LAB possess a complex proteolytic enzymatic system, because of their requirements of aminoacids (McSweeney, 2004b). They play a defining role in the degradation of casein and peptides, leading to the production of free aminoacids (FAA). The aminoacids thus produced contribute directly to the basic taste of the cheese and indirectly to cheese flavour, since they are precursors for the other catabolic reactions, giving rise to volatile aroma compounds. It is worth nothing that the products of amino acid conversion make a greater contribution to flavour than the aminoacids themselves (Fox and Wallace, 1997). Catabolic reactions and side-chain modification may yield keto acids, ammonia, amines, aldehydes, acids, and alcohols, which are essential contributors to cheese taste and aroma (Hemme et al., 1981). NSLAB possess a wide range of hydrolytic enzymes and therefore have the potential to contribute to cheese maturation (Williams and Banks, 1997). NSLAB physiology has mainly been studied with regards to lactobacilli. The addition of selected lactobacilli to the source milk for cheese determines higher FAA levels in cheese, accompanied by an increased intensity in flavour (Lane and Fox, 1996; Ur-Rehman et al., 2000); Mauriello et al. (2001) stated that mesophilic lactobacilli produce a higher number of substances than lactococci.

With this in mind, several research groups have evaluated different NSLAB strains, alone or in combination with SLAB, in order to enrich the sensory characteristics of cheese. Awad et al. (2007) tested SLAB with or without NSLAB, isolated from Egyptian dairy products, to produce experimental Ras cheeses from pasteurized milk. The highest overall score of flavour intensity, flavour and texture acceptability were obtained in the presence of adjunct cultures of *Lb. helveticus*, *Lb. paracasei* subsp. *paracasei*, *Lb. delbrueckii* subsp. *lactis* and *E. faecium*. The selected strains determined the accumulation of significantly higher amounts of FAA and free fatty acids (FFA), which both contribute directly or indirectly to Ras cheese flavour. The addition of *Lb. plantarum* to the starter culture preparation determined similar effects in Manchego cheese, increasing the content of FAA with respect to cheeses obtained with commercial starter culture alone (Poveda et al., 2004), while *Lactobacillus reuteri* influenced the volatile FFA production in reduced-fat Edam cheese (Tungjaroenchai et al., 2004). A strain of *Lb. casei* subsp. *rhamnosus*, together with *Lc. lactis* was also proved to be effective in enhancing proteolysis during ripening of low-fat Kefalograviera-type cheese (Michaelidou et al., 2003). Other NSLAB, either alone (*Lb. paracasei*) or in conjunction with one another (*Lb. paracasei* plus *Lb. plantarum*), have shown a good implantation in cheese, and have been proven to have a synergic interaction with lactococci (Ortigosa et al., 2006). However, the effect of a given NSLAB may be variable depending on the type of starter used (Skeie et al., 2008).

Regarding aroma perception, the application of *Lb. casei* subsp. *casei* as secondary adjunct cultures together with a hygienised rennet paste led to a rapid development of piquant flavour that is typical of Majorero cheese (Calvo et al., 2007). Randazzo et al. (2008) evaluated the effect of wild NSLAB strains, including *E. durans*, *Lb. rhamnosus*, and *Lb. casei*, on the volatile compounds of Pecorino Siciliano cheese. The complexity of volatile fractions was higher for experimental cheeses than control cheese processed without culture addition. Wild strains, individually or mixed with commercial strains, were able to produce 3-methil-1butanol and several ethyl esters, which are associated to typical flavours such as floral, orange and fruity notes.

Lb. paracasei subsp. *paracasei* was also tested in combination with different yeasts (*Yarrowia lipolytica* and *Debaryomyces hansenii*) to evaluate their contribution to the aroma profile of Feta cheese (Bintsis and Robinson, 2004). In the case of the last cheese, the use of yeasts is important, since Feta cheese is characterized by high salt concentration, and only a few NSLAB species may survive during ripening, in particular, *Lb. paracasei* and *Lb. plantarum* (Bintsis et al., 2000).

4. Protective effects of NSLAB

"Protective cultures" are considered to be microorganisms conferring additional safety factors which result in the microbiological stability of foods, thus reducing the risk of the growth and survival of food-borne pathogens and food spoilage organisms (Holzapfel et al., 1995). Growth of LAB itself determines inhibition of undesired microorganisms. However, their ability to produce bacteriocins is of basic importance in strategies of biological preservation of foods, namely "biopreservation" which refers to the extension of the shelf-life and improvement of the safety of foods using microorganisms and/or their metabolites (Ross et al., 2002a). In this regard, bacteriocin production plays a major role. Fundamentally, the success of any biopreservation strategy based on bacteriocins depends on the *in situ* antimicrobial efficacy (Settanni and Corsetti, 2008).

Franciosi et al. (2009b) found bacteriocin producing *E. faecalis* strains persisting longer than other NSLAB strains during Puzzone di Moena cheese ripening. This observation demonstrated that, in cheese, bacteriocin production confers a competitive advantage. Thus, this characteristic may have substantial consequences for the efficacy of NSLAB to be used as protective cultures. Although several reviews have dealt with the employment of bacteriocinogenic SLAB in cheese environments, mainly focussing on the production of nisin or other lactococcal bacteriocins (Chen and Hoover, 2003; Cotter et al., 2005; Guinane et al., 2005), only one article (Grattepanche et al., 2008) reviewed the application of a bacteriocin producing NSLAB in cheese tested by Nuñez et al. (1997).

Since then, some research papers have been aimed at studying the effect of bacteriocin producing NSLAB on the microbial composition of cheese during ripening. Giannou et al. (2009) evaluated the effect of an enterocin-producing *E. faecium* strain on *Listeria monocytogenes* in ripened Greek Graviera cheese stored in different conditions. The main findings were that bacteriocins lower the pathogen concentration, but that at low storage temperatures the survival of *L. monocytogenes* was longer. Two NSLAB (*E. faecium* FAIR-E 198 and *Lb. plantarum* ALC 01), responsible for bacteriocin production, were used in combination in Minas Frescal cheese-making (Nascimento et al., 2008). In that study, *Bacillus cereus* became susceptible to bacteriocins from the seventh day of storage onward. Some authors (Izquierdo et al., 2009) developed a new approach to employing bacteriocins produced by NSLAB within a cheese environment: the multi-bacteriocin producer *E. faecium* WHE 81 was tested as a surface culture in Munster cheese, a red smear soft cheese. This strategy was proven to have a high dairy significance since, in the presence of *E. faecium* WHE 81, *L. monocytogenes* was unable to initiate growth.

5. Health benefits of NSLAB

According to the Consensus Document issued by the European Concerted Action on Science of Functional Foods, a food may be referred to as "functional", if it has been unequivocally proven that it positively influences one or more biological function in the human body, improving the state of health and wellness, and reducing the risk of developing a disease (Diplock et al., 1999). This food category includes all products containing probiotic microorganisms defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (Araya et al., 2002). There are different studies in humans that provide evidence about the positive effects of probiotics: relief of lactose maldigestion symptoms, shortening of rotavirus diarrhoea, immune modulation, suppression of pathogenic microorganisms, blood pressure regulation among others (Ouwehand et al., 2003; Saxelin et al., 2005).

The health benefits attributable to NSLAB species involved in cheese production are discussed below.

5.1. Probiotic cultures

During recent years, consumers have paid attention towards foods containing probiotic microorganisms (Playne et al., 2003). De Vuyst (2000) reported on a general agreement about a minimal concentration of probiotic of about 10^7 cfu/g or ml of food that should be present at the moment of intake, to assure a favourable impact on consumer's health. However, the present trend for probiotic dosage is referred to a food portion: "a serving of stated size of a product should contain a minimum level of 1.0×10^9 cfu of one of the eligible microorganism(s) that is(are) the subject of the claim" (Health Canada, 2009).

Fermented dairy products have been extensively studied as functional foods enriched with probiotic bacteria (Saxelin et al., 2005). Among them, cheese has been proven to be an optimal carrier product to deliver living probiotic bacteria. Cheese is characterized by a solid matrix, higher pH, buffering capacity and fat content, which protect bacteria more efficiently than a fluid environment, such as fermented milk, during intestinal transit to the site of action (Ross et al., 2002b). da Cruz et al. (2009) and Grattepanche et al. (2008) have recently reviewed the works dealing with NSLAB. They focused on those mainly belonging to the groups of lactobacilli and enterococci which are incorporated as probiotic cultures into different cheese varieties. It is essential that probiotics maintain their viability during the manufacture and storage of the product. With this in mind, *Lb. paracasei* LPC-37 has been evaluated for its viability in Swiss-type cheeses during ripening (Aljewicz et al., 2009). The authors showed that the most intensive growth of the probiotic culture occurred during the first two weeks of ripening. The growth of another *Lb. paracasei* strain (A13) was followed in Argentinean cheeses during their manufacture and refrigerated storage (Vinderola et al., 2009). *Lb. paracasei* A13 grew a half log order at 43 °C during the manufacturing process and another half log order during the first 15 days of storage at 5 °C. Several *Lb. plantarum* strains, isolated from Bulgarian cheeses and selected for their potential probiotic properties, were tested to produce cream cheeses in which they retained high concentrations (about 10^7 cfu/g) after three months of storage at 4 °C (Georgieva et al., 2009).

The temperature of storage is a key variable for the sensorial characteristics of cheese. Vinderola et al. (2009) stated that the probiotic addition did not negatively affect the sensorial properties of Argentinean cheeses when kept at 5 °C, but if cheeses were stored at higher temperatures, the probiotic culture determined unwanted consequences on their sensorial characteristics. The same working group (Milesi et al., 2009) evaluated the contribution to cheese sensory profile of four potentially probiotic non-starter lactobacilli. All the strains maintained high concentrations during ripening. An intense post-acidification caused by *Lb. rhamnosus* decreased the sensory characteristics of cheese during ripening, whereas the addition of *Lb. casei* I90 and *Lb. plantarum* I91 did not generate any sensory defect. A review on the sensorial aspects of probiotic cheeses was published by Drake (2007).

5.2. Bioactive peptide production

Milk proteins are considered the main source of a range of biologically active peptides (bioactive peptides) defined as specific protein fragments that have a positive impact on body functions or conditions and may ultimately influence health of humans with moderate hypertension (Kitts and Weiler, 2003). Milk protein-derived bioactive peptides may function as regulatory substances, defined exorphins or formones (food hormones) (Gobbetti et al., 2004). These compounds may be liberated by LAB proteolytic systems during milk fermentation (FitzGerald and Murray, 2006). Several bioactive peptides showing hypotensive and/or angiotensin-I-converting enzyme (ACE)-inhibitory activity have been identified in milk protein hydrolysates and fermented dairy products (Korhonen and Pihlanto, 2006; Silva and Malcata, 2005). The role of SLAB in the generation of bioactive peptides is well documented (FitzGerald and Murray, 2006; Gobbetti et al., 2004).

Recently, the effects of NSLAB species in bioactive peptide production have also been investigated. Besides sensory quality aspects of proteolysis, the formation of bioactive peptides during cheese maturation, when NSLAB dominate, is being given increasing research attention. One strain of *Lb. casei*, *Lb. plantarum* and *Lb. rhamnosus* were characterized by Fuglsang et al. (2003) for *in vitro* ACE-inhibitory activity, but they were not employed in fermented milk experiments because, at the first screening, SLAB presented higher activity. A similar approach was followed to characterise several *E. faecalis* strains. Those strains were selected on the basis of their high ACE-inhibitory activity; the ones showing the best *in vitro* performances were used to produce fermented milk in order to evaluate their *in situ* efficacy (Muguerza et al., 2006). Two peptides produced by the above *E. faecalis* strains were identified as β-casein *f*(133–138) and β-casein *f*(58–76) and showed their activity when orally administered to spontaneously hypertensive rats (Quirós et al., 2007). *Lb. paracasei* was found to be associated with the production of one bioactive peptide with ACE-inhibitory activity during the fermentation of caprine whey (Didelot et al., 2006).

Now that a positive relationship between bioactive peptide potency and the extent of cheese ripening has been demonstrated (Pripp et al., 2006), bioactive peptide production by NSLAB during ageing of cheese needs to be better investigated. With this in mind, Ong et al. (2007) studied the ACE-inhibitory activity of *Lb. casei* strains, previously selected as being probiotic, in Cheddar cheese. The authors found out that the IC_{50} (concentrations of ACE needed to inhibit 50% of ACE activity) of 24-week ripened cheese obtained with lactobacilli inoculation was lower than IC_{50} of 36-week ripened cheese processed without adjunct cultures. A similar work was performed by Wang et al. (2010). In Cheddar cheese, the authors evaluated *Lb. casei* Zhang for several health beneficial effects, including final concentration of bioactive peptides with the ACE-inhibitory activity. Compared with control cheese, the estimated activity of experimental cheese increased its potential for application in the management of hypertension.

To exert their health benefit, peptides with ACE-inhibitory activity must be bioavailable; thus, survive digestion, get transported intact from the intestine to blood and there interact and inhibit ACE efficiently (FitzGerald et al., 2004). For this reason, a simulated gastrointestinal digestion of bioactive peptides is necessary to confirm that the inhibitory activities generated during cheese fermentation are resistant to a cocktail of digestive enzymes encountered during transit.

5.3. γ-Aminobutyric acid production

Current approaches in blood pressure regulation include dietary modifications and exercise, calcium-channel agonists, angiotensin II receptor blockers, diuretics and ACE inhibitors (FitzGerald and Murray, 2006). Moreover, γ-aminobutyric acid (GABA) is a non-protein amino acid that possesses these physiological functions (Guin Ting Wong et al., 2003). LAB have the capacity to synthesize GABA from L-glutamate through glutamate decarboxylase activity. During milk fermentation a high level of L-glutamate may be theoretically liberated since native caseins contain a high proportion of this amino acid. Thus, the screening of NSLAB for the ability to produce GABA is becoming increasingly important in cheese application.

Lb. buchneri MS was selected among several strains isolated from Kimchi (Cho et al., 2007). The authors found that the optimal GABA production by the strain occurred in a synthetic medium (MRS added with monosodium glutamate and modified for other components) at 30 °C for 36 h. The culture extract of *Lb. buchneri* MS partially or completely protected neuronal cells against neurotoxicant-induced cell death, showing its high potential in human health. Another *Lb. buchneri* strain (OPM-1), isolated from a naturally aged cheese, showed a similar behaviour to *Lb. buchneri* MS, in terms of GABA production (Park and Oh, 2006). Siragusa et al. (2007) performed a screening looking at its capacity to synthesize GABA on a high number of strains including SLAB and NSLAB from 22 Italian cheese varieties. Sixty-one strain showed this ability, among which some were identified as *Lb. brevis*, *Lb. paracasei* and *Lb. plantarum*. Interestingly, *Lb. paracasei* PF6 and *Lb. plantarum* C48 survived and synthesized GABA under simulated gastrointestinal conditions (medium at pH 2.0 added with bile salt fluids) in absence of L-glutamate as precursor. The same research group (Minervini et al., 2009) studied several multiple culture combinations, including SLAB and NSLAB, to manufacture fermented goats' milk products with functional properties. The inocula were selected on the basis of their GABA as well as ACE-inhibitory peptide production. A similar aim was pursued by Wang et al. (2010) using *Lb. casei* Zhang to produce Cheddar cheese. As reported above, this strain was chosen for its ACE-inhibitory activity, but its ability to generate GABA during cheese ripening made it a suitable adjunct culture with multiple potential benefits in human health.

5.4. Antigenotoxic abilities of NSLAB

One of the most modern aspects of cheese LAB is represented by their antigenotoxic properties. The ability of inhibiting DNA genotoxins is thought to be an important tool for reducing gut pathologies and colon cancer incidence. In fact, several genotoxic food related compounds may be found in the intestinal tract (Cenci et al., 2005). A close correlation between mutagenesis and carcinogenesis has been reported (Maron and Ames, 1983). LAB and bifidobacteria have been found to show antimutagenic activity (Hsieh and Chou, 2006), thus they may reduce the risk of colon cancer (Massi et al., 2004).

At present, the interest about microbial antigenotoxic–antimutagenic properties has been also directed towards NSLAB. *Lb. casei* strains isolated from traditional Italian ewe cheeses showed a high potential to this regards (Caldini et al., 2008; Corsetti et al., 2008). Those strains, in a different manner, were active *in vitro* against 4-nitroquinoline-1-oxide and N-methyl-N′-nitro-N-nitrosoguanidine, producing spectroscopic modification of genotoxins after co-incubation. The strains were also evaluated for their acid-bile tolerance in order to reach the gut in a living form to prevent genotoxin DNA damage. For this reason, this topic deserves a deeper investigation.

6. Future prospects and conclusions

The uncontrolled evolution of NSLAB may lead to variable results in cheese production, thus the selection of strains with given technological characteristics is important to maintain a certain cheese typicality. The use of protective cultures is a well accepted strategy to avoid growth of pathogenic as well as spoilage microorganisms. In addition to the traditional bacteriocin-based approaches, the conservation of cheese slices wrapped in polymeric foils activated with bacteriocins may represent a successful strategy to preserve this kind of highly requested convenience food.

The health benefits of a food provide an added value. Probiotics are not supposed to alter the sensorial characteristics of the final cheese, thus different strategies need to be developed to include probiotic cultures in cheese, e.g. encapsulation of bacteria in food-grade matrices that may also protect them during gastrointestinal transit. Also generation of bioactive peptides, GABA and antigenotoxins by NSLAB necessitates the study of different manners to enhance their health potential in cheese. All these health promoter factors may increase the requests of these kinds of dairy foods. Furthermore, it is envisaged that this trend will expand as more knowledge is gained about the multifunctional properties and physiological functions of NSLAB in cheeses.

In conclusion, the future directions of research should be oriented towards the selection of mixed cultures of NSLAB providing several beneficial effects. This process will require the adaptation of the existing production protocols and will involve food technologists in the revision of the traditional manufacture of cheese.

References

Aljewicz, M., Cichosz, G., Laniewska-Trokenheim, L., Danowska-Oziewicz, M., Lukaszuk-Kepka, W., 2009. Viability of *Lactobacillus paracasei* LPC-37 in Swiss-type cheeses during their ripening. Zywnosc-Nauka Technologia Jakosc 16, 7–15.

Antonsson, M., Molin, G., Ardö, Y., 2003. *Lactobacillus* strains isolated from Danbo cheese as adjunct cultures in a cheese model system. Int. J. Food Microbiol. 85, 159–169.

Araya, M., Morelli, L., Reid, G., Sanders, M.E., Stanton, C., Pineiro, M., Ben Embarek, P., 2002. Guidelines for the Evaluation of Probiotics in Food. Joint FAO/WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food, London (ON, Canada) April 30 and May 1. <ftp://ftp.fao.org/es/esn/food/wgreport2.pdf> (pp. 1–11).

Awad, S., Ahmed, N., El Soda, M., 2007. Evaluation of isolated starter lactic acid bacteria in Ras cheese ripening and flavour development. Food Chem. 104, 1192–1199.

Beresford, T., Williams, A., 2004. The microbiology of cheese ripening. In: Fox, P.F., McSweeney, P.L.H., Cogan, T.M., Guinee, T.P. (Eds.), Cheese: Chemistry, Physics and Microbiology. Elsevier, London, UK, pp. 287–318.

Beuvier, E., Buchin, S., 2004. Raw milk cheeses. In: Fox, P.F., McSweeney, P.L.H., Cogan, T.M., Guinee, T.P. (Eds.), Cheese: Chemistry, Physics and Microbiology. Elsevier, London, UK, pp. 319–346.

Bintsis, T., Litopoulou-Tzanetaki, E., Davies, R., Robinson, R.K., 2000. Microbiology of brines used to mature feta cheese. Int. J. Dairy Technol. 53, 106–112.

Bintsis, T., Robinson, R.K., 2004. A study of the effects of adjunct cultures on the aroma compounds of Feta-type cheese. Food Chem. 88, 435–441.

Caldini, G., Trotta, F., Corsetti, A., Cenci, G., 2008. Evidence for in vitro anti-genotoxicity of cheese non starter lactobacilli. Antonie Van Leeuwenhoek 93, 51–59.

Calvo, M.V., Castillo, I., Díaz-Barcos, V., Requena, T., Fontecha, J., 2007. Effect of a hygienized rennet paste and a defined strain starter on proteolysis, texture and sensory properties of semi-hard goat cheese. Food Chem. 102, 917–924.

Caplice, E., Fitzgerald, G.F., 1999. Food fermentations: role of microorganisms in food production and preservation. Int. J. Food Microbiol. 50, 131–149.

Cenci, G., Caldini, G., Trotta, F., 2005. Inhibition of DNA reactive agents by probiotic bacteria. Recent Res. Dev. Appl. Microbiol. Biotechnol. 2, 103–121.

Chamba, J.-F., Irlinger, F., 2004. Secondary and adjunct cultures. In: Fox, P.F., McSweeney, P.L.H., Cogan, T.M., Guinee, T.P. (Eds.), Cheese: Chemistry, Physics and Microbiology. Elsevier, London, UK, pp. 191–206.

Chao, S.-H., Tomii, Y., Sasamoto, M., Fujimoto, J., Tsai, Y.-C., Watanabe, K., 2008. *Lactobacillus capillatus* sp. nov., a motile *Lactobacillus* species isolated from stinky tofu brine. Int. J. Syst. Evol. Microbiol. 58, 2555–2559.

Chen, H., Hoover, D.G., 2003. Bacteriocins and their food applications. Compr. Rev. Food Sci. Food Saf. 2, 82–100.

Cho, Y.R., Chang, J.Y., Chang, H.C., 2007. Production of gamma-aminobutyric acid (GABA) by *Lactobacillus buchneri* isolated from Kimchi and its neuroprotective effect on neuronal cells. J. Microbiol. Biotechnol. 17, 104–109.

Coeuret, V., Gueguen, M., Vernoux, J.P., 2004. In vitro screening of potential probiotic activities of selected lactobacilli isolated from unpasteurized milk products for incorporation into soft cheese. J. Dairy Res. 71, 451–460.

Corfield, A.P., Carroll, D., Myerscough, N., Probert, C.S., 2001. Mucins in the gastrointestinal tract in health and disease. Front. Biosci. 6, 1321–1357.

Corsetti, A., Caldini, G., Mastrangelo, M., Trotta, F., Valmorri, S., Cenci, G., 2008. Raw milk traditional Italian ewe cheeses as a source of *Lactobacillus casei* strains with acid-bile resistance and antigenotoxic properties. Int. J. Food Microbiol. 125, 330–335.

Cotter, P.D., Hill, C., Ross, R.P., 2005. Bacteriocins: developing innate immunity for food. Nat. Rev. 3, 777–788.

da Cruz, A.G., Buriti, F.C.A., de Souza, C.H.B., Fonseca, J.A.F., Saad, S.M.I., 2009. Probiotic cheese: health benefits, technological and stability aspects. Trends Food Sci. Technol. 20, 344–354.

De Angelis, M., Corsetti, A., Tosti, N., Rossi, J., Corbo, M.R., Gobbetti, M., 2001. Characterization of non-starter lactic acid bacteria from Italian ewe cheeses based on phenotypic, genotypic, and cell wall protein analyses. Appl. Environ. Microbiol. 67, 2011–2020.

De Vuyst, L., 2000. Technology aspects related to the application of functional starter cultures. Food Technol. Biotechnol. 38, 105–112.

De Vuyst, L., Tsakalidou, E., 2008. *Streptococcus macedonicus*, a multi-functional and promising species for dairy fermentations. Int. Dairy J. 18, 476–485.

Didelot, S., Bordenave-Juchereau, S., Rosenfeld, E., Fruitier-Arnaudin, I., Piot, J.-M., Sannier, F., 2006. Preparation of angiotensin-I-converting enzyme inhibitory hydrolysates from unsupplemented caprine whey fermentation by various cheese microflora. Int. Dairy J. 16, 976–983.

Diplock, A.T., Aggett, P.J., Ashwell, M., Bornet, F., Fern, E.B., Roberfroid, M.B., 1999. Scientific concepts of functional foods in Europe – consensus document. Br. J. Nutr. 81, S1–S27.

Drake, M., 2007. Sensorial analysis of dairy foods. J. Dairy Sci. 90, 4925–4937.

Eneroth, Å., Christiansson, A., Brendehaug, J., Molin, G., 1998. Critical contamination sites in the production line of pasteurised milk, with reference to the psychrotrophic spoilage flora. Int. Dairy J. 8, 829–834.

FitzGerald, R.J., Murray, B.A., Walsh, D.J., 2004. Hypotensive peptides from milk proteins. J. Nutr. 134, 980–988.

FitzGerald, R.J., Murray, B.A., 2006. Bioactive peptides and lactic fermentations. Int. J. Dairy Technol. 59, 118–125.

Fitzsimons, N.A., Cogan, T.M., Condon, S., Beresford, T., 1999. Phenotypic and genotypic characterization of nonstarter lactic acid bacteria in mature cheddar cheese. Appl. Environ. Microbiol. 65, 3418–3426.

Fox, P.F., McSweeney, P.L.H., Cogan, T.M., Guinee, T.P. (Eds.), 2004. Cheese: Chemistry, Physics and Microbiology. Elsevier, London, UK.

Fox, P.F., Wallace, J.M., 1997. Formation of flavour compounds. Adv. Appl. Microbiol. 45, 17–85.

Franciosi, E., Settanni, L., Carlin, S., Cavazza, A., Poznanski, E., 2008. A factory-scale application of secondary adjunct cultures selected from lactic acid bacteria during “Puzzone di Moena” cheese ripening. J. Dairy Sci. 91, 2981–2991.

Franciosi, E., Settanni, L., Cavazza, A., Poznanski, E., 2009a. Biodiversity and technological potential of wild lactic acid bacteria from raw cows’ milk. Int. Dairy J. 19, 3–11.

Franciosi, E., Settanni, L., Cavazza, A., Poznanski, E., 2009b. Presence of enterococci in raw cow’s milk and “Puzzone di Moena” cheese. J. Food Process. Preserv. 33, 204–217.

Fuglsang, A., Rattray, F., Nilsson, D., Nyborg, N.C.B., 2003. Lactic acid bacteria: inhibition of angiotensin converting enzyme in vitro and in vivo. Antonie Van Leeuwenhoek 83, 27–34.

Georgieva, R., Iliev, I., Haertlé, T., Chobert, J.-M., Ivanova, I., Danova, S., 2009. Technological properties of candidate probiotic *Lactobacillus plantarum* strains. Int. Dairy J. 19, 696–702.

Giannou, E., Kakur, A., Matijasic, B.B., Rogelj, I., Samelin, J., 2009. Fate of *Listeria monocytogenes* on fully ripened Greek Graviera cheese stored at 4, 12, or 25 degrees C in air or vacuum packages: in situ PCR detection of a cocktail of bacteriocins potentially contributing to pathogen inhibition. J. Food Prot. 72, 531–538.

Gobbetti, M., Morea, M., Baruzzi, F., Corbo, M.R., Matarante, A., Considine, T., Di Cagno, R., Guinee, T., Fox, P.F., 2002. Microbiological, compositional, biochemical and textural characterisation of Caciocavallo Pugliese cheese during ripening. Int. Dairy J. 12, 511–523.

Gobbetti, M., Minervini, F., Rizzello, C.G., 2004. Angiotensin-I-converting-enzyme-inhibitory and antimicrobial bioactive peptides. Int. J. Dairy Technol. 57, 173–188.

Grattepanche, F., Miescher-Schwenninger, S., Meile, L., Lacroix, C., 2008. Recent developments in cheese cultures with protective and probiotic functionalities. Dairy Sci. Technol. 88, 421–444.

Guin Ting Wong, G., Bottiglieri, T., Carter Snead III, O., 2003. GABA, gammahydroxybutyric acid, and neurological disease. Ann. Neurol. 6, 3–12.

Guinane, C.M., Cotter, P.D., Hill, C., Ross, R.P., 2005. Microbial solutions to microbial problems; lactococcal bacteriocins for the control of undesirable biota in food. J. Appl. Microbiol. 98, 1316–1325.

Health Canada, 2009. The Use of Probiotic Microorganisms in Food, Section 8.7. http://www.inspection.gc.ca/english/fssa/labeti/guide/ch8ae.shtml#a8_7 (last accession 09.05.10).

Hemme, D.H., Schmal, V., Auclair, J.E., 1981. Effect of the addition of extracts of thermophilic lactobacilli on acid production by *Streptococcus thermophilus* in milk. J. Dairy Res. 51, 345–351.

Holzapfel, W.H., Geisen, R., Schillinger, U., 1995. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. Int. J. Food Microbiol. 24, 343–362.

Hsieh, M.L., Chou, C.C., 2006. Mutagenicity and antimutagenic effect of soymilk fermented with lactic acid bacteria and bifidobacteria. Int. J. Food Microbiol. 111, 43–47.

Izquierdo, E., Marchioni, E., Aoude-Werner, D., Hasselmann, C., Ennahar, S., 2009. Smearing of soft cheese with *Enterococcus faecium* WHE 81, a multi-bacteriocin producer, against *Listeria monocytogenes*. Food Microbiol. 26, 16–20.

Kandler, O., Weiss, N., 1986. Genus *Lactobacillus* Beijerinck 1901. In: Sneath, P.H.A., Mair, N.S., Sharpe, M.E. (Eds.), Bergey’s Manual of Systematic Bacteriology. Williams and Wilkins, Baltimore, MD, pp. 1209–1234.

Kitts, D.D., Weiler, K., 2003. Bioactive proteins and peptides from food sources. Applications of bioprocesses used in isolation and recovery. Curr. Pharm. Des. 9, 1309–1323.

Klaenhammer, T.R., Barrangou, R., Buck, B.L., Azcarate-Peril, M.A., Altermann, E., 2005. Genomic features of lactic acid bacteria effecting bioprocessing and health. FEMS Microbiol. Rev. 29, 393–409.

Korhonen, H., Pihlanto, A., 2006. Bioactive peptides: production and functionality. Int. Dairy J. 16, 945–960.

Lane, C.N., Fox, P.F., 1996. Contribution of starter and adjunct lactobacilli to proteolysis in Cheddar cheese during ripening. Int. Dairy J. 6, 715–728.

Maron, D.M., Ames, B.N., 1983. Revised methods for the *Salmonella* mutagenicity test. Mutat. Res. 113, 173–215.

Massi, M., Vitali, B., Federici, F., Matteuzzi, D., Brigidi, P., 2004. Identification method based on PCR combined with automated ribotyping for tracking probiotic *Lactobacillus* strains colonizing the human gut and vagina. J. Appl. Microbiol. 96, 777–786.

Mauriello, G., Moio, L., Moschetti, G., Piombino, P., Addeo, F., Coppola, S., 2001. Characterization of lactic acid bacteria strains on the basis of neutral volatile compounds produced in whey. J. Appl. Microbiol. 90, 928–942.

Mc Phee, J.D., Griffiths, M.W., 2002. Psychrotrophic bacteria, *Pseudomonas* spp. In: Roginsky, H., Fuquay, J.W., Fox, P.F. (Eds.), Encyclopedia of Dairy Sciences. Academic Press, New York, USA, pp. 2340–2351.

McSweeney, P.L.H., 2004a. Biochemistry of cheese ripening: introduction and overview. In: Fox, P.F., McSweeney, P.L.H., Cogan, T.M., Guinee, T.P. (Eds.), Cheese: Chemistry, Physics and Microbiology. Elsevier, London, UK, pp. 347–360.

McSweeney, P.L.H., 2004b. Biochemistry of cheese ripening. Int. J. Dairy Technol. 57, 127–144.

Michaelidou, A., Katsiari, M.C., Voutsinas, L.P., Kondyli, E., Alichanidis, E., 2003. Effect of commercial adjunct cultures on proteolysis in low-fat Kefalograviera-type cheese. Int. Dairy J. 13, 743–753.

Milesi, M.M., Vinderola, G., Sabbag, N., Meinardi, C.A., Hynes, E., 2009. Influence on cheese proteolysis and sensory characteristics of non-starter lactobacilli strains with probiotic potential. Food Res. Int. 42, 1186–1196.

Minervini, F., Bilancia, M.T., Siragusa, S., Gobbetti, M., Caponio, F., 2009. Fermented goats’ milk produced with selected multiple starters as a potentially functional food. Food Microbiol. 26, 559–564.

Mucchetti, G., Neviani, E. (Eds.), 2006. Microbiologia e tecnologia lattiero-casearia. Qualità e sicurezza. Tecniche nuove, Milano.

Muguerza, B., Ramos, M., Sánchez, E., Manso, M.A., Miguel, M., Aleixandre, A., Delgado, M.A., Recio, I., 2006. Antihypertensive activity of milk fermented by *Enterococcus faecalis* strains isolated from raw milk. Int. Dairy J. 16, 61–69.

Nascimento, M.S., Moreno, I., Kuaye, A.Y., 2008. Applicability of bacteriocin-producing *Lactobacillus plantarum, Enterococcus faecium* and *Lactococcus lactis* ssp. *lactis* as adjunct starter in Minas Frescal cheesemaking. Int. J. Dairy Technol. 61, 352–357.

Nielsen, D.S., Schillinger, U., Franz, C.M.A.P., Bresciani, J., Amoa-Awua, W., Holzapfel, W.H., Jakobsen, M., 2007. *Lactobacillus ghanensis* sp. nov., a motile lactic acid bacterium isolated from Ghanaian cocoa fermentations. Int. J. Syst. Evol. Microbiol. 57, 1468–1472.

Nuñez, N., Rodriguez, J.L., Garcia, E., Gaya, P., Medina, M., 1997. Inhibition of *Listeria monocytogenes* by enterocin 4 during the manufacture and ripening of Manchego cheese. J. Appl. Microbiol. 6, 671–677.

Ong, L., Henriksson, A., Shah, N.P., 2007. Angiotensin converting enzyme-inhibitory activity in Cheddar cheeses made with the addition of probiotic *Lactobacillus casei* sp. Lait 87, 149–165.

Ortigosa, M., Arizcun, C., Irigoyen, A., Oneca, M., Torre, P., 2006. Effect of lactobacillus adjunct cultures on the microbiological and physicochemical characteristics of Roncal-type ewes’-milk cheese. Food Microbiol. 23, 591–598.

Ouwehand, A.C., Salvadori, B.B., Fondén, R., Mogensen, G., Salminen, S., Sellars, R., 2003. Health effects of probiotics and culture-containing dairy products in humans. Bull. IDF 380, 4–19.

Park, K.B., Oh, S.H., 2006. Isolation and characterization of *Lactobacillus buchneri* strains with high gamma-aminobutyric acid producing capacity from naturally aged cheese. Food Sci. Biotech. 15, 86–90.

Peterson, S.D., Marshall, R.T., 1990. Non-starter lactobacilli in Cheddar cheese: a review. J. Dairy Sci. 73, 1393–1410.

Playne, M.J., Bennet, L.E., Smithers, G.W., 2003. Functional dairy foods and ingredients. Aust. J. Dairy Technol. 58, 242–264.

Poveda, J.M., Cabezas, L., McSweeney, P.L.H., 2004. Free amino acid content of Manchego cheese manufactured with different starter cultures and changes throughout ripening. Food Chem. 84, 213–218.

Pripp, A.H., Sorensen, R., Stepamak, L., Sorhaug, T., 2006. Relationship between proteolysis and angiotensin-I-converting enzyme inhibition in different cheeses. LWT-Food Sci. Technol. 39, 677–683.

Quirós, A., Ramos, M., Muguerza, B., Delgado, M.A., Miguel, M., Aleixandre, A., Recio, I., 2007. Identification of novel antihypertensive peptides in milk fermented with *Enterococcus faecalis*. Int. Dairy J. 17, 33–41.

Randazzo, C.L., Pitino, I., De Luca, S., Scifò, G.O., Caggia, C., 2008. Effect of wild strains used as starter cultures and adjunct cultures on the volatile compounds of the Pecorino Siciliano cheese. Int. J. Food Microbiol. 122, 269–278.

Ross, R.P., Morgan, S., Hill, C., 2002a. Preservation and fermentation: past, present and future. Int. J. Food Microbiol. 79, 3–16.

Ross, R.P., Fitzgerald, G., Collins, K., Stanton, C., 2002b. Cheese delivering biocultures – probiotic cheese. Aust. J. Dairy Technol. 57, 71–78.

Salminen, S., von Wright, A. (Eds.), 1998. Lactic Acid Bacteria: Microbiology and Functional Aspects. Marcel Dekker Inc., New York.

Saxelin, M., Tynkkynen, S., Mattila-Sandholm, T., de Vos, W., 2005. Probiotic and other functional microbes: from markets to mechanisms. Curr. Opin. Biotechnol. 16, 1–8.

Settanni, L., Corsetti, A., 2008. Application of bacteriocins in vegetable food biopreservation. Int. J. Food Microbiol. 121, 123–138.

Silva, S.V., Malcata, F.X., 2005. Caseins as source of bioactive peptides. Int. Dairy J. 15, 1–15.

Siragusa, S., De Angelis, M., Di Cagno, R., Rizzello, C.G., Coda, R., Gobbetti, M., 2007. Synthesis of γ-aminobutyric acid by lactic acid bacteria isolated from a variety of Italian cheeses. Appl. Environ. Microbiol. 22, 7283–7290.

Skeie, S., Kieronczyk, A., Eidet, S., Reitan, M., Olsen, K., Østlie, H., 2008. Interaction between starter bacteria and adjunct *Lactobacillus plantarum* INF15D on the degradation of citrate, asparagine and aspartate in a washed-curd cheese. Int. Dairy J. 18, 169–177.

Švec, P., Dráb, V., Sedláček, I., 2005. Ribotyping of *Lactobacillus casei* group strains isolated from dairy products. Folia Microbiol. 50, 223–228.

Tungjaroenchai, W., White, C.H., Holmes, W.E., Drake, M.A., 2004. Influence of adjunct cultures on volatile free fatty acids in reduced-fat Edam cheeses. J. Dairy Sci. 87, 3224–3234.

Turner, K.W., Lawrence, R.C., Levriere, J., 1986. A microbiological specification for milk for aseptic cheese making. N. Z. J. Dairy Sci. Technol. 21, 249–254.

Upadhyay, V.K., McSweeney, P.L.H., Magboul, A.A.A., Fox, P.F., 2004. Proteolysis in cheese during ripening. In: Fox, P.F., McSweeney, P.L.H., Cogan, T.M., Guinee, T.P. (Eds.), Cheese: Chemistry, Physics and Microbiology. Elsevier, London, UK, pp. 391–433.

Upadhyay, V.K., Huppertz, T., Kelly, A.L., McSweeney, P.L.H., 2007. Use of high pressure treatment to attenuate starter bacteria for use as adjuncts for Cheddar cheese manufacture. Innov. Food Sci. Emerg. Technol. 8, 485–492.

Ur-Rehman, S., Banks, J.M., McSweeney, P.L.H., Fox, P.F., 2000. Effect of ripening temperature on the growth and significance of non-starter lactic acid bacteria in Cheddar cheese made from raw or pasteurized milk. Int. Dairy J. 10, 45–53.

Vinderola, G., Prosello, W., Molinari, F., Ghiberto, D., Reinheimer, J., 2009. Growth of *Lactobacillus paracasei* A13 in Argentinian probiotic cheese and its impact on the characteristics of the product. Int. J. Food Microbiol. 135, 171–174.

Wang, H.K., Dong, C., Chen, Y.F., Cui, L.M., Zhang, H.P., 2010. A new probiotic Cheddar cheese with high ACE-inhibitory activity and gamma-aminobutyric acid content produced with Koumiss-derived *Lactobacillus casei* Zhang. Food Technol. Biotechnol. 48, 62–70.

Williams, A.G., Banks, J.M., 1997. Proteolytic and other hydrolytic enzyme activities in non-starter lactic acid bacteria isolated from Cheddar cheese manufactured in the United Kingdom. Int. Dairy J. 7, 763–777.

Williams, A.G., Choi, S.-C., Banks, J.M., 2002. Variability of the species and strain phenotype composition of the non-starter lactic acid bacterial population of Cheddar cheese manufactured in a commercial creamery. Food Rev. Int. 35, 483–493.

Wouters, J.T.M., Ayad, E.H., Hugenholtz, J., Smit, G., 2002. Microbes from raw milk for fermented dairy products. Int. Dairy J. 12, 91–109.