

Bio Preservation of Cheese by Lactic Acid Bacteria

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Over the last decade, more and more studies have been focusing on the bio preservation of various fermented food products, such as dairy products, in order to eliminate the use of synthetic chemical and additives as preservatives. However, the major problem is that results obtained in laboratory-scale, under well controlled experimental conditions and laboratory chemical designed medium, are not always successfully reproduced under actual industrial conditions.

For centuries, lactic acid bacteria (LAB) have been explored as starter cultures and competitive bio preservative microbiota in different types of fermented food. LAB have the ability to produce different organic acids, volatile and non-volatile and aromatic compounds which play a pivotal role on the quality of the product. They are also essential in the ripening process, and contribute to the technological and sensory properties (acidity, proteolysis, lipolysis, nutritional value, hardness, creaminess, melt ability, taste and flavour) of food products, besides their natural antimicrobial properties which enhance the safety of the product and extend the product shelf life.

LAB are ubiquitous bacteria and because of their ability to withstand heat stress and other adverse environmental conditions, they are used in various fermented dairy products [1]. In cheese, they contribute to the ripening process and to the development of aroma due to their proteolytic and lipolytic activities [2,3].

Additionally, over the last decades, research has been focusing on the application and on a better understanding of how LAB can improve the quality and safety of dairy products. Enormous work has been dedicated to the isolation and identification of beneficial LAB from different dairy products. Special attention has been given to LAB producers of antimicrobial proteins, including Bacteriocins. According to a well-accepted definition, Bacteriocins are ribosomally synthesized antimicrobial proteins, usually active against genetically related species [4]. Nonetheless, even though Bacteriocins are produced by LAB during cheese production, using milk as a suitable bacterial growth medium, their antimicrobial titers are much lower than those achieved through *in vitro* fermentations under optimal physiological and chemical conditions.

Looking at bacteriocins as antimicrobial compounds is a way to provide an alternative to chemical preservatives and to offer new and appealing food products to consumers. Countless papers have been published on bacteriocins-producing LAB from dairy origin [5,6] and on the production of cheese with bacteriocinogenic strains [7,8,9,10].

Even though numerous bacteriocins from LAB have been characterized, their commercial application as food preservatives is still very limited due to several technological or legislation barriers. The most extensively studied bacteriocins are nisin and pediocin PA-1, both having commercial applications in the food industry. Fermented dairy products' chemical and physical properties, such as pH, proteins and fat, can influence the antimicrobial activity of bacteriocins.

The production of bacteriocins is considered as primary metabolites and most LAB express bacteriocins, even if in low levels. Induction of bacteriocin production can be observed through the presence of pathogenic bacteria which can induce the production and expression of bacteriocins. However, the biochemical machinery of LAB needs sufficient nutrients for the production of bacteriocins. Some organic nitrogen sources have a critical role on bacteriocin production [5,11]. In addition, the type of carbohydrates is crucial for the growth of LAB and production of metabolites, including bacteriocins.

Considering that dairy fermented products are a peculiar environmental niche in which LAB are expected to grow, produce and express antimicrobial peptides, the important point in the screening for bacteriocinogenic LAB is addressed to the ability of LAB to grow and produce high activity bacteriocin(s) in dairy fermented products. In addition to these, it is essential to consider that LAB need to survive and grow in the presence of NaCl, usually under refrigeration temperatures, during ripening and storage processes. Under such harsh conditions, will LAB be able to grow, produce and express bacteriocin(s)? Another important key point concerns the storage temperature for most cheese (below 8°C) which is not applicable for LAB growth. So, how is it expected from them to produce bacteriocins(s)? On the other hand, pathogenic bacteria such as *Listeria monocytogenes* can survive and grow very well at cold temperatures. Then, what is the alternative? An option would be the use of semi-purified bacteriocins, previously produced by studied LAB. It is important to guarantee that bacteriocin(s) is(are) expressed in sufficient quantity under these growth/ production limiting conditions and the expressed bacteriocin(s) is(are) sufficient to show action against *L. monocytogenes* or other pathogenic or spoilage microorganisms.

The application of bacteriocinogenic LAB cultures in controlling spoilage microorganisms in dairy products cannot be expected, however, to be a magic solution to problems related to the preservation of fermented dairy products. It is important to consider that's starter and non-starter cultures need to grow in the complex matrix of the fermented dairy products. In addition, bacteriocinogenic LAB cultures need to fulfil the following criteria: Be able to produce a sufficient amount of bacteriocins; survive antagonism of other bacteria toward the producer strain; not interfere with the starter cultures; and be safe regarding the possible presence of virulence genes. Furthermore, the bacteriocin activity can be affected by the physicochemical parameters of the dairy environment, increasing or decreasing their functionality, depending on the case.

Another important point for the application of LAB in bio preservation is the strain safety. Most *Lactobacillus* spp. have a well-accepted GRAS status. However, even if GRAS is not common for *Enterococcus* spp., and some of them are considered as opportunistic pathogens, they are part of several fermented dairy products, especially from the Mediterranean area. In cheese, the positive effect of enterococci seems to be due to specific biochemical traits, such as citrate utilization, proteolytic and lipolytic activities, as well as the production of aromatic volatile compounds [12]. In particular, *Enterococcus faecium* and *Enterococcus durans* when predominating in cheese may play an essential role on aroma production [13,14]. *Enterococcus* strains isolated from water buffalo mozzarella cheese were able to use citrate, and can, therefore, produce aromatic compounds such as acetaldehyde, acetoin, and diacetyl [12]. This metabolic capability pointed by some authors resulted in the inclusion of enterococci in commercial starter cultures for mozzarella cheese [15]. Furthermore, enterococci are also used to extend the food shelf life and improve its hygienic safety, because they produce several antimicrobial substances, including bacteriocins, against several food-borne pathogens [16].

So, why would *Streptococcus thermophilus* be considered safe when other *Streptococcus* spp. do not hold the GRAS status? Although it is related to other pathogenic streptococci (e. g. *S. pneumoniae* and *S. pyogenes*), *S. thermophilus* is classified as nonpathogenic, with a GRAS status by the United States Food and Drug Administration (FDA) and has the "Qualified Presumption of Safety" (QPS) status by the European Food Safety Authority (EFSA), due to a long history of safe use in food production.

Then, it is still a challenge to develop novel bio preservative starter cultures which contribute to the product quality and, at the same time, enhance the product safety and shelf life. Besides the antimicrobial effect of LAB, it is necessary to prove that bacteriocins are effective as natural food bio preservatives, are innocuous for humans and sensitive to digestive proteases to be hydrolysed into amino acids in the intestine by α -chymotrypsin, and do not produce changes in the sensory properties of the product.

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