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# Biodiversity and the survival of autochthonous fermented products

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Many microorganisms isolated and characterized during the past century have received considerable attention from the pharmaceutical and/or food fermentation industry because of their potential biotechnological interest. Lactic acid bacteria (LAB), particularly those belonging to beneficial and non-pathogenic genera (*Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Oenococcus*, and *Streptococcus*), have traditionally been used in the food industry. They also play an essential role in the dairy industry due to the tremendous level of human consumption of several important fermented products, mainly cheese and acidified/fermented milks. In the USA, more than 4.6 million tons of cheese and more than 1.3 million tons of yogurt were produced in 2005 [1], whereas in the European Community (EU-25) more than 8 million tons of cheese and 6.9 million tons of acidified/fermented milk were produced in 2003 [3]. These figures correspond to a global sales value that surpasses US$ 28.44 billion, with the global sales value of cheese representing about 30% of total dairy-product sales [2]. Nowadays, more than 500 kinds of cheese are manufactured worldwide, and the many varieties are derived from modifications in cheese-making techniques. However, the fermentation process whereby milk is transformed into cheese has not changed to any great extent since humans discovered the process as a means of preserving food (the fermentation of milk dates back to ~3000 BC).

Cheese is a dynamic biochemical product and, unlike many processed food products for which stability is essential, it undergoes significant changes during ripening. The unripe curds of many varieties of cheese have bland and largely similar flavors. However, the sequential actions of cheese microbiota and the interactions among the different microorganisms during the ripening period result in the flavor compounds that characterize each variety. Acidifying lactococci species usually occur as dominant bacteria early in the ripening process. Afterwards their numbers decrease as they give way to lactobacilli, which, due to their higher versatility for fermentation, can survive in cheeses at stages when concentrations of major carbohydrates and free water decrease, whereas the sodium chloride load increases. Apart from the above-mentioned major genera of LAB, other bacteria, including non-starter LAB and non-lactic-acid bacteria, as well as some fungi (yeasts, moulds), staphylococci, micrococci, coryneform bacteria, and propionic acid bacteria, which develop in the product particularly during the maturation phase as secondary microbiota, are often responsible for the intensity of flavor in many dairy products. The production of a wide range of antagonistic primary and secondary metabolites, including organic acids, diacetyl, $CO_2$ and even bacteriocins, affects the complex dynamics of microorganisms in cheese. Thus, a well-founded understanding of the microbial balance in this special living ecosystem is essential to improve older varieties of cheese and to develop new ones.

Due to the dairy market's continuous growth, commercial cultures have conventionally been selected to obtain greater quantities of product, homogeneous quality of the end product, and short production times, with the final goal of satisfying the preferences of consumers. Most varieties of commercially important cheeses are manufactured worldwide by means of commercial starter cultures of generic composition (including acidifying and aromatic diacetyl-producing lactococci strains and sometimes also leuconostocs and lactobacilli strains). In Europe, Asia, Africa, and Latin America, however, there are regions where fermented products are still manufactured traditionally. In global markets, these fermented products are now highly appreciated and are considered to be of premium value because of their flavor characteristics, which are not found in varieties of cheese produced industrially. In Europe, such products are elaborated according to well-established rules governing the production of registered designations of origin. Protected designations of origin (PDOs) apply to foodstuffs that are produced, processed, and prepared in a given geographic area using recognized

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technology. In Europe, there are more than 135 different varieties of PDO cheese, and "hand-crafted" artisanal cheeses can be considered to be unique ecosystems in terms of the considerable variety of LAB types that they contain.

Strains involved as major components include more-or-less acidifying strains such as *Lactococcus lactis*, *Streptococcus thermophilus, Lactobacillus delbrueckii, Lb. helveticus*, and *Enterococcus* spp., as well as a limited range of species of non-starter lactobacilli (*Leuconostoc* spp., *Lb. casei, Lb. curvatus, Lb. brevis, Lb. plantarum, Lb. paracasei, Lb. salivarius*, and *Lb. rhamnosus*). Studies of different types of raw-milk cheeses show a great diversity of LAB at the genus and species level. For example, 12 different species of LAB were isolated and identified during the manufacture and storage of Anevato cheese, a traditional spreadable Greek cheese made from raw goat's or ewe's milk: three species of *Lactococcus* (*Lc. lactis, Lc. garviae, Lc. raffinolactis*), three species of *Leuconostoc* (*Ln. mesenteroides*, including the subspecies *mesenteroides* and *dextranicum*, *Ln. paramesenteroides*, and *Ln. lactis*) and six species of *Lactobacillus* (*Lb. plantarum, Lb. coryneformis, Lb. paracasei, Lb. brevis, Lb. bifermentans*, and *Lb. viridens*). The sources of these microorganisms have not been wholly identified, but cheese milk, cheese brines, ripening shelves, the air in ripening rooms, and human skin are involved. Interactions with microorganisms originating from environmental exposure during manufacture and ripening, as well as the initial natural diversity of the microbiota present in milk, all play a role in fermentation processes and are important in the final development of traditional dairy products.

Until recently, it was difficult to distinguish between strains of the same species, but modern molecular techniques have revealed the considerable heterogeneity of many isolates from natural cheese cultures. For example, randomly amplified polymorphic DNA (RAPD) techniques showed that *Lactobacillus helveticus*, isolated from natural starter cultures from different Italian cheeses (Monte Veronese, Provolone, and Grana cheeses), comprised different groups according to the variety of cheese. Strains of *Streptococcus thermophilus* from Italian PDO cheeses were also found to exhibit considerable heterogeneity, which partially correlated with the source of the isolate. This wide microbiological diversity raises the question whether it is desirable to elaborate traditional fermented products with undefined (generic and/or unspecific) commercial starter cultures.

At present, a small number of suppliers throughout the world provide the dairy-starter market with different strains, either in combinations or in mixtures other than the complex microbial community present in raw milk. These are ready for fermentation and are linked to the typical taste and aroma of the final products. All starter cultures currently available are derived in one way or another from natural (or artisanal) starters of undefined composition (i.e., they contain an undefined mixture of different strains and/or species). They are reproduced in the dairy industry by some form of backslopping, although the different growth ratios of the various bacterial species involved has led to shifts in the bacterial ratio, resulting in the progressive disappearance of certain strains over time. In order to conserve the "best natural starters", commercial mixed-strain starters obtained from natural cheese-milk microbiota have been developed. However, some studies have shown that the intrinsic variability of these starters is strongly reduced and that they may differ in physiological and technological properties as a result of backslopping. There is also an increasing probability that they will lose plasmid-encoded genes or become infected by specific phages. Consequently, artisans must be extremely careful when using commercial natural-starter cultures.

Although protection of the world's biodiversity is currently a topic of particular concern, little attention has been given to the preservation of microorganisms and how this is related to the survival of traditional or autochthonous products. The use of autochthonous cultures in the elaboration of artisanal or autochthonous fermented products would preserve the diversity of bacterial genera and species associated with specific products. However, many commercial companies are not involved in local master-seed production, whereas for traditional cheese-makers this is a complicated task. As a result, fermentation is achieved through backslopping or the use of undefined commercial starters, with the associated problems of unsatisfactory product performance, mainly the production of non-homogeneous cheeses or non-typical flavors.

Another point to be considered with regards to native microbiota and traditional cheese manufacturing is the effect of improved hygiene in the dairy industry and the cold storage of milk, both of which have led to low bacterial counts in milk used for foodstuff manufacture. As a result of the low bacterial counts (≤100,000 cfu/g) required by EU regulations for raw milk, it is necessary, or at least advisable, to use starters for the manufacture of raw-milk cheeses. However, only the introduction of autochthonous isolates would enable the production of a traditional fermented product with "typical" characteristics. Moreover, as a result of the intensive washing of milking equipment and preparation of the cows' udders, raw milk may contain relatively high numbers of spoilage microorganisms, including coliforms, *Listeria* spp., and *Pseudomonas* spp. Raw milk is still used in many European countries in the traditional cheese industry, but most milk destined for cheese production is pasteurized before use. Heat treatment to ensure safety for human con-

sumption alters the indigenous microbiota, inactivates indigenous milk enzymes, and causes partial denaturation of whey milk proteins, and so disturbs the natural equilibrium necessary for the elaboration of traditional products. As a result, farmhouse cheeses are no longer homogeneous and have lost some of their traditional characteristics. Under these conditions, there is an increasing risk of loss of diversity in raw-milk microbiota and the quality of traditional fermented products is seriously threatened. Traditional, good-quality, raw-milk, starter-free cheeses with their "typical" flavor, which 10–15 years ago were still easily found, have almost disappeared.

Most of the LAB strains currently used in research derive from dairy fermentation and include only a limited part of their worldwide diversity. In addition, they do not represent the total microbial diversity to be found in the existing varieties of cheese. Recent molecular studies carried out to discover more about these microorganisms, which have been in close contact with humans for thousands of years, have revealed the great versatility and potential of LAB. The high capacity of these bacteria to transform substrates through particular metabolic pathways, which were initially used to improve fermentation in foodstuffs, are meanwhile also used to inoculate silage in order to preserve moist crops, and to produce chemical and biological products including biopolymers, bulk enzymes, ethanol, and lactic acid. Furthermore, LAB are also potentially important in the health sciences because of their positive effects on the gastrointestinal microbiota of humans and animals, in addition to being used as oral delivery vehicles for vaccine antigens. More recently, genomic analyses have revealed an evolutionary trend in these bacteria toward minimization of the chromosome and elimination of unnecessary systems during adaptation to nutritionally complex environments, opening up new possibilities for industrial applications.

The role played by natural microbiota has been investigated in order to obtain local products of premium quality. However, it appears inevitable that such products will suffer the consequences of the modernization of livestock, as well as the pressures of global markets. Further knowledge of the natural microbial populations present in dairy products may help to prevent both the loss of microbial diversity in typical products and the resulting loss of a wide range of fermented products associated with local and regional traditions. International legal regulations have been developed to protect biodiversity throughout the world. In this regard, the preservation of autochthonous strains is of major interest, as they are important biological and genetic resources. It is currently believed that the use of such strains will help in meeting current and future consumer demands. Autochthonous strains represent only a small part of the world's biodiversity; but, if for no other reason, they should at least be preserved for our own benefit.

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