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Safety assessment of dairy microorganisms: The *Lactococcus* genus ☆

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Abstract

The *Lactococcus* genus includes 5 species. *Lactococcus lactis* subsp. *lactis* is the most common in dairy product but *L. garviae* has been also isolated. Their biotope is animal skin and plants. Owing to its biochemical characteristics, strains of *L. lactis* are widely used in dairy fermented products processing. Cases of human infections due to lactococci are very seldom reported even if *Lactococcus garviae* can be involved in fish diseases. Then *L. lactis* can be considered as safe and it is most commonly considered as Generally Recognized as Safe.

Keywords: *Lactococcus*; Taxonomy; Dairy use; Human safety

1. Introduction

The genus *Lactococcus* was proposed by Schleifer and colleagues in 1985 to reclassify some species of the genera *Streptococcus* (Lancefield group N lactic streptococci) and *Lactobacillus*. It has been defined on the basis of chemotaxonomic studies confirmed by 16s rRNA sequencing (Schleifer et al., 1985; Schleifer and Killper-Bälz, 1987; Collins et al., 1989). The *Lactococcus* genus includes five species, *L. garvieae* (formerly *E. serolicida*), *L. piscium*, *L. plantarum*, *L. raffinolactis* (formerly *S. raffinolactis*) and *L. Lactis*, which is differentiated into subspecies *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *hordniae* (formerly *Lactobacillus hordniae*) and *L. lactis* subsp. *lactis* (formerly *Lactobacillus xylosus*, *Streptococcus lactis*) (www.bacterio.cict.fr).

Lactococci are Gram positive cocci and belong to the group of Lactic Acid Bacteria. They are homofermentative and exclusively produce L(+) lactic acid. They are not β hemolytic and they are poorly α hemolytic.

2. Biotope and concerned food

Lactococci are generally found on plants and the skins of animals. *L. plantarum* is mainly isolated from plants, *L. garvieae* from fish, animals and milk, and *L. piscium* from salmon (Williams et al., 1990). The presence of lactococci in raw milk is due to contamination from forage during milking. The two lactococci most commonly found in raw milk, cheese and other dairy products are *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*. These two subspecies generally reach a high level ($>10^8$ CFU g^{-1}) as early as the first day of manufacturing and maintain it throughout the ripening period of many raw milk cheeses such as Camembert (Corroler et al., 1999), Serra (Macedo et al., 1996), Venaco (Casalta, 2003) and Pecorino Sardo (Ledda et al., 1996). *L. raffinolactis* has occasionally been found in raw milk and cheeses (Perez Elortondo et al., 1999; Lopez-Diaz et al., 2002). *L. garvieae* may also be isolated from raw milk (Villani et al., 2001) and raw milk cheeses: PDO Salers (Callon et al., 2004), Egyptian cheeses (El-Baradei et al., 2005), Jben cheese (Ouadghiri et al., 2005), Italian Piedmontese PDO Toma cheeses (Fortina et al., 2003). A study of 35 European artisanal dairy products indicated lactococci as the most commonly found LAB genus, accounting for 38% of the bacterial isolates identified (Cogan et al., 1997).

L. lactis subsp. *lactis* and to a lesser extent *L. lactis* subsp. *cremoris* have long been extensively used in starter cultures

☆ Contribution to the safety assessment of technological microflora found in fermented dairy products, PART II.

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for dairy fermentation (i.e. in cheeses, sour cream and butter), composed of single or multiple strains with or without other lactic acid bacteria (Beresford et al., 2001). Their main role in dairy fermentation is acidification, mainly by producing L-lactic acid. They contribute to the development of texture by producing exopolysaccharides, or to flavor by producing aromatic compounds (alcohols, ketones, aldehydes) or by citrate, amino acid or fat metabolism (Smit et al., 2005). They can also be used for food preservation due to their ability to produce organic acids and bacteriocins, nisin being the best characterized and recognized (Delves-Broughton et al., 1996). Their use as probiotics has been also considered (Ouwehand et al., 1999). The annotation of the genomes of different subspecies will undoubtedly open up new prospects for identifying new useful functions in the species (Kok et al., 2005). It will also be a great help in assessing the safety of lactococci (Kok et al., 2005).

3. Taxonomy

The identification of the most common *Lactococcus* species found in dairy products can be successfully performed by rapid and accurate molecular techniques.

PCR-DGGE (denaturating gradient gel electrophoresis) analyses can be used for differentiating *Lactococcus lactis* from other lactic acid bacteria (Coppola et al., 2001). rRNA oligonucleotide probes have been designed for identifying *L. lactis* subsp. *cremoris* (Salama et al., 1991). Several PCR or multiplex PCR reactions are now available, exploiting the diversity of sequences of 16 SrRNA genes in *L. lactis* (Pu et al., 2002) and *L. garvieae* (Zlotkin et al., 1998) and the polymorphism of the 16 S-23 S rDNA spacer region (Blaiotta et al., 2002) or other functional genes, histidine biosynthesis operon (Corroler et al., 1999), *acm*A gene (Garde et al., 1999), *sod*A gene (Fihman et al., 2006). The genetic diversity of *Lactococcus* can be analyzed by random polymorphism DNA (RAPD) (Tailliez et al., 1998) or multiple locus microsatellite analysis (Quénée et al., 2005) for *L. lactis* or by pulsed-field gel electrophoresis (PFGE) for *L. garvieae* (Vela et al., 2000).

Knowledge of the *L. lactis* genome will make it easy, in the future, to develop new genomic tools for increasingly reliable identification (Kok et al., 2005).

4. Safety assessment

Members of *Lactococcus* genus are most commonly classed as Generally Recognized as Safe (GRAS) (Salminen et al., 1998). *L. lactis* cannot be considered as an opportunist pathogen, as only two cases of endocarditis have been reported in the medical literature over a period of fifty years (Wood et al., 1955; Mannion and Rothburn, 1990).

Lactococcus garvieae has been associated with septicemic infections of fish, mainly in intensive modern aquaculture; it is the main risk factor for the Mediterranean European trout industry (Schmidtke and Carson, 2003; Vela et al., 2000; Eyngor et al., 2004). It has been also isolated from bovine mastitis (Teixeira et al., 1996). Human infections due to *L. garviae* are scarce, since only six cases has been described, all of which concerned elderly or immunosuppressed patients. *L. garvieae* has been incriminated in three cases of infection of prosthetic valves (Fihman et al., 2006), one case of native valve infection (Fefer et al., 1998), one case of osteomyelitis (James et al., 2000) and one case of bacteremia with a liver abscess (Mofredj et al., 2000). The number of cases may be underestimated as the bacterial agent responsible for endocarditis cases has not always been identified with certainty.

5. Conclusion

Lactococci are ubiquitous in the environment and in food. They are widely used as starters, with a long history of use for the sake of their technological properties, especially in dairy products, from small-scale manufacture to industrial-scale processes. Their identification no longer poses problems.

Lactococci from dairy products can be generally considered safe, in view of their extensive, daily consumption by humans and their low incidence in human infections.

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