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Safety Aspects of *Lactococcus lactis* as a Dairy Starter

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Lactococcus lactis **owing to its completely sequenced genome and biochemical characteristics is a major dairy starter used in dairy product processing. Safety of any starter is dependent on its unequivocal identification at the genus, species, and strain level along with its potential ability to transfer antibiotic resistance genes, as functional effects is strain specific. Prior to incorporating new strains into products their efficacy should be carefully assessed, and a case by case evaluation as to whether they share the safety status of traditional food-grade organisms should be made.**

Key Words: *Lactococcus lactis*; Dairy starter; Taxonomy; Antibiotic resistance; Safety aspects.

Lactococcus lactis is by far the most extensively studied lactic acid bacterium, used as a primary component of starter cultures in the dairy industry, especially in the manufacture of cheese.[1] The species are commonly isolated from plant material, but the most recognized habitat is dairy products. Isolates identified as gram positive, catalase negative, homofermentative, microaerophilic cocci, and exclusively produce L (+) lactic acid. Unlike many members of *Streptococcus thermophilus, Lactococcus lactis* species do not grow in long chains, mostly in pairs or in short chains, depending on growth conditions appears ovoid with typically 0.5 - 1.5 µM in length and unable to grow at 45 °C , pH 9.6 or in 6.5% NaCl broth. [2]

L.lactis being first lactic acid bacterium with completely sequenced genome[3,4], is an excellent microorganism for advanced analysis. Over the last decade, novel insights into metabolism and fuction of *Lactococcus lactis* has sparked renewed interest for application of a new generation of starter cultures for various dairy products, fermented foods, probiotics, and even live vaccines[5,6]. However, it also gives rise to concern because of the potential transfer of antibiotic resistance and their role in human infections. The evaluation of safety aspects of such improved or novel strains that are to be used in possible future applications is an important issue[7]. Thus an up to date analysis of safety aspects of *Lactococcus lactis* is needed to ensure consumer safety.

Function of *Lactococcus lactis* as a dairy starter

Lactococcus lactis isolates are the most important industrial dairy starter in the manufacture of a wide range of fermented products, including sour cream, butter, fresh and soft cheeses and various hard and semi hard cheeses composed of single or multiple strains with or without other lactic acid bacteria[8].

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The function of *Lactococcus lactis* is twofold; on one hand, they produce lactic acid at an appropriate rate, usually through fermentative degradation of the sugars present in the raw materials. It results in lowering of the pH that makes the medium inhospitable for most spoilage and/ or pathogenic organisms and contribute to extend the shelf life span of the food. In addition, Nisin produced by certain species of *Lactococcus lactis* is a 34-amino acid peptide and is one of the well characterized lantibiotics containing one lanthionine and four β-methyllanthionine cyclic structures [9]. Since Nisin has strong antimicrobial activity against gram positive bacteria, it has been widely used as most effective food preservative [10,11]. On the other hand, these bacteria make important contributions to development of texture by producing exopolysaccharides [12] and to the development of cheese flavour, initiating proteolysis during ripening which is believed to be result from the action of enzymes from the starter cultures[13] as acid accumulation changes the rheological and organoleptical properties of the product, a process that is complemented by the production and, in some instances, the secretion of hydrolytic enzymes, mainly proteinases, peptidases , amino acid hydrolases and, to a lower extent, lipases and esterases[14].

Safety aspects

The present review is limited to two important safety aspects.

Taxonomy and identification tools as the basis of safety evaluation

The safety of a novel starter is dependent on its unequivocal identification at the genus, species, and strain level; as functional and technological aspects is strain specific and to avoid the inclusion of potentially pathogenic microorganisms in commercial products. *Lactococcus lactis* were originally classified under the genus *Streptococcus*, but in 1985, Schleifer et al., provided evidence, based on chemotaxonomic studies confirmed by 16s rRNA sequencing, to reclassify some species of the genera *Streptococcus* (Lancefield group N lactic *streptococci*) and *Lactobacillus*, that justifies conferring genus *Lactococcus*[2]. The *Lactococcus* genus is constituted by six species, *Lactococcus chungangensis*, *L. garvieae* (formerly *E. serolicida*), *L. piscium*, *L. plantarum*, *L. raffinolactis*, (formerly *S. raffinolactis*) and *L. lactis* [15,16,17]. The *Lactococcus lactis* specie is further differentiated into three subspecies, namely *L. lactis subsp. cremoris*, *L. lactis subsp. hordniae* (formerly *Lactobacillus hordniae*) and *L. lactis subsp. Lactis*. However *L. lactis subsp. cremoris* and *L. lactis subsp. lactis* have been found more important in dairy applications while *L.lactis subsp. hordinae* has no relevance in fermented food production [18]. A combination of phenotypic traits and genotypic information has been used to investigate microbial diversity within *Lactococcus lactis* sub species[19]. *L.lactis subsp. cremoris* is distinguished from *L.lactis subsp. lactis* by its inability to (a) grow at 40°C, (b) grow in 4% NaCl, (c) hydrolyze arginine, (d) ferment ribose and (e) grow at pH 9.2 [20].

The *lactis* and *cremoris subspecies* of *L.lactis* have also been shown to be genetically distinct by highly discriminatory molecular methods, DNA-DNA homology studies, the polymorphism of the 16S-23S rDNA spacer region [21], multiple locus micro satellite analysis [22], comparison of 16S rRNA sequences[23], PCR-DGGE (Denaturating Gradient Gel Electrophoresis) analyses [24] and comparative genome hybridization using multi-strain arrays.[25]

The diversity within the species has recently been re-evaluated with molecular analyzes, conûrming that phenotypic and genotypic diversity are not coherent [18]. Currently, a clear example is commonly worldwide used laboratory strains MG1363; it displays many of *subsp. lactis* phenotypic traits, but it is usually referred to as *subsp. cremoris* due to its genetic similarity to *subsp. cremoris*[26]. Moreover, It is clear that strains used as starter in dairy industry should be identified using molecular methods and up-to-date taxonomical nomenclature and comparison of the results obtained by using different molecular methodologies (polyphasic approach) is the best way to establish strain identity. Therefore in future, the genetic based nomenclature will probably overcome the classical and phenotype-based one[27], generating a dichotomy in taxonomic procedure of identiûcation of strains at the sub species level.

Safety consideration of antibiotic resistance in *Lactococcus lactis*

The presence of antibiotic resistance

genes in the lactic acid bacteria genomic content is not a safety concern in itself, as long as the genes are not mobilized and transferred to other bacteria[28]. Thus, it is of great interest to investigate whether *Lactococcus* can act as reservoirs for antibiotic resistance genes, from which they could be spread to opportunistic or pathogenic bacteria. First strong evidence that antibiotic resistance can be spread in a food environment was found by Perrreten et al.[29] who clearly observed genes conferring resistance for streptomycin, tetracycline and chloramphenicol in *L. lactis subsp. lactis* K214 strain isolated from a raw-milk soft cheese , encoded by three different genes, located in a multi-antibiotic resistance plasmid, and these genes were almost identical to others previously found in *Staphylococcus aureus* and *Listeria monocytogene*[30]. Since then, many genes coding for proteins presenting resistance to several antibiotics, mainly tetracycline and erythromycin, have been exemplified in *Lactococcus lactis* [31], and transfer from *Lactococcus* to other bacteria, including Gram-positive pathogens, as well as the inheritance of resistance genes, has been demonstrated [32]. Further studies are needed to elucidate whether specific virulence factors are carried and expressed by the *L.lactis* clinical isolates, conferring to these strains the specific ability to cause infection in humans.

In spite of this, rare cases of human infection were reported, however, all of which concerned immuno suppressed patients[33, 34]. Yet, many open questions regarding the safety of starters remain. Thus, future, *in vivo* experiments should shed some light on the transfer events occurring from, via, or to starters. It is also difficult to assess what level of gene transfer, if any, may be considered acceptable by the community and also signiûcant reason to select strains lacking the potential to transfer genetic determinants of antibiotic resistance. There is little basis for scientiûc regulation of strains with intrinsic resistance, as little is known about the levels of intrinsic resistance in current starters and should be carefully investigated.

It is evident that potential ability of starter strains to transfer antibiotic resistances to pathogenic bacteria in the food and gut environment should be considered in the safety assessment .Also, it appears that the gastrointestinal tract may comprise a more favorable environment for antibiotic resistance transfer than conditions provided *in vitro*[35].

CONCLUSION

Despite the excellent overall safety record of *Lactococcus lactis* in human, they should be used with caution in certain specific patient groups particularly critically patients, those with immune deficiency and patient groups with increased risk for bacterial translocation due to disturbed intestinal mucosal barrier function. Taken together, the beneficial aspects of *Lactococcus lactis* clearly outweigh its rare sepsis.

Lactococcus lactis is Generally Recognized As safe (GRAS) microorganism. However, fundamental and applied clinical research is still needed to optimally explore its potential as functional starter cultures in the existing production technology to obtain quantitative and qualitative data and the host strains used for those purposes should be composed solely of DNA food-grade organism and devoid of any antibiotic resistance markers. Otherwise, they may potentially transfer to intestinal microûora in humans, there by compromising human antibiotic therapy.

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