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Safety assessment of dairy microorganisms: Coagulase-negative staphylococci ☆

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Abstract

The genus *Staphylococcus* is made up of 36 validated species which contain strains that are pathogenic, saprophytic, or used as starter cultures for the food industry. Staphylococci species used in cheese-making are novobiocin-resistant, coagulase-negative and are not usually identified at species level by routine laboratoies. A bibliographic survey was conducted to assess safety status of CNS used in fermented dairy foods. Commercial kits based on phenotypic discrimination can't reliably identify these species because of the variable expression of some phenotypic traits. Several molecular targets, including 16S rRNA, *hsp*60, *tuf*, *Sod*A and *rpo*B genes can be used for identifying species of the *Staphylococcus* genus. No coagulase-negative staphylococci isolated from milk or dairy products has ever been involved in a case of food poisoning or human pathology following ingestion of dairy products. Nevertheless, a few case of nosocomial infection caused by some species (*S. caprae, S. capitis, S. sciuri*) have been reported in patients with depressed immune systems or who have undergone long severe hospital treatments, or in the presence of an indwelling catheter or foreign materials such as cardiac prostheses. They may therefore be regarded as exceptional opportunistic pathogens in certain clinical situations.

Keywords: Coagulase-negative staphylococci; Taxonomy; Dairy use; Human safety; Antiobiotic resistance

1. Introduction

1.1. Generalities

The *Staphylococcus* genus is ubiquitously distributed in nature, with some species inhabiting specific ecological niches. Staphylococci are found living naturally on the skin and mucous membranes of warm-blooded animals and humans, but are also isolated from a wide range of foodstuffs such as meat, cheese and milk, and from environmental sources such as soil, sand, air and water (Kloos and Schleifer, 1986). All strains recognized for their technological value and involved in desirable reactions (flavor and aroma formation) during the ripening of fermented foods, especially cheeses and sausages (Irlinger et al., 1997; Blaiotta et al., 2004), are coagulase-negative species (particularly *S. xylosus*, *S. carnosus* and *S. equorum*). For this reason, coagulase-positive staphylococci are not discussed in this article.

In other circumstances, some species of coagulase-negative staphylococci can present a medical risk. For example *S. saprophyticus*, which may contribute to sausage aroma formation (Samelis et al., 1998) and help to prevent off-flavor during sausage ripening (Mauriello et al., 2004), is an ubiquitous species and is also involved in acute urinary tract infections (UTI) in young adult women (Martineau et al., 2000).

Because staphylococci are widespread in such niches as clinical environments and food manufacturing plants, it has become increasingly important to accurately identify them at genus and species level. Speciation is also crucial in human and veterinary medicine, for determining the source of an infection, characterizing the pathology and conducting epidemiological studies. Fast, accurate identification and speciation of staphylococci is therefore important not only for medical, veterinary and societal applications but also for public health.

Moreover, the high prevalence of coagulase-negative staphylococci (CNSs) and their pathogenic potential (existence of enterotoxigenic and multidrug-resistant strains) have not been clearly identified, but this is increasingly necessary for assessing the safety status of industrial CNSs in fermented dairy foods. The purpose of this review is to gather together the most relevant bibliographical data in order to assess the use of coagulase-negative staphylococci in starter cultures according to their technological usefulness.

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

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1.2. Taxonomy

Staphylococci are Gram-positive spherical bacteria that occur in microscopic clusters resembling bunches of grapes. They are aerobic or facultatively anaerobic, nutritionally undemanding and catalase-positive. Today, according to the current List of Bacterial Names with Standing in Nomenclature (Euzéby, 2004), the genus *Staphylococcus* comprises 36 species, eleven of which also contain subdivisions with named subspecies. The classification is still developing. On the basis of comparative 16S rRNA sequence studies, the genera *Staphylococcus* and *Macrococcus* belong to the Gram-positive bacteria with a low DNA G+C content. They are closely related to bacilli and other Gram-positive bacteria with low DNA G+C content such as enterococci, streptococci, lactobacilli and listeria. Combining *Staphylococcus*, *Gemella*, *Macrococcus* and *Salinicoccus* within the family *Staphylococcaceae* has been suggested (Garrity and Holt, 2001).

Most staphylococci species are coagulase-negative. Exceptions are *Staphylococcus aureus*, *Staphylococcus intermedius*, *Staphylococcus delphini*, *Staphylococcus schleiferi* subsp. *coagulans*, *Staphylococcus lutrae* and some strains of *Staphylococcus hyicus* (Kloos and Bannerman, 1995) (Table 1).

Routine laboratories do not usually identify coagulase-negative staphylococci (CNSs) at species level. However, accurate bacteriological identification at species or subspecies level is needed to develop valid epidemiological investigations, assess their pathogenic significance, and devise specific management practices.

Commercial kits based on phenotypic discrimination exist, and are rapid, but none can reliably identify the important CNSs because of the variable expression of some phenotypic traits (Monsen et al., 1998; Irlinger et al., 1997; Ligozzi et al., 2002). Multilocus enzyme electrophoresis or analysis of cellular fatty acid composition has also been used, but identification remains incomplete (Stoakes et al., 1994). To date, genotypic methods provide better results in terms of reproducibility and species discrimination. Several molecular targets have been successfully used, including 16S rRNA, *hsp*60 (De Buyser et al., 1992; Kwok and Chow, 2003) and, more recently, the *tuf*, *sod*A and *rpo*B genes (Drancourt and Raoult, 2002; Martineau et al., 2001; Poyart et al., 2001) as targets for both identification and phylogenetic studies. Unfortunately, species-specific PCR can require the use of several primer sets, which is time-consuming, expensive and sometimes labor-intensive. Although some studies have used multiplex PCR protocols that can identify the *Staphylococcus* genus and its main constituent species from food, food environment or a clinical origin, primers are not available for all species of CNS (Vannuffel et al., 1999; Mason et al., 2001; Morot-Bizot et al., 2004).

Recently, an oligonucleotide array called "Staph.Array" has been developed, which targets the manganese-dependant superoxyde dismute (*sod*A) gene (Giammarinaro et al., 2005). It is the only tool described to date that distinguishes the 36 validated staphylococcal species in one shot and allows rapid, accurate identification of staphylococcal strains from clinical and food origins.

Several different epidemiological typing methods have been used in studies of coagulase-negative staphylococci; these include biotyping, antibiotic susceptibility pattern analysis, serological typing, phage typing, slime production detection, protein profile analysis, immunoblot fingerprinting and DNA typing. The most frequently used tests are antibiotic susceptibility and extrachromosomal DNA banding patterns. Pulse Field Gel Electrophoresis of extracted chromosomal bacterial DNA, although technically demanding and expensive, is a tool which provides highly reproducible restriction profiles representing the entire bacterial genome and is far more discriminatory than RAPD for staphylococci (Maslow et al., 1993; Raimundo et al., 2002). Nevertheless none of these techniques has been applied for the differentiation of isolates belonging to the same species of coagulase-negative staphylococci but recovered from different origins (clinical, human, animals and environmental).

Table 1
Species and subspecies of staphylococci

Species and subspecies isolated from human medicine	Species and subspecies isolated from animals, environments and food
S. aureus subsp. *aureus*	*S. intermedius*
S. auricularis	*S. arlettae*
S. capitis subsp. *capitis*	*S. aureus* subsp. *anaerobius*
S. capitis subsp. *urealyticus*	*S. caprae*
S. caprae	*S. carnosus* susp. *carnosus*
S. cohnii subsp. *cohnii*	*S. carnosus* susp. *utilis*
S. cohnii subsp. *urealyticus*	*S. chromogenes*
S. epidermidis	*S. condimenti*
S. haemolyticus	*S. delphini*
S. hominis subsp. *hominis*	*S. equorum* subsp. *equorum*
S. hominis subsp. *novobiosepticus*	*S. equorum* susp. *linens*
S. intermedius	*S. felis*
S. lentus	*S. fleuretti*
S. lugdunensis	*S. gallinarum*
S. pasteuri	*S. hyicus* subsp. *chromogenes*
S. saccharolyticus	*S. hyicus* subsp. *hyicus*
S. saprophyticus subsp *saprophyticus*	*S. kloosii*
S. schleiferi subsp. *schleiferi*	*S. lentus*
S. sciuri subsp. *carnaticus*	*S. lutrae*
S. sciuri subsp. *rodentium*	*S. muscae*
S. sciuri subsp. *sciuri*	*S. nepalensis*
S. simulans	*S. piscifermentans*
S. warneri	*S. saprophyticus* subsp. *bovis*
S. xylosus	*S. schleiferi* subsp. *coagulans*
	S. sciuri subsp. *carnaticus*
	S. sciuri subsp. *rodentium*
	S. sciuri subsp. *sciuri*
	S. succinus subsp. *casei*
	S. succinus subsp. *succinus*
	S. vitulinus
	S. warneri
	S. xylosus

2. Coagulase negative staphylococci in dairy products

2.1. Cheese technology

Many investigators have noted the predominance of novobiocin-resistant coagulase negative staphylococci in cheeses, particularly in hard varieties made from ewes' or goats' milk (Delarras and Laban, 1981; Garcia et al., 1988, 1990; Massa and