Unveiling Staphylococcus aureus enterotoxin production in dairy products: a review of recent advances to face new challenges

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REVIEW PAPER

Unveiling *Staphylococcus aureus* enterotoxin production in dairy products: a review of recent advances to face new challenges

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Abstract *Staphylococcus aureus* is a major food-borne pathogen worldwide and a frequent contaminant of foodstuffs where some strains are able to produce staphylococcal enterotoxins (SE). Consumption of foods containing these SEs is responsible for staphylococcal food poisoning (SFP) outbreaks. Milk and milk products are foodstuffs commonly associated with SFP. Typical SFP symptoms are vomiting with or without diarrhoea and abdominal cramping which reduce after 12 to 72 h. Despite extensive studies, the mechanistic base of SE production is still poorly understood but appears to be quite heterogeneous among the 21 different SEs identified to date. In this review, recent data regarding *S. aureus* and SE detection and quantification in dairy products as well as data about *S. aureus* growth and SE production with regard to parameters relevant for the dairy context and the cheese industry have been summarized. Recent technological developments have allowed the detection of *S. aureus* and SEs in foodstuffs to be refined. Similarly, molecular approaches have allowed high-throughput investigations of the physiology of *S. aureus* and revealed the complexity of this multi-faceted problem. SFP control must indeed take account of the growth of *S. aureus* as well as SE production. The wealth of new available data will open up new strategies for a better risk assessment and control of this major pathogen.

金黄色葡萄球菌肠毒素对乳制品安全性的挑战

摘要 金黄色葡萄球菌是主要的食源性致病菌，经常发生由于一些葡萄球菌产生的肠毒素(SE)污染食物原料而发生的食源性葡萄球菌肠毒素中毒事件。乳和乳制品往往与葡萄球菌肠毒素中毒密切相关。葡萄球菌肠毒素中毒的症状是呕吐，部分病例会出现腹泻和腹部绞痛，一般12–72h后症状减轻。尽管对这方面已经进行过深入的研究，但是葡萄球菌肠毒素中毒的机制还是不十分清楚。到目前为止，已经鉴定出21种完全不同的葡萄球菌肠毒素。本文根据目前现有的文

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献报道，对乳制品中金黄色葡萄球菌和葡萄球菌肠毒素的定性和定量方法，以及在乳制品和干酪中金黄色葡萄球菌的生长和葡萄球菌肠毒素产生的因素进行了文献综述。当今的科学技术完全能够检测出食物中金黄色葡萄球菌和葡萄球菌肠毒素。同样，分子生物学方法能够实现对金黄色葡萄球菌的生理学进行全面的研究，并能够从不同的角度揭示出复杂生物体的性质。控制葡萄球菌肠毒素中毒首先要控制金黄色葡萄球菌的生长，也就是控制葡萄球菌肠毒素的产生。大量的研究数据将有助于对这种致病菌进行风险评估和预防。

Keywords Staphylococcus aureus · Milk · Cheese · Enterotoxin · Risk assessment

关键词 金黄色葡萄球菌 · 乳 · 干酪/肠毒素 · 风险评估

1 Introduction

Staphylococci and milk products are intimately associated with the history of food poisoning outbreaks worldwide. Especially, the identification of *Staphylococcus aureus* undoubtedly represents a landmark in the field of food safety studies. The first recorded staphylococcal food poisoning (SFP) outbreak was indeed attributed to the consumption of cheddar cheese in Michigan in 1884 (Bergdoll 1979). A few years later, in 1914, Barber (Barber 1914) demonstrated that staphylococci were the causative agents of a food poisoning due to the ingestion of bovine mastitic milk where a poisonous substance was produced when milk was left at room temperature. Despite extensive research, *S. aureus* remains a major causative agent of food-borne disease worldwide (Ikeda et al. 2005; Tirado and Schmidt 2001). Table 1 provides examples of published SFP.

SFP results from the ingestion of staphylococcal enterotoxins (SEs) produced during the growth of *S. aureus* in food. In the early 1990s, *S. aureus* was listed as low risk compared with other milk associated pathogens such as *Salmonella* sp., *Listeria monocytogenes* or enteropathogenic *Escherichia coli* (Johnson et al. 1990c). A reduction in the number of published works on recorded SFP outbreaks was observed by the end of the eighties (Table 1). Nevertheless, this is in contrast with epidemiological data in Europe over the last 20 years which have reported constant levels of SFP outbreaks: around 1,500 outbreaks were reported over a period of 6 years (1993–1998), 240 in 2006 and 258 in 2007 (EFSA and ECDC 2007; Tirado and Schmidt 2001). Over the years, *S. aureus* remains the first causative agent involved in food-borne diseases in milk and milk products (Delmas et al. 2006). It is the most frequent pathogen associated with raw milk cheeses (De Buyser et al. 2001), even though, it is assumed that SFP outbreaks are under-reported compared with other food-borne pathogens such as *Salmonella.* This is merely due to the fact that SFP symptoms are not as severe (most commonly: vomiting, diarrhoea and abdominal cramps) and remission occurs within 24 to 48 h. Thus most patients do not consult a physician. This explains the differences in the ranking of *S. aureus* when verified or possible causative agents are taken into account. In 2008, *S. aureus* was confirmed to be the second causative agent of food-borne disease outbreaks (15%) after *Salmonella* sp. but the first suspected causative agent (42.5%) in France (Institut de veille sanitaire, Données relatives aux toxi-infections alimentaires collectives déclarées en France en 2008). Another bias is the over-representation of French data on verified outbreaks (almost half of the data reported by European Food Safety Authority and European Centre for Disease Prevention and Control in 2007). This may also explain the “French particularity” on reported SFP outbreaks which represent almost 70% of all reported SFP in Europe in 2007 (EFSA and ECDC 2007).

Table 1 Examples of staphylococcal food poisoning in milk and dairy products

Country	Year	Number of cases	Food involved	SE type	Milk type	Reference
USA	1884	Unspecified	Cheese	Unspecified	Unspecified	Bergdoll (1979)
USA	1958	200	Cheese	Unspecified	Raw	Johnson et al. (1990b)
USA	1965	Unspecified	Cheese	Unspecified	Unspecified	Zehren and Zehren (1968)
Canada	1977	12	Cheese	Unspecified	Unspecified	Johnson et al. (1990b)
Canada	1980	62	Curd	SEA and SEC	Unspecified	Todd et al. (1981a)
USA	1981	16	Cheese	Unspecified	Pasteurized	Altekruse et al. (1998)
England	1983	2	Cheese	Unspecified	Pasteurized	Barrett (1986)
France	1983	20	Cheese	SEA and SED	Raw	De Buyser et al. (1985)
Scotland	1984	27	Cheese	SEA	Raw	Bone et al. (1989)
Scotland	1985	2	Goat milk	Unspecified	Unpasteurized	Sharp (1989)
USA	1985	860	Chocolate milk	SEA	Pasteurized	Evenson et al. (1988)
Israel	1987	3	Goat milk	SEB	Raw	Gross et al. (1988)
England	1988	155	Cheese	Unspecified	Unpasteurized	Maguire et al. (1991)
Brazil	1994	7	Cheese	SEH	Unspecified	Pereira et al. (1996)
France	1997	140	Cheese	Unspecified	Raw	Kerouanton et al. (2007)
France	1998	62	Cheese	Unspecified	Raw	Kerouanton et al. (2007)
France	1998	37	Semi-hard cheese	Not detected	Raw	Kerouanton et al. (2007)
Japan	2000	13,420	Milk powder	SEA and SEH	Unspecified	Asao et al. (2003), Ikeda et al. (2005)
France	2001	4	Soft cheese	SEA	Unspecified	Kerouanton et al. (2007)
France	2001	46	Semi-hard cheese	SED	Raw	Kerouanton et al. (2007)
France	2002	104	Sheep's milk cheese	SEA	Raw	Kerouanton et al. (2007)
France	2009	23	Cheese	SEE	Unpasteurized	Ostyn et al. (2010)

From De Buyser et al. 2001; Kerouanton et al. 2007

Improvement of the data received from the European reporting system will probably give a better estimation of SFP incidents.

Compared with other well-known pathogens such as *Salmonella* and *Listeria*, the risk assessment for SFP consists of an additional layer of complexity as it involves assessment of SE production and not only the presence or absence of the organisms. Risk assessment is difficult to achieve as it is necessary to establish or evaluate the probability that the *S. aureus* strain is enterotoxigenic (all strains of *S. aureus* do not carry SE genes), the type of SE and the correlation between *S. aureus* counts and SE production. Moreover, the possibility of the presence of SE even in absence of *S. aureus* counts must also be taken into account.

Current available literature on SFP has focused on epidemiological data and/or effect of (food) environment on SE production (Le Loir et al. 2003; Smith et al. 1983; Zhang and Stewart 2001) but none specifically addressed the dairy products,

apart from the review of Gilmour and Harvey published in 1990 (Gilmour and Harvey 1990). The aim of the current review was to update available data on SFP regarding (a) recent advances in *S. aureus* and SE detection in foodstuffs and especially in milk products and cheeses and (b) *S. aureus* physiology and SE production with regard to the peculiar conditions encountered in cheesemaking process. We highlight how these findings open up avenues towards risk assessment and prevention of SFP in the cheese industry.

2 Risk assessment of SFP with regard to the cheese industry

2.1 *S. aureus* detection and quantification techniques

Risk assessment regarding *S. aureus* contamination in cheese products has recently changed within the European Community. It was previously based on the quantification of coagulase-positive staphylococci (CPS) in cheese at the time of production. CPS are staphylococcal species which produce free coagulase, an enzyme capable of coagulating plasma (readily detectable by a simple Petri plate assay), and regarded as a key virulence factor. The CPS group includes *S. aureus* and other pathogenic staphylococcal species whereas, coagulase negative staphylococci are regarded as non- or moderately pathogenic (e.g. *S. xylosus* or *S. carnosus*). The so-called new hygiene package (EC regulation no. 1441/2007) takes into account the fact that SE can be produced and can remain active in foodstuffs whereas the SE-producing CPS population has declined and may no longer be detectable in the product at the time of release. Thus, the new European standards rely on controlled analyses carried out during the process at times when the CPS population is expected to be the highest and limits in CPS counts take account of the cheese technology: maximum counts (*M* values) range from 10^2 cfu·g^{-1} of product (in unripened soft cheeses made from milk or whey that has undergone pasteurization or a stronger heat treatment) up to 10^5 cfu·g^{-1} (in cheeses made from raw milk). Above those *M* values, SEs have to be investigated according to European screening methods for the detection of staphylococcal enterotoxins in milk and milk products in a community reference laboratory for CPS.

The detection and quantification of CPS in cheese does not present any difficulties. Many reliable techniques are now available and rely either on direct counts on selective media (normalized methods based on international norms) or on DNA-based techniques (polymerase chain reaction (PCR) targeting *S. aureus*-specific genes).

CPS are routinely detected and quantified on selective media. Food samples are prepared (decimal dilutions) and plated onto solid agar media. The most common selective medium is Baird-Parker (BP) medium which can be complemented with rabbit plasma fibrinogen (BP-RPF). The current international norms for *S. aureus* detection are based on these two BP and BP-RPF media. Modifications of these media have been reported such as complementation with compounds like sulphamethazine (inhibition of *Proteus* spp; (Smith and Baird-Parker 1964)), acriflavin or polymyxin (inhibition of coagulase negative staphylococci and enterococci; (Devriese 1981)), sodium azide (inhibition of waste water flora; (Lebaron and Baleux 1988)), rabbit plasma (visualization of coagulase activity; (Beckers et al. 1984)) or cycloheximide (inhibition of moulds and yeasts of cheese

surface flora; De Buyser and Hennekinne 2010). New chromogenic media are now commercially available (e.g. CHROMagarTM, CHROMagar, France or chromID *S. aureus*, BioMérieux, France) and offer rapid, selective and colour-based detection, identification and quantification of *S. aureus*. Beside normalized methods, alternative methods have been developed and are also commercially available. The use of two of them (Petrifilm™ Staph Express, 3M, and Rapid'Staph Test, BioRad) has recently been validated in France after collaborative and comparative studies. Both tests are based on selective (and chromogenic for Petrifilm™ Staph Express) media that allow presumptive identification of *S. aureus* within 24 h (instead of 48 h for current normalized methods) and propose rapid confirmatory tests.

Because *S. aureus* is a major concern in terms of nosocomial infections, great efforts have been dedicated to the development of new techniques that will allow the identification and quantification of *S. aureus* strains and even the detection of virulence associated genes (e.g. SE genes and antibiotic resistance determinants) with greater sensitivity and rapidity than the current methods (Stepan et al. 2004; Tenover 2007). Some PCR-based detection techniques have been applied to cheese and dairy products and targeted *S. aureus*-specific DNA regions (e.g. *nuc* gene encoding the thermonuclease) and some enterotoxin genes in simplex or multiplex PCR assays. For example, *nuc* gene and *sec, seg, seh* and *sei* genes were targeted in a combined PCR test to detect enterotoxigenic *S. aureus* strains in raw milk with detection limits of 10^4 to 10^7 cfu·g^{-1} (Ercolini et al. 2004). Other works using multiplex PCR on *nuc* and *sec* genes have reported sensitivity levels as high as 5 cfu·g^{-1} when applied to cheddar cheese (Tamarapu et al. 2001). More recently, a quantitative RT-PCR technique has been used for the quantification of *S. aureus* strains and was successfully applied to dairy products. Genes such as *nuc* (Alarcon et al. 2006) or SA0836, encoding a transcriptional regulator (Goto et al. 2007) were targeted and allowed quantification limits down to 10 cfu·g^{-1} in raw milk and other foodstuffs.

Compared with phenotypic methods, DNA-based molecular methods have the advantage of being independent of specific features in artificial conditions (e.g. growth in laboratory media). They are based on stable characteristics, having great discriminatory power and achieve ~100% identification since all bacteria contain DNA. Thanks to recent progress in miniaturization, automation and the lowering of costs, some of these molecular techniques are now proposed for routine applications in risk assessment within the food industry. On the other hand, DNA-based methods may also lead to the detection of DNA from dead cells and result in an overestimation of *S. aureus* counts in the samples.

2.2 Enterotoxin detection and quantification

SEs are short proteins (24–28 kg·mol^{-1}) secreted into the culture medium by some but not all *S. aureus* strains. To date, with the exclusion of variants, 21 SE types have been described from *sea* to *selv.* All possess superantigenic activity whereas only some (SEA to SEI, SER, SES and SET) have been proven to be emetic whereas some others (e.g. SElQ, SElL) have been proven to be non-emetic (Table 2). Non-emetic SEs, or the ones that have not yet been checked for emetic activity, are named "staphylococcal enterotoxin-like" (SEl) (Lina et al. 2004). SEs are soluble in water and saline solutions. They are highly stable, resist most proteolytic enzymes such as trypsin or pepsin and

thus remain active after ingestion, in the digestive tract. They also resist papain, rennin and chymotrypsin, and persist during the cheese making process. SEs are also highly heat resistant and resist conditions (heat treatment, low pH) that easily destroy *S. aureus* itself (Le Loir et al. 2003). Unlike *S. aureus* detection, SE detection and quantification in dairy products and especially in the cheese matrix are reportedly fastidious. SEs are produced in small amounts in cheeses (often <0.1 $ng \cdot g^{-1}$ of cheese product). However, cheeses are also rich in proteinaceous compounds which may interfere with the detection assays employed. Most current detection and quantification techniques are based on immunological assays. The first step of analysis involved the extraction and concentration of the SEs to eliminate as much as possible other constituents of the cheese matrix. Extraction is a crucial step since it determines the robustness of the final result. In a second step, a detection technique is applied to the samples.

Table 2 Genomic location and emetic activity of SEs

SE (gene)	Emetic activity	Genetic support	Regulation
sea	Y	ΦMu50a ΦSa3ms, ΦSa3mw, ΦNM3, and Φ252B	Phage cycle, promoter sequence, and *agr*—independent
seb	Y	SaPI3 and pZA10	*agr*- and *sar*-dependent
sec	Y	SaPIn1, SaPIm1, SaPImw2, and SaPIbov1	*agr*-, *sar*-, and *saeRS*-dependent
sed	Y	pIB485-like	*agr*-dependent
see	Y	ΦSa	–
seg	Y	*egc*1 (*v*Saβ I), *egc*2 (*v*Saβ III), *egc*3, and *egc*4	–
seh	Y	Staphylococcal chromosomal cassette	–
sei	Y (weak)	*egc*1 (*v*Saβ I), *egc*2 (*v*Saβ III), and *egc*3	–
sej	Y	pIB485-like and pF5	*agr*-independent
selk	N[a]	ΦSa3ms, SaPI5, ΦSa3mw, SaPI3, SaPI1, and SaPIbov1	–
sell	N	SaPImw2, SaPIn1, SaPIm1, and SaPIbov1	–
selm	–	*egc*2 (*v*Saβ III) and *egc*1 (*v*Saβ I)	–
seln	–	*egc*1 (*v*Saβ I), *egc*2 (*v*Saβ III), *egc*3, and *egc*4	–
selo	–	*egc*1 (*v*Saβ I), *egc*2 (*v*Saβ III), *egc*3, and *egc*4	–
selp	–[b]	ΦN315 and ΦMu3A	–
selq	N	ΦSa3ms, SaPI1, SaPI3, SaPI5, and ΦSa3mw	–
ser	Y	pIB485-like and pF5	–
ses	Y	pF5	–
set	Y	pF5	–
selu	–	*egc*2 (*v*Saβ III) and *egc*3/*egc*4	–
selv	–	*egc*4	–

For references see text

Φ phage, *p* plasmid, *SaPI Staphylococcus aureus* pathogenicity island, *v* genomic island, *egc* enterotoxin gene cluster, dash no data available

[a] Data from (Orwin et al. 2002). Lack of emetic activity is attributed to SElK by comparison with SElQ even if specific verification of emetic activity on monkeys is not specified

[b] SelP was proved to be emetic in the house musk shrew emetic assay but not on monkeys (Omoe et al. 2005)

Several commercial kits are available to detect the presence of the most commonly found SEs (SEA-SEE) in routine analysis (Table 3). All of them are based on immunological recognition of SEs by specific antibodies. In some kits, they are coated on latex beads to be used in reverse passive latex agglutination assays (e.g. SET-RPLA, Oxoid) whereas in others, they are used in enzyme-linked immunosorbent assay (ELISA)-based kits (e.g. TECRA Unique SET and TRANSIA plate SET) or enzyme linked fluorescent assay (ELFA) (e.g. VIDAS SET2, Biomérieux). Detection with ELISA and ELFA techniques is quicker than with RPLA (1.5 to 4 h, depending on the protocol; Table 3). These kits use polyvalent sera and do not allow identification of the SE type. Only the ELISA-based Ridascreen (R-Biopharm, France) and RPLA-based Oxoid kits allow identification of the SE type (Table 3). The range of detectable SE types is thus quite limited when compared with the existing 21 different SE types (see below).

Other immunoassays have recently been developed to respond to a need for real-time analysis (especially for toxins identified as potential bioterrorism weapons, which include SEB). They are examples of biosensors, which are devices that combine a biochemical recognition element with a physical transducer. Some examples of biosensor applications combine immunodetection and capture of an antigen (e.g. SE) and quantification by surface plasmon resonance sensor systems (optical sensor) (Soelberg et al. 2005). When applied to SEB detection, these devices offer high sensitivity (in the range of $ng{\cdot}mL^{-1}$) and allow real-time SEB detection (Naimushin et al. 2002; Rasooly and Herold 2006). Other techniques, such as protein array chips are based on enzyme linked sandwich immunoassay which recognize and bind virulence factors by specific antibodies (Uttamchandani et al. 2009). Recent versions of such techniques combine antigen capture with a detection of antibody-bound virulence factors (e.g. SEB) by measuring the electrical current generated by redox recycling of an enzymatically released substance. These so-called electrical protein chips provide high sensitivity (1 $ng{\cdot}mL^{-1}$ for SEB in milk, which is comparable to current commercial kits) and quick detection (within ~20 min) (Quiel et al. 2010). However, although promising, the cost of these techniques and their probable difficult use in complex food samples represent significant hurdles for their routine application in the cheese industry.

Table 3 Some features of commercial kits for staphylococcal enterotoxin detection

Detection kit	Technique	SE detected	Analysis time[a] (h)	Sensitivity ($ng{\cdot}mL^{-1}$)
TECRA Unique SET	ELISA	SEA to SEE[b]	4	0.5–1.25
RIDASCREEN	ELISA	SEA to SEE[c]	2.5	0.1–0.75
TRANSIA plate SET	ELISA	SEA to SEE[b]	1.5	0.2
Oxoid SET-RPLA	RPLA	SEA to SED[c]	20–24	0.5–1.0
Biomérieux VIDAS SET2	ELFA	SEA to SEE[b]	1.5	0.25–0.5

ELISA enzyme linked immunosorbent assay, *RPLA* reverse passive latex agglutination, *ELFA* enzyme linked fluorescent assay

[a] Extraction time is not included

[b] Staphylococcal enterotoxin types detected (but not identified) by the kit

[c] Staphylococcal enterotoxin types detected and identified by the kit

Most of the above mentioned immunoassays can lead to a false or incomplete diagnosis when used to detect SEs in food. For example, SEA and SED were shown to be undetectable (loss of serological recognition) but still active (on kitten in vivo assay) after heat treatment (Bennet 1992). Furthermore, with commercially available kits, only SEA to SEE types can be routinely detected. Yet, it is now documented that other SEs, like SEH (Jorgensen et al. 2005) or SEE (Ostyn et al. 2010), have been involved in SFP outbreaks. Besides, some newly described SEs are reportedly emetic or weakly emetic, and may have an incidence in food safety (Table 2). The use of immuno-based diagnosis cannot detect and confirm the involvement of such SEs. There is thus a need for new and improved analytical methods for SE detection and SFP diagnosis.

The recent development of high-throughput methods based on mass spectrometry now theoretically allows for the detection of any kind of SE types in complex samples. The so-called Protein Standard Absolute Quantification (PSAQ) strategy uses isotope-labelled enterotoxins as internal standards for mass spectrometry analysis. PSAQ has been applied to the detection of SEA (Dupuis et al. 2008). Compared with ELISA-based techniques, PSAQ implementation gives excellent results in terms of specificity but takes a longer time for sample preparation. For the time being, it is also twice as expensive as quantitative ELISA technique. PSAQ has been shown to give excellent results for SEA detection on coco-pearl (a coconut-based dessert) samples after an immunoaffinity enrichment step (Hennekinne et al. 2009). Mass spectrometry has also proven to be efficient when used in combination with immunomagnetic separation on magnetic beads for SEB detection on milk samples and allowed SEB detection at low-nanogram levels (detection limit, ~2 ng) (Schlosser et al. 2007).

Up to now, official detection and quantification methods are based on the immunoassay principle which suffer from severe limitations due to difficulties in obtaining specific antibodies for each type of SEs which can be incriminated or suspected in SFP outbreaks.

The development of quantitative mass spectrometry techniques will undoubtedly offer very interesting and additional information compared with the use of immunoassays.

3 Enteroxin gene expression and regulation in *S. aureus*

The increasing number of available *S. aureus* genome sequences and the concomitant development of molecular approaches of *S. aureus* physiology and virulence including transcriptomic and proteomic studies have led to a wealth of data emphasizing the complexity of virulence regulation. Here, we briefly summarize the latest data on identified enterotoxins (Thomas et al. 2007) and the control of their expression by *S. aureus* regulatory systems. From data available on sequenced *S. aureus* strains, number and type of SE genes are highly variable from strain to strain. While *S. aureus* strains NCTC8325 and Newman harbour only *sea* gene (Baba et al. 2008; Gillaspy et al. 2006), N315, Mu50 and MW2 strains hold 9, 10 and 6 *se* genes, respectively.

SE genes are found in various genetic supports and all these supports are mobile genetic elements (Le Loir et al. 2003). SE genes can be carried by plasmids (*seb*,

sed, *sej, ser, ses* and *set*) (Bayles and Iandolo 1989; Omoe et al. 2003; Ono et al. 2008; Shalita et al. 1977; Zhang et al. 1998), by phages (temperate for *sea*, defective for *see, selk, selp* and *selq*) (Betley and Mekalanos 1988; Coleman et al. 1989; Couch et al. 1988; Goerke et al. 2009), on pathogenicity islands (SaPI) (*seb, sec, selk, sell* and *selq*) (Novick and Subedi 2007) or genomic islands (*seg*, *seh*, *seli*, *selk*, *sell*, *selm*, *seln*, *selo*, *selp*, *selq, selu* and *selv*) (Baba et al. 2008; Collery et al. 2009; Holden et al. 2004; Jarraud et al. 2001; Letertre et al. 2003; Thomas et al. 2006). Some of them have been found in several genetic elements such as *seb* which was reported to be located on the chromosome, a plasmid or a transposon (Altboum et al. 1985; Shafer and Iandolo 1978; Shalita et al. 1977) and *sec,* on a plasmid or a pathogenicity island (Altboum et al. 1985; Fitzgerald et al. 2001).

The quantity of SEs produced vary with the type of SE and the strain studied making it difficult (or impossible) to generalize on SE expression from data on one type of SE. Even within a given SE type, inter-strain variations have been found. For example, it has been shown that the level of SEA production is strain-dependent, and correlates with the promoter region and the carrier phage (Borst and Betley 1993; Borst and Betley 1994a; Borst and Betley 1994b). SEA production can vary by a factor of 8 depending on the structure of the promoter region (Betley et al. 1992). SE gene transcription is also influenced by the genetic environment, in particular if they are carried by phages. Sumby and Waldor (Sumby and Waldor 2003) showed that the transcription of *sea, selg2, selk* and *sak* (staphylokinase) genes carried by phage φSa3ms depends on the phage cycle. Induction of the prophage significantly increases *sea* and *sak* expression and, to a lesser extent, *selg* and *selk* expression. Recently, Wallin-Carlquist et al. (2010) showed that genetic variability of the prophage region upstream of the *sea* gene explained the strain differences observed in *sea* expression in the presence of acetic acid at pH 5.5 and related it to an induction of the carrier prophage.

The main regulatory systems controlling virulence expression in *S. aureus* are the accessory gene regulator, *agr* (Novick 2003) and the staphylococcal accessory regulator, *sarA* (Cheung et al. 2004). The alternative sigma factor, *sigB*, is a transcriptional regulator involved in stress response and expression of virulence factors in *S. aureus* (for review, see Kazmierczak et al. 2005). Some but not all of the SE are controlled by the *agr* system. The *seb*, *sec* and *sed* genes have been demonstrated to be *agr* dependant whereas *sea* and *sej* are *agr* independent (Tremaine et al. 1993; Zhang et al. 1998). *agr* control on *sed* expression is indirect and probably mediated by another transcriptional regulator called Rot (Tseng et al. 2004) (repressor of toxins, (Cheung et al. 2004)). It has also been demonstrated that *seb* and *sec* are under positive control of *sarA* (Bronner et al. 2004) and data from *Staphylococcus aureus* microarray meta database (http://www.bioinformatics.org/sammd/; Bronner et al. 2004), while *sigB* is a negative regulator of *seb* probably acting indirectly by repressing the *agr* system (Schmidt et al. 2004). More recently, Voyich et al. (2009) showed that *sec* is under the positive control of another regulator of virulence expression, *saeRS*.

SE regulation influences their temporal expression, especially when controlled by the *agr* system or *sarA*. In fact, SEs like SEB, SEC and SED are mainly expressed during the transition from log-phase to stationary phase of growth while SEs like SEA and SEJ are expressed predominantly during the log-phase. SEA production is

only slightly affected by the culture conditions and is directly linked to the population level (Markus and Silverman 1970). Derzelle et al. (2009) followed the temporal expression of all SE and SEl genes during growth of *S. aureus* in laboratory media revealing four different patterns of expression. The mRNA abundance was unchanged for *sea, see, selj, selk selq* and *selp* while a slight decrease in transcript levels was observed over the transition from mid-exponential to late stationary growth phase for *seg, sei, selm, seln, selo* and *selu*. Conversely, expression of *seb*, *sec* and *seh* drastically increased over time or to a lesser extent for *sed, ser* and *sell*. This study constitutes a good starting point for risk evaluation of SE production based on rapid detection of *se* transcripts.

Recent molecular characterization of SE shows a great variability of production depending on the type of enterotoxin, the genetic environment and its regulation. Temporal expression of each *se* gene is controlled by an intricate network of regulation involving regulators sensing the population density (quorum sensing), the modifications of physico-chemical and nutritional conditions of the environment. These recent molecular analyses highlight the intimate link that exists between metabolism and virulence. Even if data on *se* gene regulation presented here were mainly obtained in a context far from the cheese environment, a new look on former results in the light of these recent advances might certainly help to establish new understanding of *se* regulation in the cheese context.

4 Cheesemaking-related parameters affecting SE production

Cheese is a complex, dynamic and evolving environment in which many parameters dramatically vary during the process. The following paragraphs summarize the current knowledge on physico-chemical parameters relevant to cheese making conditions that were shown to affect both *S. aureus* growth and enterotoxin production (Table 4). Finally, a paragraph is devoted to the impact of biotic parameters (i.e. the cheese ecosystems) that interact with *S. aureus* in the cheese context.

4.1 Acidification

It is generally assumed that pH is the main environmental factor that impairs the growth of *S. aureus* and SE production in cheese. Examination of cheese vats by Zheren and Zheren established a correlation between cheese acidification and SE production (Zehren and Zehren 1968). *S. aureus* growth is inhibited at pH values below 4 in aerobic conditions and 4.6 in anaerobic conditions (Mossel and Van Netten 1990). Growth rate is almost twice as low at pH 5 compared with pH 7.5 (Charlier et al. 2008; Iandolo et al. 1964). Besides, acidification rate also has a great impact on the growth of *S. aureus* with faster acidification resulting in greater inhibition of growth (Minor and Marth 1970). The nature of the acid also influences the strength of the inhibition (Domenech et al. 1992). When acidifying milk to a pH of 4.6 with lactic acid, Minor et Marth (Minor and Marth 1970) almost completely inhibited the growth of *S. aureus* and obtained the same effect with acetic acid at pH 5, citric acid at pH 4.5, phosphoric acid at pH 4.1 and chlorhydric acid at pH 4.

Table 4 Factors affecting *Staphylococcus aureus* growth and enterotoxinogenesis

Factor	Optimal growth	Growth limits	Optimal SE production	SE production limits
Temperature	35–41 °C	6–48 °C	34–40 °C	10–45 °C
pH	6–7	4–10	7–8	5–9.6
Aw	0.99	0.85≥0.99	0.99	0.86≥0.99
NaCl	0%	0–20%	0%	0–10%
Redox potential (Eh)	>+200 mV	≥200 to > +200 mV	>+200 mV	≥100 to > +200 mV
Atmosphere	Aerobic	Anaerobic–aerobic	Aerobic	Anaerobic–aerobic

From (Anonymous et al. 2010)

However, several studies on model cheeses showed that *S. aureus* can grow during the first manufacturing phase even in presence of acidifying lactic acid bacteria (LAB). The pH reached after the first hours of manufacture determines the evolution of the population of *S. aureus* during ripening (Delbes et al. 2006; Meyrand et al. 1998). Beside the growth of *S. aureus*, it was shown that SE production is optimal at pH close to neutrality and that acidic pH impairs SE synthesis (Genigeorgis and Sadler 1966). Of note, the overall pH range for SE production is narrower than that for growth limits and depends on growth conditions (Table 4). For example, the lowest pH permitting SE production is around 4.0 when *S. aureus* is grown aerobically and 5.3 when grown anaerobically (Smith et al. 1983). In the cheese context, this may induce higher SE production by *S. aureus* contamination at the surface, where aerobic conditions and higher pH values are observed, in comparison to the interior of the matrix.

4.2 Salt and water activity

Although *S. aureus* is reportedly a halotolerant bacterium compared with other pathogens or LAB used as starter in fermentation processes, salt inhibits the growth of *S. aureus*. *S. aureus* can tolerate NaCl concentrations of between 2.5% and 20%, but its growth is nevertheless dramatically impaired at high salt concentrations (Gomez-Lucia et al. 1992; Tatini 1973). Just as well, SE production decreases when salt concentration increases (Genigeorgis and Sadler 1966). However, differences exist between SEs: production of SEA and SEH was reported to be less sensitive to a decrease in water activity (a_w) than that of SEB or SEC (Regassa and Betley 1993; Sakai et al. 2008). It was not determined and remains unclear if these observations rely on differences at the gene level (different regulation for different SE types) or at the strain level (inter-strain variability in salt sensitivity). Even though most food-borne pathogenic bacteria are inhibited by a_w below 0.93, *S. aureus* is able to grow at lower values (Table 4). *S. aureus* growth inhibition occurs at a_w 0.90 in anaerobiosis, but an a_w value down to 0.84 is necessary for *S. aureus* inhibition in aerobiosis (Mossel and Van Netten 1990). Other factors such as pH, temperature and acidity interfere with the a_w effect on *S. aureus* (Iandolo et al. 1964; Notermans and Heuvelman 1983). Likewise, pH and a_w effect depends on the nature of the solute

used: NaCl, saccharose, glycerol, ethanol, etc. (Marshall et al. 1971; Stewart et al. 2005; Stewart et al. 2002; Tatini 1973; Troller 1971; Troller and Stinson 1978). The tolerance of *S. aureus* to high salt concentrations is a competitive advantage over other microbiota and its growth is favoured when the salt concentration is greater than 3.5% (Notermans and Heuvelman 1983; Peterson et al. 1964). This halotolerance is probably a key element in the explanation of the high prevalence of *S. aureus* in food-borne diseases (Hurst and Collins-Thompson 1979).

4.3 Temperature

S. aureus is capable of growth at temperatures ranging from 6 to 48 °C, even though its growth is optimal at 37 °C (Iandolo et al. 1964; Tatini et al. 1973). Similarly, SEs can be produced between 10 and 45 °C with an optimum at 40 °C (Table 4). Hence, respecting the cold chain appears to be a key point in SFP prevention: keeping the raw material (milk) and final product (cheeses) at a temperature inferior to 7–8 °C should limit *S. aureus* proliferation. Refrigeration defects during storage of milk intended for cheese manufacture are often reported as a risk factor at the origin of SFP (EFSA and ECDC 2007). Dos Santos et al. (1981) traced the presence of *S. aureus* in milks used for Minas cheese manufacture and reported that between the milking site and the manufacturing plant, milk was rarely refrigerated and resulted in high counts of *S. aureus* (Dos Santos et al. 1981). Consequently, they showed that at 37 °C (manufacturing temperature for Minas cheese), the population of *S. aureus* increased while the ferment population decreased after 2 h of manufacture. In another study, Soejima et al. (Soejima et al. 2007) concluded that milk with low contamination levels (1–2 log cfu·mL^{-1}), should not be stored for more than 6 h at 35 °C, 10 h at 25 °C and 24 h at 15 °C in order to avoid SFP upon consumption of reconstituted milk.

4.4 Aeration

S. aureus is a facultative anaerobic bacterium that can grow in the absence of oxygen even if its growth has been slowed down. The generation time of *S. aureus* during the exponential phase of growth at 37 °C in brain heart infusion medium (a rich laboratory medium) is around 35 min under aerobic conditions but takes 80 min under strict anaerobic conditions (Belay and Rasooly 2002). SE production is also higher under aerobic versus anaerobic conditions (Barber and Deibel 1972; Belay and Rasooly 2002). For example, SEH production is maximal for a pH of 7 and an aeration rate of 300 mL·min^{-1} and a strong decrease is observed in anaerobic conditions (Stewart et al. 2002). Therefore milk oxygenation during collection, after milking, or during the cheese making process can promote the proliferation of *S. aureus* while micro-anaerobic conditions in cheese (apart from the surface) should be unfavorable to SE production.

4.5 Medium composition

The development of *S. aureus* requires an organic source of nitrogen (5 to 12 amino acids) and vitamins (thiamin, nicotinic acid) (Mah et al. 1967). Nutritional

requirements vary from strain to strain but most often, *S. aureus* is reported to be auxotroph for cystein, aspartate, glutamate and even some strains can be auxotroph for valine, leucine, glycine and proline (Onoue and Mori 1997; Taylor and Holland 1989). Lysine, aspartic and glutamic acids, leucine and tyrosine are not necessary for its growth but in their absence, SE production was observed to be reduced. Overall, SEA production is less affected than SEB or SEC production by the removal of any amino acids (Onoue and Mori 1997). *S. aureus* is capable of metabolising different carbon sources such as glucose, lactose, maltose and mannitol that also influence SE production. SEB production was reported to be 7-fold lower when glucose or glutamate was added to a defined medium (Mah et al. 1967). Glucose concentration influences overall SE production, and higher glucose concentrations decrease SEA, SEB and SEC production. This repression by glucose was observed even when the pH was maintained at 6.5 (Jarvis et al. 1975; Regassa et al. 1991). Since the growth medium composition strongly influences SE production, SE production will consequently be different in milk and cheeses compared with laboratory media (Gomez-Lucia et al. 1986; Otero et al. 1993; Otero et al. 1990).

4.6 Milk ecosystem

In addition to the physico-chemical parameters that influence SE production in cheese, *S. aureus* encounters a microbial ecosystem that can restrict its growth. The presence of other flora, especially LAB, reportedly limits the growth of *S. aureus* (Mossel and Van Netten 1990). In pure cultures, the production of SE is concomitant with the growth of *S. aureus* while, in the presence of LAB, SE production and *S. aureus* growth are uncoupled for most SE types (growth without SE production) (McCoy and Faber 1966). The mechanisms of interactions between *S. aureus* and LAB have been recently reviewed in two different ecosystems, the vaginal ecosystem and the fermented foods (Charlier et al. 2009). In the context of the cheese ecosystem, a few studies have described the inhibition of SE production in the presence of LAB; however, no study has fully elicidated the mechanisms involved in such antagonism (Haines and Harmon 1973; Noleto et al. 1987; Otero et al. 1988). Alomar et al. (Alomar et al. 2008) have shown that the inhibition of *S. aureus* by *Lactococcus garvieae* in milk could not be attributed to acidification, lactate or acetate production, the production of antistaphylococcal substances, or amino acid competition, leaving the question of the mechanisms involved unresolved.

The most documented mechanisms of inhibition of the growth of *S. aureus* by LAB are the production of bacteriocins (Cotter et al. 2005; Ross et al. 2002) or hydrogen peroxide (Haines and Harmon 1973; Ito et al. 2003), competition for nutrients (Haines and Harmon 1973; Iandolo et al. 1965), and mainly, acidification (Delbes et al. 2006; Kao and Frazier 1966; Notermans and Heuvelman 1983), although the impact of the latter mechanism has been questioned (Charlier et al. 2008). Still unidentified mechanism(s) acting jointly with acidification has (have) often been suspected (Charlier et al. 2008; Daly et al. 1972; Gilliland and Speck 1974). Recently, a mathematical model of the interaction between *S. aureus* and LAB highlighted that the critical parameter was not pH or lactic acid production but more likely a critical density of the LAB population itself (Le Marc et al. 2009).

Also, nutritional competition was recognised in the early studies on *S. aureus*–LAB interactions (Haines and Harmon 1973; Iandolo et al. 1965). The importance of nutritional components in the interaction is highlighted by changes in ecosystem equilibrium when the medium is modified (Charlier et al. 2008; Daly et al. 1972). Daly et al. (Daly et al. 1972) showed that the inhibition of *S. aureus* by *L. lactis* was weaker in milk compared with that observed in TSB, a rich laboratory medium. Conversely, Charlier et al. (Charlier et al. 2008) showed that the inhibition was higher in milk compared with M17 medium (a rich laboratory medium) at regulated pH.

The inhibition of *S. aureus* by LAB is a multifactorial, complex and relatively unknown phenomenon. Indeed, it is rather difficult to draw conclusions on the effect of individual parameters (pH, H_2O_2, nutritional competition, etc.) in a mixed culture context. It is important to highlight that most of the studies were carried out in laboratory medium while it is strongly suspected that LAB antagonism in milk certainly involves phenomena which are linked to nutritional competition or the production of inhibitory metabolites.

A bacterial physiological approach through global gene expression profiling has recently become feasible due to the recent development in DNA microarrays. Species-specific microarrays have recently been developed and used to study *S. aureus*–LAB interactions in mixed culture under laboratory conditions (Even et al. 2009; Nouaille et al. 2009). These pioneer studies have shown that *L. lactis* is capable of inhibiting *S. aureus* virulence expression in conditions where the growth of *S. aureus* is hardly affected by the presence of *L. lactis*, thus demonstrating that growth and virulence expression can be decoupled in *S. aureus* in the context of bacterial interactions. The development of methodologies for RNA extraction from a cheese matrix (Ablain et al. 2009; Duquenne et al. 2010; Ulve et al. 2008) now allows for the direct study in a cheese matrix and has shown that growth and virulence inhibition remain decoupled in a model cheese matrix (Cretenet et al., *in press* in Environmental Microbiology Reports).

5 Influence of the cheese manufacturing process on enterotoxin production

The physico-chemical parameters of cheese are influenced by bacterial activity and conversely, bacterial growth depends on nutritional (e.g. nitrogen and carbon sources) and physico-chemical conditions (e.g. acidity, temperature and salinity) of the media. The cheese making process is a complex process involving several steps that affect the microbial ecosystem (including pathogenic bacteria) through mechanical and physical actions as well as the precocity and intensity of acidification. The different cheese technologies present physico-chemical parameters and bacterial communities that are more or less permissive to the growth of *S. aureus*. The behaviour of *S. aureus* in cheese depends on the cheesemaking process and, subsequently, on its capacity to resist different stresses within the cheese matrix.

5.1 Fresh cheeses

Fresh cheeses are prepared from raw or pasteurized milk and, for the large majority of these cheeses, the curd is obtained by fermentation due to the action of the starter

or natural milk flora. The pH generally decreases to around 5 and the water activity is high (a_w=0.95 to 0.97) (Anonymous et al. 2010). After manufacture, these cheeses are consumed fresh, without ripening between 5 and 30 days after preparation. Erkmen (Erkmen 1995) observed an increase in the population of *S. aureus* of 2 to 3 log in Feta cheeses during the first 24 h after an artificial contamination with *S. aureus* between 10^5 and 10^7. Then, the population of *S. aureus* decreased to the initial level of contamination after 75 days at a rate depending on salt concentration, starter activity and storage time. Conversely, in Domiati cheese, the presence of a high salt concentration (10% of sodium chloride) was advantageous to the growth of *S. aureus* when competing with LAB (Ahmed et al. 1983). In Camero cheese, Olarte et al. (2000) reported that the growth of *S. aureus* was higher in batches without starter, but they did not detect any SE. In fresh cheeses, it can be concluded that the growth of *S. aureus* (and consequently, SE production) is likely in the absence or a reduced activity of competitive flora.

5.2 Soft cheeses

Soft cheeses constitute a large and diversified category, and include cheeses such as Mont d'Or, Camembert, Brie, Sainte-Maure, Munster or Tilsit. In soft cheeses such as Camembert, the water activity is high (a_w=0.95), allowing *S. aureus* growth. Studies on the growth of *S. aureus* during soft cheese making from bovine or caprine milk have reported an increase in the population (~3 $\log_{10}$) during the first phase of the process (~22 h), from inoculation to salting (Meyrand et al. 1998; Vernozy-Rozand et al. 1998). Part of this increase in biomass (by 1 to 1.5 $\log_{10}$, in cfu·g^{-1}) can be attributed to curd draining. In general, the speed and rapidity of the draining correlate well with the concentration of microorganisms (Gay et al. 1993). After this growth period, the population of *S. aureus* usually remains stable during ripening, but depends on the ripening temperature and pH of the product. Vernozy-Rozand et al. (Vernozy-Rozand et al. 1998) observed differences in the growth of *S. aureus* between the surface and interior of the cheese during ripening, which were attributed to an increase in the pH during centripetal maturation of the cheese by non-starter flora. Favourable conditions to the growth of *S. aureus* in these cheeses also affect SE production. Accordingly, Meyrand et al. (Meyrand et al. 1998) observed a variation of SEA production of 1 to 3.2 ng.g^{-1} for an initial population of *S. aureus* of 10^3 to 10^6 cfu·mL^{-1} reaching maximal counts of 10^5 to 3.10^7 cfu·mL^{-1} at 22 h. In conclusion, soft cheeses are favourable environments to the growth of *S. aureus* that can actually involve sanitary problems especially in cases where there is an initial contamination greater than 10^3 cfu·mL^{-1}.

5.3 Semi-hard and hard cheeses

Semi-hard and hard cheeses vary in terms of their composition, format and exterior appearance (differences in the aspect of the rind or microbial flora). These cheeses are characterised by using a quick draining step (30 to 90 min) as well as having limited acidification. The risk of growth of *S. aureus* mainly depends on the application of heat treatments on the curd (e.g. 52–55°C for a maximum of 60 min for Emmental, Gruyère) or not (e.g. cheddar, St-Nectaire and Tomme).

Numerous studies are available on the growth of *S. aureus* in cheddar cheese following SFP outbreaks in the USA in the 1960s (Johnson et al. 1990a). These studies report that in cheddar technology, the population of *S. aureus* grows during the cheesemaking process until pressing and then decreases during ripening (Bachmann and Spahr 1995; Ibrahim et al. 1981a; Tatini et al. 1971). Cheddar cheese constitutes a favourable environment for the growth of *S. aureus* in the absence of an active starter (Ibrahim et al. 1981b). The activity of the competitive flora is a crucial parameter influencing the growth of *S. aureus* and SE production (Bachmann and Spahr 1995; Takahashi and Johns 1959; Tatini et al. 1971). Salting of cheddar cheese and a temperature decrease induce an increase in the population of *S. aureus*, probably due to lower starter activity in response to the increased salt concentration (Ibrahim et al. 1981a). Moreover, the duration of pressing is a critical parameter which must be taken into account when evaluating the risks of growth of *S. aureus* in cheddar cheese. SEA production in cheddar cheese depends on the size of the inoculum and the activity of the starter but is indirectly influenced by the salt concentration (via an effect on the population of *S. aureus*) (Ibrahim et al. 1981a; Koenig and Marth 1982; Reddy and Marth 1995). SEA was detected in cheddar cheese even after 3 years of ripening independently of the pH value (Tatini et al. 1971). Apart from SEA, other SEs have not been studied in cheddar cheeses.

In the Spanish Manchego-type cheeses, *S. aureus* was detected in the curd but not in the final product (Tornadijo et al. 1996). Combination of low pH values and regular decrease in the water activity during ripening to final values of 0.9 accounted for the loss of viability of *S. aureus*. As for soft cheeses, the population of *S. aureus* is concentrated during draining (by a factor of ~6) reaching a maximal population between 10^5 and 10^7 cfu·g^{-1} before a decrease being observed during ripening (Freitas and Malcata 2000; Gomez-Lucia et al. 1992; Nunez et al. 1988; Otero et al. 1993). Contradictory observations have been reported on the influence of starter on the growth of *S. aureus* in Manchego-type cheeses. Gomez-Lucia et al. (1992) observed lower counts of *S. aureus* from day 1 to the end of ripening in cheese with higher starter inocula. Conversely, Nunez et al. (1988) did not observe any differences in the counts of *S. aureus* during ripening with or without the addition of starter even though pH values were greatly different. Differences in these observations may be due to the greater or lower inhibitory effect of the initial flora in the raw milk. When SE are detected, SEA is predominant and then SEC or SED. In these studies, SE production was always positively correlated to the growth of *S. aureus* (Cosentino and Palmas 1997; Gomez-Lucia et al. 1992).

In cheeses such as Tomme de Savoie, Reblochon and St. Nectaire, the first hours of manufacture determine the growth of *S. aureus* when the media conditions are optimal for growth (Lamprell 2003). In these cheeses, the population of *S. aureus* increases during the first 6 h of manufacture as a function of the initial level of contamination in the raw milk and independently of the pH due to the slow acidification rate which permits the growth of *S. aureus*. However, the growth of *S. aureus* between 6 and 24 h depends on the pH reached at 6 h: the more acidic the curd, the lower the growth (Delbes et al. 2006). Similar results were obtained during the manufacture of other cheese types, such as Tomme de Savoie or Cantal (Lamprell 2003).

In Swiss-type cheeses, *S. aureus* grows until 24 h and can reach a maximum of 2.10^8 cfu·g^{-1} after 2 weeks, followed by a decrease during the 19 weeks of ripening (Tuckey et al. 1964). Acidification does not seem to be a critical parameter involved in the decrease of the population of *S. aureus* as SE were found in acidified cheeses (pH 5.4) (Todd et al. 1981b). Cooking of the curd can reasonably be considered to be the crucial parameter in the inhibition of *S. aureus* in this type of cheese.

6 Conclusions

Even though guidelines for herd management and hygiene during milk collection and the cheesemaking process have led to a reduced prevalence of *S. aureus*, SFP outbreaks in milk and milk products remains a persistent problem for the dairy industry.

Until recently, available literature on the growth of *S. aureus* and SE production in the cheese context has remained descriptive. The development and use of molecular approaches has now opened new perspectives with regard to the control of this major pathogen in the dairy production chain. The adaptation of *S. aureus* and SE production to a cheese environment can now be studied at the molecular level. Initial attempts have revealed that SE production responds to complex and intricate regulatory networks. The design of rational strategies for SFP management in the cheese industry has to take account of this multilayer complexity as SE production depends on SE type, and physiology and virulence expression of *S. aureus*, which are influenced by environmental conditions (and therefore cheese technology). In the future, a better knowledge of the mechanisms involved in the inhibition of *S. aureus* and SE production will hopefully provide sufficient data to implement predictive models. The evolution in the population of *S. aureus* (lag, growth, survival and death) and the effect of food-related parameters (a_w and pH) and process or storage conditions (temperature, atmosphere) have been modelled in mathematical predictive models derived from experimental data on microbial populations (Ross et al. 2005; Stewart et al. 2002; Zurera-Cosano et al. 2004). Some modelling studies have taken account of the dairy environment and notably of the interactions with LAB (Lindqvist et al. 2002). Finally, recent studies have modelled both the growth of *S. aureus* and enterotoxin (SEA only) production in liquid milk (Fujikawa and Morozumi 2006). To be understood and controlled, the behaviour of *S. aureus* must be analysed directly *in situ*, in the cheese matrix. Until now, recent high-throughput technologies, i.e. transcriptomics and proteomics, have been applied to model strains in simplified conditions. Their application to more realistic environments can now be envisioned. These future studies could increase our understanding of the growth of *S. aureus* and SE production in cheese and will open the way to the identification of new strategies for risk assessment and SFP prevention in the dairy industry.

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