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Enterotoxigenic potential of coagulase-negative staphylococci

M. Podkowik[a], **J.Y. Park**[b], **K.S. Seo**[b], **J. Bystroń**[a], **J. Bania**[a,*]

[a]Department of Food Hygiene and Consumer Health Protection, Wrocław University of Environmental and Life Sciences, Wrocław, Poland [b]Department of Basic Sciences, Mississippi State University, MS, USA

Abstract

Staphylococci are a worldwide cause of human and animal infections including life-threatening cases of bacteraemia, wound infections, pyogenic lesions, and mastitis. Enterotoxins produced by some staphylococcal species were recognized as causative agents of staphylococcal food poisoning (SFP), being also able to interrupt human and animal immune responses. Only enterotoxins produced by *Staphylococcus aureus* were as yet well characterized. Much less is known about enterotoxigenic potential of coagulase-negative species of genus *Staphylococcus* (CNS). The pathogenic role of CNS and their enterotoxigenicity in developing SFP has not been well established. Although it has been reported that enterotoxigenic CNS strains have been associated with human and animal infections and food poisoning, most of research lacked a deeper insight into structure of elements encoding CNS enterotoxins. Recent studies provided us with strong evidence for the presence and localization of enterotoxin-coding elements in CNS genomes and production of enterotoxins. Thus, the importance of pathogenic potential of CNS as a source of staphylococcal enterotoxins has been highlighted in human and animal infections as well as in food poisoning.

Keywords

Coagulase negative staphylococci; Enterotoxin; Food poisoning

1. Introduction

Staphylococcus spp. are Gram-positive and non-motile bacteria that occur as commensal colonizers of the mucocutaneous membranes of the warm-blooded animals and humans (Kloos and Bannerman, 1994; Lowy, 1998). More than 70 species and subspecies of *Staphylococcus* genus have been characterized so far (http://www.bacterio.cict.fr). According to their ability to coagulate rabbit plasma, staphylococci are traditionally divided into two groups: coagulase-positive (CPS) and coagulase-negative (CNS).

Staphylococcus aureus (*S. aureus*) being the most characterized CPS in this taxon is a well-known etiological factor of a variety of infections including superficial skin inflammations,

*Corresponding author. Tel.: +48 713205425. jacek.bania@up.wroc.pl (J. Bania).

systemic infections, septicaemia and more. It can infect almost any organ, most notably bone tissue and cardiac valves (Lowy, 1998). In immunocompromised patients suffering from chronic disorders, staphylococcal diseases usually have more severe course (Wertheim et al., 2005). By far, infections of dairy animals by *S. aureus* remain an important burden for dairy and food industry (De Buyser et al., 2001).

S. aureus is also a recognized causative agent of SFP which is one of the most common food-borne diseases (Hennekinne et al., 2012). For decades, *S. aureus* was considered the only representative of the genus *Staphylococcus* capable of producing enterotoxins. To date, 23 different *S. aureus* enterotoxins have been identified (Ortega et al., 2010; Thomas et al., 2007; Wilson et al., 2011).

Conversely, CNS have been regarded to be non-pathogenic as most of the CNS can establish a commensal relationship with human and animals (Fitzgerald and Penades, 2008; Otto, 2010). The CNS group is usually considered as positive food flora, thus being widely applied in industry. Due to their positive impact on fermentation processes and sensory characteristic of products they are frequently used as the component of meat and cheese starter cultures (Irlinger, 2008; Hugas and Monfort, 1997). However, more and more is reported about the involvement of such species as *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* and *Staphylococcus saprophyticus* in hospital infections (Crass and Bergdoll, 1986; Da Cuhna et al., 2006; Mazzariol et al., 2012; Otto, 2009; Piette and Verschraegen, 2009; Ziebuhr, 2001) as well as *Staphylococcus chromogenes*, *Staphylococcus simulans*, *Staphylococcus xylosus* in animal infections (Taponen et al., 2006; Unal and Cinar, 2012). A number of virulence factors, originally identified and characterized in *S. aureus*, such as staphylococcal enterotoxins (SEs), hemolysins α, β, δ and γ, leukocidins, exfoliative toxins A and B, and antibiotic resistance determinants, were also detected in genomes of CNS (Irlinger, 2008; Park et al., 2011; Podkowik et al., 2012).

Because of signifcant heterogeneity in pathogenicity and habitat preferences of CNS their accurate identification to the species level can have important clinical and epidemiological implications. As it was stressed by many authors equal treatment of all isolates from the CNS group might be an obstacle in research and diagnostics (Tacconelli et al., 2003). According to Supré et al. (2011) *S. chromogenes*, *S. xylosus*, *Staphylococcus cohnii* and *S. simulans* seem to affect udder health more than other CNS species. In human infection *S. epidermidis* remain the most devastating in prosthetic joint surgery (Rupp and Archer, 1994). *S. saprophyticus* is the leading cause of urinary tract infection among Gram-positives (Ronald, 2002). It has been shown that, compared to other CNS species, human infections with *Staphylococcus lugdunensis* seem to be more severe (Papapetropoulos et al., 2012; Seifert et al., 2005). On the other hand *Staphylococcus hominis* is a colonizer of human skin with low virulence and pathogenic potential (Queck and Otto, 2008). Moreover, Szymańska et al. (2011) have shown that individual CNS species can differ in antibiotic resistance.

The members of the CNS group are usually considered as positive food flora, thus being widely applied in food industry. Due to their positive impact on fermentation processes and sensory characteristic of products, they are frequently used as the component of meat and cheese starter cultures (Hugas and Monfort, 1997; Irlinger, 2008). However, in late ‘50s and

early ‘70s of the twentieth century, a few reports indicated that CNS might also produce enterotoxins in food poisoning cases (Breckindridge and Bergdoll, 1971; Omori and Kato, 1959). Several reports related to food and dairy products in recent years illuminated and ultimately attested the ability of CNS to produce enterotoxins (Valle et al., 1990; Zell et al., 2008). The presence of sequences homologous to *S. aureus* enterotoxins has been confirmed in the genomes of CNS strains used in food processing, associated with human infection and from other environments (Madhusoodanan et al., 2011; Weir et al., 2007).

Increasing clinical significance of CNS indicates that safety hazards associated with their occurrence not only in clinical environment but also in food can be higher than previously thought (Even et al., 2010; Kloos and Bannerman, 1994). Here we present an overview of the enterotoxigenic potential of CNS in human and animal infections as well as in food poisoning.

2. Staphylococcal enterotoxins

Staphylococcal enterotoxins (SEs) belong to a family of superantigens (SAgs) which were originally identified in *S. aureus*. SEs were named on the basis of their emetic activities following oral administration in a primate model. Several SEs were designated as SE-like (SEl) since they either lack emetic properties or their emetic activities have not yet been tested in this model (Lina et al., 2004). To date, in addition to the five classical and antigenically distinct SEs discovered in the 60s (SEA through SEE), 18 new types of SEs or SEl have been described (SEG-SElU, SElV2, SElX) (Hennekinne et al., 2012; Ortega et al., 2010; Thomas et al., 2007; Wilson et al., 2011). It is noteworthy that SEs as many other staphylococcal virulence factors are encoded by accessory genetic elements, *i.e.* they are not necessary for growth and multiplication of the organism. Carriage by accessory genetic elements implies horizontal transfer of virulence determinants among staphylococcal strains as well as their exchange between bacterial species (Novick et al., 2001).

SEs and SEls are water-soluble, globular, and structurally stable proteins with molecular weight ranging from 22 to 29 kDa. The common feature of SEs is high stability and resistance towards most proteolytic enzymes, such as pepsin or trypsin, allowing protection of their activity in gastrointestinal tract. Of note, SEs are highly heat-resistant as well; they are thought to be more heat-resistant in foodstuffs than in laboratory culture media (Bergdoll, 1983). SEs can be secreted by *S. aureus* into the food environment, where they function as potent gastrointestinal toxins (Balaban and Rasooly, 2000; Bergdoll, 1983; Le Loir et al., 2003).

Superantigenicity is one of the main characteristics of SEs. Unlike conventional antigens they do not need to be processed by antigen presenting cells (APCs). Most bind to the outside of the peptide binding groove of MHC II molecules on APCs and to T cell receptors (TCRs) bearing specific beta chain Vβ sequences on T cells. Binding activates APCs and extensive proliferation of T cells, resulting in an acute uncontrolled release of proinflammatory cytokines and immunomodulatory cytokines in a chronic response (McCormick et al., 2001). Its binding to T-cell receptors (TCRs) is relatively nonspecific. For most SAgs, binding occurs at a variable location on TCR β chain; hence SEs can

interact with many different lymphocyte T clones (Johnson et al., 1991; Proft and Fraser, 2003). It is estimated that SEs at picogram concentrations can stimulate nonspecifically up to 50% of all T cells in the human. These massively activated T cells secrete abnormally large amounts of cytokines and clonally proliferate. High concentrations of proinflammatory cytokines as interleukin-2 (IL-2), interferon-γ (IFN-γ), and tumour necrosis factor (TNF-α) might result in development of disease that is characterized by rapid onset of high fever, capillary leakage, and multi-organ dysfunction, altogether described as toxic shock syndrome. The suddenness and the magnitude of cytokines released determine the severity and outcome for the patient (Fraser and Proft, 2008). Following clonal proliferation, most T cells undergo activation-induced cell death, resulting in a clonal T cell deletion and survived T cells become anergy. Recent studies demonstrated that these T cells are phenotypically and functionally analogous immunosuppresive regulatory T cells (Taylor and Llewelyn, 2010). Although the immunosuppressive effect of staphylococcal infections does not apply generally to the whole organism, it is supposed that the phenomenon might facilitate the pathogen's local colonization of the host (Fraser and Proft, 2008; Proft and Fraser, 2003). Additionally, recent studies demonstrated that long-term stimulation by SAgs induces development of $CD4^+CD25^+$ regulatory T cells in humans and rodents and bovines, capable of suppressing the responses of responder T cell proliferation (Seo et al., 2007). This suggests that immunomodulation induced by SAgs has an important role in staphylococcal disease.

Emesis involves coordinated gastrointestinal activities, including contraction of abdominal muscles, forceful contractions of the stomach pylorus, and relaxation of the fundus, cardiac sphincter, and oesophagus. Studies with primates showed that SEs stimulate neural receptors in the abdomen, which transmit impulses through the vagus and sympathetic nerves, resulting in stimulation of the vomiting centre in the fourth ventricle (Sugiyama and Hayama, 1965). A recent study with the house musk shrew showed that SEA induces 5-hydroxytryptamine (5-HT) release in intestine and a pretreatment of 5-HT inhibitor or 5-HT receptor blocker inhibits SEA-induced emesis (Hu et al., 2007), suggesting 5-HT is an important neurotransmitter in SE-induced emesis. Some SE-related proteins are confirmed to lack emetic activity, suggesting that emesis is not necessarily a feature of the toxin that provides a selective advantage to staphylococci. It is more likely that enterotoxicity is a secondary effect of SE, which primarily acts as SAgs. However, still little is known about the mechanism by which SEs induce emesis.

Compared to *S. aureus*, the enterotoxigenic potential of CNS has not been well characterized. So far, only enterotoxins produced by *S. aureus* have been comprehensively described. For decades, the issue whether its counterparts, the CNS, can also be a causative agent of SFP remains to be clarified. First descriptions of acute gastroenteritis outbreaks involved with SE-producing CNS appeared in the late 50s and early 70s (Breckindridge and Bergdoll, 1971; Omori and Kato, 1959). Results of the pioneering research on CNS enterotoxigenicity were tempered with caution and definitive proof was lacking. On the other hand, since then, several reports that staphylococci other than *S. aureus* can produce SEs attracted attention from researchers.

3. Enterotoxigenic CNS in human infections

In the beginning of 80s, several findings highlighted the need of vigorous pursuit on involvement of enterotoxigenic CNS in human disease, followed by design of countermeasures preventing CNS-associated health risks. The first study on involvement of CNS in TSS was performed by Crass and Bergdoll (1986). In that study, SEs and TSST-1 were analyzed in bacterial cultures by the membrane-over-agar and optimal sensitivity plate methods. In 7 of 19 cases of TSS, SEA or TSST-producing CNS were isolated. Authors described *S. epidermidis* strains, which were able to produce TSST-1 and SEC, and other CNS species, able to produce SEA and TSST-1 (Crass and Bergdoll, 1986). In a study performed by Da Cuhna et al. (2006) on CNS from newborns, the relatively high percentage (37%) of isolates produced one or a combination of two or more classical SEs. Another study by Da Cuhna et al. (2007), focusing on detection of SEs and TSST-1 in staphylococci isolated from human clinical samples, revealed a considerable ability of CNS strains to express one or more SEs. Authors of the study on virulence factors involved in staphylococcal peritonitis postulated that CNS-derived enterotoxins, exerting superantigenic, toxic, and pyrogenic effects, can influence the course of infection in higher degree than antibiotic resistance of the pathogens (Barretti et al., 2009). This study revealed that *S. epidermidis* strains produced SEA, SEB, SEC and TSST-1 alone or in combination, whilst *S. haemolyticus* and *S. lugdunensis* isolates produced SEC (Barretti et al., 2009). PCR-detection of *see*, *seg*, *seh*, and *sei* genes and RT-PCR analysis of their expression were performed by Vasconcelos et al. (2011) on 90 clinical CNS isolates from newborns. In 29 isolates, various combinations of SE genes were found. However, only in 34% of enterotoxigenic isolates, enterotoxin mRNA was detected.

S. epidermidis strains, possessing genes homologous to *S. aureus* enterotoxins *seg* and *seh*, were found to be present in human breast milk. It might pose a risk, considering the immune status of population for which that sort of food is destined (Carneiro et al., 2004).

Coagulase-negative staphylococci can be isolated from cerebrospinal fluid from patients with meningitis with high frequency (Ataee et al., 2011). A recent survey by Ataee et al. (2011) indicates that CNS strains isolated from the cases of bacterial meningitis might be able to produce SEs. In this study, CNS strains were screened using immunoassay that enabled detection of *S. aureus* SEA–SEE enterotoxins. Meaningfully, all CNS isolated from patients with bacterial meningitis with high frequency were able to secrete at least one SE.

Collectively, these research studies denote that the enterotoxigenic properties of CNS cannot be ignored. According to Bergdoll and Chesney (1991), being aware of diversity and severity of infections caused by CNS, enterotoxigenic potential of those microorganisms should be considered harmful for human health.

4. Enterotoxigenic CNS in animal infection and carriage

Companion and farm animals seem to be well established reservoir of many staphylococcal species. Staphylococcal infections remain among the most important opportunistic infections in companion animals (Weese, 2010, 2012). The assumptive transmission between

people and animal raises unique concerns in the case of household pets. *Staphylococcus intermedius*, a representative of CPS has traditionally been identified as the primary pathogen isolated from canine pyoderma and some studies have already evidenced its enterotoxigenic potential (Becker et al., 2001a,b). The capacity of CNS from household dogs to produce enterotoxins was already demonstrated in early 80s. In a study by Adesiyun and Usman (1983), a considerable proportion (nearly 17%) of canine CNS isolates were endowed with enterotoxigenic properties. SEC was the predominantly produced SE.

Farm animals, particularly their milk, seem to be another reservoir of enterotoxigenic CNS. According to the results obtained by Valle et al. (1990) 22% of the CNS species from healthy goat including *S. chromogenes*, *Staphylococcus warneri*, *Staphylococcus sciuri*, *S. saprophyticus*, and *Staphylococcus lentus* was able to secrete enterotoxins, predominantly SEC, either solely or in combination with other toxins. CNS remain most prevalent mastitis pathogens (Bergonier et al., 2003; Taponen et al., 2006; Unal and Yildirim, 2010). Similar to *S. aureus*, the enterotoxigenic potential of CNS has been already considered in pathogenesis of ruminant mastitis. CNS strains isolated from the milk of sheep, goat and cow mastitis cases were investigated by Orden et al. (1992) who employed both double sandwich enzyme-linked immunosorbent assay (ELISA) and Western blot to detect SEC and TSST-1. Two *S. xylosus* strains were found to produce SEC. In another study on staphylococci isolated from bovine mastitis, no enterotoxigenic CNS were found (Orden et al., 1992). No genes encoding SEs were detected in a screening of 102 CNS strains associated with cases of subclinical and clinical bovine mastitis (Nemati et al., 2008). In another study, presence of 18 SE and *tst-1* genes was investigated comprehensively in 263 isolates of CNS associated with bovine intramammary infections (Park et al., 2011). This survey revealed that 82 isolates (31%) harboured at least one SAg-related gene. Remarkably, almost a half of *S. xylosus* (11/24), and all *Staphylococcus hyicus* isolates were positive for enterotoxin genes. The combination of *seb*, *seln*, and *selq* genes was the most prevalent. Of note, combination of *selm*, *selo*, *sei*, and *seg* genes, in *S. aureus* known to form together with *seln* enterotoxin gene cluster (*egc*), was not detected in any of the isolates. This indicates that genes harboured by most known *S. aureus* strains in clustered form (Thomas et al., 2006) can occur in CNS in other combinations. Presence of SE genes together with genes encoding other virulence factors was investigated in a collection of 121 CNS from cows and ewes with subclinical mastitis representing 18 different staphylococcal species (Unal and Cinar, 2012). In 46 isolates (38%) one or more SE genes were detected, with *seh* and *sej* being most common SE genes.

5. Enterotoxigenic CNS in food

According to the latest data, the 2011 estimate of foodborne illness in the USA, published by the Centers for Disease Control and Prevention (CDC), there are 31 pathogens known to cause foodborne illness. Many of them are systematically tracked by public health systems. *S. aureus* is still among top five pathogens contributing to domestically acquired foodborne illnesses being responsible for the highest number of hospitalisations each year. Reports by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) published on May 2012, give notice of 274 food-borne

outbreaks in the EU caused by *Staphylococcus* spp., concerning 941 individual cases from which 20% of patients were hospitalised.

The first food-borne disease involving staphylococci was described in Michigan (USA) in 1884 by Vaughan and Sternberg and the proof of the involvement of staphylococci in food poisoning was first brought by Barber in 1914 (Hennekinne et al., 2012). Staphylococcal food poisoning is evoked by ingestion of SEs. The characteristic features of this illness are short incubation time (from 30 min to 8 h) and its rapid course. The main symptoms include projectile vomiting and painful contractions of gastrointestinal smooth muscles, sometimes accompanied with diarrhoea, weakness and dizziness. Spontaneous remission is usually observed after 24 h in case of young healthy individuals (Le Loir et al., 2003).

Some authors argue that the risk of CNS food poisoning connected with consumption of dairy products should be low. According to the current knowledge, there is no clear evidence that CNS isolated from milk or dairy products have been unambiguously involved in food poisoning (Irlinger, 2008). On the other hand, ubiquitous prevalence of CNS in ruminants and the fact that goat and sheep milk used for cheese manufacture is not usually heat-treated raise a number of concerns about the prevalence of enterotoxigenic CNS strains, which need to be clarified (Contreras et al., 2003).

Enterotoxin-producing CNS were already identified in sheep and goat milk, sheep cheese and different anatomical sites in healthy goats (Bautista et al., 1988; Gutierrez et al., 1982; Valle et al., 1990). Bautista et al. (1988) characterized enterotoxigenic *S. cohnii*, *S. epidermidis*, *S. haemolyticus*, and *S. xylosus* strains from sheep milk using ELISA. However, significantly lower level of enterotoxigenic CNS was recorded in comparison to coagulase-positive strains. Recently, SE-producing CNS were found in Brazilian cow's raw milk concomitantly with enterotoxigenic *S. aureus* strains (Oliveira et al., 2011). Contrary to these data, Harvey and Gilmour (1985) and De Buyser et al. (1987) failed to detect enterotoxigenic staphylococci in milk of small ruminants.

Prevalence of *sea*, *seb*, *sec*, and *sed* genes was investigated in CNS isolated from Brazilian Minas cheese (Rall et al., 2010). It has been shown that a relatively high percentage of CNS strains (17 of 65 isolates) possess enterotoxin genes. However, it is noteworthy that none of them produced enterotoxins *in vitro*.

The enterotoxigenic potential of CNS from dry-cured ham was investigated by several authors (Marín et al., 1992; Rodríguez et al., 1996). Enterotoxigenic *S. epidermidis* was found in Spanish dry-cured hams (Marín et al., 1992). Production of SEC was confirmed in *S. xylosus* and *S. epidermidis* strains using RPLA. *S. xylosus* was also able to elaborate SED (Marín et al., 1992). Rodríguez et al. (1996) reported that CNS species from dry cured Iberian ham showed positive signals for *sed* and *sec* probes in DNA–DNA hybridization. However, consecutive application of immunological test gave equivocal results.

Some CNS strains, *e.g. Staphylococcus piscifermanetas*, *Staphylococcus equorum*, and *Staphylococcus succinus* subsp. *casei*, are components of food starter cultures (Hammes and Hertel, 1998). Enterotoxigenicity of these strains should pose considerable risk for food safety as the microorganisms are introduced at high concentrations in many animal-derived

products. Zell et al. (2008), using immunoblot analysis with polyclonal antibodies against SEA–SEE and SEH, detected SEs in 35 CNS strains, representing six species, isolated from food and starter cultures. Production of SEs was detectable in all species, except *Staphylococcus condimenti* and *S. succinus*. SEH was the most frequently produced toxin in this study. These results emphasize the need for evaluation of starter cultures' safety, and illustrate urgency of studies on CNS pathogenic potential.

6. Involvement of CNS in food poisoning outbreaks

Relatively little is known about involvement of CNS-derived SEs in SFP outbreaks. However, several studies support the possibility that CNS may be involved in food poisoning. It was already demonstrated that SEs produced by *S. aureus* in food involved in SFP outbreaks were detected in a range between 5 and 100 ng mL^{-1} (Bergdoll, 1989; Evenson et al., 1988). Data presented by Jay (1992) indicates that even 1 ng × g^{-1} of SE in food is enough to cause food poisoning symptoms. Similar to studies on *S. aureus*, Bergdoll (1995) found that some CNS strains also can produce SEs at concentrations reaching ng × mL^{-1} using ELISA. SEC and SElL were detected by Western immunoblotting in FRI909 *S. epidermidis* strain (Madhusoodanan et al., 2011). Analysis of staphylococcal isolates from sixteen food poisoning outbreaks in Brazil aimed to identify enterotoxin genes from both CNS and CPS isolates (Veras et al., 2008). Five CNS isolates were found to have enterotoxigenic properties. Most of the CPS strains also turn out to be potential SE producers. This study does not provide a strong evidence for involvement of CNS in food poisoning since it seems that enterotoxigenic CNS were not the only enterotoxigenic bacteria isolated from suspected foods.

In a study by Udo et al. (1999), light is shed on CNS isolates from food handlers, suggesting that those bacteria may be implicated in poisoning incidents. The staphylococcal strains were swabbed from human hands and nose, and significant majority of them were positive for SE production. The results of the sampling revealed that CNS were predominant flora swabbed from food handlers' hands, whilst *S. aureus* prevailed in nasal swabs. It can be, therefore, concluded that increased colonization of CNS on human hands can promote their transmission to foodstuffs if proper care is not taken.

7. Detection of CNS enterotoxins

Enterotoxins of CNS were investigated using molecular biology methods, *i.e.* polymerase chain reaction (PCR), nucleic acid hybridisation, and sequencing as well as using immunological approaches such as immunoblotting, immunodiffusion, RPLA, and ELISA. In a number of studies on enterotoxigenic potential of CNS a single experimental method, *i.e.* only PCR or immunodetection was used. Amplicons obtained from PCR were not usually sequenced. Results obtained from immunological methods were not simultaneously confirmed at the DNA or the RNA level. Thus, the results of some investigations should be taken with criticism.

Rozand-Vernozy et al. (1996) provide us with an important assumption that three conditions are required to prove that a CNS strain produces an SE, namely, correct identification of a

strain, confirmation of SE production by at least two immunological methods, and identification of a SE gene (Rozand-Vernozy et al., 1996). Only few studies met these three conditions (Orden et al., 1992; Rozand-Vernozy et al., 1996).

Several attempts failed to identify enterotoxigenic CNS strains (Becker et al., 2001a,b; Blaiotta et al., 2004; Rosec et al., 1997). Even et al. (2010) used a diagnostic microarray targeting 268 genes corresponding to food safety hazards. It was found that only one *S. saprophyticus* strain carried an enterotoxin homologue, namely, the *sec* gene. The authors point out that methods relying only on gene expression can provide ambiguous results, as they can be significantly influenced by environmental conditions. Since standardized laboratory conditions differ notably from those encountered in food matrices, some safety hazards might be missed.

A study performed by Seitter et al. (2011) on 32 CNS associated with food, revealed that some of them showed SE production in Western blotting, although signals in polynucleotide-based DNA microarray for corresponding genes were not detected. This inconsistency highlights the requisite of implementation of both genotypic and phenotypic examinations. PCR and hybridisation can thus serve as rapid, large-scale, and trustworthy screening tools for detection of safety traits; however, only in conjunction with phenotypic detection, they assure reliable outcomes.

Failure of SE gene detection in CNS might sometimes be explained by use of inappropriate methods of DNA preparation, in which cell wall destabilization using lysostaphin was not applied (Park et al., 2011). Another potential cause of false-negative results is that primers for CNS enterotoxin detection were based on known *S. aureus* sequences, whereas CNS sequences, despite close phylogenetic vicinity of organisms, are likely to be divergent. Therefore, the polynucleotide DNA microarrays able to detect sequence with similarities down to 70% in contrast to oligonucleotide based ones seem to be more useful in detection of CNS enterotoxins (Seitter et al., 2011). The proteins of CNS enterotoxin being presumably more related to those from *S. aureus* can be detected with cross-reacting antibodies, however, increasing the risk of false positive signals.

Similar debate over discrepancies between phenotype and genotype has concerned antibiotic resistance. Plausible reasons are the presence of silent genes that might be switched on *in vivo* or the mode of regulation of genes coding for antibiotic resistance, which makes them undetectable under standardized laboratory conditions (Perreten et al., 2005; Zhu et al., 2007). Such a phenomenon may likely occur in case of CNS enterotoxins.

The enterotoxin genes detected in some CNS isolates seem to be unstable. According to a phenomenon observed by Park et al. (2011) intensity of PCR amplicons in enterotoxigenic bovine CNS becomes weak or even completely disappeared after several passages of the bacteria. It raises a possibility that some genetic elements harbouring SAg-related genes in CNS isolates behave differently than those known from *S. aureus*. It could be explained evolutionally that CNS would be a transient status of inter- and intra-species SE gene transfer and an important reservoir of virulence-associated genes that significantly contribute to the evolution of *S. aureus* enterotoxigenicity (Kassem, 2011).

Results of recent studies provide strong evidence for presence of *S. aureus* enterotoxin homologues in genomes of CNS (Madhusoodanan et al., 2011; Weir et al., 2007). Analysis of a strain of *S. caprae* showed the presence of 439 bp DNA fragment homologous to *S. aureus* enterotoxin *selp* gene (GenBank accession number DQ641635) (Weir et al., 2007). In the most complex study on enterotoxigenic CNS, Madhusoodanan et al. (2011) determined the sequence of entire pathogenicity island SePI-1 in *S. epidermidis* strain FRI909. The authors found that SePI-1 harbours genes encoding SEC and SElL, similar to SaPIbov1 (Fitzgerald et al., 2001). *S. epidermidis* SEC and SElL were shown to display 95% and 97% identity with Mu3 *S. aureus* strains SEC3 and SElL, respectively. Moreover, the expression of these two toxins was proven by quantitative reverse transcription PCR and immunoblotting. The possibility of excision and packaging of SePI-1 by bacteriophage ϕ909 was also described. This is the first report describing pathogenicity island encoding enterotoxin-like elements in genome of non-*S. aureus* species. Importantly, Novick et al. (2001) demonstrated a transfer of SaPIbov1 to *S. xylosus* in the presence of helper phage (ϕ80), suggesting SePI-1 also can be transferred to other CNS (Chen and Novick, 2009).

The discrepancies concerning the enterotoxigenic potential of CNS accentuate the need of comprehensive studies with the use of contemporary technical armamentarium. Employing them should fuel many areas of research.

8. Production of CNS enterotoxins

Presence of SE genes does not imply their expression in any condition. Detection of SE genes without determining the status of their expression should be considered with caution. To assess real safety hazards the phenotype ought to play a crucial role, placing genotypic studies as screening tool.

The influence of food environment on SE production by CNS was evaluated by Oliveira et al. (2010). The authors used cooked ham, reconstituted skimmed milk, and cream to cultivate 10 selected CNS strains. Presence and absence of background microbiota were taken into account. Results indicate that the CNS growth in food is possible even in the presence of concurrent microflora, although SE production was unequivocally confirmed only in the case of *S. chromogenes* strain. Background microflora interfered with SE production only during cultivation on cooked ham. Detection was carried out with the use of the mini-Vidas and RPLA tests, which do not allow determination of SE concentration.

9. Concluding remarks

Coagulase-negative staphylococci have been considered as non-pathogenic bacteria in human, animals and food for many decades. However, CNS contribute to a variety of human nosocomial infections, mastitis in ruminants and even food poisoning. The importance of CNS has been recognized in human infections earlier than animal infections and food poisoning, so most CNS research has been performed in human cases. So far, enterotoxigenic potential of CNS has been generally overlooked. In addition, unlike *S. aureus*, examination of enterotoxigenicity of CNS is not included in routine food testing. However, both previous and recent studies reported that a relatively high number of CNS

isolates associated with human and animal infections, as well as food poisoning, harbour SE genes and also are able to produce some SEs at the protein level. Most cases of ruminant infections by SE-producing CNS are mainly related to a source of dairy products, milk, suggesting that it could be SFP-causing source. As yet, a pathogenic role of SE-producing CNS has not been clearly characterized in human and animal infections and especially SFP.

Staphylococcal food poisoning has been a major concern of the food industry and public health for many decades, and great advances have been made in regard to its prevention, treatment, diagnosis and aetiology. As mentioned above, SE-producing CNS have not gained attention so far, although the importance of SEs produced by *S. aureus* in SFP has been well characterized. In order to explain the significance of SEs produced by CNS in SFP, a number of problems remain to be resolved. This should primarily include the questions whether the presence of SE genes in the CNS is obviously related to their production and whether their biological activity is similar to that of *S. aureus* enterotoxins. Due to heat-sensitivity of *S. aureus*, in some SFP cases, the pathogen cannot be identified. In such cases, SFP diagnosis is confirmed solely by the detection of SEs in food remnants (Hennekinne et al., 2010). Thus, in some already described SFP cases, it might be difficult to state whether detected enterotoxins were elaborated by *S. aureus* or CNS.

Significance of genes encoding CNS enterotoxins can also be considered in light of their contribution to evolution of staphylococcal enterotoxigenicity. Coagulase-negative staphylococci are thought to be an important reservoir of virulence-associated genes that significantly contribute to the evolution of *S. aureus* in both community and hospital settings (Kassem, 2011). In *S. aureus* the enterotoxin genes are located on mobile genetic elements, potentially allowing their inter- and intra-species transfer. CNS are also ubiquitous on surfaces of items of everyday use. In complex environments, *e.g.* body mucocutaneous surfaces or food environment microorganisms evolve influencing significantly each other. Co-colonization of mucosal membranes and skin by CNS and *S. aureus* provides opportunity for horizontal transfer of mobile genetic elements. Selective pressure of environment, *e.g.* subinhibitory concentrations of chemotherapeutics triggering SOS response, can additionally drive the evolution of both commensal and pathogenic bacteria.

It is obvious that the current status of knowledge is not sufficient to draw definite conclusions on enterotoxigenic potential of CNS. Since staphylococcal enterotoxins are considered an important virulence factor in human and animal infections as well as food safety issues, more attention must be paid to SE produced by CNS. Additional research should be conducted to answer the questions for the characteristics of enterotoxigenic potential of CNS as well as their pathogenic role in regard to human and animal infections as well as SFP.

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