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Molecular analysis of *Staphylococcus aureus* pathogenicity islands (SaPI) and their superantigens combination of food samples

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ABSTRACT

Staphylococcus aureus produces a wide variety of superantigenic activity *Staphylococcal* enterotoxins (SE) and they are a major cause of food poisoning. These superantigens are associated with mobile genetic elements such as plasmids, prophages and *S. aureus* pathogenicity islands (SaPI). The presence of well-known eight SaPI integrase and 13 enterotoxin genes (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sel*, *sek*, *seq*, and *tst*) in 93 *S. aureus* strains were investigated. All *S. aureus* isolates were characterized by pulsed-field gel electrophoresis (PFGE), and the genes were detected using five sets of multiplex PCR (mPCR). The most predominant toxin genes were *sea* (19%), *seb* (15%), *sec* (54%), *sell* (48%), *selk* (46%), *selq* (52%), *seg* (22%), and *sei* (19%). Analysis showed that many *S. aureus* isolates harbored multiple toxin genes. An mPCR-based assay was developed for the determination of all SaPI and their superantigen gene combinations. Twenty three isolates revealed the gene combination *sec*, *sell* and *tst*, typical of the SaPIbov1 and SaPIn1/m1 pathogenicity islands. Twelve isolates revealed the *selk* and *selq* gene combination consistent with SaPI3. Eight isolates exhibited the *sec* and *sell* genes without the *tst* gene typical of SaPImw2. We established a correlation between superantigenic toxin genotypes in *S. aureus* in terms of combinations of toxin gene-encoding SaPI. These results provide a rapid method for determining superantigenic toxin genotypes in *S. aureus* strains. A total of 24 PFGE patterns were generated. To our knowledge, this is a first study analyzing the correlation of all known SaPI and their enterotoxins in *S. aureus* using mPCR.

1. Introduction

Staphylococcal enterotoxins (SE) are a major cause of food poisoning outbreaks worldwide (Cretenet et al., 2011). These cases are often caused by strains carrying one or more of the five classical SE (SEA-SEE), although there are a few outbreaks reported to be caused by newly described SE (Ikeda et al., 2005). To date, twenty-four *Staphylococcal* enterotoxins have been identified and divided into two groups according to their demonstrated emetic activity: classical SE and new SE. The members of group 1, classical emetic toxins designated SEA, SEB, SEC_1, SEC_{bov}, SED and SEE, are the cause of about 95% of SFP (Staphylococcal Food Poisoning) in humans and can act as superantigens (SAg) (Bergdoll, 1983). Group 2 includes toxins postulated to be involved in the remaining 5% of SFP outbreaks, i.e. other recently identified enterotoxin and enterotoxin-like superantigens (SEl) (Table 1).

All staphylococcal superantigens are encoded on mobile genetic elements, that include plasmids, prophages, *Staphylococcus aureus* pathogenicity islands (SaPI), genomic islands *ν*Sa and the staphylococcal cassette chromosome (SCC) elements (Novick et al., 2010). These mobile genetic elements have played an important role in the evolution of *S. aureus* as a pathogenic microorganism. Three *se*/*sel* genes (*sea*, *selk* and *selq*) are present together in ΦSa3ms and ΦSa3mw, while a single *se*/*sel* gene (*sea* or *selp*) is carried by other prophages (Argudin et al., 2010). The pIB485-like (*selj*, *sed*) and pF5 (*ser*, *selj*, *set* and *ses*) plasmids carry *se*/*sel* genes (Zhang et al., 1998). Enterotoxin *seh* is inserted together with a transposase in the methicillin resistance cassette SCCmec (Noto and Archer, 2006). The enterotoxin gene cluster (*egc*) containing several SE or SE-like genes (*seg*, *sei*, *sem*, *sen*, *seo* and *seu*) may be a possible source of new SE genes (Jarraud et al., 2001). Most of the known enterotoxins are located on different *S. aureus* pathogenicity islands (SaPI)(Malachowa and DeLeo, 2010). The genomes of *S. aureus* strains, MW2, Mu 50, N315 and RF 122 contain one or more SaPI, although some strains do not carry recognizable virulence genes (Novick et al., 2010). The SaPIs are inserted into a single orientation at specific sites on the chromosome. Eight important albeit different SaPIs carrying six superantigen genes (*tst*, *selk*, *selq*, *seb*, *sec*, and *sell*) and other pathogenicity factors (*ear*, encoding a penicillin-binding protein; *eta*, encoding the exfoliatin A; and *bap*, encoding a biofilm-associated protein) have been identified to date (Novick et al., 2010). Most of these pathogenicity islands have been previously identified in clinical *S. aureus* strains (SaPI1, SaPI2, SaPI3, SaPI4, SaPI5, SaPIn1/m1 and SaPI1mw2), and two of them in a *S. aureus* strain isolated from a bovine mastitis case (SaPIbov1 and SaPIbov2) (Novick et al., 2010).

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Table 1
Staphylococcus aureus enterotoxins.

Enterotoxin	Gene	Emetic activity	ORF length (bp)	Reference
SEA	*sea*	Yes	774	Jarraud et al. (2001)
SEB	*seb*	Yes	801	Bergdoll (1983)
SEC[b]	*sec*	Yes	801–816	Ikeda et al. (2005)
SED	*sed*	Yes	777	Jarraud et al. (2001)
SEE	*see*	Yes	774	Bergdoll (1983)
SEG[c]	*seg*	Yes	729–777	Argudin et al. (2010)
SEH	*seh*	Yes	726	Argudin et al. (2010)
SEI	*sei*	Weak	729	Smyth et al. (2005)
SEIJ	*selj*	Yes[a]	806	Herron-Olson et al. (2007)
SEIK	*selk*	Yes[a]	729	Baba et al. (2002)
SEIL	*sell*	No	723	Omoe et al. (2005)
SEIM	*selm*	Yes[a]	722	Omoe et al. (2005)
SEIN	*seln*	Yes[a]	720	Omoe et al. (2005)
SEIO	*selo*	Yes[a]	783	Omoe et al. (2005)
SEIP	*selp*	Yes[a]	783	Omoe et al. (2005)
SEIQ	*selq*	No	729	Omoe et al. (2005)
SER	*ser*	Yes	600	Kuroda et al. (2001)
SES	*ses*	Yes	774	Herron-Olson et al. (2007)
SET	*set*	Weak	651	Smyth et al. (2005)
SEIU	*selu*	Yes[a]	786	Diep et al. (2006)
$SEIU_2$(SEW)	$selu_2$	Yes[a]	771	Argudin et al. (2010)
SEIV	*selv*	Yes[a]	720	Argudin et al. (2010)
SEIX	*selx*	Weak	612	Wilson et al. (2011)
SEF or TSST	*tst*	No	705	Argudin et al. (2010)

[a] Emetic activity demonstrated in animal, but not in a primate model.
[b] Enterotoxin C (SEC) exists in multiple variants: C_1, C_2, C_3, C_{bovine} and C_{sheep}.
[c] Enterotoxin G (SEG) exists in two different variants: G_2 and G_v.

The aims of this study were to determine the distribution of thirteen SAg genes and eight chief SaPI integrase genes of *S. aureus* strains isolated from various food products and furthermore to investigate the genetic relatedness of enterotoxigenic isolates by using PFGE. In addition, we investigated the presence of enterotoxin genes and their correlation with *S. aureus* pathogenicity islands (SaPI). The genotypes of the foodborne isolates were compared with data on the structure of other typical SaPIs in order to determine the enterotoxin gene combination with the characterized SaPI.

2. Materials and methods

2.1. Bacterial strains

Ninety three *S. aureus* strains previously obtained from various food samples were used in this study (Alibayov et al., 2014). The *S. aureus* strains were isolated from milk ($n = 57$), meat products ($n = 7$), fish ($n = 9$ s), confectionery products ($n = 6$), sausage and ham ($n = 7$) and other food products ($n = 7$). Strains were collected from different districts of the Czech Republic and were characterized using standard microbiological procedures such as Gram-staining, hemolytic activity on sheep blood agar, catalase production, oxidase test, growth in Baird–Parker agar supplemented with egg yolk, coagulase tube test and phosphatase activity on the selective chromogenic culture medium SaSelect ™ Medium (Bio-Rad, USA) (Bania et al., 2006), and also molecular identification of *S. aureus* strains confirmed by PCR (Martineau et al., 1998). Strains were stored at −80 °C in Trypticase Soy Broth (TSB, Merck, Germany) in 25% *v/v* glycerol for further characterization.

Fourteen reference strains were used as positive controls in PCR reactions (Table 2). These strains were kindly provided by Prof. Jiří Doškař and by Dr. Renata Karpiskova (Brno, Czech Republic).

2.2. DNA isolation

Total genomic DNA was extracted as previously described by Valihrach et al. (2009). Working cultures were prepared in tubes containing 5 ml TSB incubated at 37 °C for 24 h. After incubation, 1 ml of the culture was centrifuged at 12,000 ×*g* for 10 min, and the supernatant was removed. The pellet was resuspended in 200 μl of deionized water (dH_2O) and heated at 100 °C for 20 min. The heated suspension was subjected to centrifugation at 12,000 ×*g* for 6 min. The supernatant was transferred to a new tube and used as the DNA template for the PCR assay. After extraction, DNA concentration was measured using a nanophotometer (Implen, Germany). DNA preparations were stored at −20 °C until used for PCR amplifications.

2.3. PCR primer design

The nucleotide sequences of all PCR primers used in this study and their respective amplified products are listed Table 3. Fourteen PCR primers specific for SE and SaPI integrase genes were designed using the online Primer3 (http://wwwprimer3.sourceforge.net/), Primer Premier 5.0 (PREMIER Biosoft International, USA) and NCBI Primer-BLAST program (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). The primers for the SEA-SEJ enterotoxins were sourced from Monday and Bohach (1999) and Lovseth et al. (2004). Oligonucleotides ranging from 18 to 24-mers were selected from the published sequences available in the GenBank database.

2.4. Multiplex PCR detection SaPI integrase and enterotoxin genes

The presence of 13 enterotoxin genes was assessed using multiplex PCR assays to *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *selk*, *sell*, *selq*, and *tst*. The combinations of primer sets and reaction conditions for the multiplex PCR were optimized to ensure that all PCR products of target genes were satisfactorily amplified and that PCR production was efficient in each mPCR reaction. The primers were combined into five (a,

Table 2
S. aureus reference strains used in this study.

No.	Strain	Enterotoxin gene(s)	Pathogenicity islands	Reference
1	STA054	*selk*, *selq*, *tst-1*	SaPI1	This study
2	RF122	*seb*, *sec*, *sell*, *tst-1*	SaPIbov1, SaPI2	Herron-Olson et al. (2007)
3	MSSA476	*selq*, *selk*, *seh*	no SaPI integrase gene	Holden et al. (2004)
4	MRSA252	No SE gene	SaPI4	Novick et al. (2010)
5	Newman	No SE gene	SaPI4	Baba et al. (2002)
6	N315	*seb*, *sec*, *seg*, *sei*, *sell*, *selm*, *seln*, *selo*, *selp*, *tst-1*	SaPIn1/m1	Omoe et al. (2005)
7	USA300	*selq*, *selk*	SaPI3, SaPI5	Diep et al. (2006) and Highlander et al. (2007)
8	Mu50	*sea*, *seb*, *sec*, *seg*, *sei*, *sell*, *selm*, *seln*, *selo*, *tst-1*	SaPIn1/m1	Kuroda et al. (2001)
9	NCTC8325	*selk*	SaPI5	This study
10	COL	*sea*, *seb*, *seh*, *selk*, *selq*, *tst-1*	SaPIbov1, SaPI3, SaPI5	Gill et al. (2005) and Smyth et al. (2005)
11	CCM 3953 (ATCC 25923)	*sec*	SaPIn1/m1, SaPI4	This study
12	FRI137	*seb*, *sec*, *seg*, *seh*, *sei*, *selk*, *sell*, *selm*, *seln*, *selu*	SaPIbov1	Bania et al. (2006)
13	FRI361	*seb*, *sec*, *sed*, *seg*, *sei*, *sej*, *sell*, *selm*, *seln*, *selo*, *selr*	SaPI4	This study
14	MW2	*sea*, *seb*, *sec*, *seh*, *selk*, *sell*, *selq*	SaPImw2	Baba et al. (2002)