

Characterization of enterotoxin A-producing *Staphylococcus aureus*

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Purpose: This study aims to characterize the wild-type staphylococcal enterotoxin A (SEA)-producing *Staphylococcus aureus*.

Materials and methods: We identified 29 wild-type *sea*-positive *S. aureus* isolates from dairy and meat samples, as well as from patients, measured the amount of SEA produced under favorable cultivation conditions using enzyme-linked immunosorbent assay and *sea* mRNA transcriptional level and investigated the phage type as well as genetic diversity by means of pulsed-field gel electrophoresis and multilocus sequence typing.

Results: Among 29 *sea*-positive isolates, 22 were from food sources (including one outbreak case) and seven from clinical patients. Five enterotoxin gene profiles, namely, *sea* (14 isolates), *sea+sec* (9 isolates), *sea+seb* (4 isolates), *sea+seb+sec* (1 isolate) and *sea+seb+sed* (1 isolate), were identified. Multilocus sequence typing generated sequence type (ST)1 (13 isolates), ST6 (5 isolates), ST59 (3 isolates), ST239 (3 isolates), ST5 (2 isolates), ST188 (2 isolate) and ST15 (1 isolate). The amount of SEA per 10⁸ colony-forming unit (CFU) after 24 h of incubation was 1.1–33.5 (mean, 8.74; SD, 7.7) ng/10⁸ CFU. The amount of SEA per hour incubation in the log growth phase was 0.1–12.0 (mean, 2.37; SD, 3.06) ng/10⁸ CFU. Overall, 54.2% of SEA was produced in the log growth phase. Both the transcriptional level of *sea* mRNA and the amount of SEA in the log growth phase correlated well with the amount of SEA after 24 h of cultivation. Four isolates, namely, SA-212, SA-217, SA-340 and SA-341, were categorized to be of high SEA production (877–1,109 ng/mL, 24 h). The total amount of SEA was mainly based on the amount of SEA in 10⁸ CFU, not the relatively fixed bacterial cell counts (21.1–43×10⁸ CFU/mL). Seven isolates from patients all carried the ΦMu3A phage, whereas 21 of the 22 isolates from the environmental sources all carried the ΦSa3ms phage.

Conclusion: The present study exhibits varied SEA production capacity of the wild *sea*-positive *S. aureus* strains. An apparent boundary in phage types between strains from the clinical samples and strains from the environment was also identified.

Keywords: *Staphylococcus aureus*, staphylococcal enterotoxin A, multilocus sequence typing, phage, SEA production capacity

Introduction

Staphylococcal food poisoning (SFP) typically exhibits sudden onset of nausea, violent vomiting, abdominal cramps and sometimes diarrhea. It is one of the most common forms of bacterial foodborne outbreaks worldwide and has been reported to be the fourth most common type of foodborne outbreaks in Europe in 2011.¹ The major causative agents of SFP are staphylococcal enterotoxins (SEs). SEs are often produced in food contaminated with enterotoxin-producing *Staphylococcus aureus* (*S. aureus*) due to improper handling and storage. Classical SEs are classified into five types, namely, staphylococcal entero-

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toxin A (SEA), SEB, SEC, SED and SEE, by the serological method. Meanwhile, 23 SE types are known, but it is not yet known how relevant some of these are for SFPs.² Up to now, commercial assays only measure types A–E. The five classical SEs contribute to 95% of the SFP outbreaks.³ In particular, SEA is viewed to be most frequently associated with SFP and is detected in >50% of relevant outbreaks.^{4,5} SEA is encoded by the *sea* gene existing on two types of phages.⁶ Previous studies have proved that the minimal toxic dose of SEA for humans ranges from 20 to 100 ng.^{7,8}

A number of experimental studies have been carried out to characterize the diverse SE gene profiles in strains from various food sources and have found that over a half of the *S. aureus* isolates from meat or milk are enterotoxigenic.^{9,10} Despite ubiquitous presence of *S. aureus* in the environment, whether an SFP will occur is determined largely by enterotoxin levels in contaminated food. However, studies exploring the differential enterotoxin production ability in wild-type *S. aureus* strains are scant. Only limited knowledge on impacts of temperature, lactic acid and processed pork meat in standard strains is available.¹¹

To investigate the SEA production capacity of *S. aureus* isolates harboring the *sea* gene, we identified 29 wild-type *sea*-positive *S. aureus* isolates that were isolated from dairy and meat samples, as well as from patients, measured the amount of SEA produced in the log growth phase and after 24 h of incubation under favorable cultivation conditions using enzyme-linked immunosorbent assay (ELISA) and relevant transcriptional level of *sea* mRNA and investigated the phage types, as well as genetic diversity by means of pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST).

Materials and methods

Staphylococcus aureus isolates

The *S. aureus* isolates used in this study were recovered from SFP outbreaks, dairy and meat samples as well as from clinical samples as part of the routine hospital laboratory procedure. The isolates were identified using standard laboratory techniques including Gram stain, plasma coagulase test, biochemical reactions by the Vitek 2 Gram-positive identification card (bioMérieux, Marcy l'Etoile, France) and polymerase chain reaction (PCR) detection of the *nuc* gene.¹²

DNA extraction and PCR amplification

Genomic DNA of *S. aureus* isolates was extracted using bacterial genomic DNA extraction kits (TaKaRa MiniBEST, Ver.3.0; TaKaRa, Kawasaki, Japan). The *sea*, *seb*, *sec*, *sed*

and *see* genes were amplified according to the methodologies reported in a previous study.¹³ *Staphylococcus aureus* ATCC25923 and a *sea*-positive *S. aureus* proved by sequencing were used as a negative and a positive control for the *sea* gene, respectively. Isolates with the *sea* gene were used to determine the SEA production capacity.

MLST and PFGE

MLST was conducted on *sea*-positive *S. aureus* isolates by means of amplifying seven house-keeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi* and *yqiL*) listed on the *S. aureus* MLST website (<http://saureus.mlst.net>).¹⁴ The allelic profile of a *S. aureus* isolate appearing as an array of seven allele numbers was derived by sequencing internal fragments of these seven house-keeping genes (Sangon Biotech Company, Shanghai, China). The sequence types (STs) were assigned on the basis of the array of seven allele numbers using the same website.

PFGE was conducted to investigate genetic relatedness of the *sea*-positive *S. aureus* isolates according to the PulseNet standardized protocol.¹⁵ All image patterns were normalized against the pattern of the *Salmonella* Braenderup H9812 reference standard. Image data were transferred into software BioNumerics 7.1 (Applied Maths, Sint-Martens-Latem, Belgium) and processed to identify clusters of isolates. Dendrograms of the PFGE patterns were constructed using the unweighted pair-group method.

Culture conditions for the determination of SEA

Favorable cultivation was done in brain heart infusion (BHI) broth or on BHI agar at 37°C as described previously with some modifications.¹¹ *S. aureus* isolates with the *sea* gene were transferred from a stock to BHI agar for overnight incubation, and one colony was inoculated into 10 mL of broth for overnight culture prior to the experiment. Portions of 10 mL culture were inoculated into 50 mL of broth at a final cell concentration of 10⁵ colony-forming unit (CFU)/mL and cultivated with shaking at 150 rpm. Culture was taken at 5 h of growth time from the 50 mL inoculated broth and used as a seed culture for the following assay. Then, the seed culture was inoculated into 50 mL of broth under the same conditions. In this way, we could ensure to obtain culture almost in the log growth phase of bacteria. Cultures were taken after 4 h, 6 h and 24 h of incubation to determine the SEA concentration. At 6 h cultivation, bacterial growth almost entered the stationary phase. Bacterial cells were counted by colony counting on plates after serial 10-fold dilutions of the bacterial cultures and were expressed as CFU/mL.

ELISA for the determination of SEA

The concentration of extracellular SEA was determined for the *sea*-positive isolates using Ridascreen SET A, B, C, D and E (R-Biopharm, Darmstadt, Germany) following the manufacturer's instructions. In short, *S. aureus* liquid cultures were centrifuged at $3,500 \times g$ at 10°C for 10 min. The supernatant was diluted to 1:100, 1:200 and 1:500 (v/v) with 0.85% NaCl solution to ensure that the resulting values were within the detection limit of the ELISA kit. SEA standard (0.1 ng/mL; Toxin Technology Inc., Sarasota, FL, USA) and culture supernatants were added onto the plate (100 μL /well) at appropriate dilutions. The optical density (OD_{405}) of each well was measured using a microplate reader (Multiskan Ascent, Thermo Electron Corporation, Waltham, MA, USA). OD_{405} values were plotted against SEA concentrations. SEA concentrations for each isolate were calculated from linear regression. Three replicate measurements were obtained to ensure the reliability of results.

Transcriptional level of *sea* mRNA in the log growth phase

The total RNA of culture in the log growth phase was extracted using TRIzol (Thermo Fisher Scientific, Waltham, MA, USA) according to the product manual. Pelleted RNA was resuspended in RNA storage solution (TaKaRa). RNA purity and concentration were verified by electrophoresis and $\text{OD}_{260}/\text{OD}_{280}$ absorption ratio >1.95 using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific). First-strand cDNA was synthesized from the suspended RNA extraction (1 μg) in two separate reverse transcription assays using specific reverse primers for the *sea* gene and the 16S rRNA gene (Table 1). Real-time PCR amplification was conducted using quantitative PCR Direct SYBR Premix (TaKaRa) on Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific). Each reaction contained 15 μL of quantitative

PCR premix, 1 μL of each primer ($c=10 \mu\text{M}$), 2 μL of cDNA template and 11 μL of water. The amplification conditions for *sea* and 16S rRNA genes were initial denaturation at 98°C for 1 min, followed by 40 cycles of 95°C for 15 s, 60°C for 15 s and 72°C for 45 s. The cycle threshold (C_t) values for *sea* and 16S rRNA genes were determined. The relative *sea* expression was obtained by the ΔC_t method for each isolate by comparing the *sea* C_t value to the 16S rRNA C_t value.¹⁶ The relative expression ratio was calculated for each isolate. Three experimental replicates were performed for each reaction.

Parameters for assessing the SEA production capacity

To assess the capacity of SEA production, four parameters as previously described with some modifications,¹⁷ ie, total amount of SEA (ng/mL) and amount of SEA per 10^8 CFU (ng/ 10^8 CFU) after 24 h of incubation, amount of SEA per 10^8 CFU per hour and transcriptional level of *sea* mRNA in the log growth phase between 4 h and 6 h of incubation were determined to compare the SEA production ability between isolates. The amount of SEA per 10^8 CFU was calculated as follows: amount of SEA divided by bacterial count at the same time with unit of ng/ 10^8 CFU. The amount of SEA per hour in the log growth phase was calculated as following: amount of SEA after 6 h of incubation minus that after 4 h of incubation, then divided by 2. The mRNA expression ratio of *sea* gene was *sea* C_t value to 16S rRNA C_t value as previously described.¹⁶

Amplification of gene encoding prophage tail protein

To determine two types of phages (referred to as $\Phi\text{Mu}3\text{A}$ and $\Phi\text{Sa}3\text{ms}$), we designed two sets of primers according to the highly varied regions of gene encoding phage tail protein, ie, from 1,120 bp to the end of 1,385 bp-long gene of $\Phi\text{Mu}3\text{A}$

Table 1 Primers used in this study

Gene	Primers (5'→3')	Amplicon size (bp)	Accession number	References
For genes encoding phage tail protein				
$\Phi\text{Mu}3\text{A}$	$\Phi\text{Mu}3\text{A}$ -1: GTAAATTC TTTGTGCAGCTTT $\Phi\text{Mu}3\text{A}$ -2: TGAACAACAAACAATTCCTT	325	NC_009782	This study
$\Phi\text{Sa}3\text{ms}$	$\Phi\text{Sa}3\text{ms}$ -1: CATCAAAGTATGCAAACCAAT $\Phi\text{Sa}3\text{ms}$ -2: CAAATAGACAATAAAACCATTG	307	NC_002953	This study
For SEA mRNA expression analysis				
<i>sea</i>	<i>sea</i> -1: CCTTTGGAAACGGTTAAACG <i>sea</i> -2: TCTGAACCTTCCCATGAAAAAC	127		13
16S rRNA	16sRNA-1: GTGTAGCGGTGAAATGC 16sRNA-2: GTTTACGGCGTGGACTA	115		This study

Abbreviation: SEA, staphylococcal enterotoxin A.

(accession no. NC_009782) and the end of 1,391 bp-long gene of Φ Sa3ms (accession no. NC_002953). For each reaction of 50 μ L, 25 μ L of PCR Master Mix (Promega Corporation, WI, USA), 2 μ L of each primer ($c=10 \mu$ M), 2 μ L of DNA template and 21 μ L of water were added. PCR cycling conditions included the following: 5 min hot start at 95°C, followed by 30 amplification cycles (denaturation at 95°C for 45 s, annealing at each set of primer-specific annealing temperature for 45 s and elongation at 72°C for 45 s), a final elongation step at 72°C for 10 min and a cooling step. Target-specific amplicons were confirmed by electrophoresis using a 1% agarose gel.

Results

A total of 29 wild-type *S. aureus* isolates with the *sea* gene were identified from 168 *S. aureus* isolates. These *sea*-positive isolates comprised 22 from food sources (including one outbreak case) and seven from patient samples (Table 2). The following five SE profiles were identified in 29 isolates: *sea* (14 isolates), *sea+sec* (9 isolates), *sea+seb* (4 isolates), *sea+seb+sec* (1 isolate) and *sea+seb+sed* (1 isolate), revealing 55% of isolates having more than one of five classical SE genes.

Seven STs were identified in 29 isolates, including ST1 (13 isolates), ST6 (5 isolates), ST59 (3 isolates), ST239

Table 2 SEA production capability of 29 *sea*-positive *Staphylococcus aureus* in terms of amount of SEA per hour in the log growth phase and after 24 h of cultivation

Strains	ST	SE profile	Source	Phage	CFU ($\times 10^6$ /mL)		SEA (ng/ 10^8 CFU)		Total SEA (ng/mL, 24 h)	Expression ratio at the log growth phase
					6 h	24 h	Per hour at the log growth phase	24 h		
SA-2	ST188	A	Dairy	Φ Sa3ms	16.2	33.2	0.1	1.1	36	Baseline
SA-16	ST6	A	Meat	Φ Sa3ms	21.1	36.1	2.1	11.2	404	3.2
SA-19	ST188	A	Dairy	Φ Sa3ms	19.0	37.0	0.6	2.1	77	3.1
SA-56	ST6	AC	Meat	Φ Sa3ms	18.1	34.6	1.5	5.9	204	5.6
SA-83	ST6	AC	Meat	Φ Sa3ms	16.0	31.1	1.2	4.1	128	1.7
SA-112	ST1	AC	Meat	Φ Sa3ms	31.1	43.0	3.1	8.3	357	1.0
SA-148	ST1	ABD	Meat (SFP)	Φ Sa3ms	14.5	30.5	2.8	8.1	247	2.2
SA-187	ST59	ABC	Patient	Φ Mu3A	24.0	41.1	0.1	2.0	82	1.4
SA-189	ST59	A	Dairy	Φ Mu3A	18.0	33.2	0.2	3.2	106	1.4
SA-212	ST6	AC	Dairy	Φ Sa3ms	16.1	34.9	11.1	30	1,047	9.8
SA-217	ST15	A	Dairy	Φ Sa3ms	14.2	33.1	12	33.5	1,109	14.1
SA-227	ST59	AB	Patient	Φ Mu3A	22.0	39.0	0.2	3.5	136	1.1
SA-230	ST5	A	Patient	Φ Mu3A	17.9	33.5	1.3	7.1	238	2.7
SA-239	ST6	A	Meat	Φ Sa3ms	19.2	36.7	0.3	3.7	136	1.2
SA-240	ST1	A	Meat	Φ Sa3ms	19.1	36.8	1.2	6.7	247	1.4
SA-253	ST1	AC	Dairy	Φ Sa3ms	20.0	35.6	0.4	7.0	249	2.3
SA-254	ST1	AC	Dairy	Φ Sa3ms	19.0	32.0	2.9	9.2	294	6.8
SA-255	ST1	AC	Dairy	Φ Sa3ms	18.8	34.1	1.2	8.1	276	2.5
SA-256	ST1	AC	Meat	Φ Sa3ms	16.0	33.2	0.5	5.2	173	2.6
SA-262	ST5	AC	Patient	Φ Mu3A	15.0	32.9	1.3	5.4	178	1.9
SA-265	ST1	A	Meat	Φ Sa3ms	11.2	26.6	0.4	4.5	120	2.4
SA-282	ST1	AB	Dairy	Φ Sa3ms	9.8	25.3	3.1	8.9	225	3.5
SA-283	ST1	AB	Dairy	Φ Sa3ms	11.0	26.1	2.0	7.8	204	2.8
SA-284	ST1	AB	Dairy	Φ Sa3ms	8.8	21.1	1.7	6.1	129	3.1
SA-335	ST1	A	Meat	Φ Sa3ms	18.0	38.0	3.9	10.1	384	2.1
SA-337	ST239	A	Patient	Φ Mu3A	22.3	39.2	1.1	5.1	200	3.1
SA-340	ST239	A	Patient	Φ Mu3A	29.9	41.0	7.2	21.4	877	10.1
SA-341	ST239	A	Patient	Φ Mu3A	26.7	42.1	4.1	18.1	762	6.9
SA-346	ST1	A	Meat	Φ Sa3ms	16.7	35.9	1.2	6.1	219	1.8
Mean					18.26	34.37	2.37	8.74	305	3.64
SD					5.26	5.12	3.06	7.70	281	3.17
Maximum					31.1	43.0	12.0	33.5	1,109	14.1
Minimum					8.8	21.1	0.1	2.0	36	1.0

Abbreviations: CFU, colony-forming unit; SE, staphylococcal enterotoxin; SEA, staphylococcal enterotoxin A; SFP, staphylococcal food poisoning; ST, sequence type.

(3 isolates), ST5 (2 isolates), ST188 (2 isolates) and ST15 (1 isolate). The genetic typing results demonstrated a predominant *sea*-positive ST1 lineage. PFGE also proved a similar cluster around the ST1 lineage. Especially, the only one outbreak-related isolate also belonged to ST1 (Figure 1 and Table 2).

The values of bacterial cell counts after 24 h of incubation showed limited variation, most focusing on $29\text{--}39 \times 10^8$ CFU/mL. After 6 h of incubation, on the average, bacterial cell counts accounted for 53% (18.26/34.37) of the maximal cell counts of 24 h of incubation (Table 2).

The amount of SEA after 24 h of incubation ranged from 1.1 to $33.5 \text{ ng}/10^8$ CFU, with an average of 8.74 and most values at lower part of the range (Table 2 and Figure 2A). The amount of SEA per hour in the log growth phase ranged from 0.1 to

$12.0 \text{ ng}/10^8$ CFU, with an average of 2.37 and most values also at lower part of the range (Table 2 and Figure 2A). Upon comparing the amount of SEA after 24 h of incubation with the amount of SEA of the 2 h log growth phase (amount of SEA per hour $[2.37] \times 2$), it was found that 54.2% (4.74/8.74) of SEA was produced in the log growth phase. This value was very close to the proportion (53%) of bacterial cell counts in the log growth phase. Two linear regressions illustrated that both the transcriptional level of *sea* mRNA and the amount of SEA in the log growth phase correlated well with the 24-h amount of SEA. When arbitrarily setting the 24-h amount of SEA that was over the median value of the amount of SEA range as a cutoff value for high SEA production, four isolates, namely, SA-212, SA-217, SA-340 and SA-341, were categorized to be of high SEA production (Table 2).

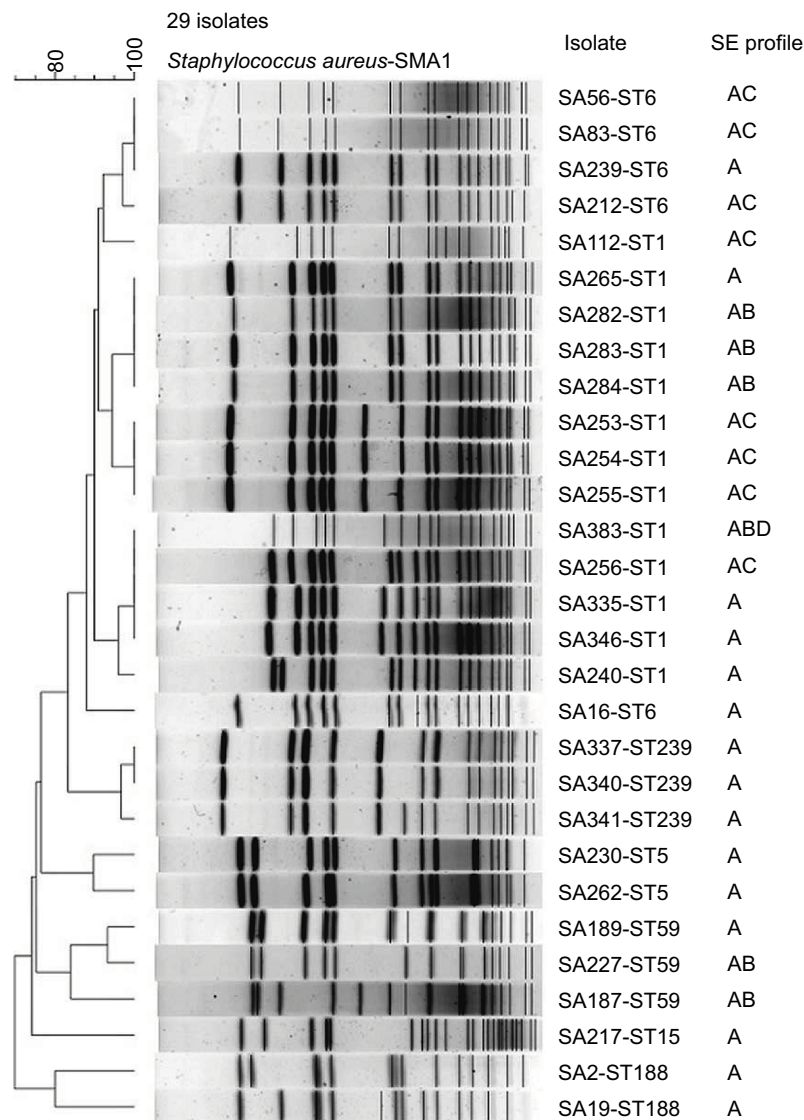


Figure 1 PFGE, MLST and SE profiles of 29 *sea*-positive *Staphylococcus aureus* isolates.

Abbreviations: MLST, multilocus sequence typing; PFGE, pulsed-field gel electrophoresis; SE, sequence type.

As a measure indicating the production speed of SEA, the amount of SEA per hour in the log growth phase correlated well with the concentration of SEA at 24 h (Figure 2A). Similar correlation existed with the SEA expression level in the log growth phase and the amount of SEA at 24 h (Figure 2B). Upon integrating the bacterial cell counts, the amount of SEA in 10^8 CFU at 24 h and total amount of SEA at 24 h, we could see that the total amount of SEA (y axis in Figure 2C and D) was determined largely by the amount of SEA in 10^8 CFU at 24 h (x axis in Figure 2C) and almost no apparent correlation with the bacterial cell counts (x axis in Figure 2D).

As to types of phages that carry the *sea* gene, seven isolates carried the Φ Mu3A phage and 22 isolates carried the Φ Sa3ms phage (Table 2). It is important to note that the isolates from patients all carried the Φ Mu3A phage and 21 of the 22 isolates from environmental sources carried the Φ Sa3ms phage. This phenomenon generated a distinct boundary of phage type between two types of isolation sources.

Discussion

The present study on the SEA production capacity of wild *sea*-positive *S. aureus* isolates yielded the following findings: although all isolates produced SEA at varied levels, isolates with high production capacity accounted for a small proportion of all isolates; production speed in the log growth phase decided the final SEA concentration, which further decided the total amount of SEA; transcriptional level of *sea* mRNA in the log growth phase could also well reflect the SEA production capacity; and there was an apparent boundary of phage types between isolates from clinical samples and isolates from the environment.

As shown by PFGE and MLST distribution, wild *sea*-positive *S. aureus* isolates were found to focus on limited lineages genetically. Isolates of ST1 (n=13) and ST6 (n=5) accounted for most of the environmental isolates (82%, 18/22) (Figure 1 and Table 2). ST6 was previously reported to be the most dominant clone in China.¹⁸ ST5 and ST188 are also among the most found STs in SE-producing strains

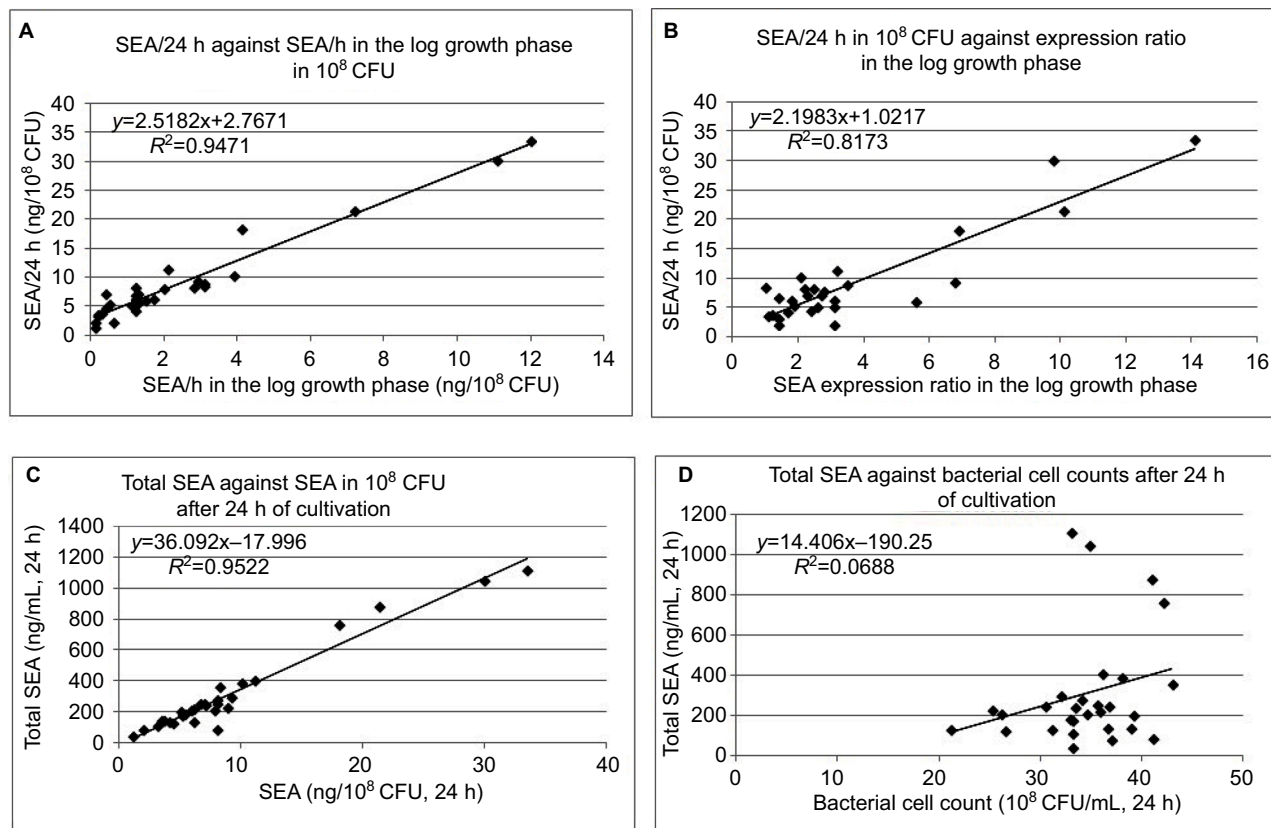


Figure 2 Relationship between SEA production after 24 h of incubation and per hour production in 10^8 CFU (A), and relevant expression ratio in the log growth phase (B) as well as after 24 h of incubation relationship between total amount of SEA and amount of SEA in 10^8 CFU (C), and relevant bacterial cell counts (D). (C) and (D) show that total amount of SEA is mainly based on the amount of SEA in 10^8 CFU, not the relatively fixed bacterial cell counts.

Abbreviations: CFU, colony-forming unit; SEA, staphylococcal enterotoxin A.

both from foodborne cases and environmental sources.^{19,20} However, the remarkably large proportion of ST1 isolates in this study indicates a clustering trend in such strains. Regarding the hospital isolates in this study, ST239, ST5 and ST59 were most found STs.

This study proved that after 24 h of incubation all wild *sea*-positive *S. aureus* isolates could produce SEA at varied levels of amount. Several previous studies were performed concerning the influential factors such as temperature, prophage induction and pH.^{11,17} The results of these studies varied greatly due to such different influential factors on SEA production. Under favorable cultivation conditions at 37°C for 24 h, it has been observed that 50–300 ng/mL of SEA is reached,¹¹ and another report showed that ~608–739 ng/mL has been detected on pork sausage,²¹ which is very close to the results of this study (average: 305 ng/mL; range: 362–1,109 ng/mL) after 24 h of incubation. In this study, the amount of SEA produced after 24 h of incubation by four highest SEA-producing isolates, namely, SA-212, SA-217, SA-340 and SA-341, was 10 times that produced by four lowest SEA-producing isolates, namely, SA-2, SA-19, SA-187 and SA-189. This gap was much smaller than a reported one that was 4 log units and with a range of 2–10,378 ng/mL.¹⁷ Moreover, even without induction, substantial SEA could be detected in all isolates after 24 h of incubation. As a whole, the isolates with high production capacity accounted for a small proportion of all isolates (Table 2 and Figure 2). Especially, although SA-148 from an outbreak of 52 cases with a distinctive SE pattern of three SE genes, *sea*, *seb* and *sed*, did not produce very high amount of SEA, it still caused a serious foodborne outbreak probably due to its SED production. This proves the previous report that SEA along with SED is more prone to cause foodborne outbreaks.²² Taken into consideration the SEA production levels and genetic distribution, differential SEA production capacity existed among isolates of different genetic types. Most of the ST59 and ST188 isolates had low production capacity which could only be vaguely detected at the early incubation period and could only be safely proved toward the end of extended time of incubation.

We demonstrated that more than half (54.2%, 4.74/8.74) of SEA was produced toward the end of the log growth phase, which was highly proportional to the bacterial cell count during the same period (Table 2). This phenomenon suggests that SEA production is substantially proportional to cell growth. However, in food where *S. aureus* grows much more slowly compared with growing under optimal experimental conditions, SEA production lasts for days.²³ Both the

transcriptional level of *sea* mRNA and the amount of SEA in the log growth phase correlated well with the 24-h amount of SEA, indicating that the high SEA-producing isolates began to express and produce SEA at the early phase of growth. The production speed in the log growth phase would decide the final SEA concentration and the total amount of SEA.

Here, we gained an interesting finding that the distinctive distribution of types of *sea*-carrying phages existed between isolates from environmental sources and those from clinical sources. Twenty-one of the 22 isolates from environmental sources carried the ΦSa3ms phage, whereas seven from hospital sources all carried the ΦMu3A phage. Moreover, one hospital-originated SA-341 carried both. Among over 80 phages found in *S. aureus*, only five types harbor *sea* gene, which can be typed into integrase group Sa3, serogroup Fa/Fb and holigroup 255a/255b.⁶ However, these typing methods overlap results between two distinct *sea* structures. In this study, we used the highly varied region of the end of phage tail gene and clearly differentiated two structurally different phages. More important, the method could generate a distinct boundary between environmental strains and hospital strains. As to the SEA production capacity, SA-212 and SA-217 with ΦSa3ms and SA-340 and SA-341 with ΦMu3A had high SEA-producing capacity, indicating that both phages had the ability to produce large amounts of SEA, although only in a few strains (Table 2).

Conclusion

The present study exhibits varied SEA production capacity of the wild *sea*-positive *S. aureus* strains in terms of SEA concentration and total amount of SEA after 24 h of incubation, as well as production speed and transcriptional level in the log growth phase. We also identified a distinctive boundary of phage types between strains from clinical samples and the environment.

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Disclosure

The authors report no conflicts of interest in this work.

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