FEMS Microbiology Letters, 362, 2015, fnv024

doi: 10.1093/femsle/fnv024
Advance Access Publication Date: 16 February 2015
Research Letter

RESEARCH LETTER – Food Microbiology

Temporal expression of the staphylococcal enterotoxin D gene under NaCl stress conditions

Henna-Maria Sihto, Taurai Tasara, Roger Stephan and Sophia Johler*

Institute for Food Safety and Hygiene, University of Zurich, CH-8057 Zurich, Switzerland

*Corresponding author: Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich Winterthurerstr. 272 CH-8057 Zurich, Switzerland. E-mail: sophia.johler@uzh.ch

One-sentence summary: This piece of research shows a strain-specific variation in the expression of the enterotoxin D gene in *Staphylococcus aureus* under NaCl stress conditions and its control by the regulatory elements *agr*, *sarA* and *sigB*.

Editor: Wolfgang Kneifel

ABSTRACT

Staphylococcus aureus is one of the most osmotolerant food-borne pathogens. While its growth is repressed by competing bacteria, the organism exhibits a growth advantage at increased salt concentrations. Staphylococcal enterotoxin D leads to vomiting and diarrhea upon ingestion. To date, the effect of NaCl on both *sed* expression and its regulatory control are unclear. We determined the impact of NaCl stress on *sed* expression and the influence of *agr, sarA* and *sigB* on *sed* expression under NaCl stress. The temporal expression of *sed* in LB and LB with 4.5% NaCl was compared, as well as *sed* expression of wild-type (wt) strains and isogenic Δ*agr*, Δ*sarA* and Δ*sigB* mutants. In general, NaCl stress led to decreased *sed* expression. However, one strain exhibited a trend towards increased *sed* expression under NaCl stress. No significant effect of *agr* on *sed* expression was detected and only one Δ*sigB* mutant showed a significant decrease in *sed* expression in the early stationary phase under NaCl stress. One Δ*sarA* mutant showed decreased *sed* expression in the early stationary and another increased *sed* expression in the stationary growth phase under NaCl stress. These findings suggest high strain-specific variation in *sed* expression and its regulation under NaCl stress.

Key words: *Staphylococcus aureus*; *sed*; NaCl stress; regulation; stress response

INTRODUCTION

Staphylococcus aureus is the most prevalent pathogen implicated in food-borne intoxications worldwide. Strains can produce one or several of the five major staphylococcal enterotoxins (SE), designated SEA-SEE. Upon consumption of SE preformed in food, patients suffer from violent vomiting, diarrhea and abdominal cramping. In the US alone, an estimated 240 000 cases of staphylococcal food poisoning occur each year (CDC), leading to hopitalization in 1000 of cases and to six deaths (Scallan *et al.* 2011). While its growth is repressed by competing bacteria in most food matrices, *S. aureus* has a crucial growth advantage under NaCl stress (Chapman 1945; Troller 1986). The organism is able to grow at a_w values < 0.90 (2.6 M NaCl), rendering it one of the most osmotolerant food-borne pathogens (Troller 1986). While the osmoadaptive process in *S. aureus* is comparable to that in other pathogens, regulatory mechanisms controlling NaCl stress response do not seem to follow common patterns. For instance, NaCl stress induces the expression of the alternative sigma factor σ^B in *Bacillus subtilis*, whereas 1 M NaCl was shown to repress σ^B in *S. aureus* (Chan *et al.* 1998).

Several studies evaluated SE expression under NaCl stress using immunological methods (Genigeorgis and Sadler 1966; McLean, Lilly and Alford 1968; Genigeorgis *et al.* 1971; Troller 1971; Ewald and Notermans 1988). For a comprehensive review on enterotoxin production under stress conditions, see Schelin *et al.* (2011). However, the value of these data is limited, because it was later reported that loss of serological recognition does not equal loss of emetic activity (Bennett 2005). It has therefore

Received: 14 January 2015; **Accepted:** 11 February 2015

been suggested that the evaluation of enterotoxin expression on mRNA level would represent a more useful tool that allows to determine the risk of *S. aureus* food poisoning (Lee *et al.* 2007).

Staphylococcal enterotoxin D (SED) is frequently detected in *S. aureus* strains associated with intoxications (Wieneke, Roberts and Gilbert 1993; Kérouanton *et al.* 2007). Transcription of *sed* is moderately increased in the post-exponential growth phase when no stress is applied (Tseng, Zhang and Stewart 2004; Derzelle *et al.* 2009). Investigating the effect of various regulatory factors on *sed* expression, Tseng, Zhang and Stewart (2004) were able to show that *sed* promoter activity is increased by the accessory gene regulator (Agr) and the staphylococcal accessory regulator (SarA) and decreased by σ^B and the repressor of toxins. The function of the regulatory factors has also been shown to be highly connected (Yarwood and Schlievert 2003; Hsieh, Tseng and Stewart 2008). The post-exponential increase in *sed* transcription was shown to result from an Agr-dependent decrease in Rot activity rather than from a direct effect of Agr on *sed* expression (Tseng, Zhang and Stewart 2004). It has been suggested that SED is only partially upregulated by RNAIII and that high concentrations of the enterotoxin can be produced independently of *agr* (Yarwood and Schlievert 2003).

To date, both the effect of NaCl stress on *sed* expression and the regulatory mechanisms controlling *sed* expression under NaCl stress remain unclear. Therefore, we aimed to (i) determine *sed* mRNA levels during growth of *S. aureus* under NaCl stress conditions encountered during food production and preservation and (ii) evaluate the influence of *agr, sarA* and *sigB* on *sed* expression under NaCl stress.

MATERIALS AND METHODS

Bacterial strains

All *S. aureus* strains used in this study are listed in Table 1. Five *sed*$^+$ isolates (BW10, KLT8, RKI1, RKI2, SAI48) were selected. Three of these strains were used to create regulatory mutants and to assess the influence of *agr, sarA* and *sigB* on *sed* expression under NaCl stress. Strains RN27, NM518, BB1385 and LR12 were kindly provided by Brigitte Berger-Bächi (University of Zurich). Regulatory knockout mutants were obtained by transduction of the *agr, sarA* and *sigB* knockouts from NM518, BB1385 and LR12 to the isolates RKI1, RKI2 and SAI48, using phage 80α and protocols previously described (Charpentier *et al.* 2004). Antibiotics were used at the following concentrations to screen for presumptive regulatory knockout mutants using selective plates: tetracycline at 10 μg mL^{-1} and erythromycin at 10 μg mL^{-1} (Sigma). Correct deletion of the respective regulatory genes in putative mutants grown on selective plates was evaluated by PCR screening for *agr, sarA* and *sigB*. In addition, characteristic changes in phenotype associated with loss of the regulatory element were observed such as changes in hemolysis pattern or loss of pigmentation.

Bacterial growth and collection of samples

S. aureus strains were grown in LB broth (Bertani 1959) and LB broth adjusted to 4.5% NaCl (0.8 M NaCl, a_w = 0.97). These conditions were chosen to mimic NaCl levels encountered during production or preservation of foods. Media ingredients were obtained from Difco laboratories (Detroit, MI), Oxoid (Cambridge, UK), Becton Dickinson (Allschwil, Switzerland) and Sigma (Buchs, Switzerland). Growth phases were determined using plate count and DMFit 3.0 (Baranyi and Roberts 1994).

Frozen stock cultures were resuscitated by plating on 5% sheep blood agar and incubated at 37°C over night. Single colonies were grown in 5 mL of LB broth for 18 h (37°C, 225 rpm shaking) to reach stationary phase. Aliquots of 1 mL of the overnight cultures were centrifuged (6000 × g for 10 min) and washed twice with 0.8% NaCl to remove residual media components. We prepared 10-fold dilution series and used 50 μL of a 10^{-3} dilution to inoculate a 50 mL day culture of LB or LB adjusted to 4.5% NaCl. For RKI2Δ*sarA*, the 10^{-2} dilution was used instead of the 10^{-3} dilution to account for an extended lag phase in this strain. Day cultures were grown at 225 rpm and 37°C to early exponential (T1), mid-exponential (T2), early stationary (T3) and stationary growth phase (T4). Cells were harvested by centrifugation at 8000 × g for 5 min and resuspended in 500 μL RNA protect bacteria reagent (Qiagen, Hombrechtikon, Switzerland), incubated at room temperature for 5 min and harvested again by centrifugation (3000 × g for 5 min). Subsequently, cell pellets were stored at −80°C. This procedure was repeated to gain two independent samples of each strain grown under control conditions (LB), as well as NaCl stress conditions (LB adjusted to 4.5% NaCl).

RNA extraction and reverse transcription

Cell lysis, RNA extraction and reverse transcription were performed as previously described (Sihto *et al.* 2014). RNA integrity numbers determined by Bioanalyzer (Agilent Technologies, Waldbronn, Germany) ranged from 7.2 to 9.9.

Real-time quantitative PCR (qPCR)

Single peaks in the melting curve analyses and single product bands on agarose gels confirmed target-specific amplifications for all primers (Table 2). Real-time PCR experiments were performed using LightCycler480 (Roche). A total reaction volume of 10 μL was used, including 4 μL cDNA template (dilution 1:100), 250 nM of each primer and the LightCycler480 SYBR Green I master mix (Roche). Water (no template) and RT minus samples were used as controls. An inter-run calibrator sample was included as a control for variation in cDNA synthesis and amplification. PCR cycling conditions included 8 min at 95°C, 45 amplification cycles (95°C for 10 s, the respective annealing temperature for 15 s, 72°C for 20 s, 78°C for 1 s with a single fluorescence measurement), a melting curve (60–95°C at 2.2°C s^{-1}, and a continuous fluorescence measurement) and a final cooling step. Standard curves based on genomic DNA were generated to determine the efficiency of target gene amplification for each strain. All samples were amplified in triplicates. Expression levels of *sed* were compared using relative expression ratios, which were normalized using *rho* and *rplD* as reference genes, previously shown to be suitable for normalization of *S. aureus* qPCR data in LB and LB adjusted to 4.5% NaCl (Sihto *et al.* 2014). The effect of NaCl on *sed* expression was assessed by calculating fold changes in *sed* expression in early exponential, mid-exponential, early stationary and stationary phase relative to the mean *sed* expression level at T1 in LB. The effect of *agr, sarA* and *sigB* on *sed* expression was assessed by calculating fold changes in *sed* expression in isogenic regulatory mutants relative to mean *sed* expression in the wt at T1 in LB.

Statistical analysis

Statistical analysis was performed using SPSS Statistics 22 (SPSS Inc., Chicago, IL). Results were considered significant at

Table 1. *S. aureus* wt and mutant strains used in this study.

Strain ID	Relevant characteristics	Source (reference)
RKI1	Clinical strain associated with food-borne outbreak, CC8/t648, *sea+, sed+, sej+*	Robert Koch Institute, Germany
RKI2	Clinical strain associated with food-borne outbreak, CC8/t008, *sea+, sed+, sej+*	Robert Koch Institute, Germany
SAI48	Clinical strain isolated from *S. aureus* infection, CC5/t002, *sec+, sed+, sej+*	Institute of Medical Microbiology, University of Zurich, Switzerland
KLT8	Clinical strain associated with foodborne outbreak, CC5/t8017 *sea+, sed+, sej+, egc cluster*	Cantonal Laboratory Thurgau, Switzerland
BW10	Clinical strain associated with foodborne outbreak, CC45/t383, *sec+, sed+, sej+*	Medical Department of the German Federal Armed Forces, Germany
NM518	RN4220_*agr*::*ermB*	Brigitte Berger-Bächi (McCallum, Hinds and Ender 2010)
LR12	RN4220_*sarA*::*tetL*	Brigitte Berger-Bächi (McCallum, Hinds and Ender 2010)
BB1385	BB255_rsbUVWσ^B::*ermB*	Brigitte Berger-Bächi (Kullik, Giachino and Fuchs 1998)
RN27	80α lysogen	Brigitte Berger-Bächi (Novick 1963)
RN4220	Restriction deficient mutant of strain NCTC8325-4 that accepts foreign DNA	Brigitte Berger-Bächi (Kreiswirth *et al.*1983)
RKI1Δ*agr*	RKI1 with *ermB* replacing *agr*	This work
RKI1Δ*sigB*	RKI1 with *ermB* replacing *sigB*	This work
RKI1Δ*sarA*	RKI1 with *tetL* replacing *sarA*	This work
RKI2Δ*agr*	RKI2 with *ermB* replacing *agr*	This work
RKI2Δ*sigB*	RKI2 with *ermB* replacing *sigB*	This work
RKI2Δ*sarA*	RKI2 with *tetL* replacing *sarA*	This work
SAI48Δ*agr*	SAI48 with *ermB* replacing *agr*	This work
SAI48Δ*sigB*	SAI48 with *ermB* replacing *sigB*	This work
SAI48Δ*sarA*	SAI48 with *tetL* replacing *sarA*	This work

Table 2. Primer pairs, including amplicon sizes, E-values and annealing temperatures for the target and reference genes used in this study.

Gene	Function	Primer pair (5′–3′)	Amplicon size (bp)	E(%)	Annealing T (°C)	Reference
sed	Staphylococcal enterotoxin D	GTG GTG AAA TAG ATA GGA CTG C ATA TGA AGG TGC TCT GTG G	384	90–8	62	(Monday and Bohach 1999)
rho	Transcription termination factor	GAA GCT GCT GAA GTC G CGT CCA TAC GTG AAC CC	319	97–8	57	(Sihto *et al.* 2014)
rplD	Ribosomal protein L4	TTC GGA CCA ACT CCA AGA CGA GCA CCT CCT CAA C	352	93–6	57	(Sihto *et al.* 2014)

$P < 0.05$. Growth parameters such as lag phase duration, exponential phase growth rate and maximum cell density in stationary phase were compared using one-way ANOVA. Log transformed relative expression ratios of *sed* were also compared using one-way ANOVA.

RESULTS

Effect of NaCl stress on growth

Bacterial growth of the five wt strains was compared in LB and LB adjusted to 4.5% NaCl (Fig. 1). Respective isogenic *agr, sarA* and *sigB* mutant strains of three wt strains grew similarly with exception of RKI2Δ*sarA* growing more slowly under NaCl stress (Fig. S1, Supporting Information). OD values at sampling time points were as follows: 0.01 (T1), 0.15 (T2), 1.80 (T3) and >2.00 (T4). In the wt strains grown under NaCl stress, the mean lag phase duration was prolonged ($\Delta 1.02 \pm 0.73$ h; $P = 0.01$), the growth rate was decreased ($\Delta 0.15 \pm 0.10$ lg CfU mL^{-1} h^{-1}; $P = 0.02$) and cultures reached lower maximum cell densities ($\Delta 0.72 \pm 0.42$ lg CfU mL^{-1}; $P = 0.00$). Detailed data on the effect of NaCl stress on lag phase duration, growth rates and maximum cell densities for *agr, sarA* and *sigB* mutant strains is provided as supplemental material (Table S1, Supporting Information).

Effect of NaCl stress on *sed* expression

The impact of 4.5% NaCl on temporal *sed* expression is presented in Fig. 2 and fold change values are provided in Table 3. In both LB and LB adjusted to 4.5% NaCl, highest *sed* expression was generally observed in the stationary growth phase. However, in strain

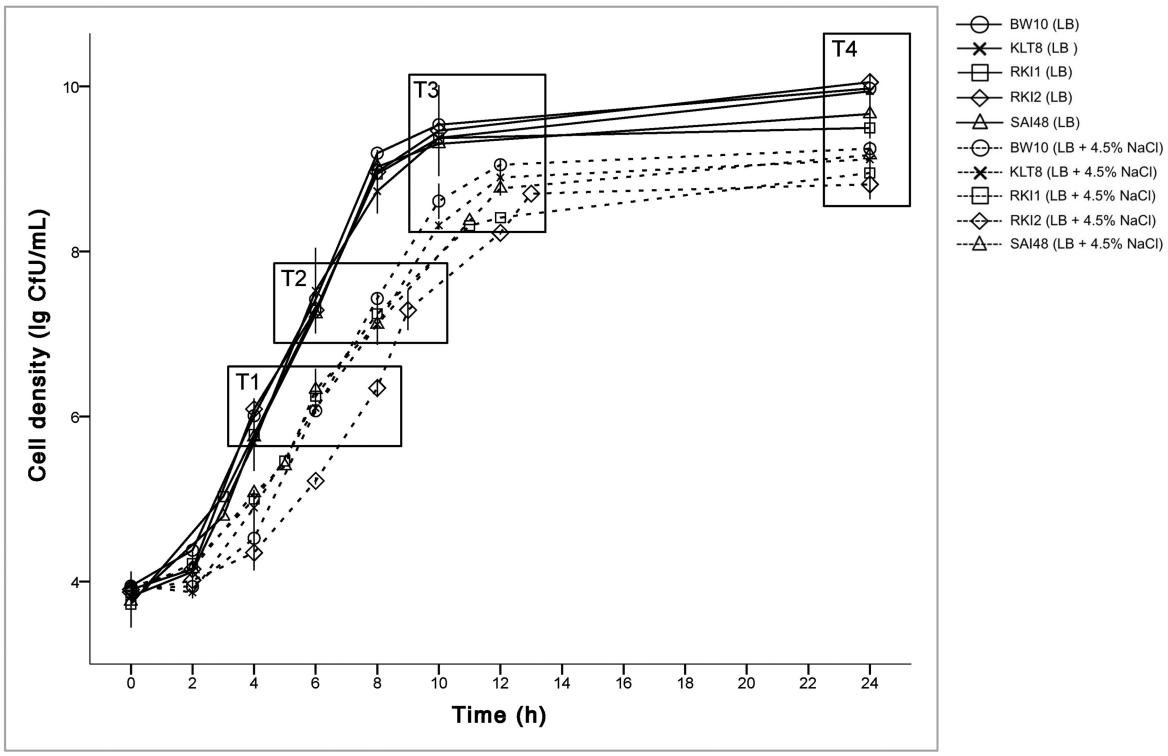

Figure 1. Growth of five *S. aureus* strains (BW10, KLT8, RKI1, RKI2, SAI48) was compared in LB and LB adjusted to 4.5% NaCl. Cells were harvested in early exponential (T1), mid-exponential (T2), early stationary (T3) and stationary growth phase (T4).

Table 3. Effect of NaCl stress on *sed* expression. Fold change of *sed* expression in early exponential (T1), mid-exponential (T2), early stationary (T3) and stationary phase (T4) is indicated relative to the mean expression level at T1 in LB. Statistically significant changes in *sed* expression in LB + 4.5% NaCl compared to LB only are marked by an asterisk.

Strain	LB				LB + 4.5% NaCl			
	T1	T2	T3	T4	T1	T2	T3	T4
BW10	1.0 (± 0.7)	3.3 (± 1.7)	26.4 (± 3.0)	337.3 (± 3.0)	1.6 (± 0.2)	2.2 (± 0.9)	21.2 (± 6.1)	16.1 (± 4.2)* ↓
KLT8	1.0 (± 0.6)	1.0 (± 0.3)	12.7 (± 6.4)	56.4 (± 13.3)	0.5 (± 0.2)	0.5 (± 0.0)	3.2 (± 1.0)* ↓	9.3 (± 2.3)* ↓
RKI1	1.0 (± 0.4)	1.9 (± 2.1)	7.0 (± 5.4)	98.9 (± 70.0)	0.6 (± 0.2)	0.7 (± 0.6)	6.0 (± 1.4)	13.3 (± 6.0)* ↓
RKI2	1.0 (± 0.8)	1.7 (± 2.0)	6.9 (± 4.9)	209.3 (± 124.7)	0.9 (± 0.7)	0.9 (± 0.6)	4.7 (± 2.4)	19.5 (± 13.3)* ↓
SAI48	1.0 (± 0.8)	4.5 (± 4.9)	17.5 (± 10.6)	97.9 (± 53.7)	1.1 (± 0.3)	3.3 (± 2.4)	33.1 (± 20.4)	128.7 (± 54.9)

BW10, *sed* expression under NaCl stress peaked in early stationary phase.

In all growth phases, relative *sed* expression values tended to be lower under NaCl stress compared with no-stress conditions. Still, only in the stationary growth phase, a statistically significant reduction in *sed* expression under NaCl stress was observed for four out of five tested strains ($P = 0.00$). Interestingly, one strain (SAI48) exhibited a trend towards increased *sed* expression under NaCl stress in the stationary growth phase.

Effect of *agr*, *sarA*, *sigB* on *sed* expression in LB

Temporal relative expression ratios of *sed* were determined for wt strains and their isogenic Δ*agr*, Δ*sarA* and Δ*sigB* knockout mutant strains. Fold change in *sed* expression was calculated relative to the mean *sed* expression level at early exponential phase in the respective wt (Table 4). No significant changes in *sed* expression were found when comparing *sed* expression in wt strains and isogenic Δ*agr* mutants. This was the case for both growth in LB and growth in LB adjusted to 4.5% NaCl. As for the effect of NaCl on *sed* expression in the Δ*agr* mutants, NaCl stress only led to a significant reduction in *sed* expression in the stationary growth phase for one of the three tested strains (RKI2Δ*agr*, $P = 0.00$). When assessing the influence of *sarA* on *sed* expression, the effect of Δ*sarA* mutations was dependent of the growth phase and the strain. In SAI48 at early stationary phase under NaCl stress, a statistically significant decrease in *sed* expression was present in the Δ*sarA* mutant compared to the wt ($P = 0.02$). By contrast, in RKI1 at stationary phase under NaCl stress, a statistically significant increase in *sed* expression in the Δ*sarA* mutant compared to the wt ($P = 0.02$) was observed. As regards the effect of NaCl on *sed* expression in the Δ*sarA* mutants, NaCl stress only led to a significant reduction in *sed* expression in the early stationary growth phase for one of the three tested strains (RKI1Δ*sarA*, $P = 0.01$). When assessing the effect of Δ*sigB*, a statistically significant decrease in *sed* expression in the early stationary phase under NaCl stress in the Δ*sigB* mutant of SAI48 compared to the SAI48 wt ($P = 0.02$) was observed.

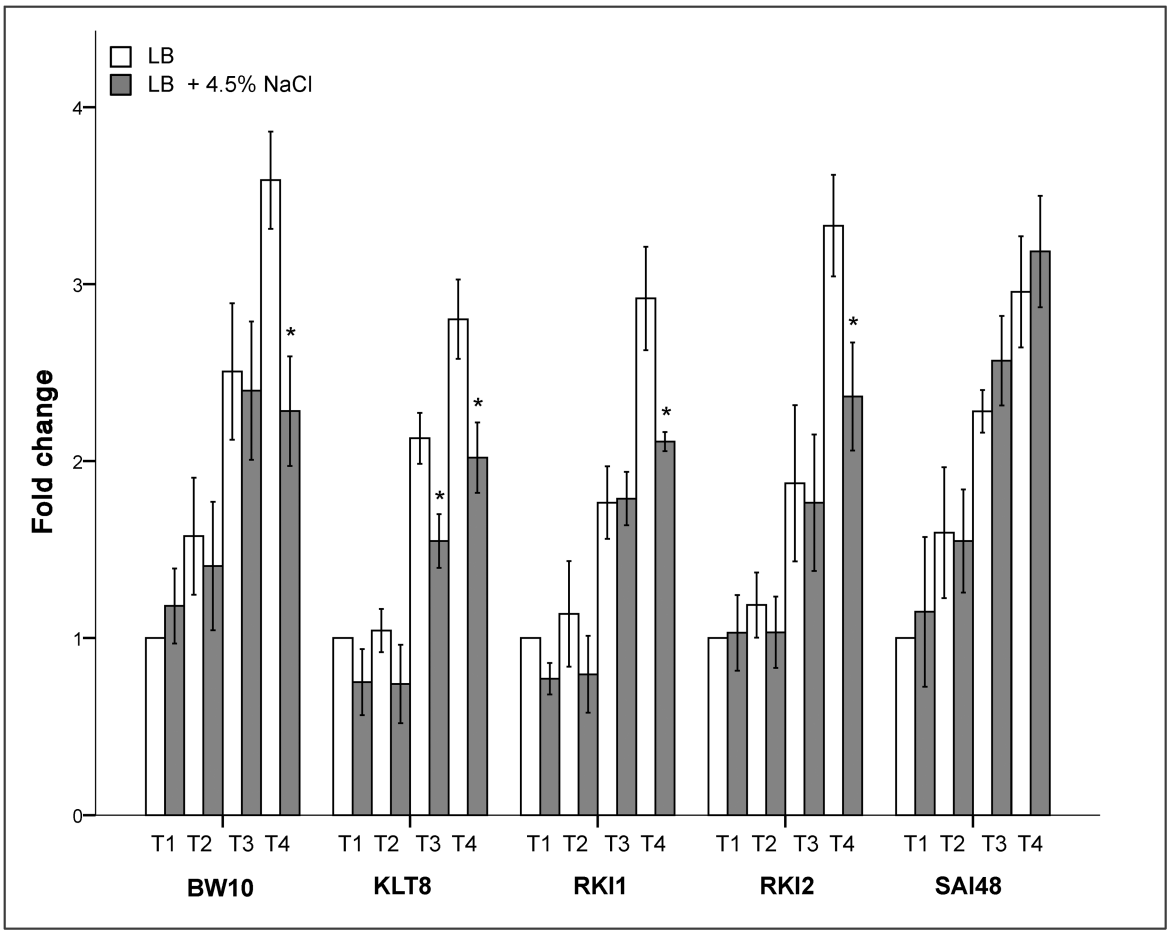

Figure 2. Effect of NaCl stress on *sed* expression in five *S. aureus* strains (BW10, KLT8, RKI1, RKI2, SAI48). Fold change of *sed* expression in early exponential (T1), mid-exponential (T2), early stationary (T3) and stationary phase (T4) was calculated relative to the mean expression level at T1 in LB. Statistically significant changes in *sed* expression in LB adjusted to 4.5% NaCl compared to LB ($P < 0.05$) are marked by an asterisk.

Table 4. Effect of *agr*, *sarA* and *sigB* on *sed* expression. Fold change of *sed* expression in wt strains and isogenic regulatory mutants in early exponential (T1), mid-exponential (T2), early stationary (T3) and stationary phase (T4) is presented relative to mean *sed* expression in the wt at T1 in LB. Statistically significant changes in *sed* expression between wt and mutants are marked by an asterisk.

Mutation	Strain	LB				LB + 4.5% NaCl			
		T1	T2	T3	T4	T1	T2	T3	T4
Δ*agr*	RKI1	1.0 (± 0.8)	2.0 (± 1.2)	14.7 (± 11.9)	26.7 (± 18.6)	0.9 (± 0.7)	1.4 (± 0.8)	5.5 (± 3.9)	21.0 (± 16.8)
	RKI2	1.0 (± 0.2)	0.9 (± 0.3)	14.1 (± 13.5)	335.5 (± 181.1)	0.4 (± 0.1)	0.5 (± 0.2)	3.4 (± 2.6)	6.2 (± 2.8)
	SAI48	3.1 (± 2.0)	5.9 (± 3.2)	47.7 (± 24.7)	130.1 (± 20.1)	4.2 (± 2.3)	4.4 (± 1.6)	28.4 (± 13.9)	49.6 (± 39.0)
Δ*sarA*	RKI1	0.5 (± 0.3)	0.6 (± 0.0)	7.9 (± 1.0)	78.3 (± 5.7)	0.3 (± 0.0)	0.5 (± 0.2)	1.8 (± 0.6)	53.3 (± 12.2)* ↑
	RKI2	0.4 (± 0.2)	0.7 (± 0.0)	15.4 (± 11.0)	103.2 (± 43.6)	0.3 (± 0.3)	0.6 (± 0.4)	2.9 (± 0.6)	48.9 (± 16.3)
	SAI48	0.7 (± 0.3)	1.6 (± 0.6)	12.1 (± 2.0)	186.4 (± 53.5)	2.0 (± 1.4)	1.6 (± 0.6)	5.9 (± 1.5)* ↓	161.9 (± 68.2)
Δ*sigB*	RKI1	0.5 (± 0.3)	1.3 (± 0.8)	5.1 (±1.2)	129.6 (± 51.1)	0.5 (± 0.2)	0.6 (±0.2)	1.7 (± 0.8)	28.1 (± 17.0)
	RKI2	2.2 (± 3.7)	1.1 (± 1.3)	4.0 (± 2.6)	46.0 (± 17.8)	0.7 (± 0.8)	1.1 (± 1.5)	3.7 (± 3.8)	21.5 (± 7.2)
	SAI48	2.5 (± 1.0)	2.2 (± 0.7)	17.4 (± 6.4)	292.9 (± 153.8)	1.5 (± 0.8)	1.3 (± 0.2)	5.8 (± 2.1)* ↓	41.5 (± 13.7)

Concerning the effect of NaCl on *sed* expression in the Δ*sigB* mutants, NaCl stress led to a significant reduction in *sed* expression in the stationary growth phase for two of the three tested strains (RKI1Δ*sigB* and SAI48Δ*sigB*, $P \leq 0.02$).

DISCUSSION

This is the first study evaluating the effect of NaCl stress on *sed* expression in *S. aureus*. The selected NaCl level mimics stress conditions encountered during production and preservation of many food items, including various raw ham or cheese products. These stress conditions grant *S. aureus* a competitive growth advantage over other microorganisms present in the food matrix. Under 4.5% NaCl stress, we detected prolonged lag phases, as well as a decrease in growth rates and maximum cell densities. This is consistent with previous studies showing a linear correlation between growth of *S. aureus* and NaCl concentration (Ewald and Notermans 1988; Genigeorgis 1989).

Many genes coding for secreted proteins are upregulated at the end of the exponential growth phase, followed by downregulation of housekeeping and facultative genes during the transition to stationary growth phase and preparation of cells for long-term survival during full stationary growth phase (Derzelle *et al.* 2009). In both LB broth and LB broth adjusted to 4.5% NaCl, highest *sed* expression was generally observed in the stationary phase of growth showing a moderate post-exponential increase. In contrast to the plasmid encoded *sed*, expression of the chromosomally encoded *agr*-regulated enterotoxin genes *seb* and *sec* has been shown to exhibit a more pronounced post-exponential increase (Derzelle *et al.* 2009).

NaCl has been reported to affect production of SEA, SEB and SEC. In this study, NaCl stress resulted in decreased *sed* expression. However, one strain exhibited a trend towards increased *sed* expression under NaCl stress. Considering the pronounced strain-specific variation detected in this study, this suggests that NaCl may induce *sed* expression in a subset of *S. aureus* strains.

In this study, no statistically significant alterations in *sed* expression were observed in Δ*agr*, Δ*sarA* or Δ*sigB* mutants compared to their isogenic wt, when strains were grown without NaCl stress. Previous studies suggested that *sed* expression is increased by Agr and SarA, and decreased by σ^B and Rot (Bayles and Iandolo 1989; Tseng, Zhang and Stewart 2004). However, these studies used derivatives of NCTC8325, a strain that was shown to exhibit an 11-base deletion in *rsbU*, a gene encoding an indirect positive regulator of σ^B (Gertz *et al.* 1999). As σ^B influences other global regulators including *sar* and *agr*, these findings may be misleading (Bischoff and Entenza 2001). In addition, pronounced strain-specific variation in *S. aureus* is well described, with a study reporting strain-specific variation in Rot levels (Jelsbak *et al.* 2010). As the *agr* effect on *sed* expression was suggested to be Rot-dependent (Tseng, Zhang and Stewart 2004), this may contribute to differences in *sed* expression.

Under the NaCl stress conditions tested in this study (0.8 M NaCl), no significant effect of *agr* on *sed* expression was detected. Similar findings were reported for enterotoxin C (Regassa and Betley 1993), for which a decrease in *sec* mRNA levels under NaCl stress conditions (1.2 M NaCl) compared to control conditions (0 M NaCl) was shown, independent of an intact *agr* allele. In Δ*sarA* mutants grown under NaCl stress, strain-specific variation was observed. One Δ*sarA* mutant showed decreased *sed* expression in the early stationary and another Δ*sarA* mutant increased *sed* expression in the stationary growth phase. Blevins *et al.* (2002) reported strain-specific variation in the regulatory role of *agr* and *sarA*. These findings suggest that this may also be the case for the *agr* and *sarA* regulation under NaCl stress conditions. As for the effect of *sigB* on *sed* expression under NaCl stress, a statistically significant decrease in *sed* expression in the early stationary phase was detected in one of the three tested Δ*sigB* mutant strains. High levels of NaCl were shown to release and activate σ^B (Betley, Borst and Regassa 1992; Miyazaki *et al.* 1999), which in turn influences the regulators SarA and RNAIII (Bischoff and Entenza 2001).

CONCLUSIONS

The data generated in this study indicate that *sed* expression under NaCl stress is controlled by a complex intertwined network of regulatory elements. Therefore, deletion of a single regulatory element does not necessarily alter *sed* expression. While NaCl stress generally leads to decreased *sed* expression in *S. aureus*, our results indicate that it may induce *sed* expression in some strains. As our findings are based on experiments in culture media, additional experiments investigating *sed* expression in the food matrix are needed to determine the effect of stressors encountered during food production and preservation on *sed* expression.

SUPPLEMENTARY DATA

Supplementary data is available at FEMSLE online.

ACKNOWLEDGEMENTS

We express our gratitude to Brigitte Berger-Bächi for providing strains.

FUNDING

This work was supported by grants from the Swiss National Research Programme 69 (40690_145211/1) and a Forschungskredit of the University of Zurich (grant no. FK-13-059).

Conflict of interest statement. None declared.

REFERENCES

Baranyi J, Roberts TA. A dynamic approach to predicting bacterial growth in food. *Int J Food Microbiol* 1994;**23**:277–94.

Bayles KW, Iandolo JJ. Genetic and molecular analyses of the gene encoding staphylococcal enterotoxin D. *J Bacteriol* 1989;**171**:4799–806.

Bennett RW. Staphylococcal enterotoxin and its rapid identification in foods by enzyme-linked immunosorbent assay-based methodology. *J Food Protect* 2005;**68**:1264–70.

Bertani G. Studies on lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. *J Bacteriol* 1959;**62**:293–300.

Betley M J, Borst DW, Regassa LB. Staphylococcal enterotoxins, toxic shock syndrome toxin and streptococcal pyrogenic exotoxins: a comparative study of their molecular biology. *Chem Immunol* 1992;**55**:1–35.

Bischoff M, Entenza JM. Influence of a functional *sigB* operon on the global regulators *sar* and *agr* in *Staphylococcus aureus*. *J Bacteriol* 2001;**183**:5171–9.

Blevins JS, Beenken KE, Elasri MO, *et al.* Strain-dependent differences in the regulatory roles of *sarA* and *agr* in *Staphylococcus aureus*. *Infect Immun* 2002;**70**:470–80.

Chan PF, Foster SJ, Ingham E, *et al.* The *Staphylococcus aureus* alternative sigma factor σ^B controls the environmental stress response but not starvation survival or pathogenicity in a mouse abscess model. *J Bacteriol* 1998;**180**:6082–9.

Chapman GH. The significance of sodium chloride in studies of staphylococci. *J Bacteriol* 1945;**50**:201–3.

Charpentier E, Anton AI, Barry P, *et al.* Novel cassette-based shuttle vector system for gram-positive bacteria. *Appl Environ Microb* 2004;**70**:6076–85.

Derzelle S, Dilasser F, Duquenne M, *et al.* Differential temporal expression of the staphylococcal enterotoxins genes during cell growth. *Food Microbiol* 2009;**26**:896–904.

Ewald S, Notermans S. Effect of water activity on growth and enterotoxin D production of *Staphylococcus aureus*. *Int J Food Microbiol* 1988;**6**:25–30.

Genigeorgis CA. Present state of knowledge on staphylococcal intoxication. *Int J Food Microbiol* 1989;**9**:327–60.

Genigeorgis C, Foda MS, Mantis A, *et al.* Effect of sodium chloride and pH on enterotoxin C production. *Appl Microbiol* 1971;**21**:862–6.

Genigeorgis C, Sadler WW. Effect of Sodium Chloride and pH on Enterotoxin B Production. *J Bacteriol* 1966;**92**:1383–7.

Gertz S, Engelmann S, Schmid R, *et al.* Regulation of σ^B-dependent transcription of *sigB* and *asp23* in two different *Staphylococcus aureus* strains. *Mol Gen Genet* 1999;**261**:558–66.

Hsieh HY, Tseng CW, Stewart GC. Regulation of Rot expression in *Staphylococcus aureus*. *J Bacteriol* 2008;**190**:546–54.

Jelsbak L, Hemmingsen L, Donat S, *et al.* Growth phase-dependent regulation of the global virulence regulator Rot in clinical isolates of *Staphylococcus aureus*. *Int J Med Microbiol* 2010;**300**:229–36.

Kérouanton A, Hennekinne JA, Letertre C, *et al.* Characterization of *Staphylococcus aureus* strains associated with food poisoning outbreaks in France. *Int J Food Microbiol* 2007;**115**:369–75.

Kreiswirth BN, Löfdahl S, Betley MJ, *et al.* The toxic shock syndrome exotoxin structural gene is not detectably transmitted by a prophage. *Nature* 1983;**305**:709–12.

Kullik I, Giachino P, Fuchs T. Deletion of the alternative sigma factor sigma B in *Staphylococcus aureus* reveals its function as a global regulator of virulence genes. *J Bacteriol* 1998;**180**:4814–20.

Lee Y-D, Moon B-Y, Park J-H, *et al.* Expression of enterotoxin genes in *Staphylococcus aureus* isolates based on mRNA analysis. *J Microbiol Biotechn* 2007;**17**:461–7.

McCallum N, Hinds J, Ender M. Transcriptional profiling of XdrA, a new regulator of *spa* transcription in *Staphylococcus aureus*. *J Bacteriol* 2010;**192**:5151–64.

McLean RA, Lilly HD, Alford JA. Effects of meat-curing salts and temperature on production of staphylococcal enterotoxin B. *J Bacteriol* 1968;**95**:1207–11.

Miyazaki E, Chen JM, Ko C, *et al.* The *Staphylococcus aureus rsbW* (orf159) gene encodes an anti-sigma factor of SigB. *J Bacteriol* 1999;**181**:2846–51.

Monday SR, Bohach GA. Use of multiplex PCR to detect classical and newly described pyrogenic toxin genes in staphylococcal isolates. *J Clin Microbiol* 1999;**37**:3411–4.

Novick RP. Analysis by transduction of mutations affecting penicillinase formation in *Staphylococcus aureus*. *J Gen Microbiol* 1963;**33**:121–36.

Regassa LB, Betley MJ. High sodium chloride concentrations inhibit staphylococcal enterotoxin C gene (*sec*) expression at the level of *sec* mRNA. *Infect Immun* 1993;**61**:1581–5.

Scallan E, Hoekstra RM, Angulo FJ, *et al.* Foodborne illness acquired in the United States - major pathogens. *Emerg Infect Dis* 2011;**17**:7–15.

Schelin J, Wallin-Carlquist N, Cohn MT, *et al.* The formation of *Staphylococcus aureus* enterotoxin in food environments and advances in risk assessment. *Virulence* 2011;**2**:580–92.

Sihto H-M, Tasara T, Stephan R, *et al.* Validation of reference genes for normalization of qPCR mRNA expression levels in *Staphylococcus aureus* exposed to osmotic and lactic acid stress conditions encountered during food production and preservation. *FEMS Microbiol Lett* 2014;**356**:134–40.

Troller JA. Effect of water activity on enterotoxin B production and growth of *Staphylococcus aureus*. *Appl Microbiol* 1971;**21**:435–9.

Troller JA. Water relations of foodborne bacterial pathogens - an updated review. *J Food Protect* 1986;**49**:656–70.

Tseng CW, Zhang S, Stewart GC. Accessory gene regulator control of staphyloccoccal enterotoxin D gene expression. *J Bacteriol* 2004;**186**:1793–801.

Wieneke AA, Roberts D, Gilbert RJ. Staphylococcal food poisoning in the United Kingdom, 1969-90. *Epidemiol Infect* 1993;**110**:519–31.

Yarwood JM, Schlievert PM. Quorum sensing in *Staphylococcus* infections. *J Clin Invest* 2003;**112**:1620–5.