Journal of Food Protection 86 (2023) 100127

Contents lists available at ScienceDirect

Journal of Food Protection

journal homepage: www.elsevier.com/locate/jfp

Research Paper

Glucose stress lowers staphylococcal enterotoxin C production independently of Agr, SarA, and SigB

Danai Etter [1,2], Céline Jenni [2], Veronica Edwards [2], Mariella Greutmann [2], Tabea Waltenspül [2], Taurai Tasara [1], Sophia Johler [1,*]

[1] Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 272, 8057 Zurich, Switzerland
[2] Institute for Food, Nutrition and Health, ETH Zurich, Schmelzbergstrasse 9, 8092 Zurich, Switzerland

ARTICLE INFO

Keywords:
Foodborne intoxication
sec variants
Staphylococcal food poisoning
Stress response
Superantigen
Virulence gene regulation

ABSTRACT

Staphylococcal enterotoxin C (SEC) can cause staphylococcal food poisoning, one of the most prevalent foodborne intoxications. It is produced by *Staphylococcus aureus* during growth in the food matrix. While the surrounding bacteria in food matrices usually repress the growth of *S. aureus*, the organism possesses a remarkable growth advantage under stressful conditions encountered in many foods. Examples for such food matrices are pastry and bakery products with their high sugar content that lowers water availability. While *S. aureus* can still grow in these challenging environments, it remains unclear how these conditions affect SEC expression. Here, the influence of 30% glucose on *sec* mRNA in a qPCR assay and SEC protein expression was investigated for the first time in an ELISA. In addition, regulatory knockout mutants *Δagr*, *ΔsarA*, and *ΔsigB* were generated to investigate regulatory gene elements in glucose stress. In five out of seven strains, glucose stress led to a pronounced decrease in *sec* mRNA transcription and SEC protein levels were substantially lower under glucose stress. It could be shown that key regulatory elements *Δagr*, *ΔsarA*, and *ΔsigB* in strain SAI48 did not contribute to the pronounced downregulation under glucose stress. Based on these findings, glucose effectively lowers SEC synthesis in the food matrix. However, the mechanism by which it acts on toxin expression and regulatory elements in *S. aureus* remains unclear. Future studies on other regulatory elements and transcriptomics may shed light on the mechanisms.

Staphylococcus aureus is one of the most common causative agents of foodborne intoxications. The EU reported 74 staphylococcal food poisoning (SFP) outbreaks resulting in 1,400 cases and 141 hospitalizations in 2019 (EFSA, 2021). SFP is caused by staphylococcal enterotoxins (SEs) that are preformed in the food matrix during growth of *S. aureus* (Fisher et al., 2018). Typical symptoms include the onset of violent vomiting shortly after ingestion of contaminated foodstuff, diarrhea, nausea, abdominal pain, fever, and fatigue (Argudín et al., 2010). Since most symptoms fade after 12–24 h, cases are likely underreported (Hennekinne et al., 2012). Foods commonly associated with SFP are diverse such as meat and dairy products, convenience, bakery and pastry foods, seafood, fish, and vegetables (EFSA, 2018). While the surrounding bacteria in food matrices usually repress growth of *S. aureus*, the organism possesses a remarkable growth advantage under stressful conditions encountered in many foods such as low a_w values (Fetsch & Johler, 2018; Oberhofer & Frazier, 1961). Only limited data are available on how these stress conditions influence SE expression. Reduced *seb* promoter activity and slightly reduced *sed* mRNA transcription were previously described under food-related low a_w conditions achieved by addition of 30% glucose (Sihto et al., 2017, 2016). However, results from other SEs cannot be extrapolated.

Currently, 25 SEs including the classical SEA-SEE and the newly described SEG-SE*l*Z have been characterized (Etter et al., 2020; Fetsch & Johler, 2018). SEC is of particular interest because this toxin is produced in up to 10 times higher amounts than other SEs (Spaulding et al., 2013). In addition, SEC exists in four human (SEC_{1-4}) and two animal variants (SEC_{ovine}, SEC_{bovine}), further complicating the characterization of SEC expression (Etter et al., 2020). The expression of SEC is regulated by multiple and often overlapping pathways. *S. aureus* uses a combination of quorum-sensing and other two-component systems as well as trans-acting regulatory proteins to respond to alterations in the environment. SEC is regulated by the quorum-sensing system of the accessory gene regulator (Agr) that uses autoinducing peptides (AIPs). It acts on toxin transcription indirectly

* Corresponding author.
E-mail address: sophia.johler@uzh.ch (S. Johler).

https://doi.org/10.1016/j.jfp.2023.100127
Received 16 April 2023; Accepted 1 July 2023
Available online 4 July 2023

via RNAIII that represses the repressor of toxins (Rot) (Bronesky et al., 2016; Hsieh et al., 2008; Novick et al., 1995; Regassa & Betley, 1993; Regassa et al., 1991). Additionally, SarA, SigB, and SaeRS play a role in the regulation of SEB and SEC when environmental factors change (Fisher et al., 2018; Liu et al., 2016; Regassa & Betley, 1993).

In this study, the SEC expression of seven strains from different origins and with different SEC variants and *sec* gene promoters (promoter variants v1–v4, Table 1) at mRNA and protein level under 30% glucose stress as encountered in foods high in sugar such as pastry or bakery products was investigated. The objectives were to 1) quantify SEC expression under glucose stress using qPCR and an ELISA assay; 2) determine the influence of three regulatory elements Agr, SarA, and SigB on *sec* expression under glucose stress in strain SAI48.

Materials and Methods

Bacterial strains, growth conditions, and sample collection for *sec* mRNA and SEC protein quantification. All *S. aureus* strains and their respective SEC variants in this study are listed in Table 1. The strains were grown in LB medium (BD) (nonstress control conditions) and in LB supplemented with 30% glucose (Sigma-Aldrich). All media were sterile filtered and stored at 4°C. Strains BW10, NB6, SAI3, SAI48, SAR1, SAR38, and OV20 were grown and sampled according to procedures previously described (Etter et al., 2021). All experiments were performed in biological triplicates and technical duplicates.

RNA extraction. RNA extraction was performed with the RNeasy mini Kit Plus (Qiagen) as previously described (Sihto et al., 2014) and quantified with Quantifluor (Promega). Quality control was performed by the Agilent 2100 Bioanalyzer (Agilent Technologies). Samples were included in the study if they met the inclusion criteria of RNA integrity numbers >6.0. RNA integrity numbers ranged from 6.1 to 9.1.

Reverse transcription and quantitative real-time PCR. All RNA samples were subjected to reverse transcription and qRT-PCR as previously described (Etter et al., 2021). Relative expression of the target gene *sec* was normalized using the housekeeping genes *rho* and *rplD* (Sihto et al., 2014). Ct values were determined using the Lightcycler®Software version 1.1.0.1320 (Roche). Data were expressed as Δct values (target-reference).

Protein quantification. An Enzyme-linked Immunosorbent Assay (ELISA) was performed as previously described (Etter et al., 2021). The protocol was based on Poli et al. (2002) with some modifications according to Wallin-Carlquist et al., 2010. Antibodies and reference toxins were obtained from Toxin Technology Inc.

Generation of knockout mutants. Knockout mutants were generated using 80α bacteriophages. RN4220 deletion mutants served as donor strains for *Δagr::tet*, *ΔsarA::tet,* and *ΔsigB::erm*. RN4220 mutants were obtained from previous study (Sihto et al., 2015). Transduction protocols (Krausz & Bose, 2016; Olson, 2016) were adapted as follows for phage transduction to transfer knockout elements from donor strain RN4220 to SAI48.

Overnight RN4220 strains were resuspended in 3 mL TSB (Sigma-Aldrich) with 5 mM $CaCl_2$ (Sigma-Aldrich). Titer determination and phage propagation were performed on the RN4220 strain using the double-layer agar technique. Phages were harvested and soft agar and cell residues were removed by centrifugation and filtration through 0.2 μm filters (Huberlab). Phage infection was performed on a liquid culture of the donor strain with 1 mL $CaCl_2$ and 10 μL (10^{10}) 80α phages and incubated for 4–5 h at 30°C while shaking and then left overnight at room temperature. The suspension was then filtered through 0.2 μm filters. Liquid overnight cultures of recipient strain SAI48 were centrifuged and resuspended in TSB, 10 mg/mL $CaCl_2$ and 500 μL of phage solution. The mix was incubated at room temperature for 10 min, then at 30°C for 35 min without shaking. One mL Na-citrate (0.02 M) was added to the culture before centrifugation (4,500 × *g*, 10 min) and washing two times in 5 mL TSB. The bacteria were subsequently incubated for 1.5 h at 37°C at 125 rpm. After centrifugation (4,500 × *g*, 10 min) and the addition of 0.5 mL TSB, 0.1 mL of bacterial phage suspension was plated on TSA plates containing 10 μg/μL erythromycin (Sigma-Aldrich Chemie GmbH) and grown at 37°C for 48 h.

Replication and statistical analysis. RNA and protein analysis experiments were always carried out in biological triplicates and technical duplicates. mRNA and protein data were log transformed and analyzed via two-way ANOVA and posthoc Tukey's multiple comparisons (RStudio 1.3.1093 and GraphPad Prism 9.2.0). Results were regarded as significant if $p < 0.05$.

Results and Discussion

Glucose stress lowers *sec* mRNA transcription. At exponential (4 h), early stationary (10 h), and late stationary phase (24 h), *sec* mRNA levels were measured across seven *S. aureus* strains (Fig. 1) and expressed normalized to growth of the respective strain (Supplementary Fig. 1). SFP and human isolates BW10, NB6, SAI48 (all SEC_2, v1), and SAI3 (SEC_1, v3) had significantly decreased *sec* mRNA transcription at all time points (Fig. 1). Ovine strain OV20 (SEC_{ovine}, v4) only exhibited decreased *sec* mRNA levels at 10 h. Transcription of *sec* mRNA was not affected in both bovine isolates SAR1 and SAR38 (both SEC_{bovine}, v2) by glucose stress. Interestingly, the reduction of *sec* mRNA transcription was most pronounced in the two high-level SEC producers BW10 and SAI48 (see control conditions, Table 2).

In the early 1990s, first experiments were conducted to measure *sec* expression in the presence of glucose (Regassa et al., 1991). However, very low sugar concentrations of between 14 and 20 mg/mL (1.4–2%) were used that did not lead to osmotic stress. In addition, *sec* expression was measured in northern blots and the SEC variants were not specified. The study revealed reduced *sec* expression and *agr* activity when low amounts of glucose were present in the medium at pH 5.5 (Regassa et al., 1991). When 30% glucose stress was previously applied to two reference strains, *seb* promoter activity was significantly reduced (Sihto et al., 2017). Another study showed decreased *sed* expression in late stationary phase for one strain (RKI2) under 30%

Table 1
Overview of *S. aureus* strains used in this study including their SEC variants, origin, and assignment to clonal complexes

Strain	Protein variant	Sec promoter variant	Origin	Clonal complex	Reference
BW10	SEC_2	sec_p v1	SFP	CC45	(Johler et al., 2011)[1]
NB6	SEC_2	sec_p v1	SFP	CC45	(Johler et al., 2011)[2]
SAI3	SEC_1	sec_p v3 (H-EMRSA-15)	Human infection	CC8	(Wattinger et al., 2012)
SAI48	SEC_2	sec_p v1 (79_S10)	Human infection	CC5	(Wattinger et al., 2012)
SAR1	SEC_{bovine}	sec_p v2	bovine mastitis milk	CC151	(Johler et al., 2011)
SAR38	SEC_{bovine}	sec_p v2	bovine mastitis milk	CC151	(Johler et al., 2011)
OV20	SEC_{ovine}	sec_p v4	ovine	CC133	(Guinane et al., 2010)

[1] BW10 = SFP18.
[2] NB6 = SFP12.

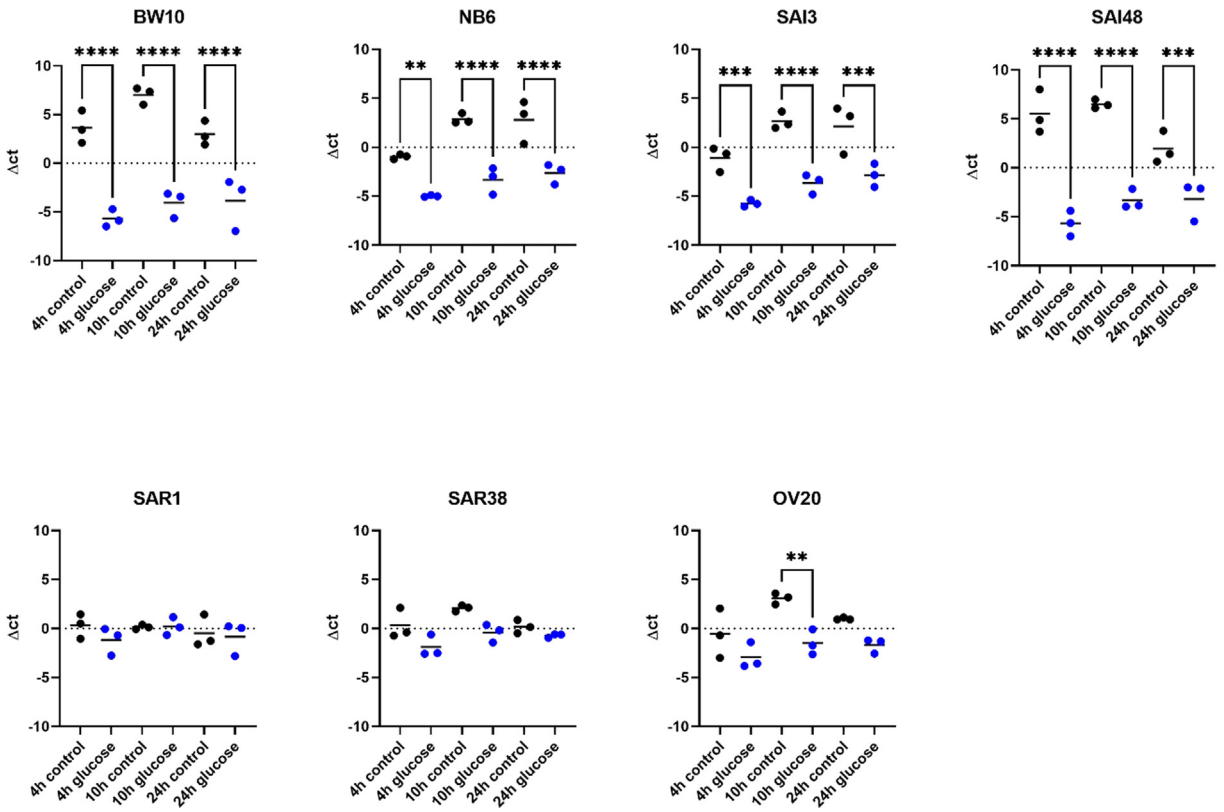

Figure 1. Effect of glucose stress on sec mRNA levels in seven S. aureus strains (BW10, NB6, SAI3, SAI48, SAR1, SAR38, and OV20) measured by qRT-PCR. qPCR Δct values in exponential (4 h), early stationary (10 h), and late stationary phase (24 h) in LB and LB + 30% glucose for each time point and strain ($n = 3$). Control conditions in black, glucose stress conditions in blue. Target mRNA (sec) was normalized to two reference genes rho and rplD. Statistically significant changes identified by ANOVA with Tukey's multiple comparisons in sec mRNA levels in LB + 30% glucose compared to LB ($p < 0.05$) are marked by brackets and asterisks ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$). Δct = 0 is indicated by a dotted line.

Table 2
Effect of glucose on SEC protein expression as measured by an ELISA assay. Values are given in ng/mL and the effect of glucose is calculated in % relative to the control condition ($n = 3$)

Strains		BW10	NB6	SAI3	SAI48	SAR1	SAR38	OV20
SEC produced under glucose stress (ng/mL)	4 h control	461.8 ± 307.3	6.9 ± 2.9	5.5 ± 1.6	379.9 ± 220.4	16.3 ± 1.8	15.3 ± 9.9	6.9 ± 2.6
	10 h Control	3324.8 ± 360.1	57.6 ± 29.3	39.9 ± 9.9	5987.5 ± 1317.4	212.7 ± 86.7	227.6 ± 27.3	328.3 ± 87.5
	24 h Control	3410.3 ± 561.9	54.5 ± 12.3	114.5 ± 59.5	9867.7 ± 4687.8	189.1 ± 64.7	253.6 ± 7.4	344.9 ± 33.5
	4 h glucose	2.1 ± 0.1	0.5 ± 0.1	0.2 ± 0	1.7 ± 0.3	51.4 ± 6.2	84.1 ± 14.8	0.7 ± 0.4
	10 h glucose	9.7 ± 3.4	3.3 ± 2.6	0.1 ± 0.1	7.4 ± 2.1	12.7 ± 2.1	18.1 ± 1.2	1.5 ± 0.9
	24 h glucose	6.3 ± 0.8	14.4 ± 9.1	1.4 ± 0.6	16 ± 2	23.4 ± 10.9	18.5 ± 4.1	12.1 ± 6.5
Effect of glucose [%]	4 h	−99.54	−93.36	−95.6	−99.54	216.24	448.8	−89.27
	10 h	−99.71	−94.19	−99.73	−99.88	−94.04	-92.04	−99.54
	24 h	−99.82	−73.53	−98.76	−99.84	−87.61	−92.72	−96.5
	Sum	−299.07	−261.08	−294.09	−299.25	34.59	264.03	−285.3

glucose stress (Sihto et al., 2016). One of three strains included by Sihto and coworkers, SAI48 that harbors *sec* and *sed*, was also used in the experiments presented in this study. For this strain, the reduction of *sec* under glucose stress was much more pronounced, while *sed* was not significantly affected by this stress condition. Consequently, it was shown for the first time that each SE can respond differently to an applied stressor and strains expressing multiple SEs may up- and downregulate them at the same time. This suggests the involvement of multiple regulatory mechanisms (Zeaki et al., 2019). Comparison of different SEs might enhance our understanding of the complex and intertwined network of regulators involved in their expression.

Glucose stress effectively reduces SEC protein concentration. In addition to *sec* mRNA transcript levels, SEC protein concentrations were measured by an in-house ELISA in exponential (4 h), early stationary (10 h), and late stationary phase (24 h). Consistent with the transcriptional reduction, glucose stress led to an overall decrease in SEC concentration for most strains (Fig. 2, Table 2). SFP strains BW10, NB6 (both SEC2, v1), human infection isolates SAI3 (SEC_1, v2), SAI48 (SEC_2, v1), and ovine mastitis strain OV20 (SEC_{ovine}, v4) had significantly lower SEC concentrations under glucose stress in all growth phases. Only bovine strains SAR1 and SAR38 (both SEC_{bovine}, v3) showed an increase in SEC protein expression at 4 h before SEC levels were significantly reduced, as well. There was no

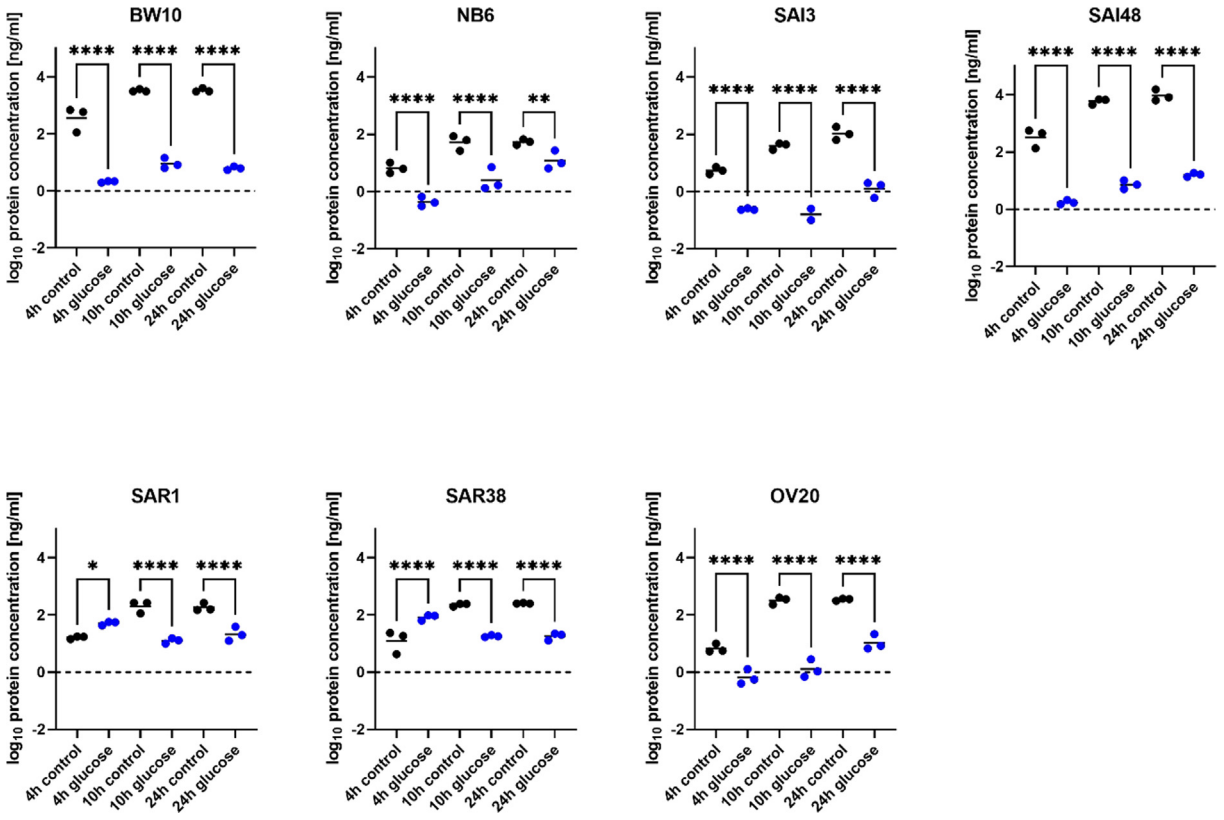

Figure 2. Effect of glucose stress on SEC protein levels in seven S. aureus strains (BW10, NB6, SAI3, SAI48, SAR1, SAR38, and OV20) measured by ELISA. log10 values of protein concentration in ng/mL in exponential (4 h), early stationary (10 h), and late stationary phase (24 h) in LB and LB + 30% glucose for each time point and strain ($n = 3$). Control conditions in black, glucose stress conditions in blue. Statistically significant changes identified by ANOVA with Tukey's multiple comparisons in SEC protein levels in LB + 30% glucose compared to LB ($p < 0.05$) are marked by brackets and asterisks ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$). Log(1) is indicated by a dotted line to signify when protein concentrations fell below 1 mg/mL.

relation between the higher SEC concentration in SAR1 and SAR38 at 4 h and their *sec* mRNA transcription patterns. Except for these two isolates, protein expression data reflected the downregulation in *sec* mRNA.

Older studies had demonstrated a decrease in SEB and SEC via western blot in two strains when glucose (approx. 1.8%) was present in the growth medium, while SEA production was not affected by glucose (Jarvis et al., 1975). As stated in the previous paragraph, this could indicate that each SE reacts differently on protein level, as well. Another study found that cultures containing amounts of 1.4–2% glucose exhibited less extracellular SEC compared with control cultures (Regassa et al., 1991). However, the glucose concentrations used were significantly lower than 30% and absolute quantification was lacking. Therefore, direct comparison of the data is challenging. SEC expression under 30% glucose stress conditions has not been assessed before.

Overall, the addition of glucose seems to provide a suitable strategy to reduce SEC concentration in foods as it showed a pronounced effect on all the investigated strains.

Agr, SarA, and SigB are not responsible for the lower *sec* expression under glucose stress. To determine the effect of the three regulatory genes (*agr*, *sarA,* and *sigB*), *sec* expression of SEC-overproducer wild type strain SAI48 was compared to *sec* expression of the three knockout mutants (Fig. 3). While the effect of *agr* had already been investigated by Regassa et al., (1991) in a limited number of strains with no information on the respective SEC variants, no other regulators have ever been targeted in regulatory experiments regarding enterotoxin expression under glucose stress. In all experiments, expression patterns of knockouts deviated from the wild type, but to different extents. For Δ*agr* and Δ*sarA*, significant differences to the wild type were only seen under control conditions. Compared to the wild type, Δ*agr* showed significantly reduced *sec* expression during early stationary growth, while Δ*sarA* exhibited significantly higher *sec* mRNA levels in late stationary phase. The late exponential decrease in *sec* expression in the Δ*agr* mutant is consistent with the conclusion of Regassa et al., (1991) that maximal *sec* expression in *S. aureus* requires an intact *agr* gene. In contrast, under glucose stress, no significant differences were found for Δ*agr* and Δ*sarA* mutants compared to the SAI48 wild type. Contrary to control conditions, these regulatory elements lose importance under glucose stress, highlighting that other regulatory processes might bypass them. An intact *agr* was also not required for the glucose-related effect seen by Regassa et al., 1991. An Agr-strain produced more SEC and had more steady-state *sec*+ mRNA when grown in medium that lacked glucose compared with medium that contained glucose (Regassa et al., 1991).

For the Δ*sigB* mutant, on the other hand, significant changes were only observed under glucose stress, with *sec* expression being significantly higher during early exponential and late stationary phases. This finding underscores the important role of SigB during stress response (Jenul & Horswill, 2019). The significantly increased *sec* levels in the late exponential and late stationary phases indicate a downregulating effect, which may arise partly from its antagonistic action to Agr (Bischoff et al., 2001).

In conclusion, each of the three regulators studied has a significant influence on *sec* mRNA expression in SAI48, but none is solely responsible for the strong reduction in expression that was observed under glucose stress. This suggests that either a combination of or other regulatory mechanisms must be at work.

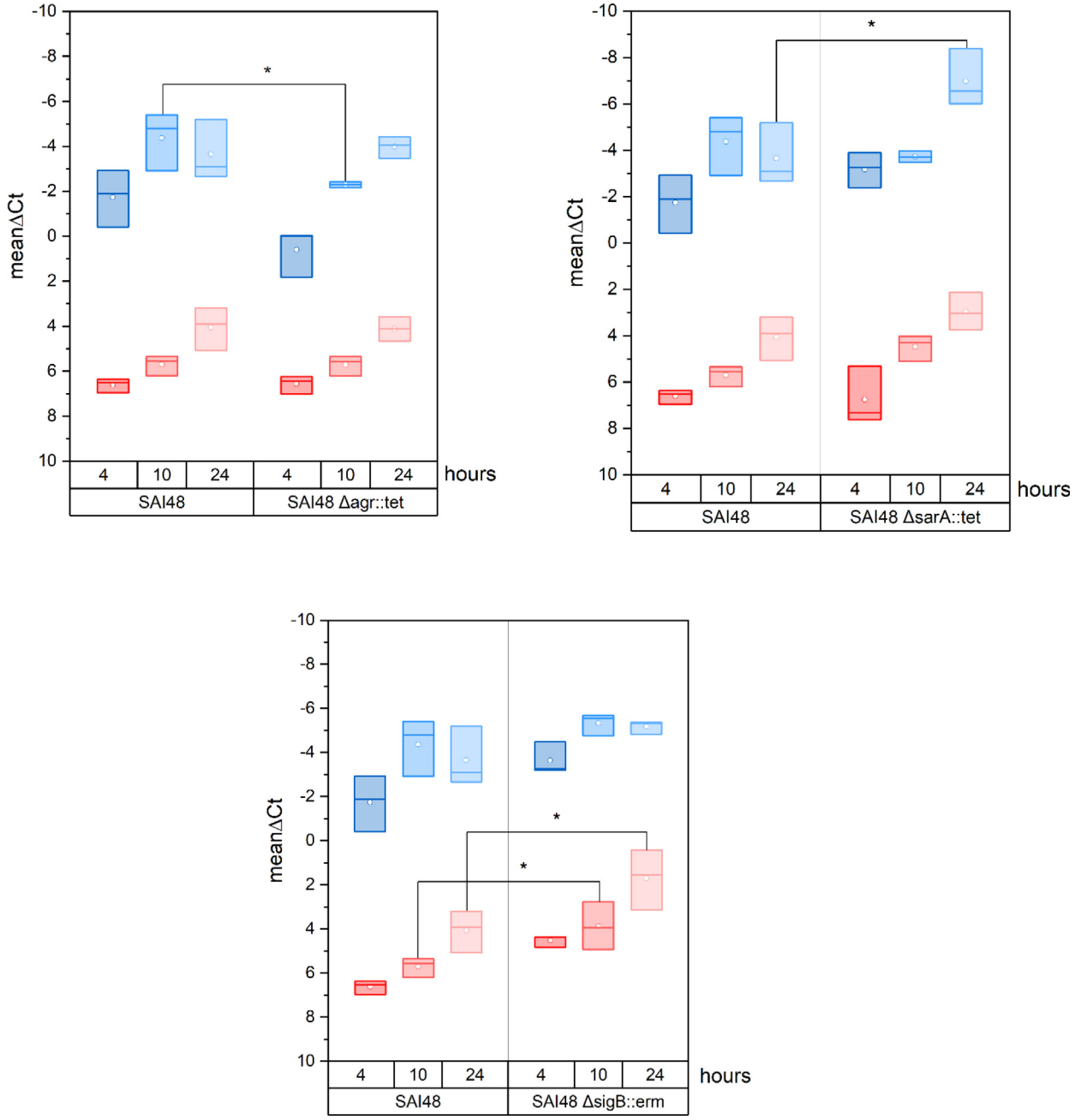

Figure 3. Effect of glucose stress on sec mRNA expression, expressed in terms of ΔCt in SAI48 wild type and Δagr, ΔsarA, and ΔsigB knockout mutants in exponential (4 h), late exponential/early stationary (10 h), and late stationary phase (24 h). Control conditions in blue, glucose stress conditions in red. mRNA values of sec were normalized to reference genes rho and rplD. Statistically significant differences ($\alpha = 0.05$) between glucose stress and control conditions are indicated with asterisks ($^{**}p < 0.01$).

Conclusions

The fact that bovine isolates SAR1 and SAR38 (both SEC_{bovine}, v2, CC151) were not affected under glucose stress could point to strain-specific differences in SEC regulation. Possibly, the strain background contributes to the resilience of these strains under glucose stress. A larger strain set of diverse origins including more bovine and nonbovine isolates could shed light on the influence of the strain origin of this behavior. Investigation of strain SAI48 expressing multiple toxins revealed differential expression of toxins under the same stress condition. It is likely that an intricate interplay of multiple regulatory mechanisms is controlling SE expression under stress conditions encountered in food. Only SAR1 and SAR38 showed elevated protein levels at 4 h, before SEC levels were decreased in later growth phases. Again, this could point toward a strain-specific resilience toward glucose stress. Considering the pronounced reduction of SEC under glucose stress, this food ingredient seems a suitable compound to reduce SEC in foods. None of the studied regulatory gene elements were able to explain the pronounced downregulation of *sec* expression. Likely, a combination or other regulatory circuits or posttranscriptional modifications are involved in this process. Transcriptomic analyses might uncover

some of these processes in the future. These findings contribute to a better understanding of matrix-pathogen interaction and shed light on regulatory mechanisms involved in enterotoxin regulation under stress conditions.

Funding source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Danai Etter: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft. **Céline Jenni:** Data curation, Formal analysis, Investigation, Validation. **Veronica Edwards:** Data curation, Formal analysis, Investigation, Validation. **Mariella Greutmann:** Data curation, Formal analysis, Investigation, Validation. **Tabea Waltenspül:** Data curation, Formal analysis, Investigation, Validation. **Taurai Tasara:** Methodology, Supervision, Writing – review & editing. **Sophia Johler:** Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors want to express their gratitude toward Prof. Roger Stephan for supporting the project by providing the necessary infrastructure and resources.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfp.2023.100127.

References

Argudín, M. Á., Mendoza, M. C., & Rodicio, M. R. (2010). Food poisoning and *Staphylococcus aureus* enterotoxins. *Toxins, 2*(7), 1751–1773. https://doi.org/10.3390/toxins2071751.

Bischoff, M., Entenza, J. M., & Giachino, P. (2001). Influence of a functional *sigB* operon on the global regulators *sar* and *agr* in *Staphylococcus* aureus. *Journal of Bacteriology, 183*(17), 5171–5179. https://doi.org/10.1128/JB.183.17.5171-5179.2001.

Bronesky, D., Wu, Z., Marzi, S., Walter, P., Geissmann, T., Moreau, K., Vandenesch, F., Caldelari, I., & Romby, P. (2016). *Staphylococcus aureus* RNAIII and its regulon link quorum sensing, stress responses, metabolic adaptation, and regulation of virulence gene expression. *Annual Review of Microbiology, 70*(1), 299–316. https://doi.org/10.1146/annurev-micro-102215-095708.

Efsa (2018). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *EFSA Journal, 16*(12), e05500.

Efsa (2021). The European Union One Health 2019 Zoonoses Report. *EFSA Journal, 19*(2), e06406.

Etter, D., Jenni, C., Tasara, T., & Johler, S. (2021). Mild lactic acid stress causes strain-dependent reduction in SEC protein levels. *Microorganisms, 9*(5), 1–10. https://doi.org/10.3390/microorganisms9051014.

Etter, D., Schelin, J., Schuppler, M., & Johler, S. (2020). Staphylococcal enterotoxin C-An update on SEC variants, their structure and properties, and their role in foodborne intoxications. *Toxins, 12*(9), 584. https://doi.org/10.3390/toxins12090584.

Fetsch, A., & Johler, S. (2018). *Staphylococcus aureus* as a foodborne pathogen. *Current Clinical Microbiology Reports, 5*(2), 88–96. https://doi.org/10.1007/s40588-018-0094-x.

Fisher, E. L., Otto, M., & Cheung, G. Y. C. (2018). Basis of virulence in enterotoxin-mediated staphylococcal food poisoning. *Frontiers in Microbiology, 9*(MAR), 436. https://doi.org/10.3389/fmicb.2018.00436.

Hennekinne, J.-A., de Buyser, M.-L., & Dragacci, S. (2012). *Staphylococcus aureus* and its food poisoning toxins: Characterization and outbreak investigation. *FEMS Microbiology Reviews, 36*(4), 815–836. https://doi.org/10.1111/j.1574-6976.2011.00311.x.

Hsieh, H.-Y., Tseng, C. W., & Stewart, G. C. (2008). Regulation of Rot expression in *Staphylococcus aureus*. *Journal of Bacteriology, 190*(2), 546–554. https://doi.org/10.1128/JB.00536-07.

Jarvis, A. W., Lawrence, R. C., & Pritchard, G. G. (1975). Glucose repression of enterotoxins A, B and C and other extracellular proteins in staphlyococci in batch and continuous culture. *Journal of General Microbiology, 86*(1), 75–87. https://doi.org/10.1099/00221287-86-1-75.

Jenul, C., & Horswill, A. R. (2019). Regulation of *Staphylococcus aureus* virulence. *Microbiology Spectrum, 7*(2), 1–21. https://doi.org/10.1128/microbiolspec.GPP3-0031-2018.

Krausz, K. L., & Bose, J. L. (2016). Bacteriophage transduction in Staphylococcus aureus: Broth-based method. *Methods in Molecular Biology (Clifton, N.J.), 1373*, 63–68. https://doi.org/10.1007/7651_2014_185.

Liu, Q., Yeo, W.-S., & Bae, T. (2016). The SaeRS two-component system of *Staphylococcus aureus*. *Genes, 7*(10). https://doi.org/10.3390/genes7100081.

Novick, R. P., Projan, S. J., Kornblum, J., Ross, H. F., Ji, G., Kreiswirth, B., Vandenesch, F., & Moghazeh, S. (1995). The *agr* P2 operon: An autocatalytic sensory transduction system in *Staphylococcus aureus*. *MGG Molecular & General Genetics, 248*(4), 446–458. https://doi.org/10.1007/BF02191645.

Oberhofer, T. R., & Frazier, W. C. (1961). Competition of *Staphylococcus aureus* with other organisms. *Journal of Milk and Food Technology, 24*(6), 172–175. https://doi.org/10.4315/0022-2747-24.6.172.

Olson, M. E. (2016). Bacteriophage transduction in *Staphylococcus aureus*. *Methods in Molecular Biology (Clifton, N.J.), 1373*, 69–74. https://doi.org/10.1007/7651_2014_186.

Regassa, L. B., & Betley, M. J. (1993). High sodium chloride concentrations inhibit staphylococcal enterotoxin C gene (*sec*) expression at the level of *sec* mRNA. *Infection and Immunity, 61*(4), 1581–1585. https://doi.org/10.1128/iai.61.4.1581-1585.1993.

Regassa, L. B., Couch, J. L., & Betley, M. J. (1991). Steady-state staphylococcal enterotoxin type C mRNA is affected by a product of the accessory gene regulator (*agr*) and by glucose. *Infection and Immunity, 59*(3), 955–962. https://doi.org/10.1128/iai.59.3.955-962.1991.

Sihto, H.-M., Stephan, R., Engl, C., Chen, J., & Johler, S. (2017). Effect of food-related stress conditions and loss of *agr* and *sigB* on *seb* promoter activity in *S. Aureus*. *Food Microbiology, 65*(March), 205–212. https://doi.org/10.1016/j.fm.2017.03.006.

Sihto, H.-M., Tasara, T., Stephan, R., & Johler, S. (2014). 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 Microbiology Letters, 356*(1), 134–140. https://doi.org/10.1111/1574-6968.12491.

Sihto, H.-M., Tasara, T., Stephan, R., & Johler, S. (2015). Temporal expression of the staphylococcal enterotoxin D gene under NaCl stress conditions. *FEMS Microbiology Letters, 362*(6), 24. https://doi.org/10.1093/femsle/fnv024.

Sihto, H.-M., Tasara, T., Stephan, R., & Johler, S. (2016). Growth behavior and temporal enterotoxin D expression of *Staphylococcus aureus* strains under glucose and lactic acid stress. *Food Control, 62*, 69–73. https://doi.org/10.1016/j.foodcont.2015.10.008.

Spaulding, A. R., Salgado-Pabón, W., Kohler, P. L., Horswill, A. R., Leung, D. Y. M., & Schlievert, P. M. (2013). Staphylococcal and streptococcal superantigen exotoxins. *Clinical Microbiology Reviews, 26*(3), 422–447. https://doi.org/10.1128/CMR.00104-12.

Wallin-Carlquist, N., Cao, R., Márta, D., Da Silva, A. S., Schelin, J., & Rådström, P. (2010). Acetic acid increases the phage-encoded enterotoxin A expression in *Staphylococcus aureus*. *BMC Microbiology, 10*, 147. https://doi.org/10.1186/1471-2180-10-147.

Zeaki, N., Johler, S., Skandamis, P. N., & Schelin, J. (2019). The role of regulatory mechanisms and environmental parameters in staphylococcal food poisoning and resulting challenges to risk assessment. *Frontiers in Microbiology, 10*(June), 1307. https://doi.org/10.3389/fmicb.2019.01307.