Deciphering staphylococcal enterotoxin expression and its regulatory control under stress encountered during food production and preservation

Background:
Ingestion of staphylococcal enterotoxins (SEs) such as SEB, SEC, and SED leads to staphylococcal food poisoning, the most prevalent food-borne intoxication worldwide. Patients suffer from acute signs of gastroenteritis including violent vomiting, diarrhea, cramps, and fever. As the symptoms result in pronounced electrolyte imbalances and dehydration, the intoxication is particularly dangerous to children and the elderly, with a fatality rate of 4.4%. SEs are formed during growth of *S. aureus* in food. While growth of the organism is repressed by competing bacteria in most food matrices, *S. aureus* exhibits a crucial competitive growth advantage in foods with high salt or sugar concentrations or low pH. To date, both the influence of stress conditions on enterotoxin production, as well as the regulation of enterotoxin production under stress are poorly understood. Control strategies are focused on hygiene measures to avoid food contamination and limit *S. aureus* growth. To effectively minimize SE formation during food production and preservation, additional data on enterotoxin expression and its regulatory control under stress conditions is needed.

Project 1: Expression of *sec* under stress conditions

Hypothesis: Stress conditions encountered during food production and preservation such as lactic acid, glucose, NaCl, and nitrite stress influence SEC expression.

A total of seven *S. aureus* strains from different sources and representing different SEC variants were grown under conditions simulating stress during food production and preservation. The temporal expression of *sec* under stressed and non-stressed growth conditions was compared by quantifying *sec* mRNA levels by RT-qPCR and SEC protein levels by ELISA. Growth media mimicked food-related stress conditions and exhibited 150 mg/L nitrite, 30% glucose, lactic acid (pH 6.0), and 4.5% NaCl, respectively. The effect of the different stressors on *sec* transcription and translation was time and strain dependent. While overall, a trend towards decreased *sec* expression under stress was observed on transcriptional and translational level, this was not always the case and nitrite stress increased SEC protein levels in some strains. Glucose had a higher inhibitory effect on toxin production than lactic acid and NaCl stress.
Conclusion: Our results suggest that glucose, lactic acid and NaCl can be used to control SEC formation in foods. These findings indicate that stressors encountered in food production and preservation influence *sec* expression in a way that cannot be predicted based exclusively on viable cell counts.

- Etter et al. (2021): Mild lactic acid stress causes strain-dependent reduction in SEC protein levels. Microorganisms, 9, 1014. https://doi.org/ 10.3390/microorganisms9051014

Project 2: Regulation of *sec* expression

Hypothesis: Regulatory elements other than the global virulence regulator agr play a key role in sec expression under stress conditions.

Regulatory knockout mutants (*Δagr*, *ΔsarA*, *ΔsigB*) were constructed and *sec* expression was investigated at mRNA level under nitrite stress and control conditions. In addition, strains with known *sec* expression patterns under stress (see project 1) were whole genome sequenced using Illumina sequencing. While *Δagr* mutants exhibited lower *sec* mRNA transcription levels than wt strains, this response was not stress specific. The *ΔsigB* mutants displayed the opposite *sec* expression behavior under stress conditions compared to control conditions. WGS analysis revealed a defective *agr* element in one strain that did however not influence *sec* transcription or SEC protein synthesis. Δ*agr* mutants expressed less *sec* than wildtype (wt) strains. Loss of *sarA* did not significantly influence *sec* transcription.

Conclusion: While *σB* plays a crucial role in *sec* expression under nitrite stress conditions, the relevance of Agr may have been overestimated in the past as this regulator could be bypassed in our experiments.

- Paper submitted

Project 3: Expression of *seb* on single cell level

Hypothesis: S. aureus strains exhibit a substantial amount of strain-specific variation in enterotoxin expression. We hypothesize that single cells of S. aureus also express highly different levels of enterotoxins.

project ongoing