Expression properties of the novel *Staphylococcus aureus* extacellular protease Jep under infection-relevant stress conditions

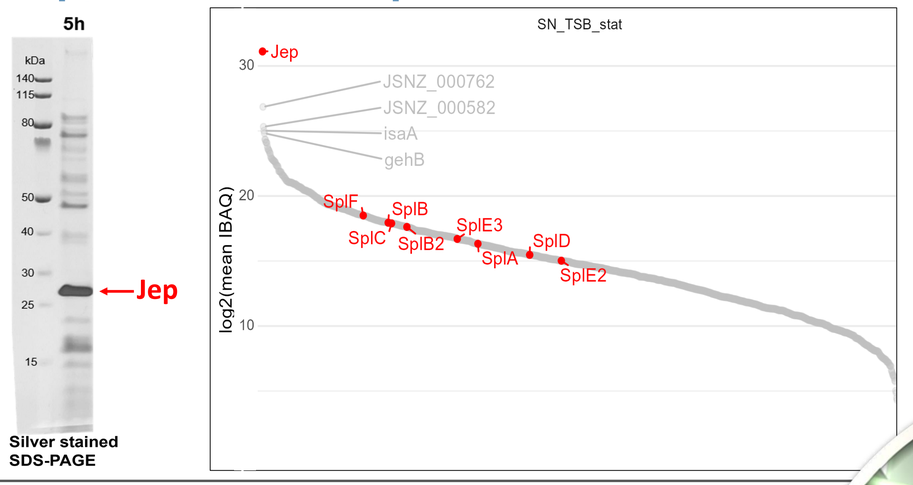
J.Tebben[1], G. Wockenfuß[1], H. Wolfgramm[1], L.M. Busch[1], S.S. Peringathara[2], E.M. Bludau[2]
M. Schaffer[1], A. Reder[1], U. Mäder[1], K. Surmann[1], B.M. Bröker[2], S. Holtfreter[2], U. Völker[1]
[1] Department of Functional Genomics, University Medicine Greifswald [2] Institute of Immunology, University Medicine Greifswald

A family of proteases

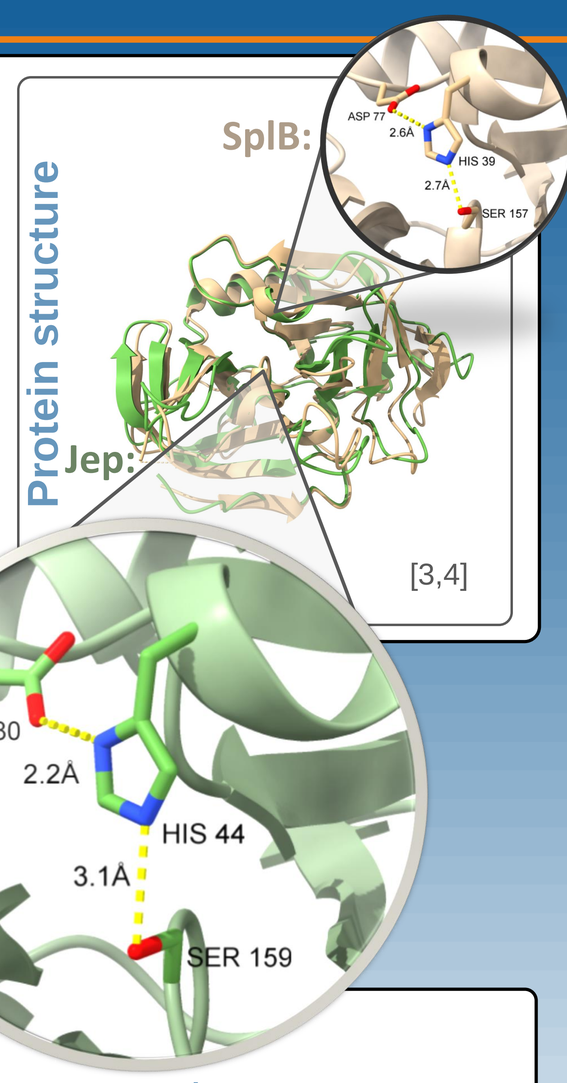
Extracellular proteases are important virulence factors in *Staphylococcus aureus*. This also comprises a set of serine protease-like proteins (Spls) whose role in infection is still poorly understood. In the mouse-adapted *S. aureus* strain JSNZ, we recently identified a closely related protease, JSNZ extracellular protease (Jep) [1,2]. It shares significant sequence homology and a conserved catalytic triad with the Spls, making it an interesting candidate for investigating the role of serine proteases in murine *S. aureus* infection models.

Here, we characterize *jep*-expression in JSNZ under different stress conditions. Furthermore, we optimize a system for inducible *jep*-expression to analyze the effect of the protease in different genetic backgrounds.

Jep, the dominant protein of the secretome



Protein structure



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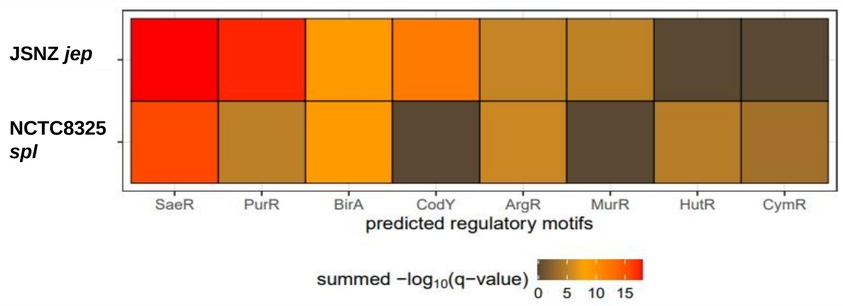
Regulator prediction



- Analysis performed with *in silco* tool “FIMO” [5], regulatory motifs from “RegPrecise” [6]
- 300 bp upstream – 200 bp downstream of *jep* and *spl* start codon

Growth phase and stress-dependent *jep* expression

Infection relevant conditions:

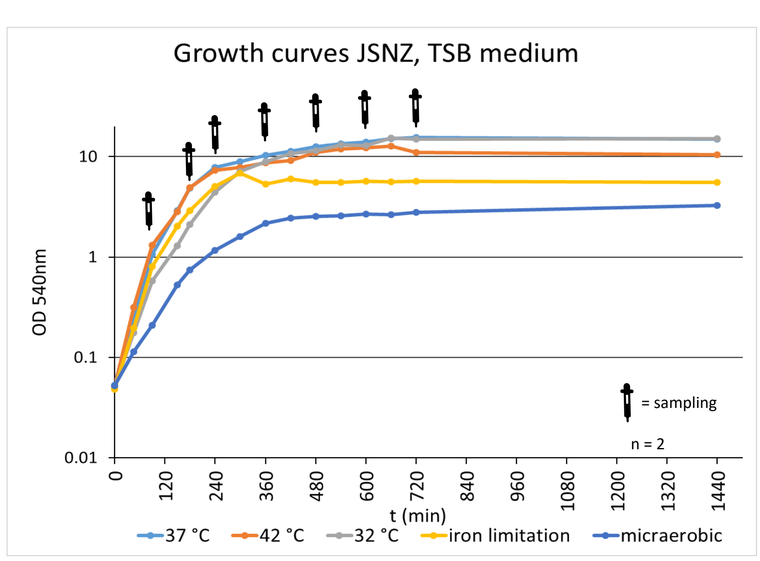
ctrl. 37 °C, body temperature

42 °C: high fever

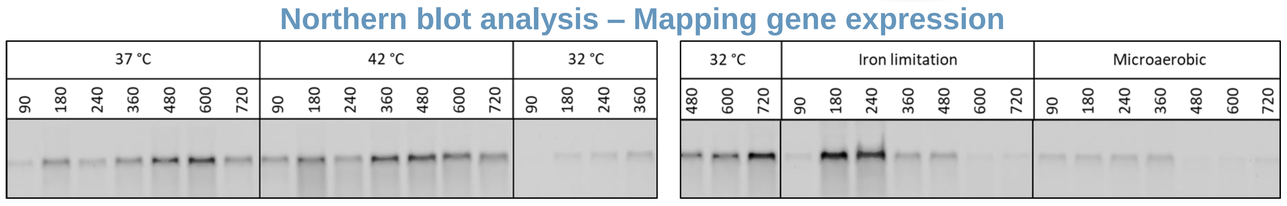
32 °C: nasal colonization, severe sepsis

~~Fe^{2+}~~ Iron limitation: low iron availability in host organism

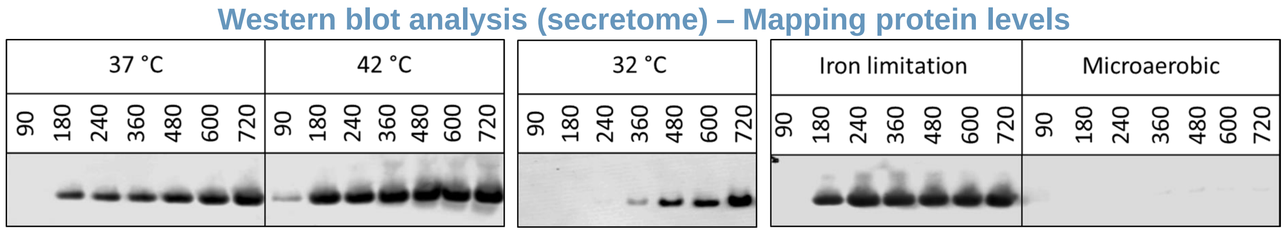
~~O_2~~ Microaerobic: low oxygen availability host organism



Northern blot analysis – Mapping gene expression



Western blot analysis (secretome) – Mapping protein levels

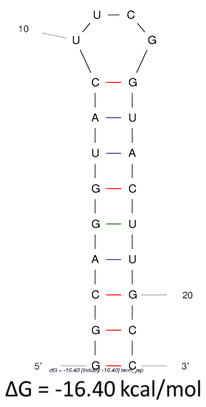


Results

- Expression of *jep* starts in exponential phase and increases until stationary phase
- The protein accumulates in the supernatant
- Iron limitation and heat stress lead to earlier and higher expression
- Oxygen limitation decreases expression drastically

Inducing *jep* expression in a human adapted strain

Identification of potential terminator → Cloning of *jep* sequence with and without terminator → Induced *jep* expression → SDS-PAGE of the supernatant → Northern blot analysis (*jep*)



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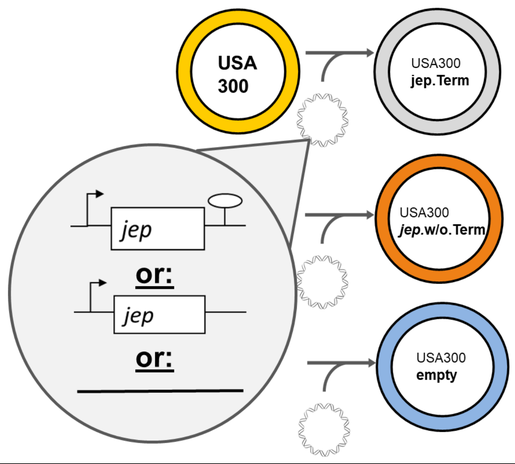

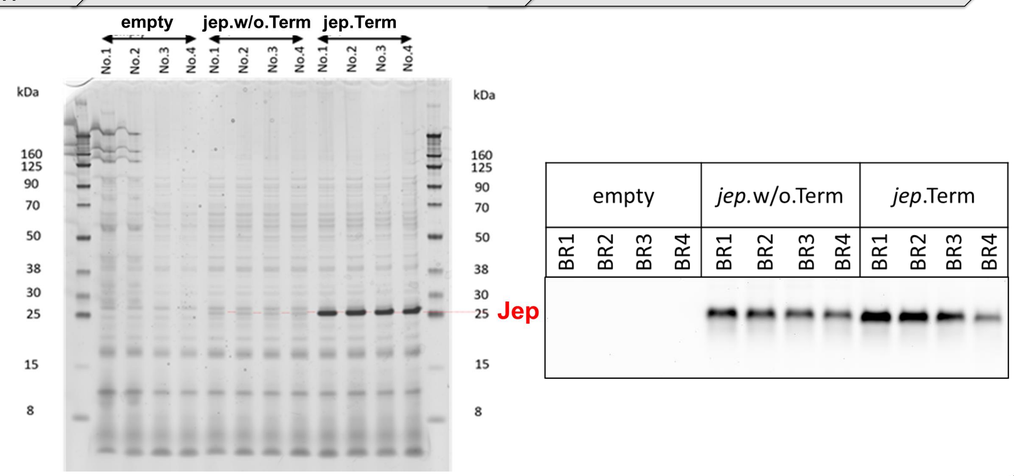

Summary

- Jep is the most abundant protein in JSNZ stationary phase supernatant indicating a central role in JSNZ lifestyle
- Sequence and structure homology of Jep and Spls: Jep could give insights to the role of Spls in human infection
- Potential regulatory motifs connect *jep*-expression to virulence
- Protein accumulates in supernatant: indicates high stability of the protease
- Iron limitation and heat stress lead to earlier *jep*-expression, oxygen limitation decreases expression: potential adaptation to diverse niche environments in the host organism
- System for inducible *jep*-expression optimized

Outlook

- Comparison of JSNZ intra- and extracellular proteome under stress conditions shown above via mass spectrometry
- Characterization of *jep*-expression under oxidative stress

References

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Center for Functional Genomics of Microbes

Corresponding author: joeran.tebben@stud.uni-greifswald.de