



University of Colorado
Anschutz Medical Campus

Effect of *Staphylococcus epidermidis* proteases on *Staphylococcus aureus* biofilms

A. Cao, M. Bhattacharya, PhD, K. Keim, A. Horswill, PhD

Department of Immunology and Microbiology, University of Colorado Anschutz Medical Campus, Aurora, Colorado, 80045, USA

Background

- Staphylococcus epidermidis* (SE) and *Staphylococcus aureus* (SA) are two of the most common bacteria isolated from human skin.¹
- Atopic dermatitis (AD) lesions are often colonized by both SE and SA, and SA biofilms have been found to colonize the eccrine ducts of AD skin. Staphylococcal biofilms can also affect keratinocyte differentiation, apoptosis, or cytokine secretion.²
- Both species can behave as pathogens and form biofilms. SE can assist in SA biofilm formation, but their complex interactions are not fully understood.
- SE accumulation associated protein (Aap) and its ortholog in SA, *S. aureus* surface protein G (SasG), are important for biofilm formation and are surface-associated adhesins that must be proteolytically cleaved to be activated.³
- SA SasG expression is repressed by the global regulator, MgrA, and prior work has shown that SasG is upregulated in a $\Delta mgrA$ mutant.⁴
- SE produces the proteases Esp (serine protease), SepA (metalloprotease), and Ecp (cysteine protease). SepA has been shown to cleave Aap.

Hypothesis

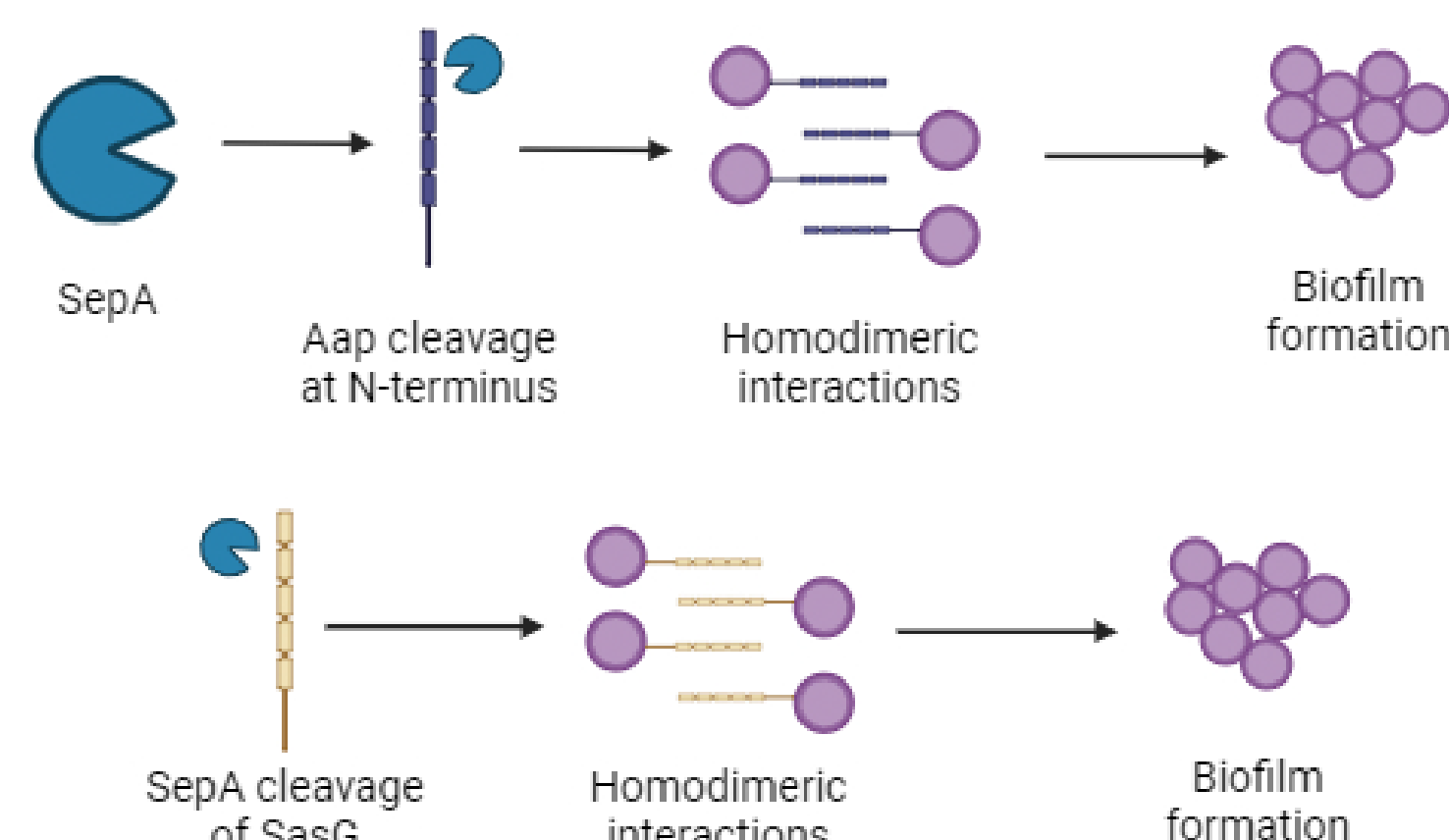


Figure 1. Hypothesized regulation of biofilm formation in SA and SE.

Figure 2. Effect of SE single protease mutants on SA biofilms. SA biofilms increased when incubated with supernatant from SE *sepA* and *ecp* mutants relative to incubation with SE WT supernatant. No significant differences were observed with Δesp supernatant incubation. N=4, *** P <0.001, **** P <0.0001 via ANOVA.

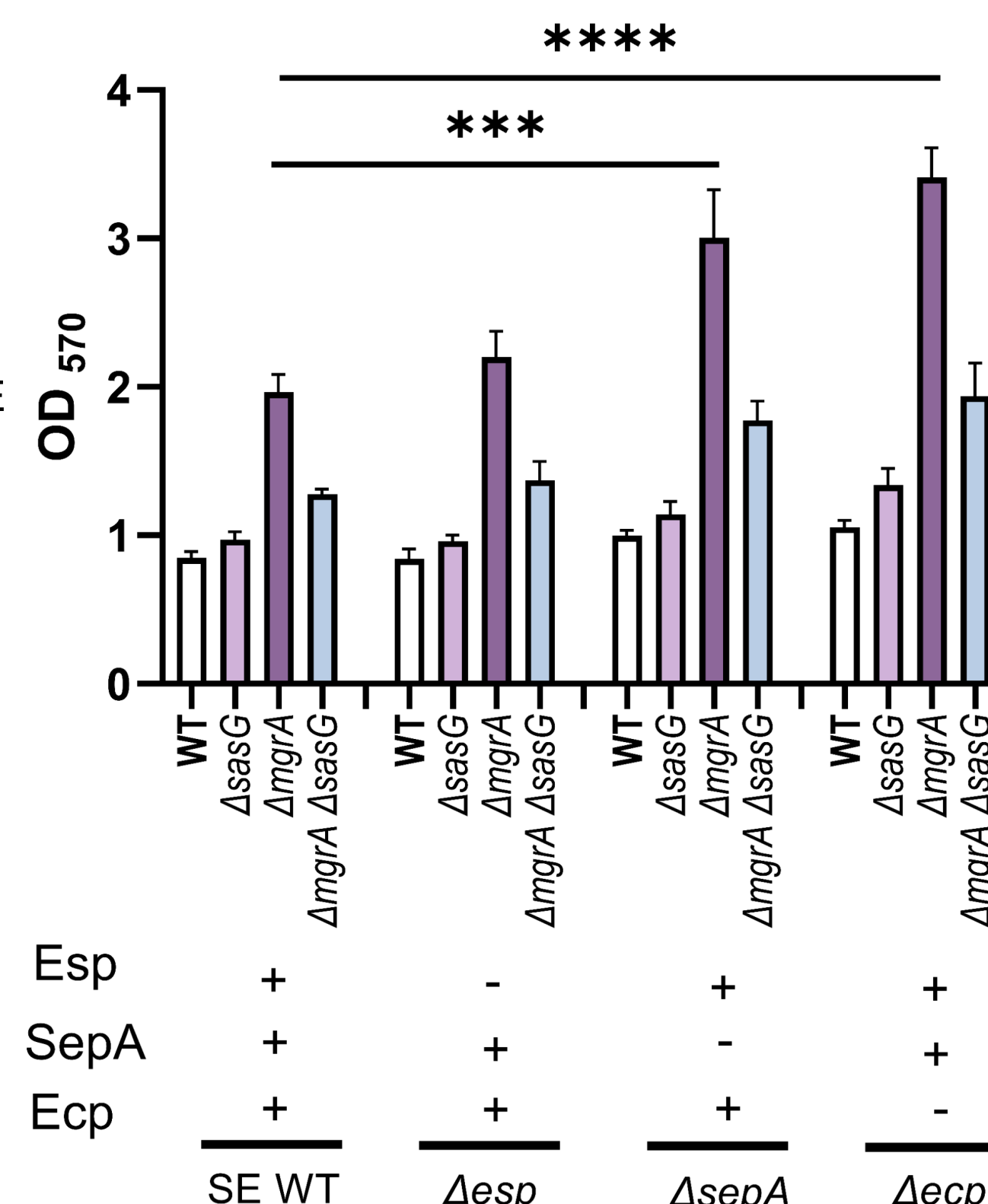
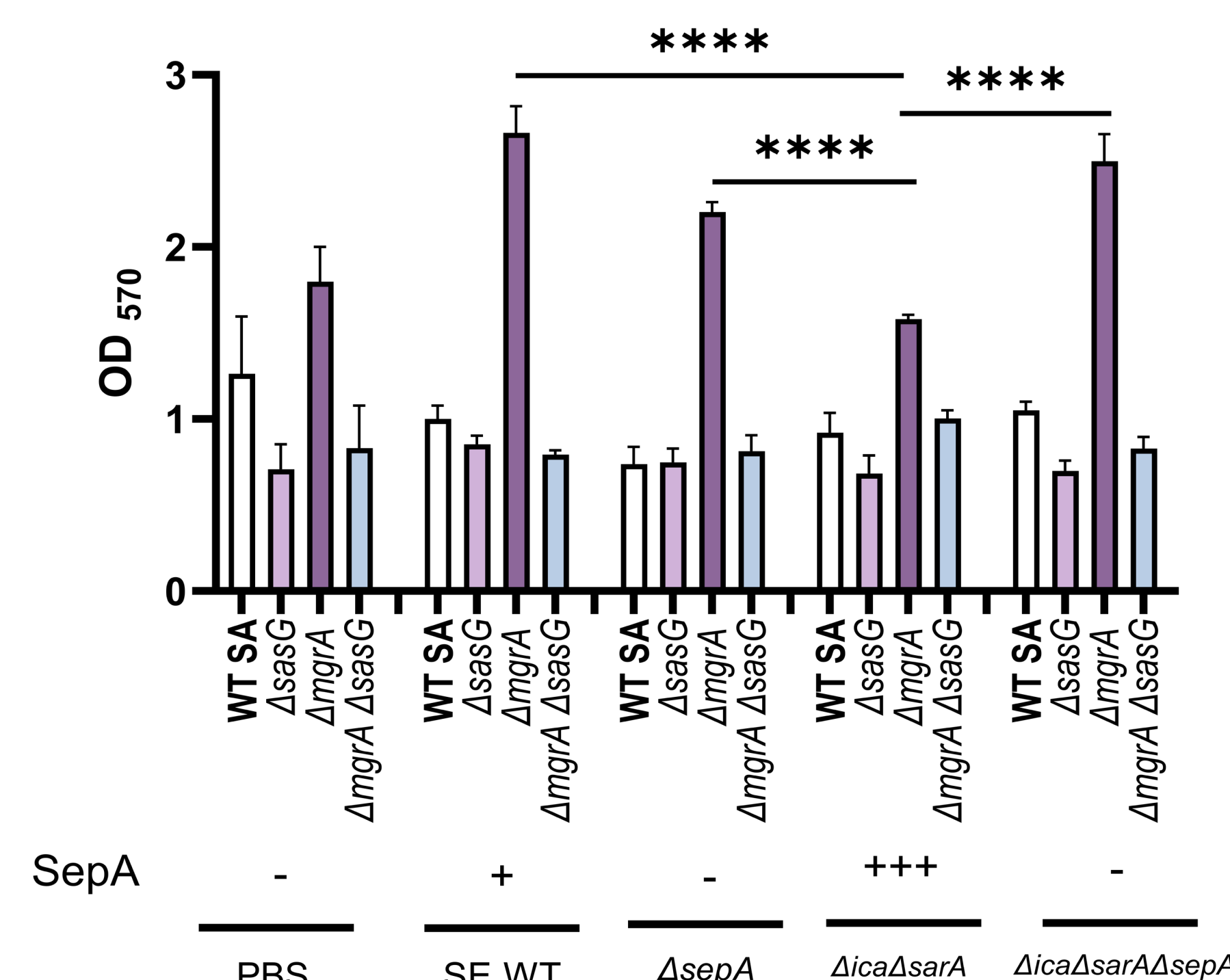


Figure 3. SE SepA overproduction decreases SA biofilms. SA biofilms were decreased when incubated with supernatant containing excess SepA relative to incubation with either SE wildtype (WT) and $\Delta sepA$ supernatants. N=3, **** P <0.0001 via ANOVA.



Results

Figure 4. SE triple protease mutant enhances SA biofilms. SA biofilms increased when incubated with supernatant from SE triple protease mutant relative to incubation with either SE WT or single protease mutant supernatants. N=3, ** P <0.01 via ANOVA.

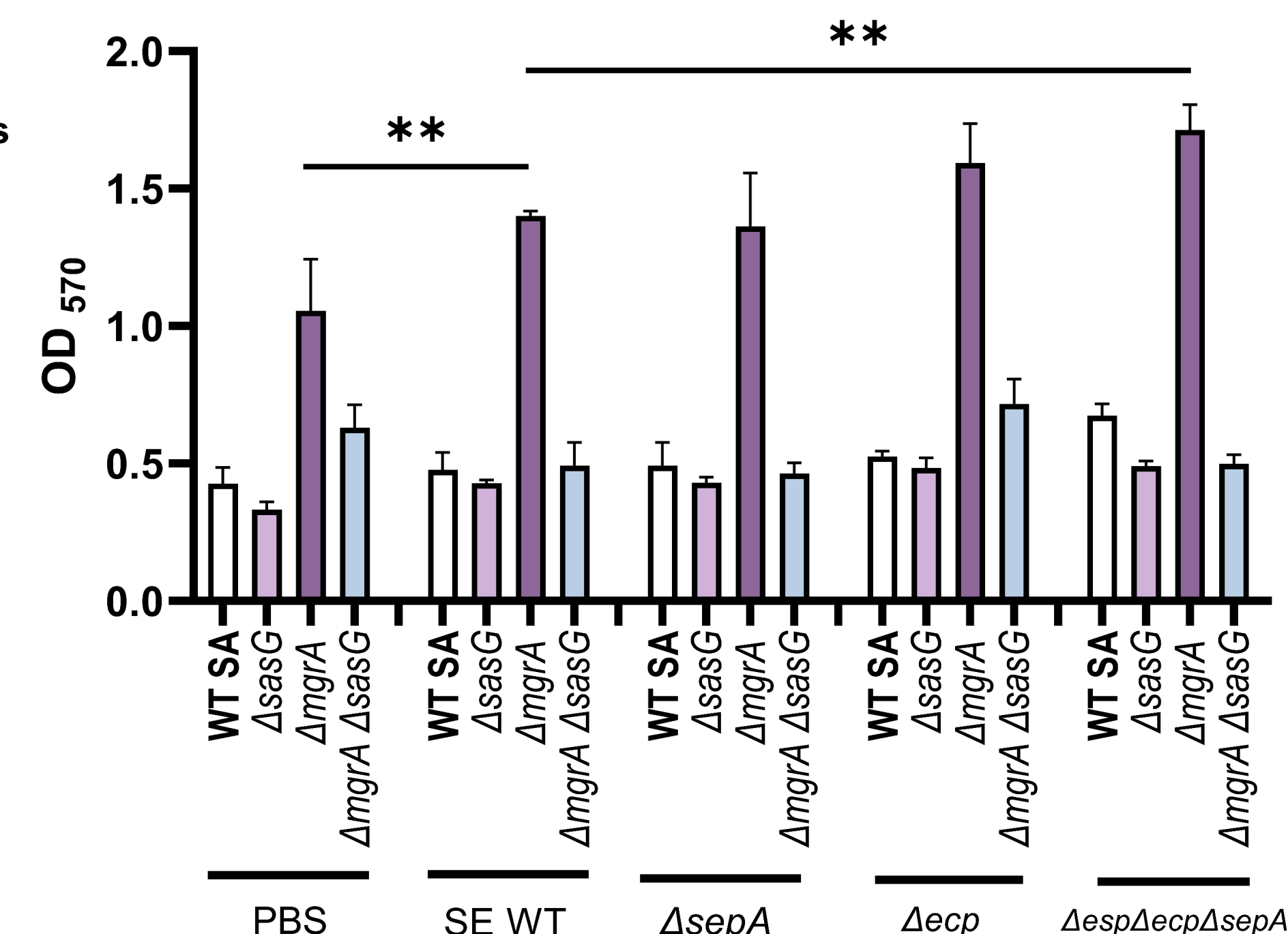
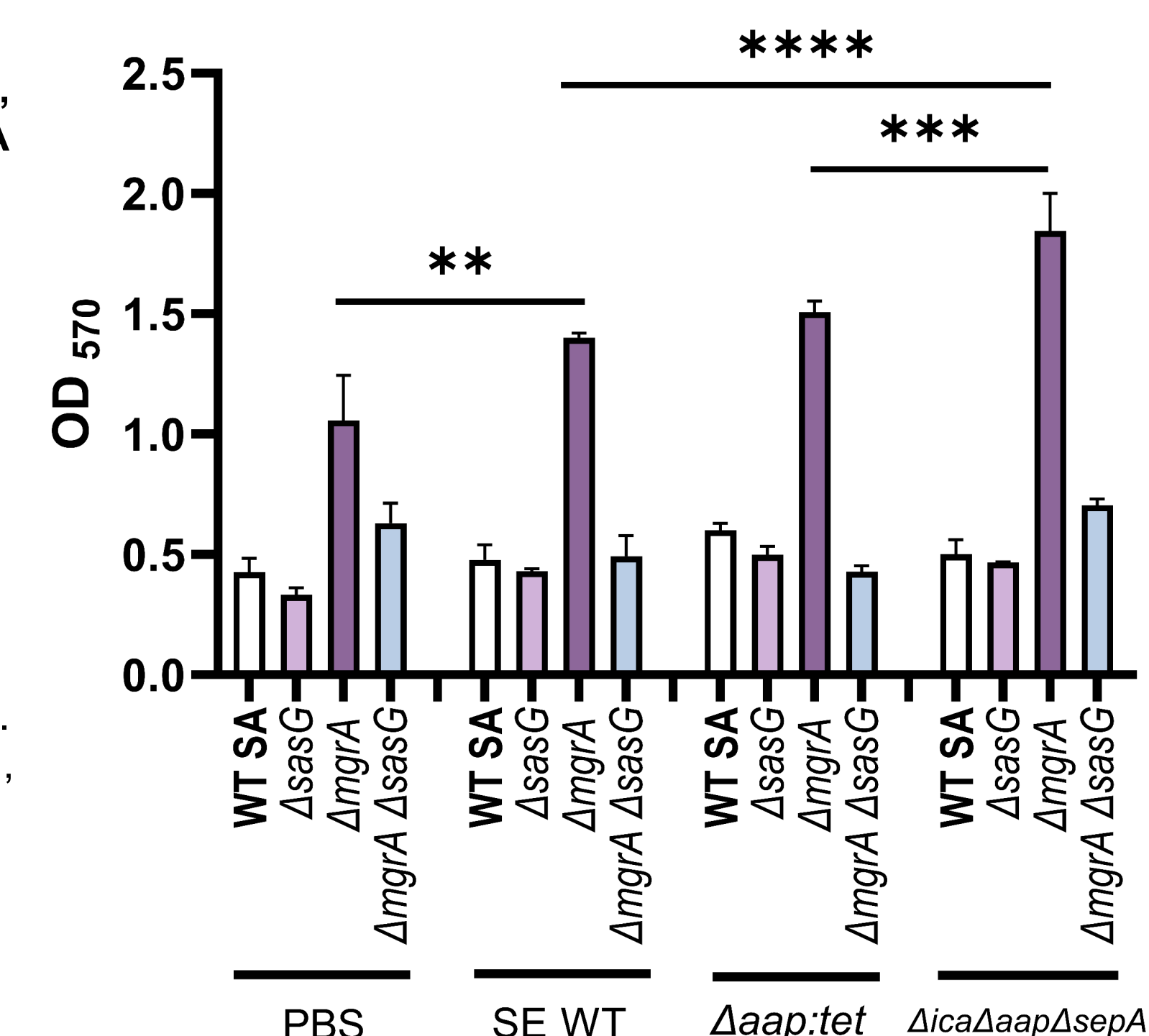
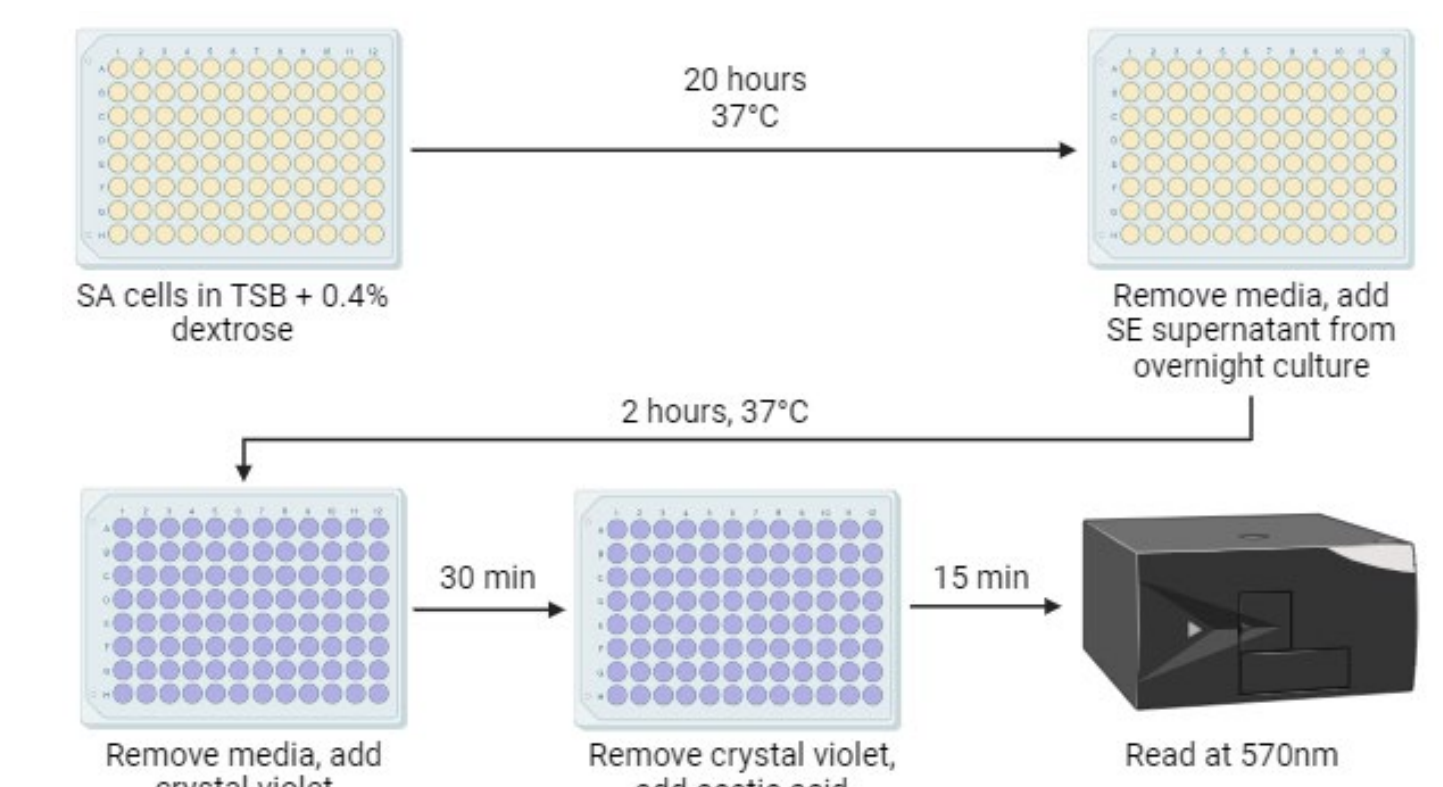


Figure 5. Aap, Ica, and SepA triple mutant enhances SA biofilms. SA biofilms increased in a SasG-dependent manner when incubated with supernatant without Aap, Ica, and SepA. N=3, ** P <0.01, *** P <0.001, **** P <0.0001 via ANOVA.



Methods

Standard crystal-violet based biofilm assays were employed to assess if SepA could affect SA biofilms.



Conclusions

- SE SepA reduces SA biofilm formation.
- SepA, Ecp, and Esp have an inhibitory effect on SA biofilms.
- Aap alone does not significantly affect SA biofilms, but when combined with *ica* and *sepA* gene deletions, increased SA biomass.
- Future experiments involve understanding the mechanisms of SepA inhibition of SA biofilms and potential roles for Ecp and Esp in SasG-mediated biofilm formation.

References

- Otto M. Staphylococcus colonization of the skin and antimicrobial peptides. *Expert Rev Dermatol.* 2010;5(2):183-195. doi:10.1586/edm.10.6
- Gonzalez T, Biagini Myers JM, Herr AB, Khurana Hershey GK. Staphylococcal Biofilms in Atopic Dermatitis. *Curr Allergy Asthma Rep.* 2017;17(12):81. Published 2017 Oct 23. doi:10.1007/s11882-017-0750-x
- Corrigan RM, Rigby D, Handley P, Foster TJ. The role of Staphylococcus aureus surface protein SasG in adherence and biofilm formation. *Microbiology (Reading).* 2007;153(Pt 8):2435-2446. doi:10.1099/mic.0.2007/006676-0
- Jenul C, Horswill AR. Regulation of Staphylococcus aureus Virulence. *Microbiol Spectr.* 2019;7(2):10.1128/microbiolspec.GPP3-0031-2018. doi:10.1128/microbiolspec.GPP3-0031-2018
- All figures created with BioRender.com. All graphs made with GraphPad Prism.
- We would like to thank Dr. Paul Fey (University of Nebraska Medical Center) who kindly provided the $\Delta esp \Delta ecp \Delta sepA$ SE strain.