



Establishing a Co-infection Model between Seasonal Human Coronaviruses and *Staphylococcus aureus*

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February 24th, 2025

Acknowledgements

We acknowledge with respect the Lekwungen-speaking peoples on whose traditional territory the university stands, and the Songhees, Esquimalt, and WSÁNEĆ peoples whose historical relationships with the land continue to this day.

Thank you to the Goncheva lab for your support. This research was supported by the Jamie Cassels Undergraduate Research Awards, University of Victoria, and supervised by Mariya Goncheva.

Background

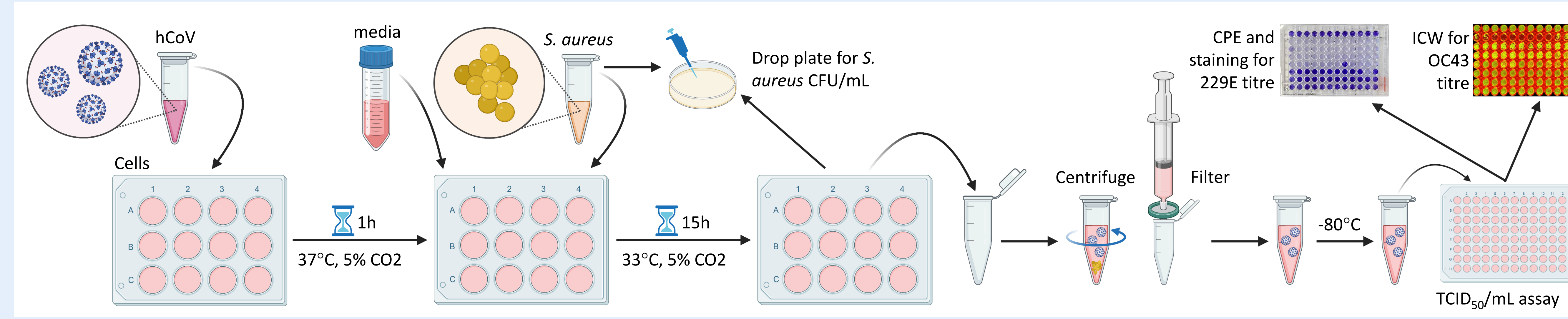
A significant complication of severe COVID-19 disease, caused by SARS CoV-2, has been co-infection with the bacterium *Staphylococcus aureus*¹. While co-infection does not impact *S. aureus* growth, it leads to significant pro-viral effects¹. The *S. aureus* iron-regulated surface determinant A (isdA) protein contributed to the increase in viral titre by altering JAK2-STAT3 levels and modifying host transcription¹.

229E and OC43 are two common seasonal human coronaviruses (hCoVs), which are generally responsible for mild upper respiratory tract infections². Co-infection with *S. aureus* is frequently detected following hCoV infection, but little is known about the impacts of *S. aureus* on viral replication, or vice versa³.

Aims

1. Establish and characterize a new *in vitro* co-infection model with hCoVs and *S. aureus*.
2. Assess the impact of *S. aureus* co-infection on hCoV titre at 33°C and 5% CO₂, with a focus on the isdA protein.
3. Assess *S. aureus* proliferation during hCoV co-infection at 33°C and 5% CO₂.

Methods



Results

Staphylococcus aureus co-infection increases 229E replication in HuH cells, in an isdA-dependent manner.

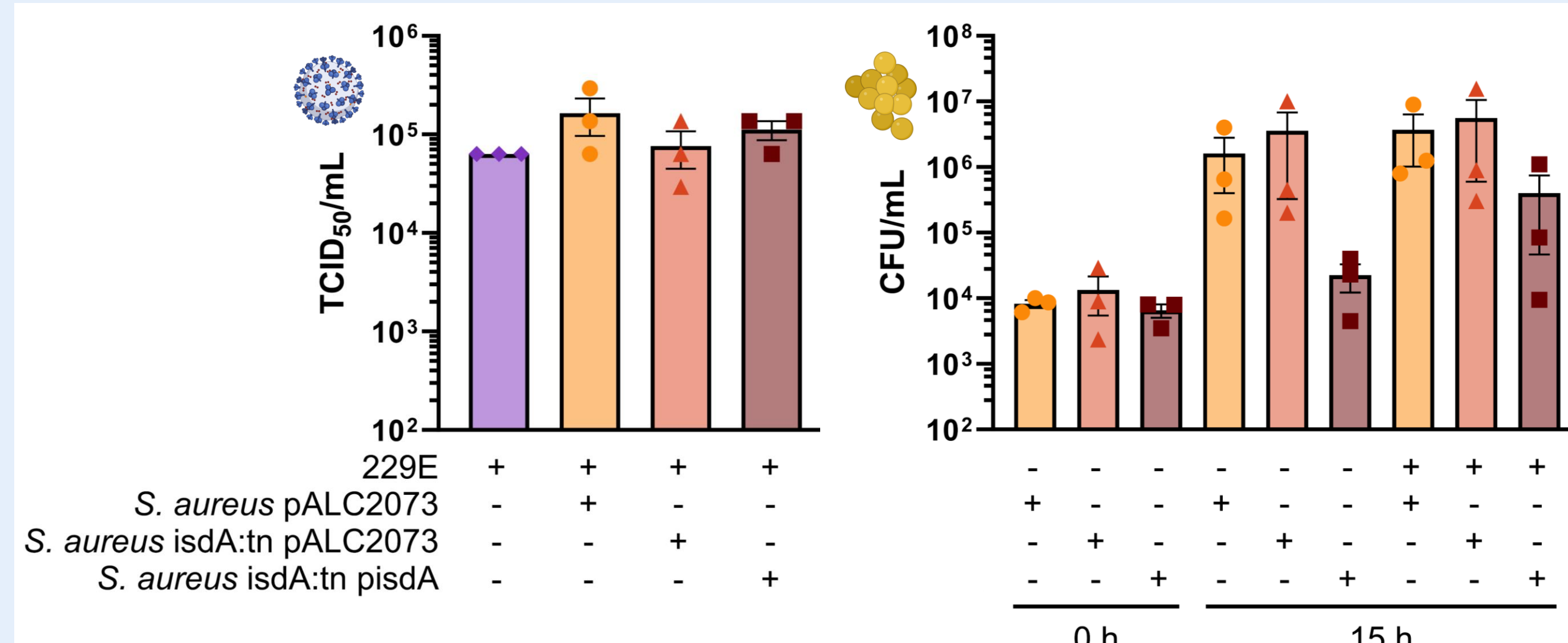


Figure 1. *S. aureus* co-infection increases the replication of 229E in HuH cells, while viral replication does not impact *S. aureus* growth. HuH cells were infected with 229E (MOI 3) for 1 hour at 37°C, 5% CO₂. After inoculum removal, 10² CFU *S. aureus* were added, and the cells were incubated at 33°C, 5% CO₂ for 15 hours. Data shown are mean ± SEM of 3 independent experiments. Virus titre (left) was quantified by TCID₅₀/mL on HuH cells. Bacteria (right) were quantified by drop plating on TSA plates.

Staphylococcus aureus co-infection increases 229E replication in nasal epithelial cells independently of isdA.

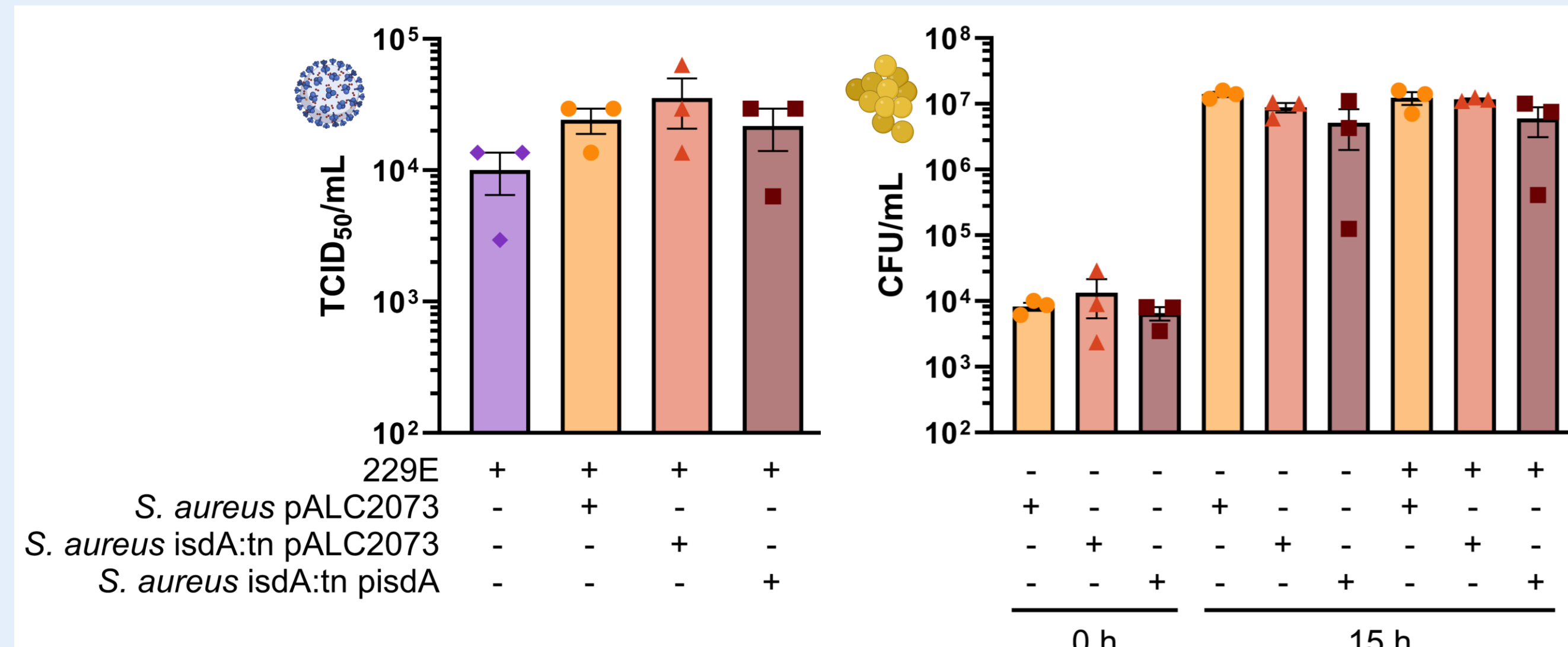


Figure 2. *S. aureus* co-infection increases the replication of 229E in CCL30 cells, while viral replication does not impact *S. aureus* growth. CCL30 cells were infected with 229E (MOI 3) for 1 hour at 37°C, 5% CO₂. After inoculum removal, 10² CFU *S. aureus* were added, and the cells were incubated at 33°C, 5% CO₂ for 15 hours. Data shown are mean ± SEM of 3 independent experiments. Virus titre (left) was quantified by TCID₅₀/mL on HuH cells. Bacteria (right) were quantified by drop plating on TSA plates.

Staphylococcus aureus isdA could impact OC43 growth in Vero cells.

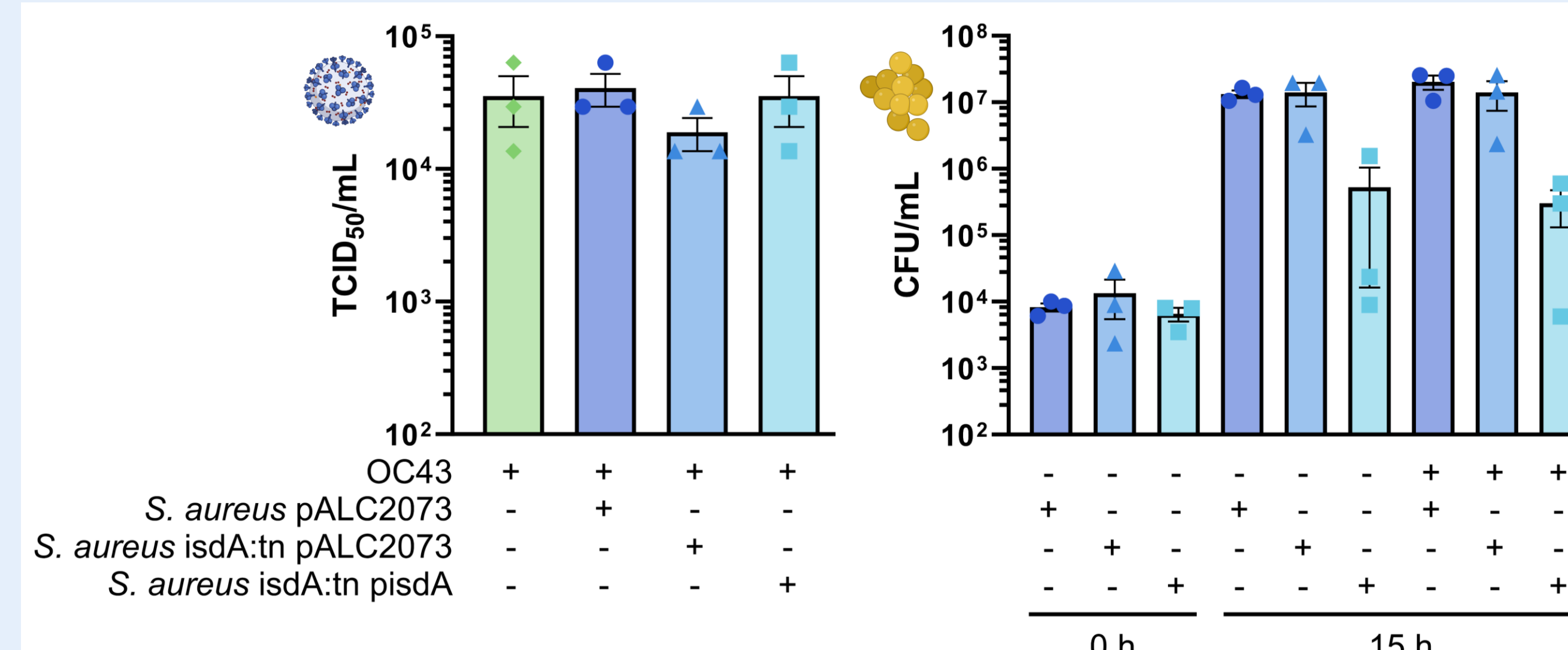


Figure 3. *S. aureus* co-infection may impact the replication of OC43 in Vero cells, but viral replication does not impact *S. aureus* growth. Vero cells were infected with OC43 (MOI 3) for 1 hour at 37°C, 5% CO₂. After inoculum removal, 10² CFU *S. aureus* were added, and the cells were incubated at 33°C, 5% CO₂ for 15 hours. Data shown are mean ± SEM of 3 independent experiments. Virus titre (left) was quantified by TCID₅₀/mL on Vero cells. Bacteria (right) were quantified by drop plating on TSA plates.

Overexpression of *Staphylococcus aureus* isdA inhibits OC43 growth in nasal epithelial cells.

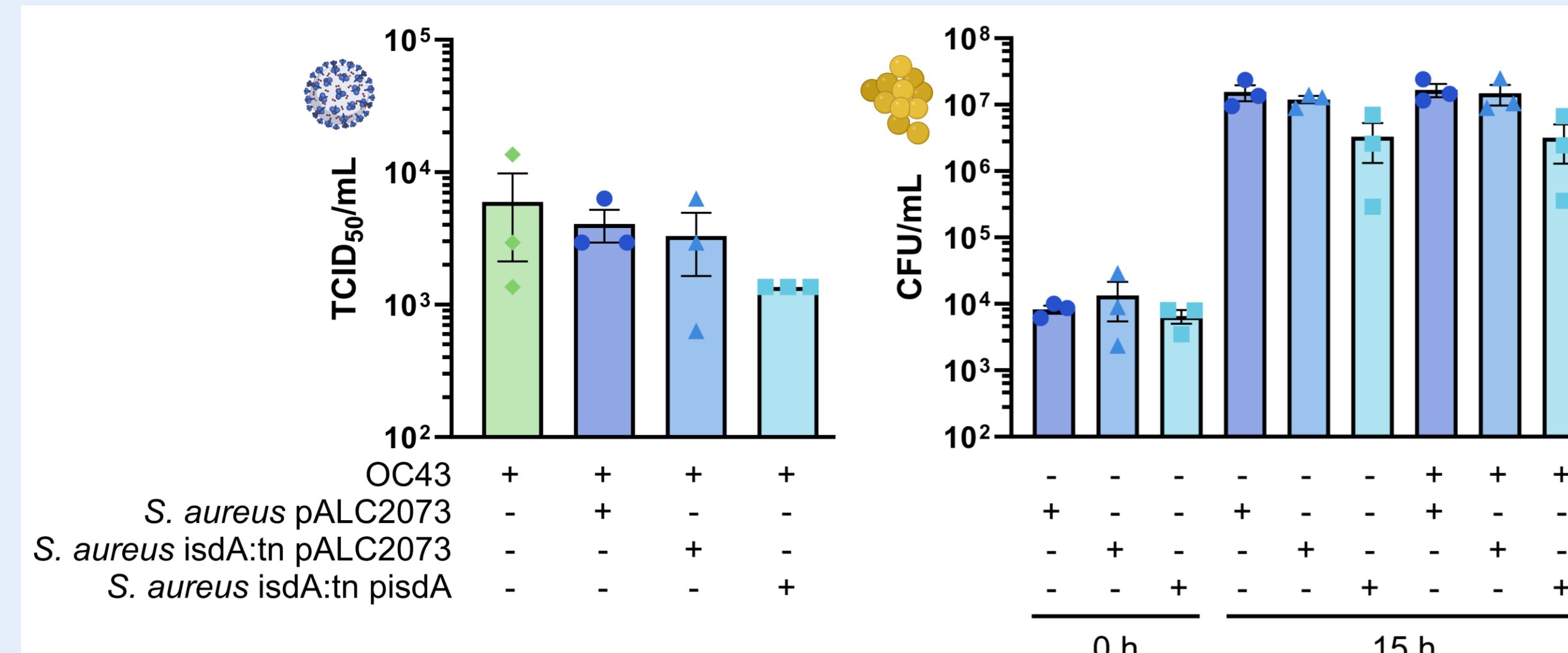


Figure 4. *S. aureus* co-infection does not increase the replication of OC43 in CCL30 cells, nor does viral replication impact *S. aureus* growth. CCL30 cells were infected with OC43 (MOI 3) for 1 hour at 37°C, 5% CO₂. After inoculum removal, 10² CFU *S. aureus* were added, and the cells were incubated at 33°C, 5% CO₂ for 15 hours. Data shown are mean ± SEM of 3 independent experiments. Virus titre (left) was quantified by TCID₅₀/mL on Vero cells. Bacteria (right) were quantified by drop plating on TSA plates.

Conclusions

1. Both hCoVs and *S. aureus* can be grown in the CCL30 nasal epithelial cell line, HuH cell line, and Vero cell line.
2. A pro-viral effect was observed in *S. aureus* and 229E co-infection at 33°C, 5% CO₂. This effect was isdA-dependent in HuH cells, and isdA-independent in CCL30 cells.

Loss of isdA expression inhibited OC43 growth in Vero cells, but isdA overexpression inhibited OC43 growth in CCL30 cells. This suggests that isdA may have a positive effect on viral growth, but only when expressed at appropriate levels.

3. isdA overexpression hindered *S. aureus* replication, especially in HuH and Vero cells at 33°C, 5% CO₂. No pro-bacterial effects were observed with hCoV co-infection.

Future Directions

Future directions include completing co-infection assays at 37°C, 5% CO₂ to mimic the conditions of a lower respiratory tract infection. We will compare the proliferation of *S. aureus* and the hCoVs of an upper respiratory tract infection (33°C) with those of a lower respiratory tract infection (37°C).

References

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2. V'kovski P, Kratzel A, Steiner S, Stalder H, Thiel V. Coronavirus biology and replication: implications for SARS-CoV-2. *Nat Rev Microbiol*. 2021 Mar;19(3):155–70.
3. Choi WI, Kim IB, Park SJ, Ha EH, Lee CW. Comparison of the clinical characteristics and mortality of adults infected with human coronaviruses 229E and OC43. *Sci Rep*. 2021 Feb 24;11(1):4499.

Methods graphic created in <https://BioRender.com>