Playing Dirty: How does *S. aureus* clear its competition?

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1. Introduction and aims

Staphylococcus aureus is a common skin bacterium that can cause blood poisoning and other illnesses[1]. It readily acquires antibiotic resistance, resulting in 'superbugs' like MRSA[2].

S. aureus uses machinery called the **Type VII Secretion System (T7SS)** to secrete antibacterial toxins like **TspA** across its cell envelope and into its surroundings.

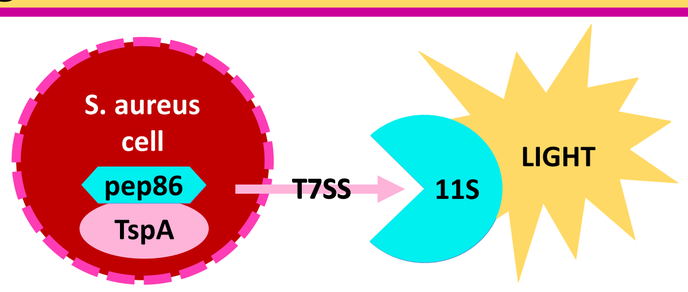
These toxins help *S. aureus* kill competitor bacteria that limit its invasion of the body[3], so understanding their secretion could help us control *S. aureus* infections.

This project investigated whether three **partner proteins SACOL2603, SACOL2602 and SACOL2601** support TspA secretion via a luminescence assay and chromatographic purification.

2. Measuring secretion

Figure 1: NanoLuc luminescence assay

The pep86 tag on TspA combines with the 11S protein fragment once outside the cell and catalyses a luminescent reaction using furimazine. **More luminescence** shows **greater secretion**[4].



3. Purification

TspA and SACOL2603 were N-terminally His-tagged and twinstrep-tagged respectively.

TspA and the partners were then overexpressed in *Escherichia coli* M15 cells and purified via His-trap and twinstrep **affinity chromatography (AC)** followed by **size exclusion chromatography (SEC)**.

4. Results

Figure 2: The TspA locus and SACOL2603-1 locus.

The gene loci containing TspA and the partners were identified from the COL strain of *S. aureus*. Both are distant on the bacterial chromosome.

The forward (**+**) strand of the chromosome encodes TspA followed by multiple DUF443 proteins, including the TsaI immunity protein that inhibits TspA for self-protection.

The reverse (**–**) strand contains genes for the three partners beginning with *SACOL2603.*

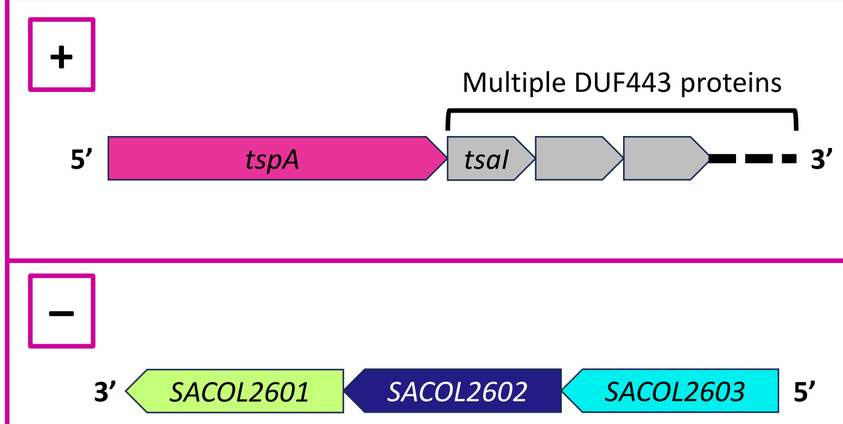


Figure 3: Partner proteins maintain TspA stability and support its secretion.

SACOL2603 or *SACOL2602* gene deletion reduces TspA secretion with an approximately equal effect for each partner. Removal also destabilises TspA, most significantly for *ΔSACOL2603*, lowering cytoplasmic concentration.

SACOL2601 deletion had an inconsistent effect during the project, with a slight net increase in secretion and cytoplasmic concentration.

Secretion and cytoplasmic levels were compared between each strain via NanoLuc assays.
Tagged TrxA (non-secreted) or T7SS-deficient strains were used as controls (not shown) to confirm that TspA leaves via the T7SS only.

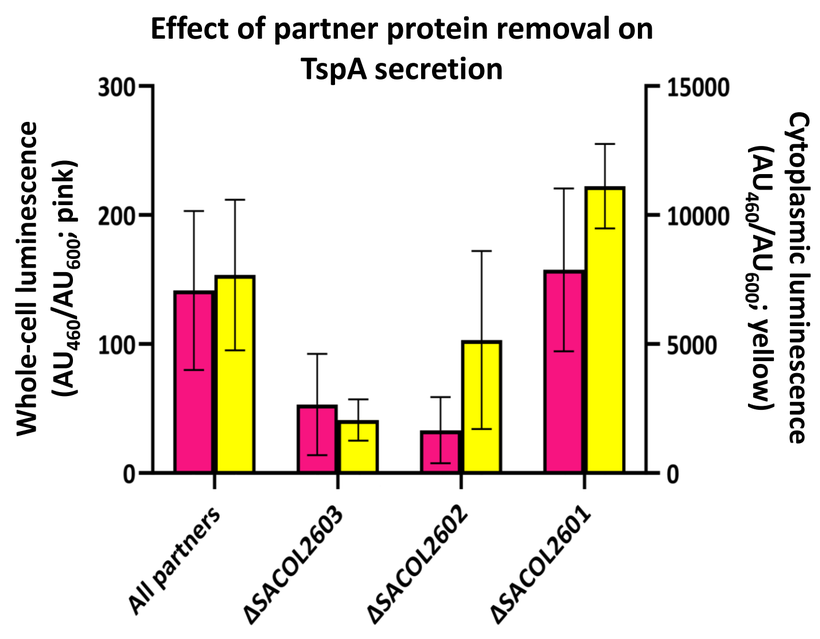


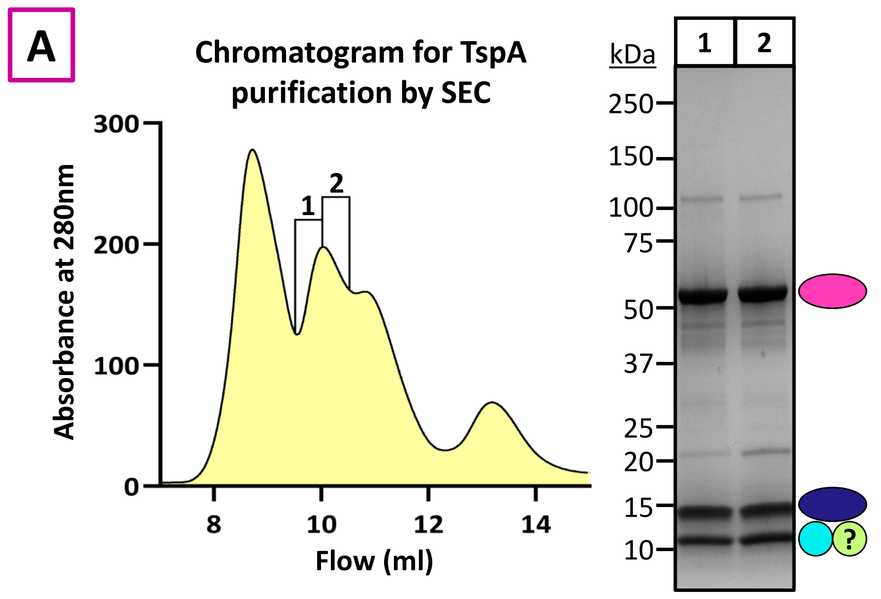
Figure 4: The partner proteins co-purify in a complex with TspA

A. Following AC and SEC (left), proteins were separated by SDS-PAGE and Coomassie-stained (right).

TspA and at least two of the partners co-purify in AC, forming an unstable equilibrium of different complexes and degradation products in SEC.

B. The complex structure, predicted by ColabFold[5], was modelled in ChimeraX (bottom). Using this, a possible secretion mechanism is summarised in **C.** based on a model for a similar T7SS toxin complex[6].

SACOL2603 and SACOL2602 form an N-terminal rod on TspA, and SACOL2601 sits at the toxic C-terminus. This matches other T7SS toxin-partner complexes like EsaD[7].



Key

- TspA (pink) = ~52.5kDa, tagged
- SACOL2603 (cyan) = ~12.7kDa, tagged
- SACOL2602 (dark blue) = 14.8kDa
- ? SACOL2601 (green) = 11.6kDa, unclear

His = N-terminal His-tag on TspA
TS = N-terminal twinstrep tag on SACOL2603

5. Conclusions

Both **SACOL2603 and SACOL2602 promote TspA secretion**. Additionally, SACOL2602 and especially SACOL2603 **maintain the stability of TspA** within the cell.

Furthermore, these partners co-purify with TspA, indicating that **the partners form a pre-secretion complex** like EsaD and other T7SS toxins. This could enable TspA to be targeted to the T7SS machinery and secreted.

However, further investigation is required to confirm how these partners promote secretion and stability. For example, substitution mutations could be used to identify a targeting sequence.

Additionally, **the role of SACOL2601 is uncertain** due to ambiguous NanoLuc assay and chromatography results. Future work could examine how this protein interacts with TspA and confirm how it impacts secretion.

These findings underscore the requirement of toxin-partner protein complexes to facilitate secretion by the T7SS. This could be targeted by future treatments of *S. aureus* infections or exploited to use TspA against *S. aureus*.

6. References

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