O240

1-hour Oral Session

Antimicrobial tolerance of biofilms: mechanisms and solutions

Enzymes catalyzing the urea and TCA cycles are important determinants of biofilm formation in methicillin-resistant *Staphylococcus aureus* USA300

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Objectives: Among methicillin-resistant *S. aureus* (MRSA), the USA300 clone is a major cause of serious infections worldwide. We earlier identified a USA300 isolate, UAS391, as a prolific biofilm former. Transcriptomics on the biofilm and planktonic phenotypes of UAS391 detected 10−178 fold upexpression of genes involved in the urea and tricarboxylic acid (TCA) cycle during biofilm formation. Their role was further analysed in this study.

Methods: The erythromycin-sensitive derivative of UAS391 (UAS391-EryS), and transposon (Tn, *bursa aurealis*-bearing) insertion mutants of USA300-JE2 (NARSA, http://www.beiresources.org/) were utilized for transduction experiments. Transducing phage Φ11 recovered from the culture supernatant of *S. aureus* RN0451 was propagated on RN0450, and utilized for infecting Tn-bearing JE2 mutants. After infection of cultures of the recipient UAS391-EryS, transductants were selected on LB with 0.05% sodium citrate containing 5 mg/L erythromycin. Log phase cultures of UAS391, UAS391-EryS, and the generated transductants were studied in duplicate on a continuous flow assay (Bioflux, Fluxion). 17h-biofilms were stained (SYTO9, Life Technologies) and fluorescence intensity was quantified by microscopy (Zeiss, ImageJ).

Results: To study the effect of gene knockouts in UAS391, we generated a set of Tn-interrupted UAS391-EryS mutants by transduction from the JE2 background. Tn inactivated genes included those encoding argininosuccinate lyase (*argH*::Tn*),* succinyl-CoA synthetase (*sucC*::Tn), succinate dehydrogenase (*sdhA*::Tn and *sdhB*::Tn), aconitase (*acnA*::Tn), and isocitrate dehydrogenase (*icd*::Tn). The *argH*::Tn mutant produced 3-fold less biofilms than the parent UAS391-EryS (P=0.007) **(Fig. A, B).** Biofilm formation also decreased by 1.6−2-fold in *sdhA*::Tn (P=0.036), *acnA*::Tn (P=0.003), and *icd*::Tn (P=0.006) compared to UAS391-EryS (**Fig. A, B**). However, no significant decrease in biofilm formation was detected in the *sucC*::Tn and *sdhB*::Tn mutants (P≥0.1071) (**Fig. A, B**). As part of the urea cycle, argininosuccinate lyase catalyzes the conversion of L-arginino-succinate to arginine and fumarate (KEGG database, **Fig. C**). Arginine is not only an essential amino acid during biofilm growth but its catabolic by-product, ammonia, is required for pH homeostasis, while fumarate feeds into the TCA cycle (**Fig. C**). Deletion of the functionally non-redundant *acnA* and *icd* enzymes allowed estimating the net contribution of the TCA cycle to biofilm formation.

Conclusions: Genetic analysis of a MRSA USA300 strain revealed a key role of *argH* and of genes encoding different steps of the TCA cycle (*sdhA*, *acnA*, and *icd)* in biofilm formation.

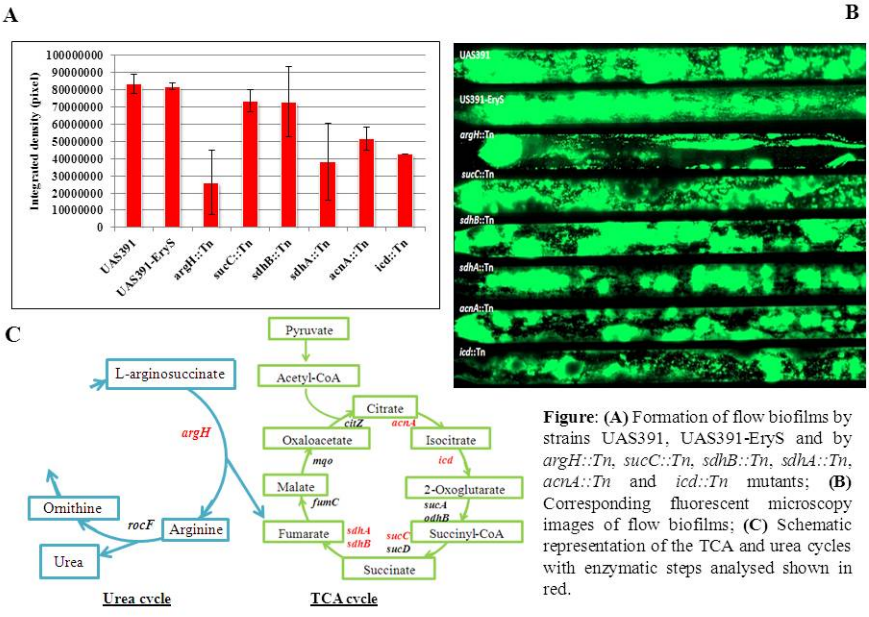

Figure: **(A)** Formation of flow biofilms by strains UAS391, UAS391-EryS and by *argH::Tn, sucC::Tn, sdhB::Tn, sdhA::Tn, acnA::Tn* and *icd::Tn* mutants; **(B)** Corresponding fluorescent microscopy images of flow biofilms; **(C)** Schematic representation of the TCA and urea cycles with enzymatic steps analysed shown in red.