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O0242 TCA cycle intermediate malate stimulates biofilm formation in methicillin-resistant Staphylococcus aureus USA300

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Background: Urea and tricarboxylic acid (TCA) cycle intermediates, especially argininosuccinate, are important for biofilm formation. We have previously shown that an argininosuccinate lyase (ArgH) transposon (Tn) mutant is defective in biofilm formation. ArgH is central to both TCA and urea cycles (**Fig. A**). It converts L-arginino-succinate to arginine − whose catabolic by-product in the urea cycle, ammonia, is required for pH homeostasis − and to fumarate, which feeds into TCA cycle. Here, we attempted to estimate the net contribution of urea and TCA cycle intermediates to the biofilm phenotype in a highly prolific biofilm-forming strain of methicillin-resistant Staphylococcus aureus (MRSA) USA300, UAS391.

Methods: Tn (*bursa aurealis*-bearing) insertion mutants of USA300-JE2 (http://www.beiresources.org/) in fumarate hydratase (*fumC*::Tn), citrate synthase (*gltA*::Tn), and arginase (*rocF*::Tn) (**Fig. A**) were used for transduction into the erythromycin-sensitive derivative of UAS391 (UAS391-Ery^S). Transducing phage Φ11 recovered from RN0451, was propagated on RN0450, utilized for infecting UAS391-Ery^S, and Tn mutants were studied by static (96-well microtiter plate) and continuous flow (Bioflux, Fluxion) biofilm assays in triplicate. Bacterial growth rates were analysed (http://bellinghamresearchinstitute.com) by repetitive kinetic turbidimetric measurements at 600nm (Multiskan™GO spectrophotometer, Thermo Scientific). Total RNA from 17h-old static biofilms was extracted (Masterpure™Complete DNA & RNA purification kit, Epicentre) and converted into cDNA (Reverse Transcription System, Promega) with random primers. Gene expression levels were studied by quantitative reverse-transcriptase PCR (StepOnePlus™, Applied Biosystems®).

Results: The *fumC*::Tn mutant (OD_{492} 0.355; integrated density (ID) $2.1x10^7$) produced 2-4 fold less biofilm than the parent UAS391-Ery^S (OD_{492} 0.787, P<0.001; ID $8.2x10^7$; P<0.001), while *gtlA*::Tn (OD_{492} 0.774, P=0.399; ID $6.4x10^7$, P=0.059) and *rocF*::Tn (OD_{492} 0.753, P=0.515; ID $7.6x10^7$, P=0.076) showed no significant change in biofilm mass production under both flow and static conditions (**Fig. B&C**). No pleiotropic effect on overall growth rates (UAS391-Ery^S 0.161 min^{-1}) was observed in any of the mutants: *fumC*::Tn 0.162 min^{-1}, *rocF*::Tn 0.163 min^{-1}, and *GtlA*::Tn 0.157 min^{-1} (P=0.452). All three mutants showed a 2-3 fold downexpression of the corresponding mutated gene as compared to UAS391-Ery^S (P<0.001), which confirmed the Tn insertion.

Conclusions: Using Tn knockouts, we show that the TCA cycle intermediate, malate, is important for biofilm formation by MRSA USA300.

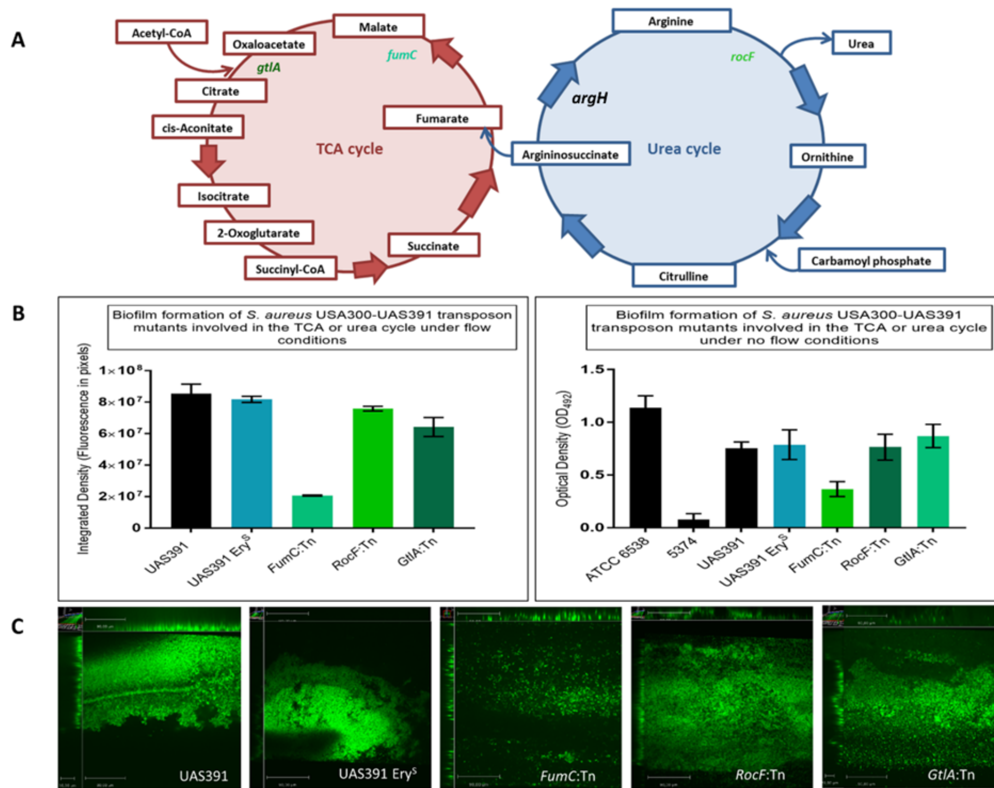

Figure: (**A**) Schematic representation of the TCA and urea cycle with argininosuccinate as a central mediator and their observed effect on biofilm formation by *S. aureus* USA300-UAS391. Knock-out mutants encoding different steps (*fumC*:Tn, *rocF*:Tn and *gtlA*:Tn) are represented in green. (**B**) In the static assay (right), biofilms were stained with Hucker's crystal violet (2%) for mathematical quantification of optical densities (OD_{492}) and compared with simultaneously run MRSA ATCC reference strains 6538 and 5374. In the dynamic assay (left), 17h-biofilms were stained (SYTO9, Life Technologies) and fluorescence intensity was quantified by (**C**) microscopy (Zeiss, ImageJ). Error bars depict 95% confidence interval.