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Enzymes catalyzing the urea and TCA cycles are important determinants of biofilm formation in MRSA USA300

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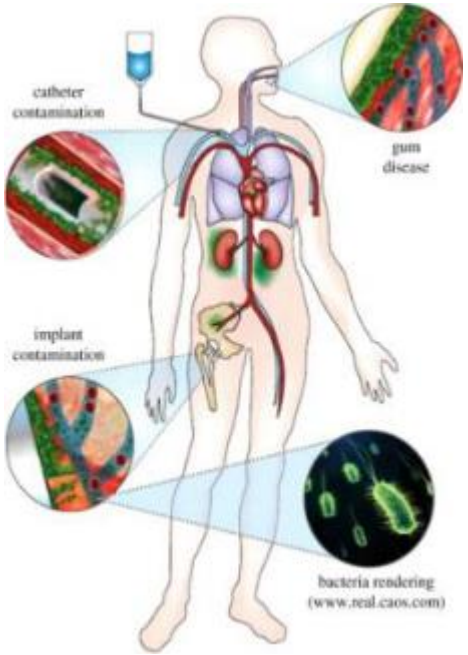
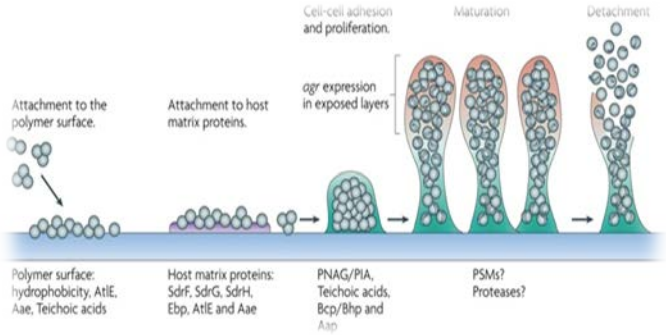
Biofilm formation by *S. aureus*

- The ability of *Staphylococcus aureus* to form biofilms is an important characteristic which complicates infections, especially those associated with foreign materials such as catheters and implants
- *S. aureus* in biofilms exhibit altered gene expression that correlates with phenotypic alterations such as antibiotic resistance, persistence etc.
- Insights on biofilm-specific mechanisms are needed to be able to develop preventive or disruptive interventions



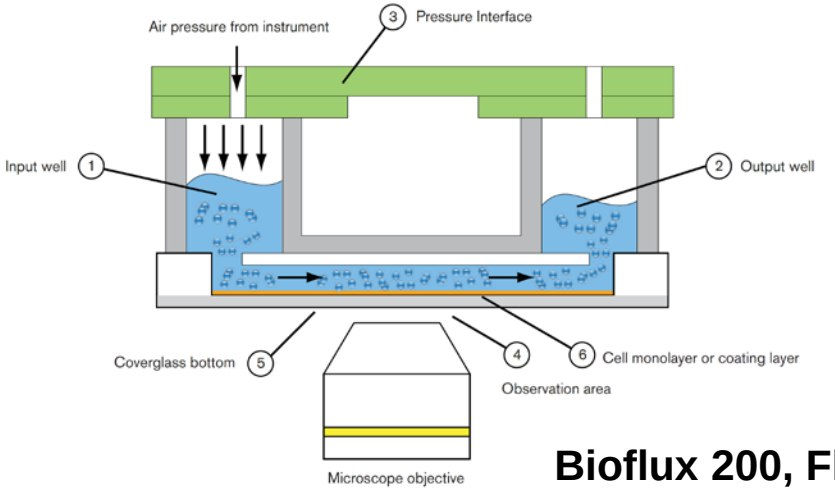


Otto, Nat. Rev. Microbiol. 2009

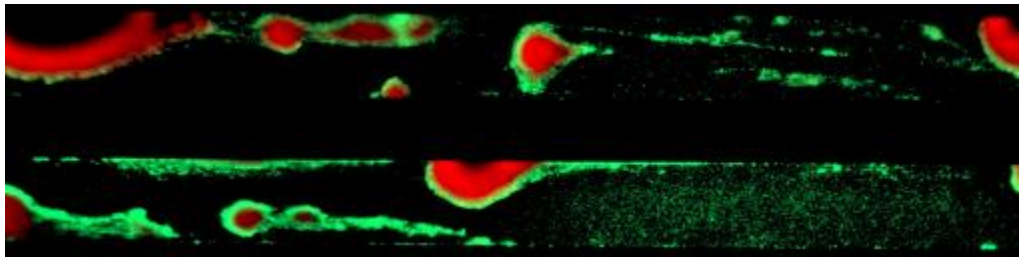
Biofilm formation by major MRSA clonal lineages

Static model

Shear flow model



Bioflux 200, Fluxion



Biofilm formation by MRSA under shear flow

MRSA clone characteristics and biofilm formation in static and shear flow assays

MRSA clone (No. of strains)	Clonal complex	*SCCmec*	Sequence type	Presence of *pvl/ACME*	Biofilm formation in static assay			Biofilm formation in shear flow assay	
					Strong formers* (n)	Moderate formers (n)	Weak formers (n)	+ (n)	– (n)
Southern Germany (6)	5	I	228/5	–	1	2	3	3	3
New York/Japan (4)	5	II	5/496	–	0	4	0	3	1
Iberian (4)	8	I	247/336	–	1	2	1	2	2
Hungarian/Brazilian (12)	8	III	239/241	–	5	3	4	8	4
EMRSA-16 (4)	30	II	36	–	1	2	1	3	1
USA600 (3)	45	II	45	–	0	0	3	3	0
Pediatric (5)	5	IV	5	–	3	1	1	0	5
USA500 (8)	8	IV	8	–	8	0	0	3	5
USA300 (3)	8	IV	8	*pvl/ACME*	3	0	0	3	0
EMRSA-15 (7)	22	IV	22	–	7	0	0	5	2
South-West Pacific (2)	30	IV	30	–	0	1	1	0	2
South-West Pacific (3)	30	IV	30	*pvl*	0	1	2	0	3
Berlin (11)	45	IV	45	–	3	6	2	5	6
European (4)	80	IV	80	*pvl*	3	1	0	1	3

* Defined cut-offs for strong (OD_{492}: > 0.027, moderate: 0.027–0.009, and weak biofilm formers: <0.009)

Utilizing ‘omics’ to identify differential gene expression in USA300 biofilms and planktonic cultures

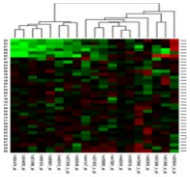
Culture planktonic bacteria in flasks with shaking and change of medium for 72 hrs

Culture biofilm cells under flow in a continuous-flow bioreactor for 72 hrs

Extract RNA (Ambion), check RNA quality and concentration (Bioanalyzer, Agilent)

Enrich mRNA (ExpressArt Bacterial mRNA amplification Micro kit, AmpTec Gmbh, Germany)

Perform microarray hybridizations (*S. aureus* Genome arrays, Affymetrix)



Analyze expression data

Genes upregulated in USA300 UAS391 biofilms

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Highly upregulated genes in USA300-S391 biofilms

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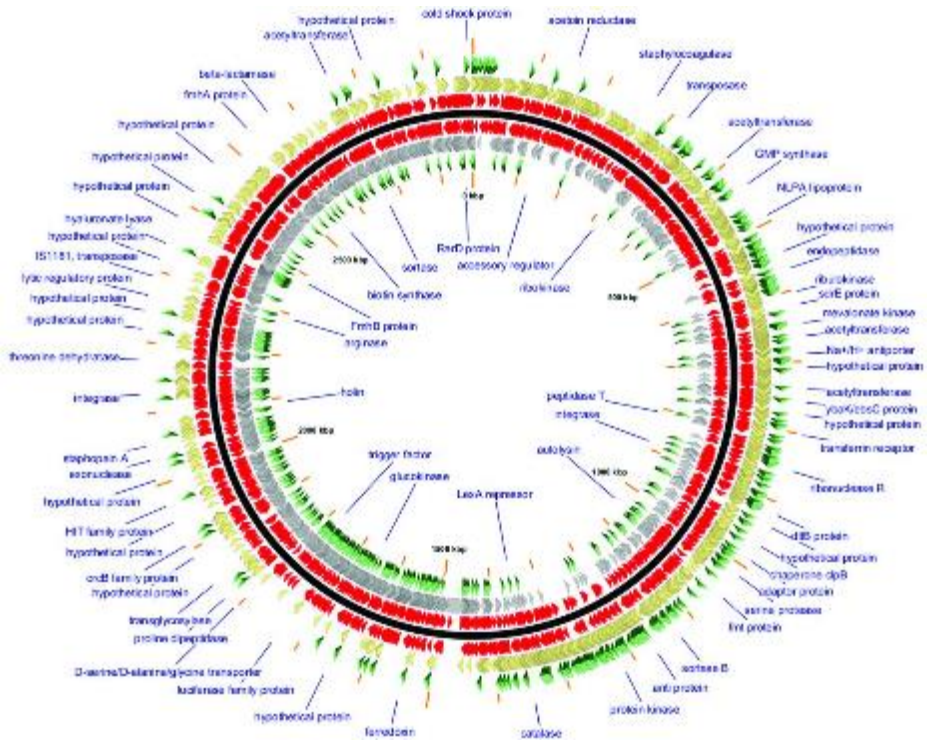
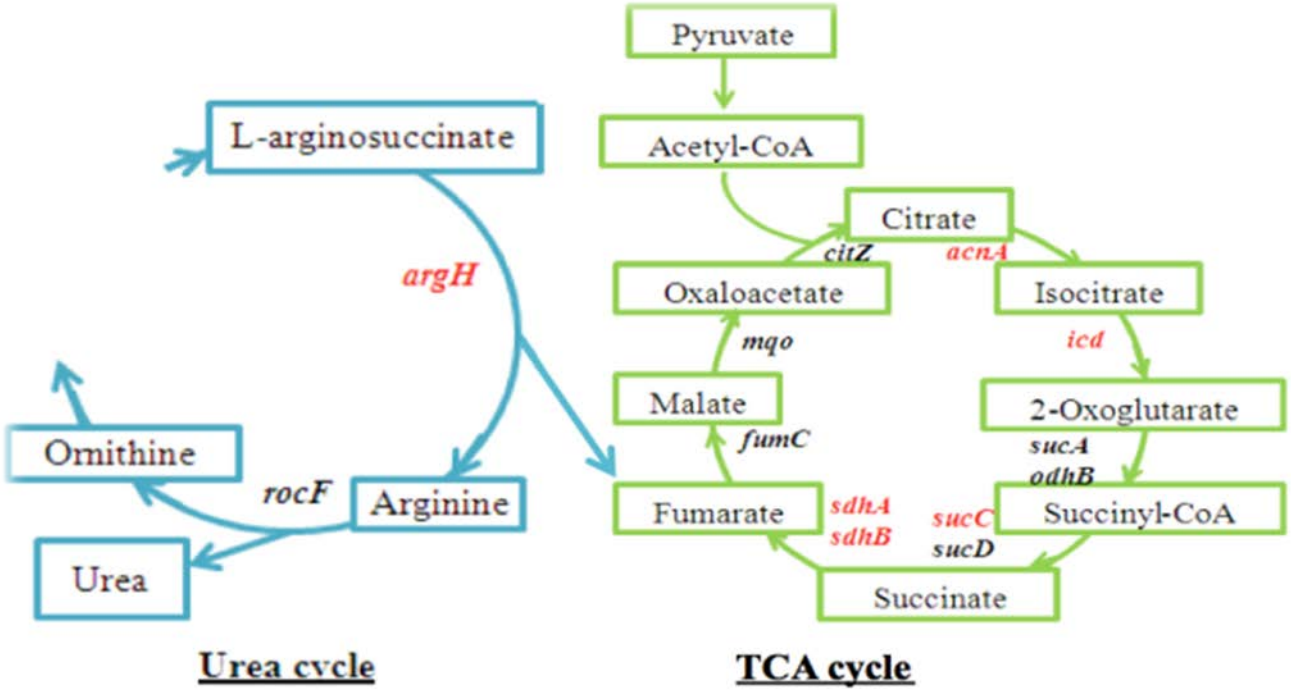
Gene knockout in UAS391 by transduction

- Erythromycin-sensitive derivative of UAS391 (UAS391-EryS) and transposon (*bursa aurealis*) insertion mutants of USA300-JE2 (NARSA)
- Transducing phage ϕ11 was utilized to infect the Tn-bearing JE2 and culture supernatant to infect the recipient UAS391-EryS
- UAS391-EryS transductants were selected on LB agar with erythromycin



Fitzgerald MRSA protocols, 2007; Fey et al. mBio 2013

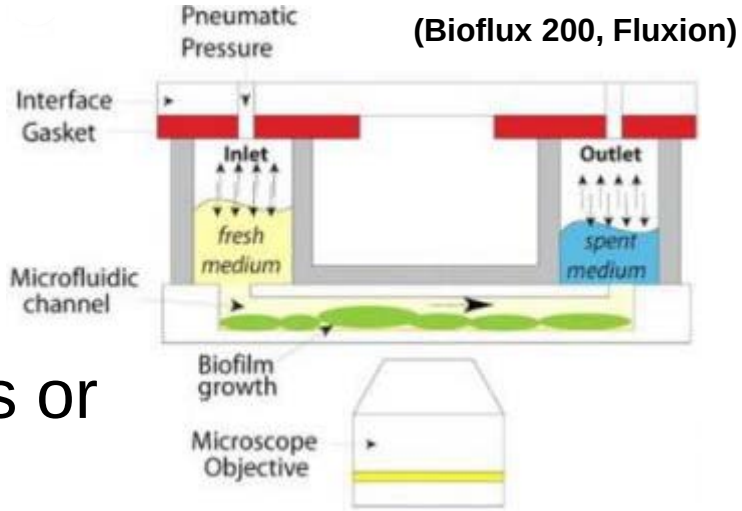
UAS391 Tn insertion mutants



In vitro biofilm models

- Limited nutrient and aeration
- High-throughput
- Rapid quantification of biofilm mass

- Fresh nutrients
- Control of parameters such as shear forces
- Medium-throughput
- Direct rapid quantification of mass or viable cells



VanHommerig et al., PLOS One, 2014

UAS391 Tn insertion mutants show decreased biofilm formation

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argH: Argininosuccinate lyase

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UAS391 Tn insertion mutants show decreased biofilm formation

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The TCA cycle

- Plays a central role in metabolism
  - Nutrients, like sugars, amino acids, and fatty acids, are metabolized into TCA intermediates and enter this cycle at several points
  - Intermediates can be removed from the cycle for use in biosynthetic pathways
  - NADH produced by the TCA cycle feeds into the electron transport pathway >> energy/ATP production
  - TCA cycle activity associated with different effects like survival, virulence, and **!! the production of the main biofilm slime substance, polysaccharide intercellular adhesin (PIA)**

UAS391 biofilms are PIA-independent

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Why is the TCA cycle upregulated in USA300 biofilms?

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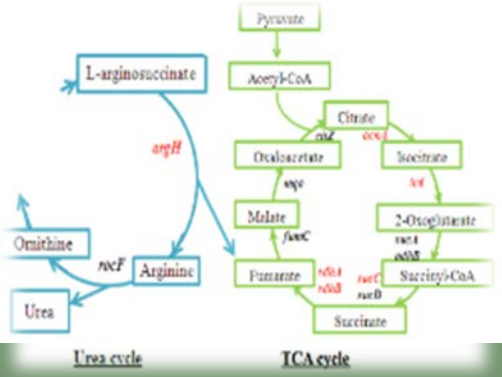
Gaup et al., J. Bact, 2010

Why is the TCA cycle upregulated in USA300 biofilms?

- To make better use of traces of oxygen
- To allow better metabolism of excreted fermentation products like acetic or lactic acid
- To permit better catabolism of amino acids
- TCA upregulation highly advantageous to bacterium under oxygen-nutrient limited condtions
- Urease cycle upregulation for pH homeostasis



Conclusions

- Comparative genomics and transcriptomics are the first steps in identifying (novel) genes involved in biofilm formation in MRSA
  - Deletion of the functionally non-redundant *sdhA*, *acnA* and *icd* genes allowed to investigate contribution of the TCA cycle to biofilm formation
- Large scale screenings are likely to yield clinically relevant ‘hits’ for validation phenotypic and genotypic *S. aureus* variants to confirm commonality of the target mechanisms and on animal models and in clinical samples

Thank you for your attention