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Microbial pathogenesis reloaded

The role of mecR1 in biofilm formation by methicillin-resistant Staphylococcus aureus USA300

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Background: 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. Comparison of the transcriptome of UAS391 generated under flow-biofilm and planktonic conditions showed the MecR1 β-lactam sensor/signal transducer protein carried on the *SCCmec* mobile genetic element to be 60-fold up-expressed under biofilm conditions as compared to planktonic conditions. Utilizing transposon mutagenesis, we further explored the role of *MecR1* on the biofilm phenotype of UAS391.

Material/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 to construct a *MecR1*::Tn mutant. 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 5mg/L erythromycin. Antibiotic susceptibility of UAS391, UAS391-EryS and *MecR1*::Tn was tested using E-tests for penicillin, oxacillin and cefoxitin. 17h-old biofilms of UAS391, UAS391-EryS, and *MecR1*::Tn were studied *in vitro* in a static biofilm assay (96-well microtiter plate) and in a continuous flow assay (Bioflux, Fluxion) in triplicate. In the static assay, biofilms were stained with Hucker's crystal violet (2%) for mathematical quantification of optical densities (OD_{492}) and compared with simultaneously run reference *S. aureus* strains ATCC 6538 and 5374. In the dynamic assay, 17h-biofilms were stained (SYTO9, Life Technologies) and integrated density of the biofilm mass was quantified by fluorescence microscopy (Zeiss, ImageJ).

Results: The *MecR1*::Tn knockout mutant remained resistant to β-lactam antibiotics with MIC-values for penicillin, oxacillin and cefoxitin ranging from 3µg/ml, 16µg/ml, and 16µg/ml, respectively. No significant difference in methicillin resistance was detected in comparison to the plasmid-cured UAS391-EryS strain with MIC-values 4µg/ml, 16µg/ml, and 24µg/ml, respectively. After 17h of growth, *MecR1*::Tn formed 1.5-fold less biofilm (OD=0,202) when compared to UAS391-EryS (OD=0,278; P=0,005) and UAS391 (OD=0,287; P=0,002) under static conditions (Fig.1A). *MecR1*::Tn displayed 7-fold decrease of total biofilm area under flow conditions (integrated density = 24.267.974) when compared to UAS391 (integrated density = 175.959.473; P<0,001) and UAS391-EryS (integrated density = 159.425.594; P<0,001) (Fig.1B&C).

Conclusions: We report here for the first time that *MecR1* plays an important role in biofilm formation by USA300. The fact that the *MecR1* gene was highly up-regulated in USA300 biofilms in the absence

of a β-lactam and without a concomitant up-expression of *mecA*, indicates an as yet undiscovered non-resistance related regulatory function of this signal transducer.

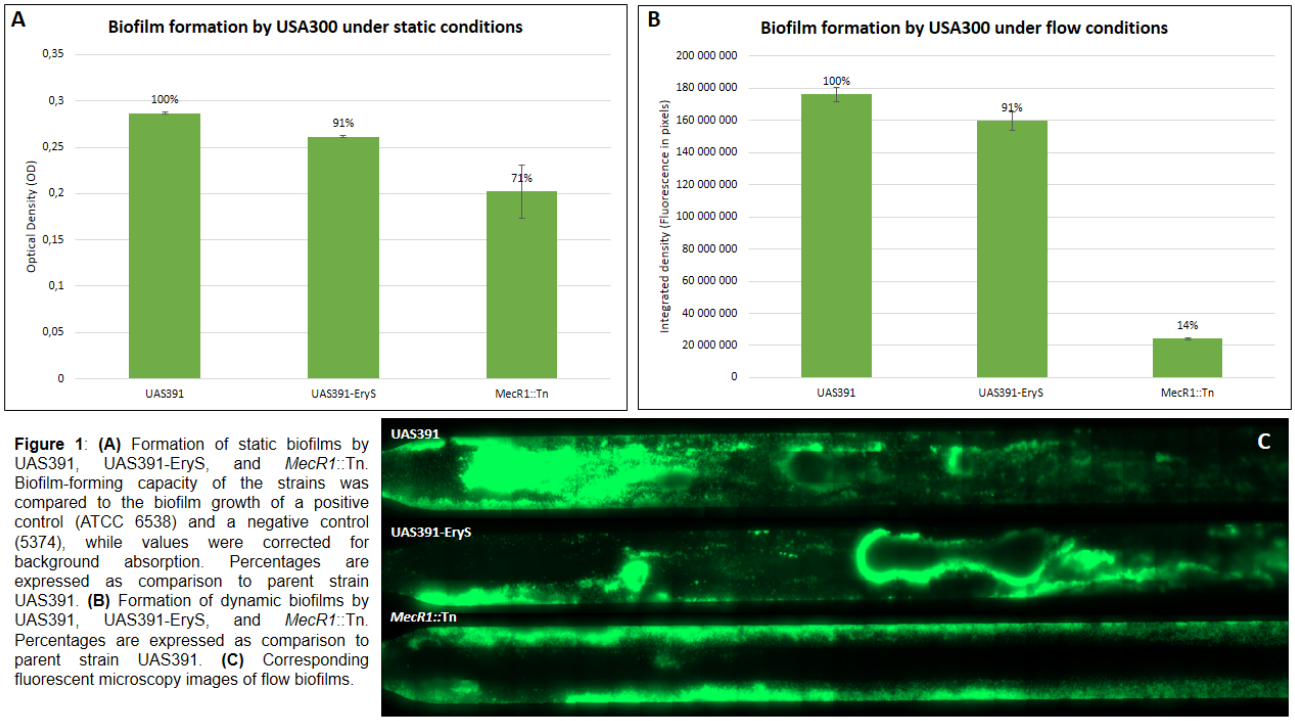

Figure 1: **(A)** Formation of static biofilms by UAS391, UAS391-EryS, and *MecR1*::Tn. Biofilm-forming capacity of the strains was compared to the biofilm growth of a positive control (ATCC 6538) and a negative control (5374), while values were corrected for background absorption. Percentages are expressed as comparison to parent strain UAS391. **(B)** Formation of dynamic biofilms by UAS391, UAS391-EryS, and *MecR1*::Tn. Percentages are expressed as comparison to parent strain UAS391. **(C)** Corresponding fluorescent microscopy images of flow biofilms.