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The role of *MecR1* in biofilm formation by methicillin-resistant *Staphyloccocus aureus* USA300

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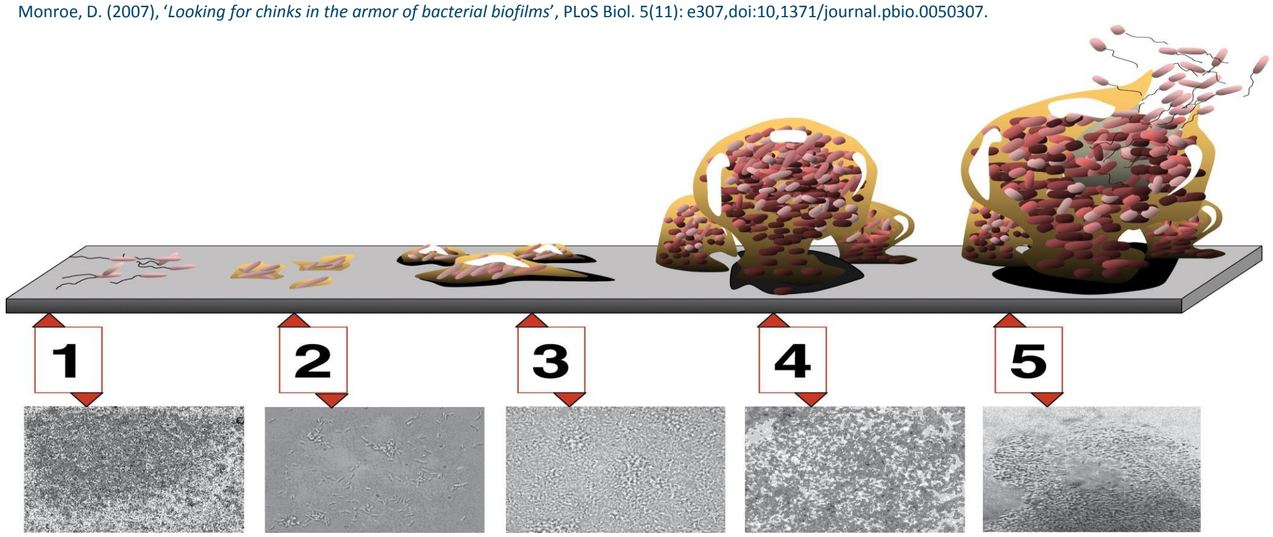
Introduction

Staphylococcus aureus causes infections ranging from minor skin infections to life-threatening diseases, such as pneumonia. It's success can be partly attributed to prolonged persistence of methicillin-resistant *S. aureus* (MRSA) infections linked to the formation of biofilms *in vivo* (1). We analysed the transcriptome of USA300_UAS391 to study the underlying basis of biofilm formation (2) (unpublished data). Among other genes, the MecR1 β-lactam sensor/signal transducer protein, which is involved in regulation of PBP2a-mediated β-lactam resistance by *mecA*, was 60-fold up-expressed under biofilm conditions as compared to planktonic conditions. We further explored the role of *MecR1* on the biofilm phenotype of UAS391.

Fig. 1: Five stages of biofilm development: (1) initial attachment, (2) irreversible attachment, (3) maturation I, (4) maturation II, and (5) dispersion. Adapted from Monroe, D. (2007), '*Looking for chinks in the armor of bacterial biofilms*', PLoS Biol. 5(11): e307,doi:10,1371/journal.pbio.0050307.



Materials & 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. 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) (3).

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.3A). *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.3B & 4).

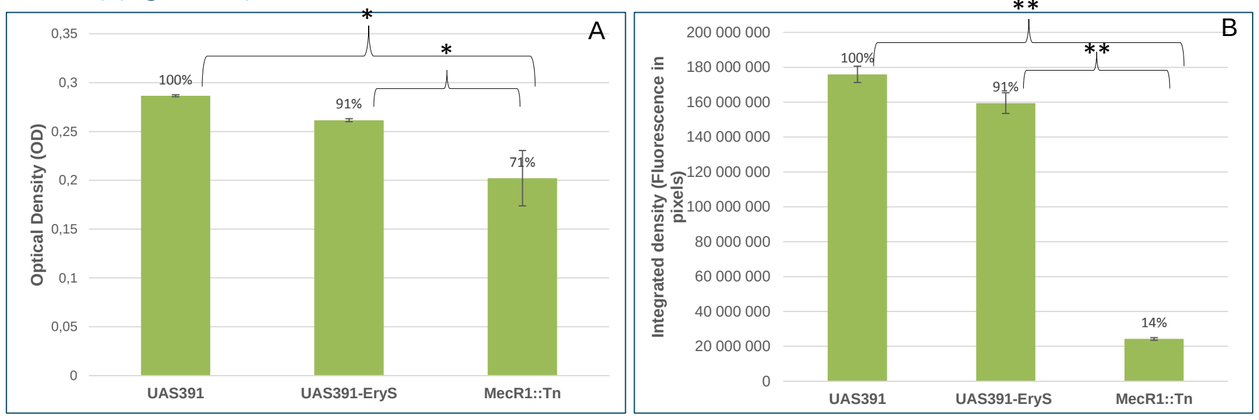


Fig. 3: (A) Formation of static biofilms by UAS391, UAS391-EryS, and *MecR1*::Tn. Biofilm-forming capacity of the strains was compared to the biofilm growth of a positive control (ATCC 6538) and a negative control (5374), while values were corrected for background absorption. Percentages are expressed in comparison to parent strain UAS391. **(B)** Formation of dynamic biofilms by UAS391, UAS391-EryS, and *MecR1*::Tn. Percentages are expressed in comparison to parent strain UAS391. Error bars represent 95% CI. ** shows statistically significant p-values <0.001, * shows statistically significant p-values <0.05.

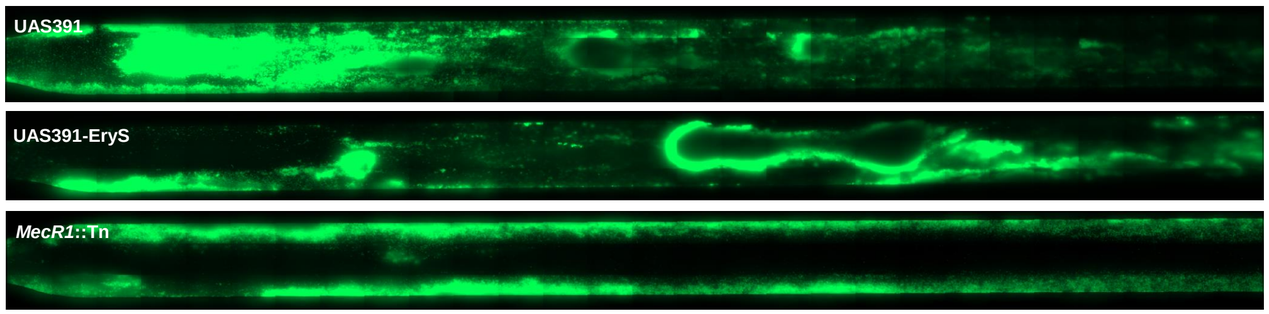


Fig. 4: Corresponding fluorescent microscopy images of flow biofilms.

CONCLUSIONS: We report for the first time that the β-lactam sensor/signal transducer protein MecR1 is an important mediator in biofilm formation by USA300_UAS391. The fact that the *MecR1* gene was highly up-regulated in the absence of a β-lactam and without concomitant up-expression of *MecA*, indicates an as yet undiscovered non-resistance related regulatory function of this signal transducer.

References:

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