

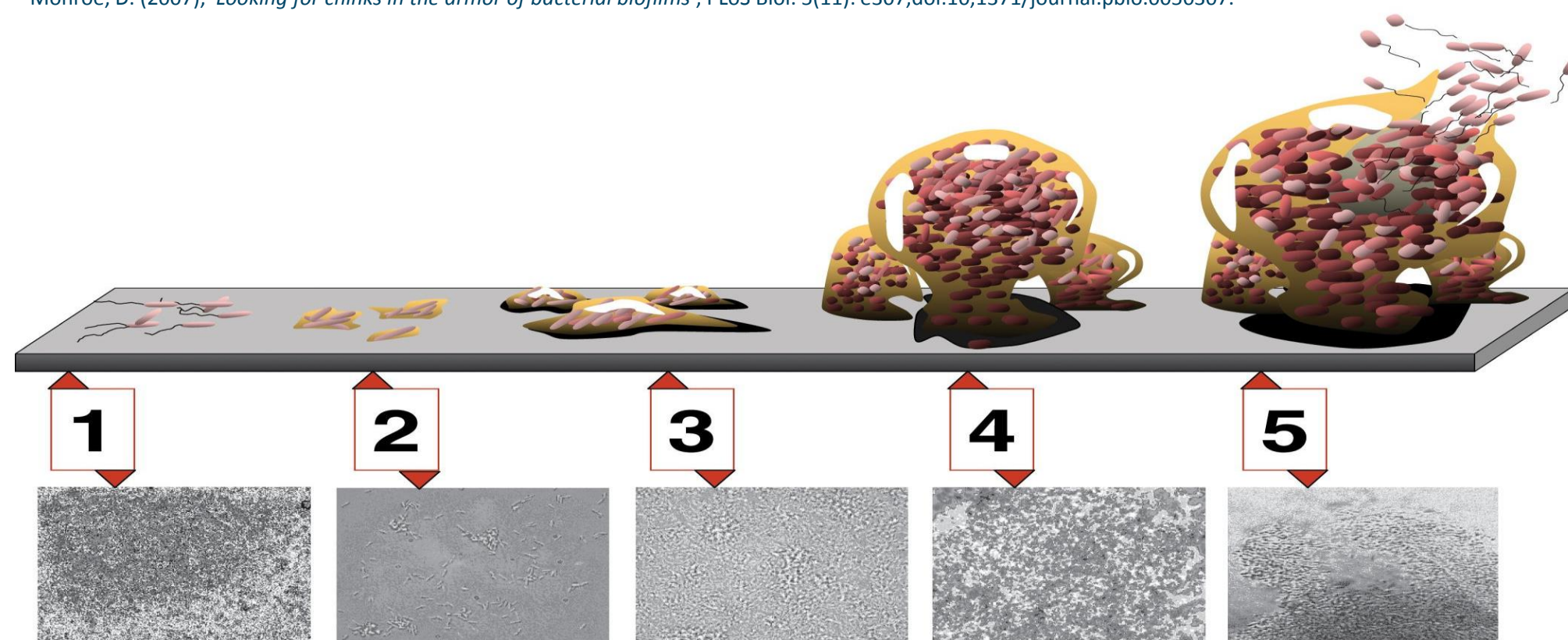
Sarah de Backer¹, Ines de Pauw¹, Jean-Pierre Hernalsteens², Julia S. Sabirova¹, Henri De Greve^{3,4}, Herman Goossens¹, Surbhi Malhotra-Kumar¹

1 – Department of Medical Microbiology, Vaccine & Infectious Disease Institute, Universiteit Antwerpen, Antwerp, Belgium; 2 – Viral Genetics Research Group, Vrije Universiteit Brussel, Brussels, Belgium; 3 – Structural & Molecular Microbiology, Structural Biology Research Center, VIB, Brussels, Belgium; 4 – Structural Biology Brussels, Vrije Universiteit Brussel, 1050 Brussels, Belgium.

Introduction

Staphylococcus aureus causes infections ranging from minor skin infections to life-threatening diseases, such as pneumonia. Its 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 We analysed the transcriptome of USA300_UAS391 to study the underlying basis of biofilm formation ⁽²⁾ (unpublished data).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 Φ 11 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⁾.

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:

- (1) Archer, N. K., Mazaitis, M. J., Costerton, J. W., Leid, J. G., Powers, M. E., & Shirtliff, M. E., (2011), 'Staphylococcus aureus biofilms: properties, regulation and roles in human disease', Virulence, 2 (5): 445-459.
- (2) Sabirova, J. S., Xavier, B. B., Hernalsteens, J., De Greve, H., Ieven, M., Goossens, H., & Malhotra-Kumar, S., (2014), 'Complete genome sequences of two prolific biofilm-forming Staphylococcus aureus isolates belonging to USA300 and EMRSA-15 clonal lineages', Genome Announc., 2(3): e00610-14.
- (3) Vanhommerig, E., Moons, P., Pirici, D., Lammens, C., Hernalsteens, J., De Greve, H., Kumar-Singh, S., Goossens, H., & Malhotra-Kumar, S., (2014), 'Comparison of biofilm formation between major clonal lineages of methicillin resistant Staphylococcus aureus', PLoS ONE, 9(8): e104561, doi:10.1371/journal.pone.0104561.
- (4) Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk susceptibility tests; approved standard – Twelfth edition. CLSI document M02-A12 (ISBN 1-56238-985-8 Print; ISBN 1-56238-986-6 Electronic). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2015.

Results

The *MecR1::Tn* knockout mutant remained resistant to β -lactam antibiotics with MIC-values for penicillin, oxacillin and cefoxitin ranging from 3 μ g/ml, 16 μ g/ml, and 16 μ g/ml, respectively. No significant difference in methicillin resistance was detected in comparison to the plasmid-cured UAS391-EryS strain with MIC-values 4 μ g/ml, 16 μ g/ml, and 24 μ g/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).

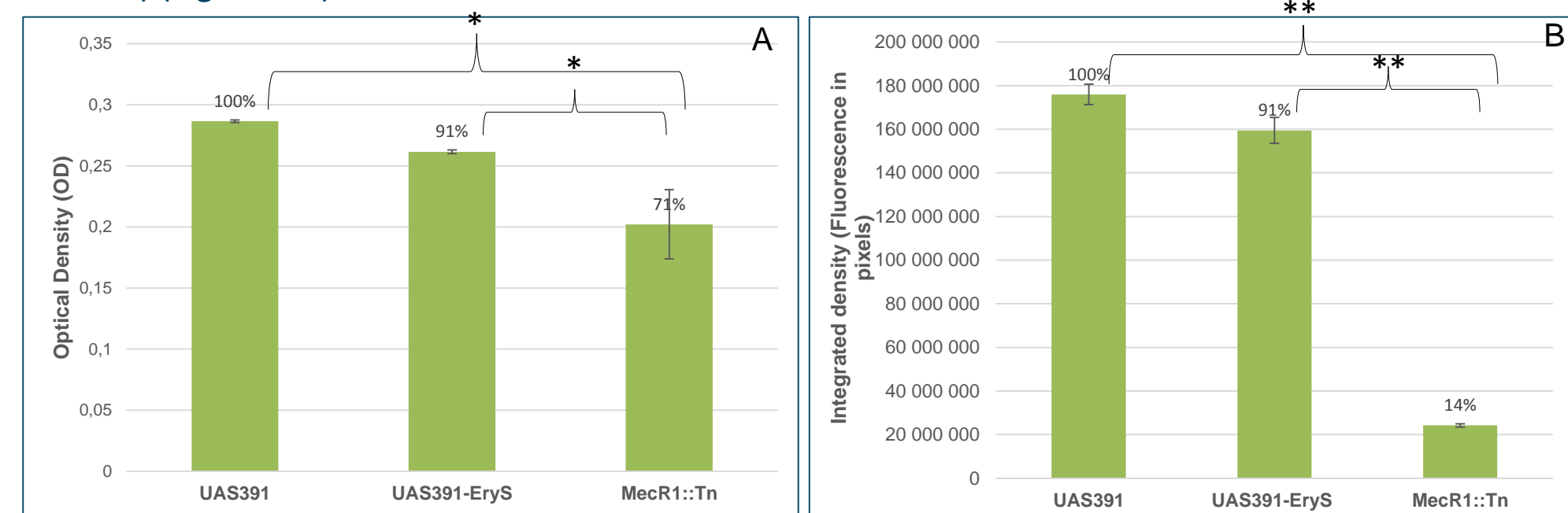


Fig. 3: (A) Formation of static biofilms by UAS391, UAS391-EryS, and *MecR1::Tn*. Biofilm-forming capacity of the strains was compared to the biofilm growth of a positive control (ATCC 6538) and a negative control (5374), while values were corrected for background absorption. Percentages are expressed 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.

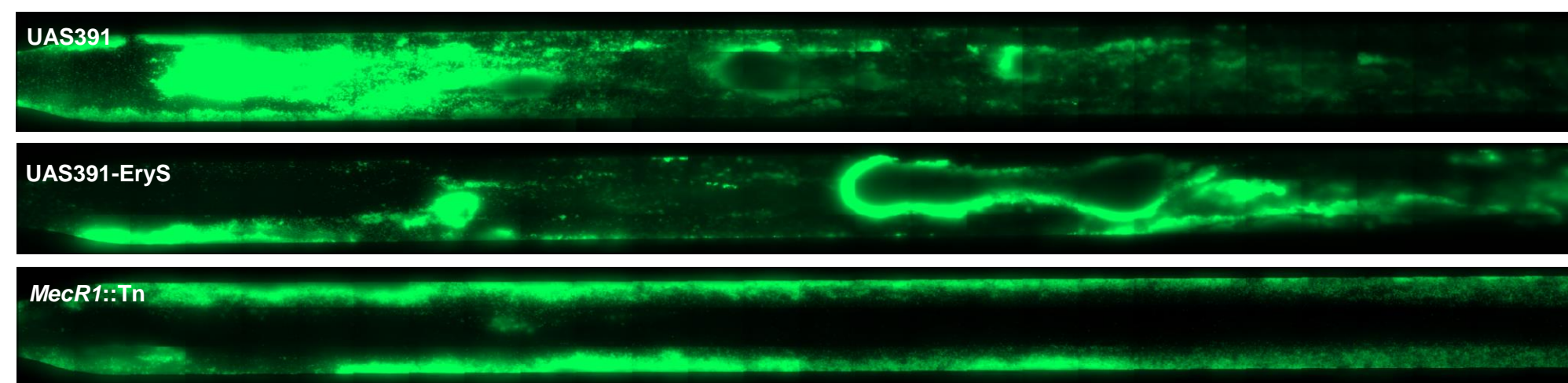


Fig. 4: Corresponding fluorescent microscopy images of flow biofilms.