Effect of stress hormone epinephrine on Pseudomonas aeruginosa biofilm formation

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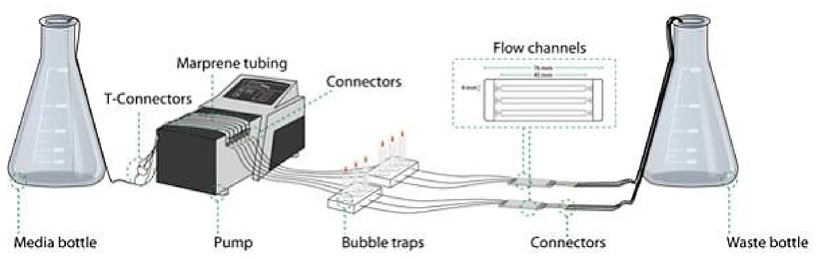
Laboratory of Microbiology Signals and Microenvironnement LMSM EA 4312,
Normandie Université, Univ. Rouen Normandie, Evreux, France. ***www.lmsm-lab.fr***

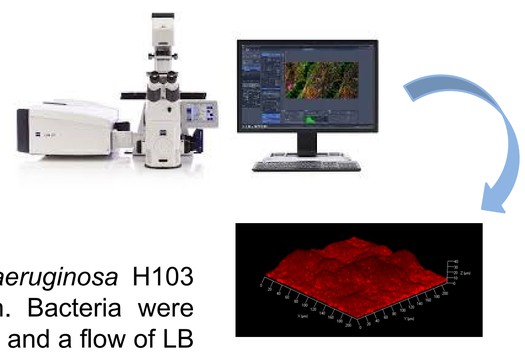
Introduction

Host signals such as hormones, neurotransmitters or immune system molecules have been shown to modulate bacterial physiology. Among them, catecholamines hormones epinephrine/norepinephrine, released by stress, physical effort or used therapeutically as inotrope were shown to affect bacterial behaviors of various Gram-negative bacteria. *Pseudomonas aeruginosa* is an opportunistic pathogen, often involved in nosocomial infections and responsible for chronic infections in immunocompromised patients, which is likely to be in presence of these hormones in human body.
Biofilm formation of *P. aeruginosa* is closely related to its virulence and is often implicated in chronic infections. We therefore decided to evaluate the effect of various concentrations of epinephrine on *P. aeruginosa* biofilm formation.

Methods

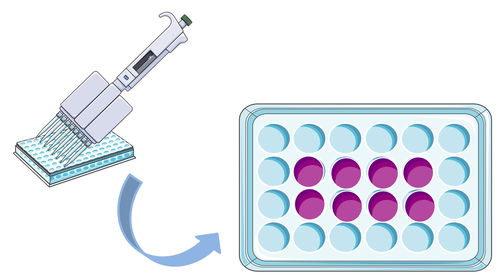
Dynamic Biofilms





Effect of epinephrine at different concentrations was examined on *P. aeruginosa* H103 biofilm formation under hydrodynamic conditions in a flow-cell system. Bacteria were allowed to attach to the glass side for 2h in presence or not of epinephrine, and a flow of LB medium (containing epinephrine) was then applied for 24h. Biofilm biovolume and architecture were monitored using Confocal Laser Scanning Microscopy (CLSM).

Static Biofilms



Adhesion and biofilm formation were also examined in static conditions. A 2h adhesion step was performed in presence or not of epinephrine in a saline solution. LB medium was then added and the plate was incubated 6h at 37 °C. Adhered cells or biofilm were stained with Cristal Violet and quantified by 595 nm absorbance reading.

Results

✦ Epinephrine enhanced *P. aeruginosa* adhesion

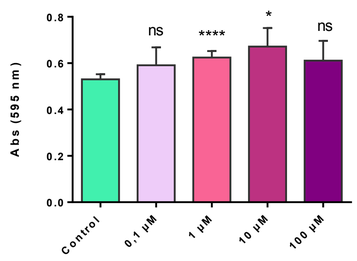


Fig. 1 : **Adhesion of *P. aeruginosa* H103 in presence of 0.1 to 100 µM of epinephrine.** Two-tailed *t*-test (* $p < 0.05$; *** $p < 0.001$; ns: not significant). Adhesion in microplate and crystal violet staining.

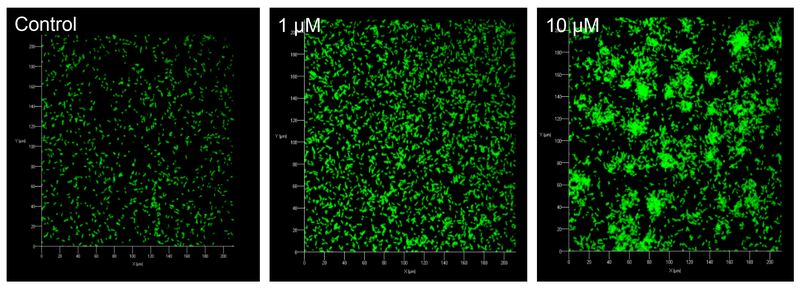


Fig. 2 : **Top views of *P. aeruginosa* H103 adhesion performed in flow cell system and exposed to 1 or 10 µM of epinephrine for 2h.** CLSM observations.

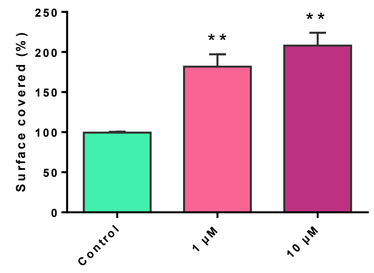


Fig. 3 : ***P. aeruginosa* H103 adhesion in presence of 1 or 10 µM of epinephrine.** Images analysis by ImageJ. Two-tailed *t*-test (** $p < 0.01$)

☞ *P. aeruginosa* H103 adhesion was significantly increased only when exposed to 1 and 10 µM of epinephrine. *P. aeruginosa* H103 displayed a coverage surface more important when treated with 1 µM of epinephrine (181% compared to control). With 10 µM, the coverage surface raised up to 207%, compared to the untreated bacteria.
Moreover, *P. aeruginosa* H103 seems to form aggregates when exposed to 10 µM of epinephrine

✦ *P. aeruginosa* biofilm formation was improved by epinephrine

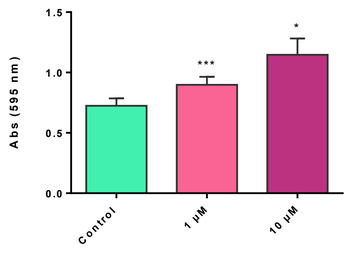


Fig. 4 : **Effect of epinephrine on *P. aeruginosa* H103 biofilm formation.** 6h biofilm performed in static conditions and crystal violet staining. Two-tailed *t*-test (* $p < 0.05$; *** $p < 0.001$).

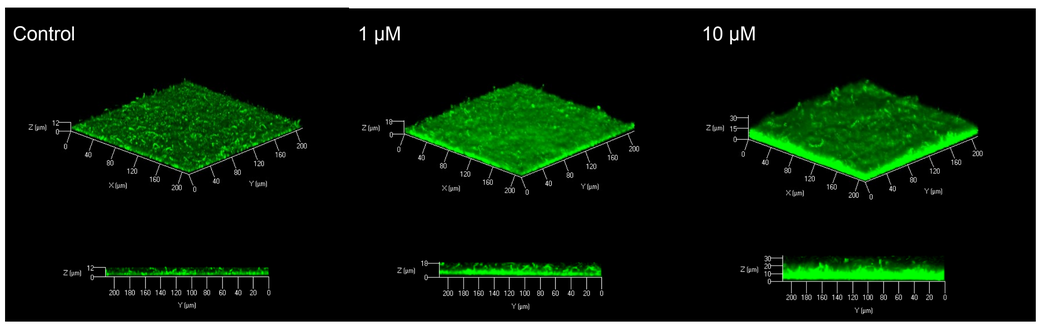


Fig. 5 : **3D and side views of *P. aeruginosa* H103 biofilms performed in dynamic system and exposed to 1 or 10 µM of epinephrine for 24h.** CLSM observations.

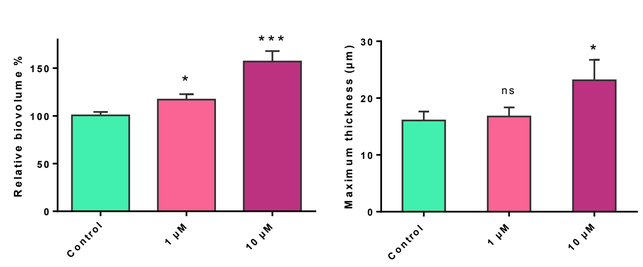


Fig. 6 : **Effect of epinephrine exposure on *P. aeruginosa* H103 biofilm biovolume and maximum thickness.** Images analysis by COMSTAT. Two-tailed *t*-test (* $p < 0.5$; *** $p < 0.001$; ns: not significant)

☞ Biofilm formation of *P. aeruginosa* H103, was found to be increased when continuously exposed to epinephrine and this effect depended on the hormone concentration used. A two-fold higher biovolume of the biofilm was seen when the bacteria were exposed to 10 µM of epinephrine. Moreover, microscopic image analysis also showed that epinephrine modified *P. aeruginosa* H103 adhesion on abiotic surface by inducing cells aggregation.

Conclusion

In this work, *P. aeruginosa* H103 seems to sense epinephrine stress hormone and respond by increasing its biofilm formation capacity. This result suggests that increase of epinephrine concentration in case of acute stress could promote biofilm formation of *P. aeruginosa*. A better understanding of these mechanisms and the identification of *P. aeruginosa* adrenergic putative sensor may be an interesting pathway to develop new antibacterial strategies against this clinical pathogen.

Contacts: sophie.rodrigues@univ-rouen.fr
nathalie.connil@univ-rouen.fr

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