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Adhesion and biofilms of *Pseudomonas aeruginosa* are increased under epinephrine stress

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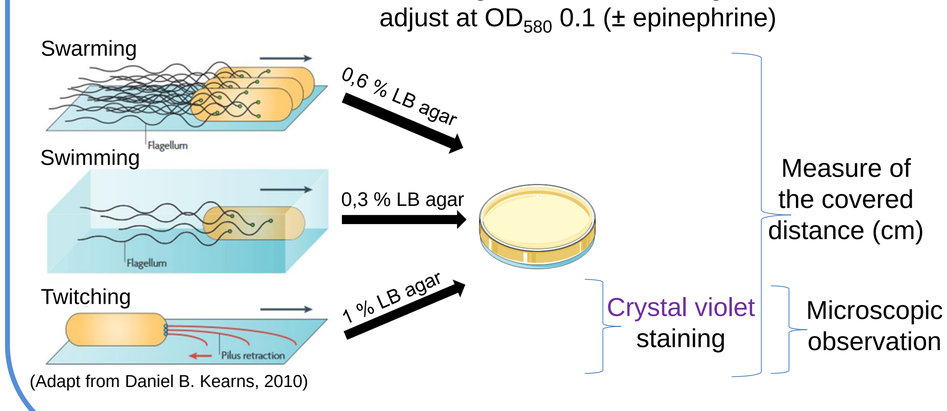
❖ Introduction

Microbial endocrinology is, nowadays, a well-known field, highlighted by Mark Lyte in the nineties. He showed that eukaryotic molecules, and notably catecholamines, were able to modulate the physiological behavior of the bacteria inside the human body. Since then, a lot of studies have shown that various substances can act on bacteria as hormones (Calam *et al*., 1980), neurotransmitters (Chevrot *et al*., 2006), and molecules of the immune system (Porat *et al*., 1991). Catecholamines, defined as hormones/neurotransmitters, comprise dopamine, norepinephrine and epinephrine. These molecules are released in case of stress, physical efforts and sometimes used in medical treatment. It has been shown that they are able to modify the mobility of some bacteria as *Vibrio harveyi* (Yang *et al*., 2014) and modulate the bacterial capacity to form biofilms in various species and mostly in *Escherichia coli* (Bansal *et al*., 2007). *Pseudomonas aeruginosa* is an opportunistic pathogen often found in hospital acquired-infections, responsible for many deaths in patients suffering from cystic fibrosis, and well-known for its antibiotics resistance, notably because of its great capacity to form strong biofilms. In this study, we have investigated the effect of the epinephrine stress molecule, on *P. aeruginosa* H103 motilities, adhesion, and biofilms formation in static and dynamic conditions.

1 ❖ Methods

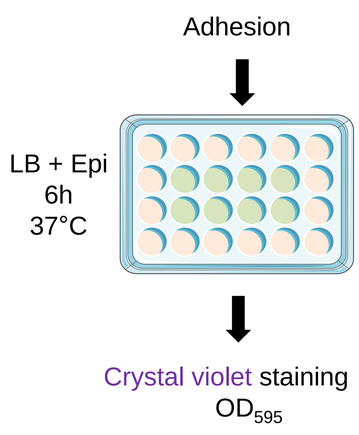
Motilities

From an overnight culture of *P. aeruginosa* H103 adjust at OD_{580} 0.1 (± epinephrine)

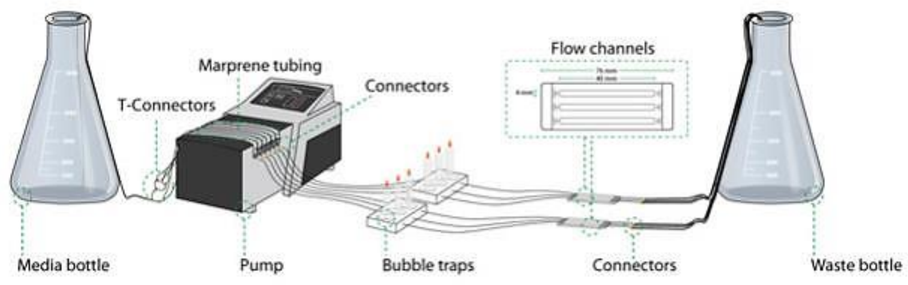


(Adapt from Daniel B. Kearns, 2010)

Biofilm in static condition

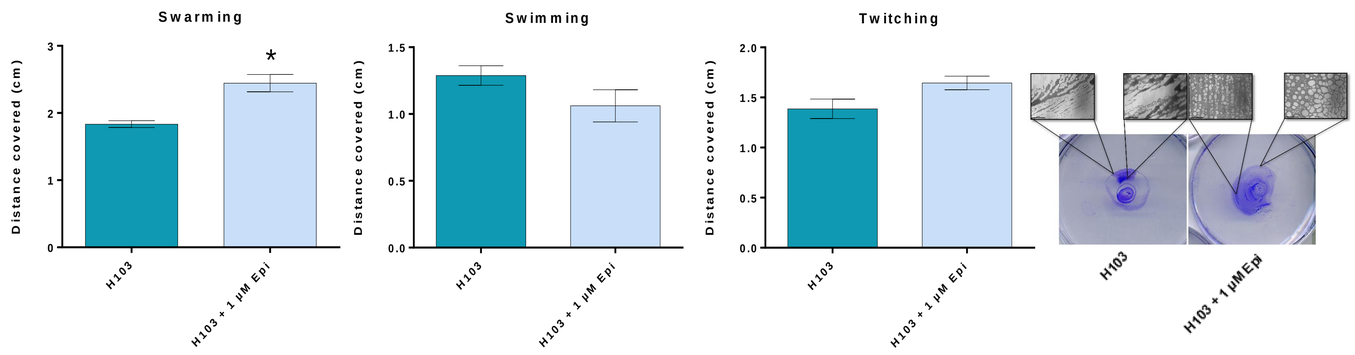


Flow-cell device for adhesion and biofilm in dynamic condition



Observation with confocal microscopy and analysis using COMSTAT2

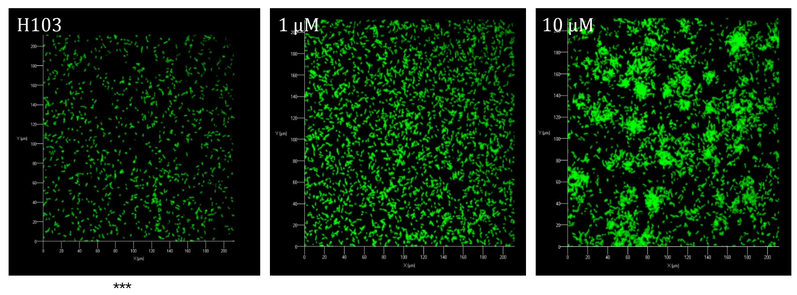
2 ❖ Motilities of P. aeruginosa exposed to epinephrine

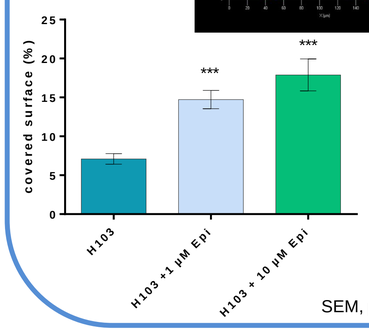


After exposure to 1 µM epinephrine, swarming of *P. aeruginosa* H103 was increased. Conversely, no significant variation of the distance covered by the bacteria were observed for swimming and twitching motilities, but the aspect of twitching changed. These both motilities (swarming and twitching), affected by epinephrine, involved the type IV pili, which is also necessary for adhesion, the first step of biofilm formation.

SEM, paired t-test : * *p-value* <0,05

3 ❖ Adhesion after epinephrine treatment

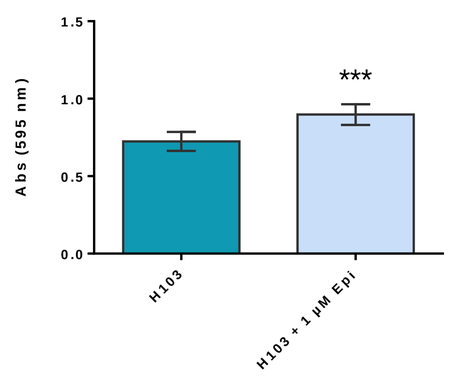




Epinephrine (1 and 10 µM) increased the adhesion of *P. aeruginosa* H103. The covered surface by the bacteria was almost twice more important when exposed to 1 µM compared to the control, and increased by 2.5 fold with 10 µM of epinephrine.

SEM, paired t-test : *** *p-value* <0,001

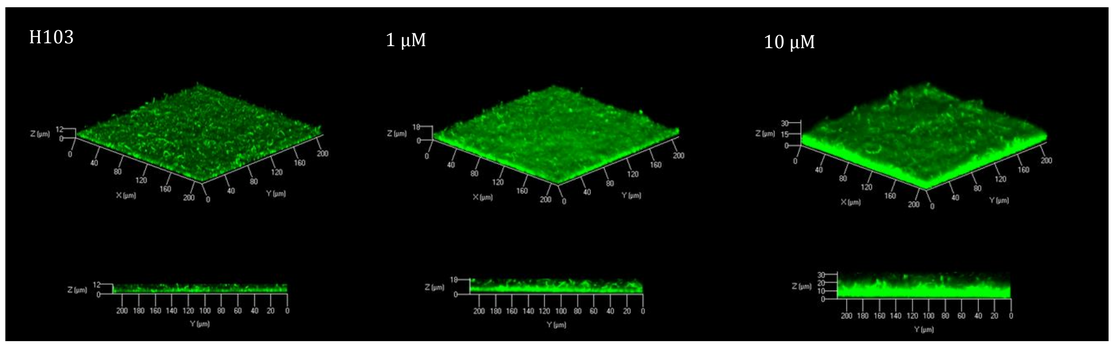
4 ❖ Biofilms in static condition

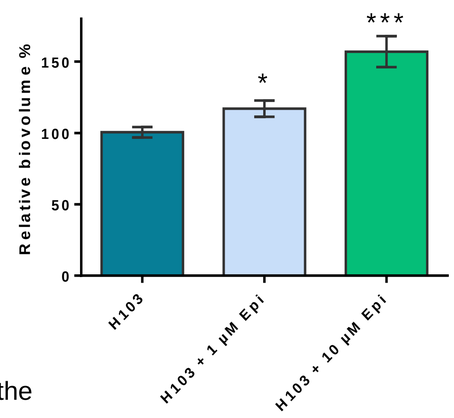


Epinephrine treatment (1 µM) led to an increase of the early biofilm production (6h) in *P. aeruginosa* H103.

SEM, paired t-test : *** p-value <0,001

5 ❖ Biofilms in dynamic condition





Biofilms in dynamic condition were used to mimic the *in vivo* environment. Epinephrine led to an increase of the biofilms relative biovolume of 117% with 1 µM of the molecule and 157% with 10 µM. The thickness of the biofilms was also modified.

SEM, paired t-test : * *p-value* < 0,05 *** *p-value* <0,001

❖ Conclusion

This study showed that epinephrine can modulate the physiology of the opportunistic pathogen *P. aeruginosa* H103. Motilities involved in its pathogenicity were impacted by epinephrine treatment. The type IV pili are involved in swarming and twitching motilities and have an important role in the adhesion of this strain. In dynamic condition with continuous flow, an increase of the biovolume and thickness of *P aeruginosa* H103 biofilms was observed, mostly with 10 µM of epinephrine. Thus, *P aeruginosa* H103 seems to be able to sense epinephrine and may possesses putative sensors. Potential candidates homologous to QseC, the adrenergic sensor of *E.coli* have been found by bioinformatic analysis (data not shown). Further experiments will aim to decipher the molecular mechanism implicated in epinephrine sensing and biofilm formation in *P aeruginosa* H103.

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