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Effect of stress hormone Epinephrine on *Pseudomonas aeruginosa* biofilm formation

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**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 an 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**

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 slide 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).

**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.

*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 (18%) compared to control. With 10 µM, the coverage surface raised up to 267% 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. Biofilm performed in static conditions and crystal violet staining. Two-tailed t-test (*p* > 0.05; ***p* < 0.001).

Biofilm formation of *P. aeruginosa* H103, was found to be increased when continuously exposed to epinephrine and this effect depend 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.

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