



HAL
open science

The absence of OprF increased biofilm formation through variation of the c- di-GMP level in *Pseudomonas aeruginosa*

Emeline Bouffartigues, Joana A Moscoso, Rachel Duchesne, Thibaut Rosay, Laurène Fito-Boncompte, Gwendoline Gicquel, Olivier Maillot, Magalie Bénard, Alexis Bazire, Gerald Brenner-Weiss, et al.

► To cite this version:

Emeline Bouffartigues, Joana A Moscoso, Rachel Duchesne, Thibaut Rosay, Laurène Fito-Boncompte, et al.. The absence of OprF increased biofilm formation through variation of the c- di-GMP level in *Pseudomonas aeruginosa*. Pseudomonas Conference, 2017, Washington, United States. hal-02366394

HAL Id: hal-02366394

<https://hal-normandie-univ.archives-ouvertes.fr/hal-02366394>

Submitted on 15 Nov 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

The absence of *OprF* increased biofilm formation through variation of the *c*-di-GMP level in *Pseudomonas aeruginosa*.

Emeline Bouffartigues¹, Joana A. Moscoso², Rachel Duchesne¹, Thibaut Rosay¹, Laurène Fito-Boncompte¹, Gwendoline Gicquel¹, Olivier Maillot¹, Magalie Bénard³, Alexis Bazire⁴, Gerald Brenner-Weiss⁵, Olivier Lesouhaitier¹, Patrice Lerouge⁶, Alain Dufour⁴, Nicole Orange¹, Marc Feuilleley¹, Joerg Overhage⁵, Alain Filloux² and Sylvie Chevalier¹

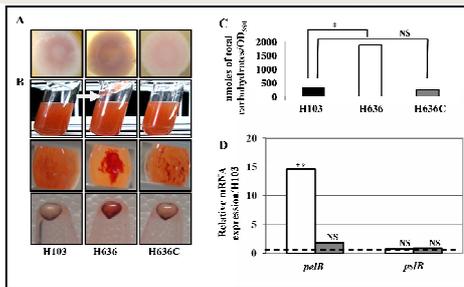
¹Laboratory of Microbiology Signals and Microenvironment, LMSM EA4312, University of Rouen (France)
²Centre for Molecular Microbiology and Infection, Division of Cell and Molecular Biology, Imperial College, London (UK)
³Cell Imaging Platform of Normandy (Primacen), IRiB, Faculty of Sciences, University of Rouen (France),
⁴Laboratory of Marine Biotechnology and Chemistry, EA 3884, South Britain University, IUEM, Lorient (France),
⁵Karlsruhe Institute of Technology (KIT), Karlsruhe (Germany),
⁶Glyco MeV Laboratory, University of Rouen, Normandie University (France).

Introduction

Pseudomonas aeruginosa is well known to be a human opportunistic pathogen able to adapt to many environmental conditions. The first cellular structure in contact with the environment is the outer membrane. In *Pseudomonas* genus, *OprF* is the major outer membrane protein. It is an aspecific porin allowing the diffusion of several types of small polar molecules. *OprF* is also a pleiotropic protein involved in other numerous cellular functions, as adhesion on biotic or abiotic surfaces, cell wall resistance and virulence of *P. aeruginosa*. Through a multiphenotypic study, we show here that the lack of *OprF* increased biofilm formation through modulation of the *c*-di-GMP level.

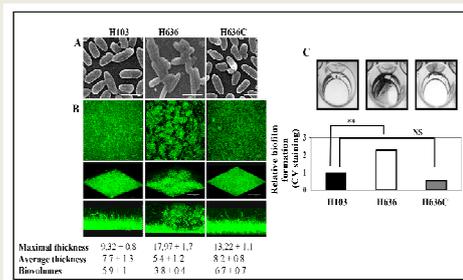
The lack of *OprF* affected biofilm-related phenotypes

The lack of *OprF* (H636 strain) increased exopolysaccharide (EPS) production



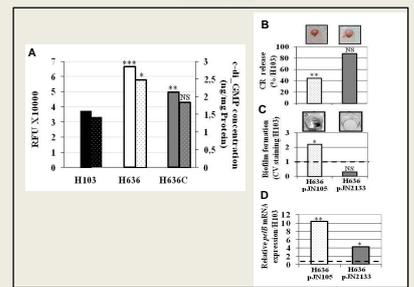
(A) Colony morphology observed on Congo Red containing LB agar plates
 (B) H103, H636 and H636C were grown in LB containing Congo Red for 24 h at 37 °C
 - Top images: A slime production is indicated by a grey arrow in case of H636
 - Middle images: Congo Red colored aggregates were observed at the bottom of the cultures
 - Bottom images: Congo Red binding of pelleted cells (10⁹)
 (C) Cell-associated carbohydrates were quantified by gas chromatography.
 (D) Relative *pel* and *psI* mRNA expression in H636 (white bars) and H636C (grey bars) relatively to H103 (dashed black line).
 Each experiment was performed at least three times. For quantitative assay, statistics were done by unpaired t test. **p*<0.05, ***p*<0.01, NS not significant.

The lack of *OprF* increased biofilm formation.



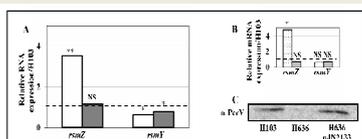
(A) Scanning electron micrograph images of H103, H636 and H636C (enlargement: 4 500 x; white bars represent 2 µm).
 (B) Biofilms of H103, H636 and H636C grown in flow cells for 24 h and examined by CLSM. Top images, top views (x, y, z-plane); intermediate images, cross section views; bottom images, 3D-modelizations (x, y, z, axes). Maximal, average thicknesses (in µm), and biovolumes (in µm³/µm²) were determined by COMSTAT analyses. The averages and standard deviations were calculated from 10 samples.
 (C) Microtiter grown biofilm formed by H103, H636 and H636C. Biofilms were quantified by measuring absorbance at 595 nm after crystal violet staining. At least six assays were performed for each strain. Statistics were achieved by unpaired t test. ***p*<0.01, NS not significant.

The *c*-di-GMP level is increased in the *oprF* mutant.



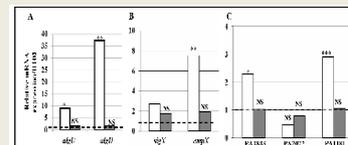
c-di-GMP level evaluation using the *cdiA-gfp* reporter fusion (full bars; Rybtke *et al.*, 2010), and LC/MS *c*-di-GMP level measurement (dotted bars) in H103 (black bars), H636 *oprF* mutant (white bars), and H636C (grey bars).
 (B) Exopolysaccharide production by H636 harboring pJN105 (dotted white bar) or pJN2133 (grey bar), using the Congo red release assay. H103 EPS production has been fixed at 100%.
 (C) Biofilms formed by H636 relatively to H103 harboring pJN105 (dotted white bar) or pJN2133 (grey bar), were quantified by crystal violet staining.
 (D) qRT-PCR assays on *pelB* expression in H636 harboring pJN105 (dotted white bars) or pJN2133 (grey bars) relatively to H103.
 (B), (C), (D): Results are given as the ratio H636/H103. Dashed black line: H103 biofilm production (C) or *pelB* expression (D) were fixed at 1.
 Each experiment was performed at least three times. Statistics were achieved by unpaired t test: **p*<0.05, ***p*<0.01, ****p*<0.001, NS not significant.

The lack of *OprF* increased *rsmZ* expression through modulation of the *c*-di-GMP level.



(A) Relative mRNA expression of the small non coding RNAs *rsmZ* and *rsmY* in H636 mutant (white bars) and *oprF* complemented mutant strain H636C (grey bars), relatively to H103 (dashed black line).
 (B) Relative expression of *rsmZ* and *rsmY* in H636 *oprF* mutant strain harboring pJN105 (dotted white bars) or pJN2133 (grey bars) relatively to H103 (dashed black line).
 (C) Western blot analyses on T3SS α-PcrV in *P. aeruginosa* H103, H636 and H636 harboring pJN2133.
 Each experiment was performed at least three times. Statistics were done by unpaired t test. **p*<0.05, ***p*<0.01, NS not significant.

The ECF sigma factor *SigX* is active in the *oprF* mutant.



Relative mRNA expression of *algU*, *algD* (A), *sigX* and *cmpX* (B), and of the three putative *SigX*-dependent diguanylate cyclases encoding genes (C) in *P. aeruginosa* H636 (white bars) and H636C (grey bars) relatively to H103 (dashed black line).
 Each experiment was performed at least three times. Statistics were achieved by unpaired t test. **p*<0.05, ***p*<0.01, ****p*<0.001, NS not significant

Conclusions

We have shown that the absence of *OprF* led to increase biofilm formation and EPS production. This phenotype was associated to *pel*, but not *psI* overexpression. Accordingly, the *c*-di-GMP pool level was elevated. By artificially decreasing the *c*-di-GMP level, we observed that both the biofilm formation and *pel* expression were restored in the *oprF* mutant. In addition we observed that expression of PA4843 and PA1181, encoding diguanylate cyclases, was strongly increased in the *oprF* mutant at least partly through enhanced activity of the extracytoplasmic function sigma factor *SigX*. Finally, expression of *rsmZ*, but not *rsmY* was increased in the *oprF* mutant through *c*-di-GMP level alteration.