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

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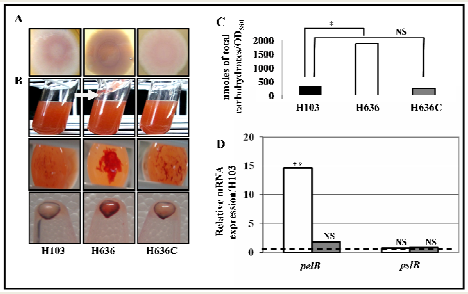
Introduction

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.**

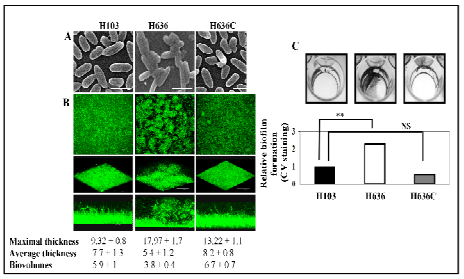
The lack of OprF affected biofilm-related phenotypes

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



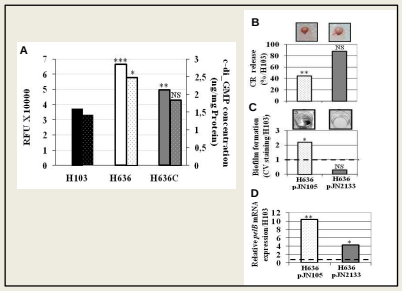
(A) Colony morphology observed on Congo Red containing LB agar plates
(B)H103, H636 and H636C were grown in LB containing Congo Red for 24 h at 37 C
- Top images: A slime production is indicated by a grey arrow in case of H636
- Middle images: Congo Red colored aggregates were observed at the bottom of the cultures
- Bottom images: Congo Red binding of pelleted cells (10^9)
(C) Cell-associated carbohydrates were quantified by gas chromatography.
(D) Relative *pel* and *psl* 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.



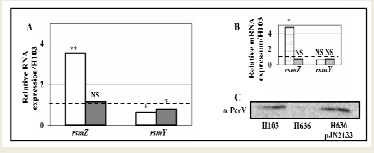
(A) Scanning electron micrograph images of H103, H636 and H636C (enlargement: 4 500 x; white bars represent 2 µm).
(B) Biofilms of H103, H636 and H636C grown in flow cells for 24 h and examined by CLSM. Top images, top views (x, y-plane); intermediate images, cross section views; bottom images, 3D-modelizations (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.



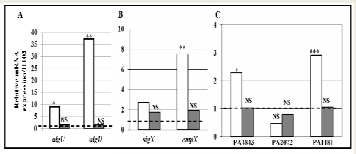
c-di-GMP level evaluation using the cdrA-gfp reporter fusion (full bars; Rybtke *et al*., 2010), and LC/MS c-di-GMP level measurement (dotted bars) in H103 (black bars), H636 *oprF* mutant (white bars), and H636C (grey bars).
(B) Exopolysaccharide production by H636 harboring pJN105 (dotted white bar) 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 cristal 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 pel 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 *psl* 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.