Type VI secretion system and expression of flagellar class IV genes in the Pseudomonas fluorescens MFE01 strain

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INTRODUCTION

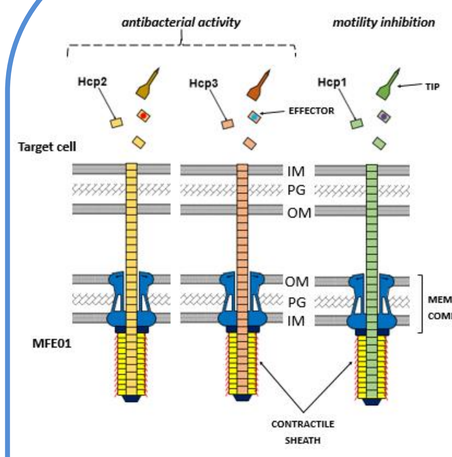


Figure n°1 : Schematic representation of *P. fluorescens* MFE01 T6SS (adapted from **Gallique M. *et al.*, 2017b**).

Type 6 Secretion Systems (T6SS) are contractile nanomachines involved in the secretion of effectors directly in prey cells (bacteria or eukaryotic cells). These systems are composed of a membrane complex, a baseplate, and a contractile tail essential for the T6SS effectors/toxin loading . Effectors are transported by a tube with overhead tip that is propelled by a tail contraction. Nowadays, the most studied T6SS effectors exhibit antibacterial activity, by acting on peptidoglycan, lipid membrane or DNA (**Durand *et al.*, 2014**).

Pseudomonas fluorescens MFE01 is an environmental strain, that possess a functional T6SS. The genes encoding the membrane complex, baseplate and sheath (*tssB/C*) of this contractile nanomachine are clustered in contrary to genes encoding the tube. There are at least three different orphan genes encoding this tube (*hcp1*/*2* and *3*) involved in the formation of different variations of T6SS (**Figure 1**). Two different *hcp* genes (*hcp2* and *hcp3*) are involved in antibacterial activity (**Decoin *et al.*, 2014** ; **Gallique *et al.*, 2017a**), unlike the other one (*hcp1*) that acts on bacterial motility (**Decoin *et al.*, 2015**).

Bacterial motility is allowed thanks to an extracellular appendage, the flagella. This complex structure consists of basal body and long filament (polymer of flagellin) connected by a "hook" (**Figure 2**). The expression of the flagellum filament occurs after the hook reaches a correct length. In this case, the specific sigma factor FliA, is released from FlgM protein, that is secreted throught an incomplete flagellar apparatus. The free FliA protein is able to link the RNA polymerase to permit expression of the flagellar class IV genes including flagellin monomer **(Dasgupta *et al.*, 2003)**.

We have already show that the deletion of *hcp1* gene (tube protein) or *tssC* gene (sheath protein), lead on the first hand, to the loss of swimming motility and flagellin secretion. On the second hand, MFE01 is able to inhibit *P. fluorescens* MFN1032 target cell motility, thanks to its T6SS formed by Hcp1 (**Decoin *et al.*, 2015**). This work focuses on identification and characterization of new T6SS effector, associated with Hcp1 (tube protein).

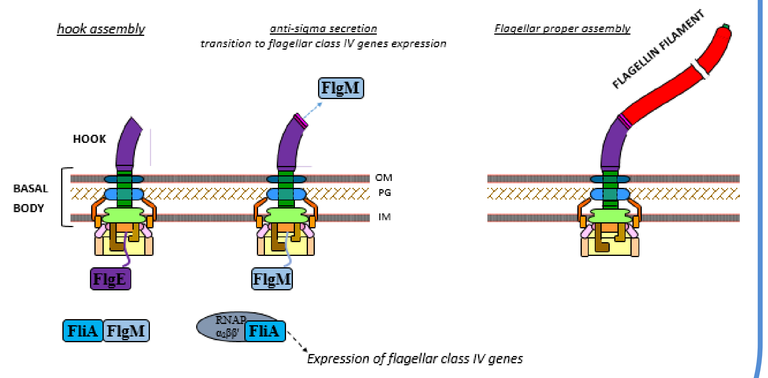


Figure n°2 : Schematic representation of flagellar class IV genes expression switch (unpublished).

1 ELECTRONIC MICROSCOPY

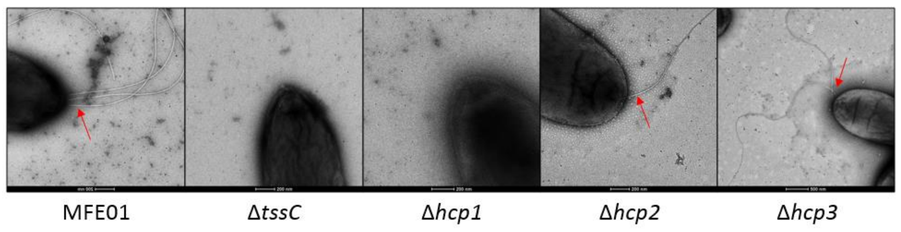


Figure n°3 : Electronic microscopy of MFE01 and different T6SS deletion mutants. Red arrows show flagellar filament (**Gallique *et al.***, in progress).

MFE01, Δ*hcp2* and Δ*hcp3* possess flagella, contrary to Δ*tssC* and Δ*hcp1*

↳ The lack of T6SS formed with Hcp1 disturbs flagella formation.

2 TRANSCRIPTOMIC ANALYSIS

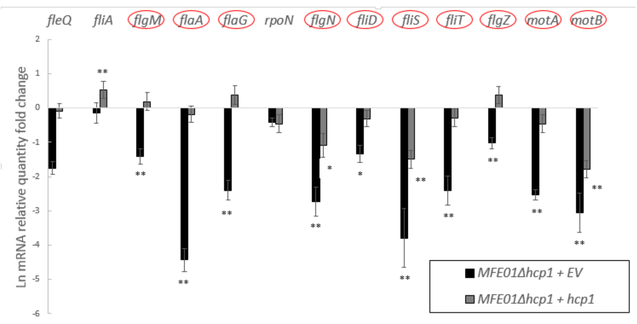


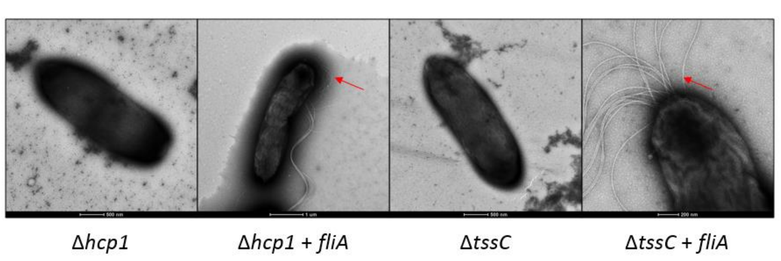
Figure n°4 : Expression of flagellar genes in MFE01 Δ*hcp1* +EV/+*hcp1* compared to wild type strain containing empty vector (EV). Data shown represent the mean ± SEM. Significances of differences between mean values were assessed using Mann-Whitney test (n=6) with significance set as *P<0.05; **P<0,005. Red circles represent genes that are expressed by FliA sigma factor. (**Gallique *et al.***, in progress).

The expression of flagellar class IV genes is disturbed in MFE01Δ*hcp1.*

↳ The lack of T6SS formed with Hcp1 inhibits the sigma factor FliA activity.

3 IMPACT OF THE OVEREXPRESSION OF SIGMA-FACTOR (FLIA)

A.



B.

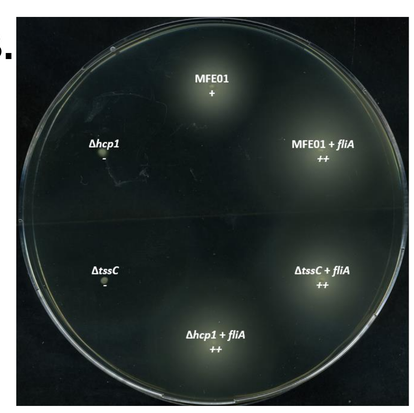


Figure n°5 : Phenotypes analysis of Δ*hcp1* or Δ*tssC* with or without *fliA* overexpression. (**A.**) Electronic microscopy. Red arrows show flagellar filament **(B.)** Motility on LB 0,3% agar plate (**Gallique *et al.***, in progress).

The overexpression of *fliA* gene restores the production of flagella in Δ*hcp1/tssC* and by the way the "swimming" motility.

↳ Concerning motility, only FliA activity is disturbed by the lack of T6SS formed with Hcp1.

4 FLGM SECRETION

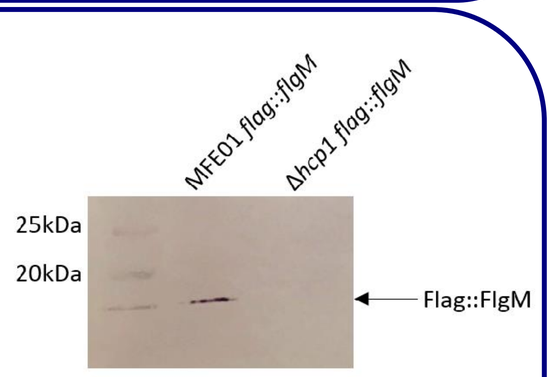


Figure n°6 : Western blot on supernatant proteins of MFE01 or Δ*hcp1* after a translational fusion of *flag* sequence with *flgM* gene. The anti-Flag primary antibody, and a secondary antibody coupled with alkaline phosphatase were used to highlight Flag-FlgM protein. (unpublished).

FlgM is secreted only in wild type supernatant and this result is confirmed by LC-MS-MS analysis.

↳ The lack of T6SS formed with Hcp1 blocks the secretion of the anti-sigma factor FlgM. FliA is not free in the cytoplasm of Δ*hcp1* mutant, and there is no expression of flagellar class IV genes.

CONCLUSION

Here we show that the deletions of *hcp1* or *tssC* genes induce the loss of flagella and decrease the expression of flagellar class IV genes, that is regulated by the sigma factor FliA. The overexpression of this sigma factor, in Δ*hcp1* or Δ*tssC* strains, restores the wild type phenotype such as flagellin secretion (data not shown), flagella proper assembly and by the way "swimming" motility. By western blot and LC-MS-MS analysis, we show that the anti-sigma factor FlgM is not secreted by Δ*hcp1* deletion mutant and could impact free FliA in the cytoplasm. This result may explain the less expression of flagellar class IV genes.

On the first hand, we assume that anti-sigma factor FlgM could be secreted through the T6SS formed with Hcp1 in *Pseudomonas fluorescens* MFE01. This hypothesis will be checked by analyzing the effect of FlgM overexpression on prey bacteria motility using strains that are immobilized when they are co-cultivated with MFE01 (**Decoin V. *et al.*, 2015**). On the other hand, we forecast that the complex "sigma-factor FliA/anti-sigma-factor FlgM" is stabilized by T6SS deletion mutation. In order to test this hypothesis we will study phenotypes of the double mutant Δ*hcp1*- Δ*flgM*. To identify the toxin that is secreted through T6SS formed by Hcp1 protein, we will perform interactomic studies using translational fusion of *flag* sequence on *hcp1* and *flgM* genes.

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Supported by :

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