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

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INTRODUCTION

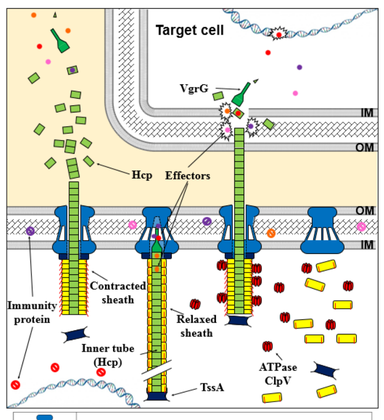

Figure n°1 : Schematic representation of T6SS (adapted from **Gallique M. *et al.*, 2017b**).

T6SSs are macromolecular machineries that are utilized by bacteria to inject diverse effectors into prokaryotic and/or eukaryotic cells by using a contractile sheath (TssB/C). These systems are constituted by a membrane complex, a baseplate and a tip that is expelled in association with effectors (**figure n°1**). Nowadays, the most studied effectors exhibit antibacterial activity acting on peptidoglycan, lipid membrane or DNA.

The *Pseudomonas fluorescens* MFE01 strain possesses a functional T6SS (**Decoin *et al.*, 2014**). All genes encoding T6SS membrane complex, baseplate and the sheath are clustered. The presence of three different orphan genes encoding the tube (*hcp1, hcp2*, *hcp3*) allows to provide various declinations of T6SS. The T6SSs containing Hcp2 or Hcp3 exhibit an antibacterial activity (**Decoin *et al.*, 2014**; **Gallique *et al.*, 2017**), contrary to the T6SS formed by Hcp1 (**Decoin *et al.*, 2015**) that acts on bacterial motility. We already showed that the deletion of *hcp1* gene suppresses MFE01Δ*hcp1* motility and by the way no flagellin is secreted in this deletion mutant.

Flagella are extracellular appendages, formed by a basal body and a long filament (polymer of flagellin) connected by a hook (FlgE) (**figure n°2**) that are assembled in a four-tiered transcriptional regulatory in *Pseudomonas* (**Dasgupta N. *et al*, 2003**). Briefly, genes that are clustered in class I to class III permit the expression of the basal body and the hook, whereas class IV genes are involved in the filament and flagellar motor production. In order to obtain a functional flagella, the switch between the expression of class III to class IV genes occurs when the hook achieves a correct length. In that case, the anti-sigma factor (FlgM) is secreted through incomplete flagellar apparatus, releasing the sigma factor FliA, that permits the expression of class IV genes. Here we study different deletion mutants (phenotypes, proteomic and transcriptomic analysis) to identify T6SS and flagellar cross-talk.

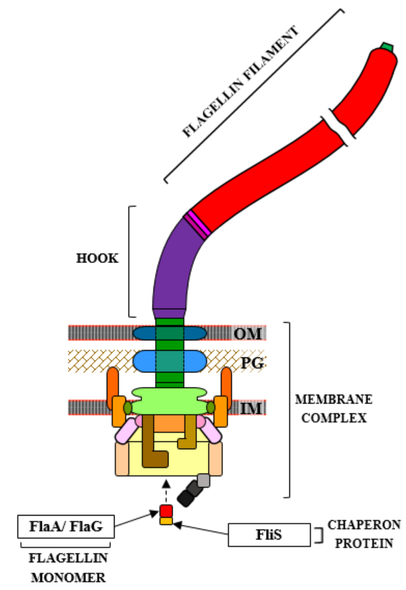

Figure n°2 : Schematic representation of flagellum (unpublished).

1 ELECTRONIC MICROSCOPY

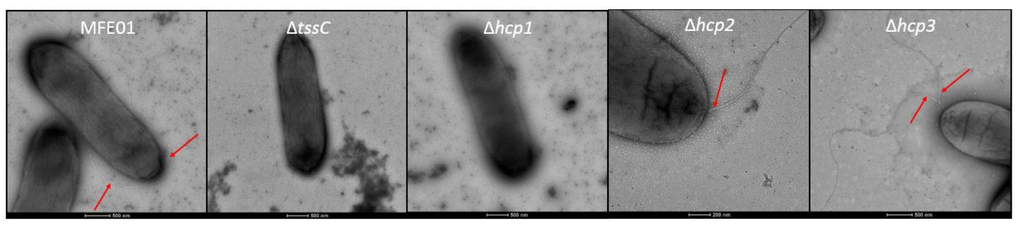

Figure n°3 : Electronic microscopy of MFE01, and different T6SS deletion mutants. Red arrows show flagellar filament (**Gallique *et al.***, in progress).

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

The lack of T6SS formed with Hcp1, in Δ*hcp1* or Δ*tssC* deletion mutants, disturbs flagellar formation.

2 TRANSCRIPTOMIC ANALYSIS

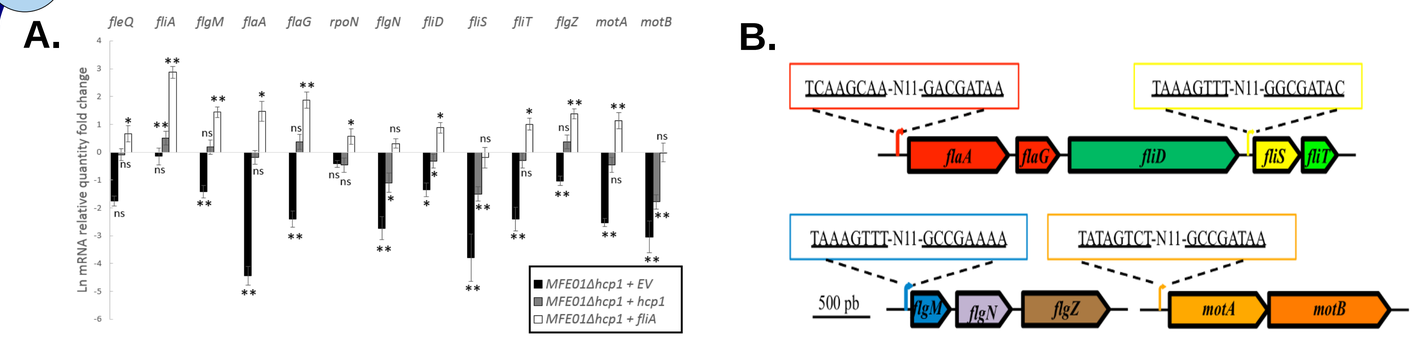

Figure n°4 : Expression of flagellar genes in MFE01 Δ*hcp1* +EV/+*hcp1*/ +*fliA* in comparison to wild type strain containing empty vector (EV; **A.**). Data shown represent the mean ± SEM. Significances of differences between mean values were assessed using Mann-Whitney test (n=6) with significance set as ∗P < 0.05; **P<0,005; ns : no significance. Identification of genes regulated by the sigma-factor FliA (**B.**) (**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)

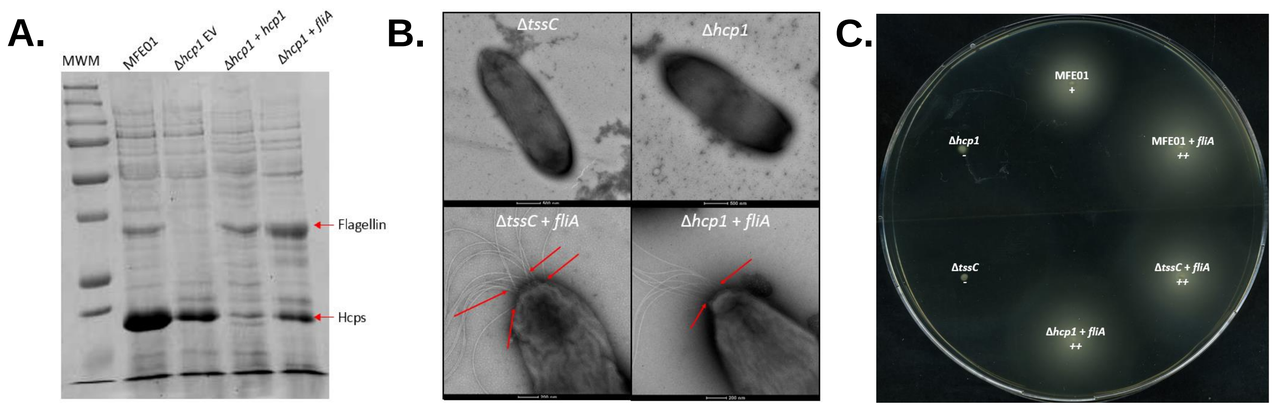

Figure n°5 : Phenotypes analysis of Δ*hcp1* or Δ*tssC* that overexpress *fliA.* (**A.**) SDS-PAGE on supernatant proteins (**B.**) Electronic microscopy. Red arrows show flagellar filament **(C**.) Motility on LB 0,3% agar plate (**Gallique *et al.***, in progress).

The overexpression of *fliA* gene restores the secretion of flagellin in Δ*hcp1* supernatant, allowing the production of flagella 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

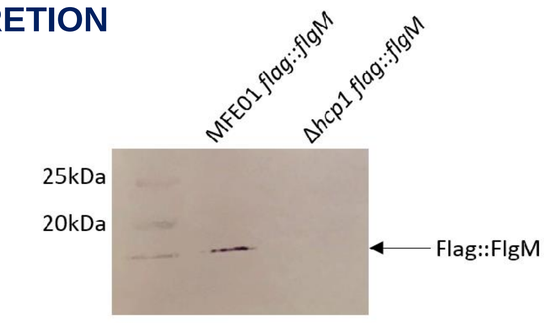

Figure n°6 : Western blot on supernatant protein of MFE01 or Δ*hcp1* after a translational fusion of *flag* sequence with *flgM* gene. The anti-Flag primary antibody, and a secondary antibody coupled with alkaline phosphatase was used to highlight Flag-FlgM protein. (unpublished).

FlgM is secreted only in wild type supernatant and this result has been upheld by LC-MS-MS.

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, 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 that 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*.

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Supported by

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