

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

Mathilde Bouteiller, M. Gallique, A. Kosta, J. Hardouin, Y. Konto-Ghiorghi, Corinne Barbey, X. Latour, A. Chane, M.G.J Feuilloley, A. Mérieau

▶ **To cite this version:**

Mathilde Bouteiller, M. Gallique, A. Kosta, J. Hardouin, Y. Konto-Ghiorghi, et al.. Type VI secretion system and expression of flagellar class IV genes in the Pseudomonas fluorescens MFE01 strain. Bacterial Protein Export 2018, Sep 2018, Louvain, Belgium. hal-02386911

HAL Id: hal-02386911

https://normandie-univ.hal.science/hal-02386911

Submitted on 29 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.

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

Bouteiller Mathilde[1], Gallique Mathias[1], Kosta Artemis[2], Hardouin Julie[3], Konto-Ghiorghi Yoan[1], Barbey Corinne[1], Latour Xavier[1], Chane Andréa[1], Feuilloley Marc[1] and Merieau Annabelle[1]

[1]Laboratory of Microbiology Signals and Microenvironnement LMSM EA 4312, Normandie Université, Univ. Rouen Normandie, Evreux, France. www.lmsm-lab.fr

[2] Plateforme de Microscopie de l'Institut de Microbiologie de la Méditerranée IMM FR 3479, Institut

[3]PISSARO proteomics facility, Université de Rouen, F-76821, Mont-Saint-Aignan, France

INTRODUCTION

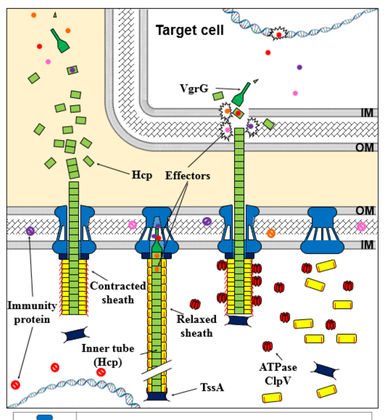


Figure n°1 : Schematic representation of T6SS (adapted from **Gallique M. *et al.*, 2017b**).

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.

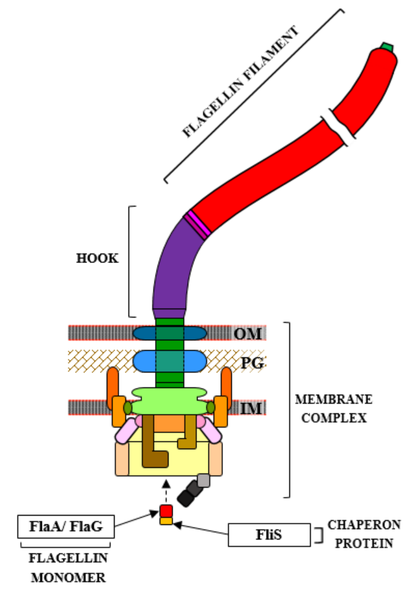


Figure n°2 : Schematic representation of flagellum (unpublished).

1 ELECTRONIC MICROSCOPY

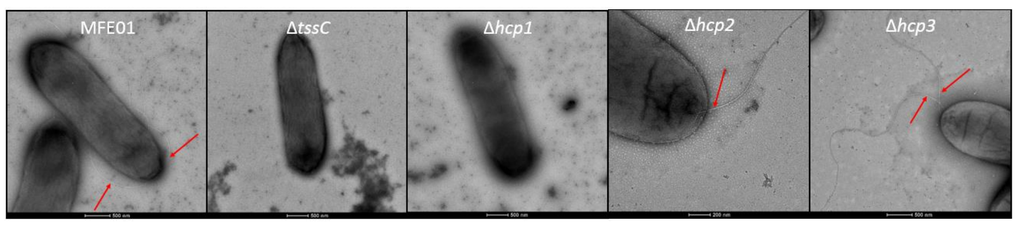


Figure n°3 : Electronic microscopy of MFE01, and different T6SS deletion mutants. Red arrows show flagellar filament (**Gallique *et al.***, in progress).

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

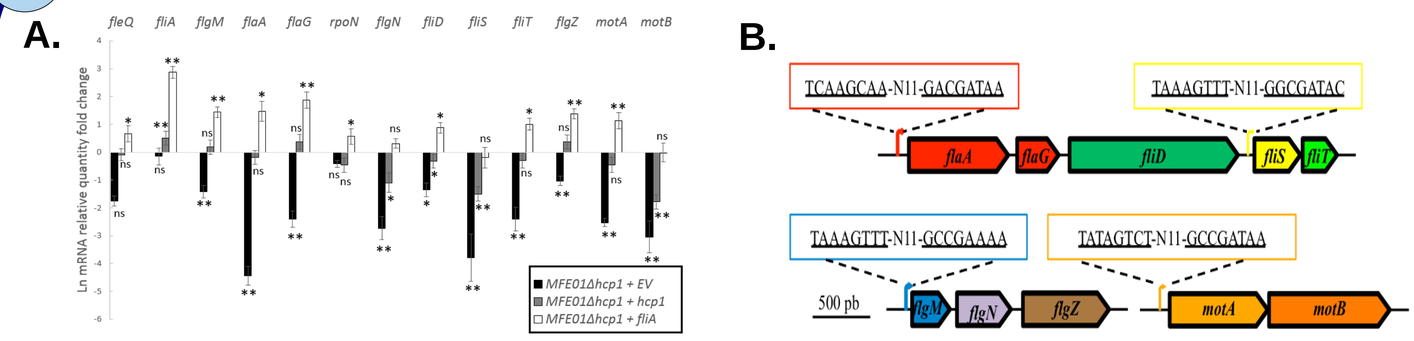


Figure n°4 : Expression of flagellar genes in MFE01 Δ*hcp1* +EV/+*hcp1*/ +*fliA* in comparison to wild type strain containing empty vector (EV; **A.**). Data shown represent the mean ± SEM. Significances of differences between mean values were assessed using Mann-Whitney test (n=6) with significance 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)

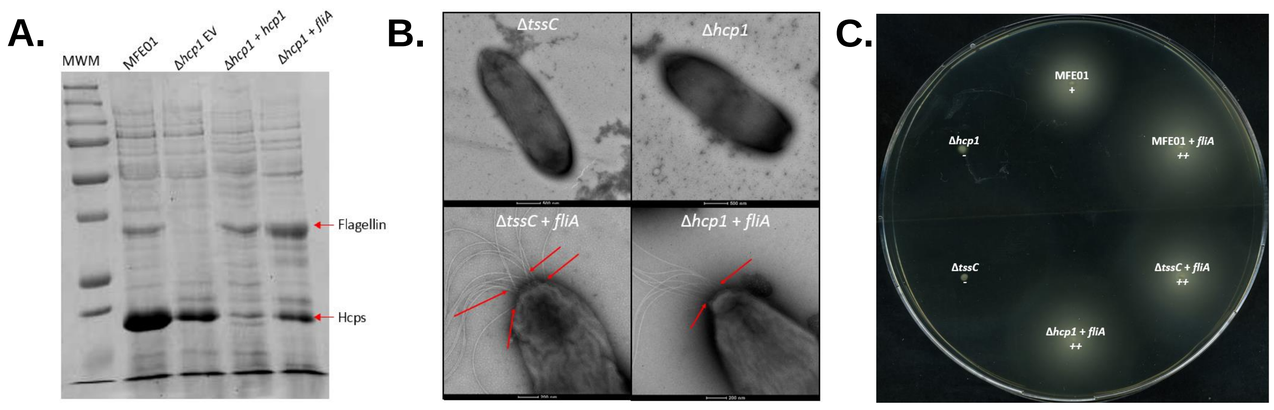


Figure n°5 : Phenotypes analysis of Δ*hcp1* or Δ*tssC* that overexpress *fliA.* (**A.**) SDS-PAGE on supernatant proteins (**B.**) Electronic microscopy. Red arrows show flagellar filament **(C**.) Motility on LB 0,3% agar plate (**Gallique *et al.***, in progress).

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

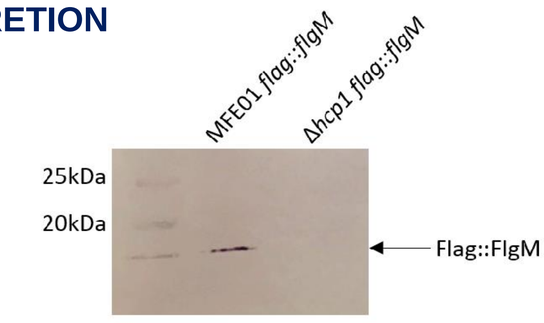


Figure n°6 : Western blot on supernatant protein of MFE01 or Δ*hcp1* after a translational fusion of *flag* sequence with *flgM* gene. The anti-Flag primary antibody, and a secondary antibody coupled with alkaline phosphatase was used to highlight Flag-FlgM protein. (unpublished).

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*.

Contacts: annabelle.merieau@univ-rouen.fr
mathilde.bouteiller@etu.univ-rouen.fr

Supported by

References :

Dasgupta N. *et al.*, A four-tiered transcriptional regulatory circuit controls flagellar biogenesis in *Pseudomonas aeruginosa. Molecular microbiology* **50**, (2003)
Decoin V. *et al.*, A Type VI Secretion System Is Involved in *Pseudomonas fluorescens* Bacteriam Competition. *PLOS ONE* **9**, e89411 (2014).
Decoin V. *et al.*, A *Pseudomonas fluorescens* type 6 secretion system is related to mucoidy, motility and bacterial competition. *BMC Microbiol* **15**, (2015)
Gallique M. *et al.*, Contribution of the *Pseudomonas fluorescens* MFE01 Type VI Secretion System to Biofilm Formation. *PLOS ONE* **12**, (2017a)
Gallique M. Bouteiller M., Merieau A., The Type VI Secretion system : A Dynamic System for Bacterial Communication ? *Front Microbiol* **8**, (2017b)