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

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. 22ième Edition des Journées de l’école doctorale EdNBISE, Mar 2019, Caen, France. hal-02387221

HAL Id: hal-02387221
https://normandie-univ.hal.science/hal-02387221
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

Boutelle Mathilde1, Gallique Mathias1, Kosta Artemis2, Hardouin Julie3, Konto-Ghiorgh Yoan1, Barbey Corinne1, Latour Xavier1, Chane Andrea1, Feuilloloe Marc1 and Merieu Annabelle1

1Laboratory of Microbiology Signals and Microenvironment LMSM EA 4312, Normandie Université, Univ. Rouen Normandie, Evreux, France. www.lmsm-lab.fr
2Plateforme de Microscopie de l'Institut de Microbiologie de la Méditerranée IMM FR 3479, Institut PSSARO proteomics facility, Université de Rouen, F-76821, Mont-Saint-Aignan, France

**INTRODUCTION**

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 overhanging 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 possesses a functional T6SS. The genes encoding the membrane complex, baseplate and sheath (tssB/C) of this contractile nanomachine are clustered together 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 through 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 flagellar secretion. On the second hand, MFE01 is able to inhibit *P. fluorescens* MFE01032 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).

**ELECTRONIC MICROSCOPY**

MFE01, Δhsp2 and Δhsp3 possess flagella, contrary to ΔtssC and Δhsp1

The lack of T6SS formed with Hcp1 disturbs flagella formation.

**TRANSCRIPTOMIC ANALYSIS**

The expression of flagellar class IV genes is disturbed in MFE01 Δhsp1.

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

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

A. Δhsp1 Δhsp2 Δhsp3 FlIA

The overexpression of FlIA gene restores the production of flagella in Δhsp1/tssC and by the way the “swimming” motility.

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

B. Δhsp1 Δhsp2 Δhsp3 FlIA

**FLG M SECRETION**

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 Δhsp1 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 Δhsp1 or ΔtssC 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 Δhsp1 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-sigma factor FlIA/anti-sigma-sigma factor FlgM” is stabilized by T6SS deletion mutation. In order to test this hypothesis we will study phenotypes of the double mutant Δhsp1-ΔFlgM. To identify the toxin that is secreted through T6SS formed by Hcp1 protein, we will perform interactive studies using translational fusion of flag gene sequence on hcp1 and flgM genes.

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

References:


Decoin V. et al., A Type VI Secretion System Is Involved in Pseudomonas fluorescens Bacterium Competition. PLOS ONE 9, e89411 (2014).


Gallique M. et al., Contribution of the Pseudomonas fluorescens MFE01 Type VI Secretion System to Biotin Formation. PLOS ONE 12, (2017a)