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Identification of a new type 6 secretion system effector of *Pseudomonas fluorescens* MFE01 involved in bacterial motility inhibition.

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Introduction

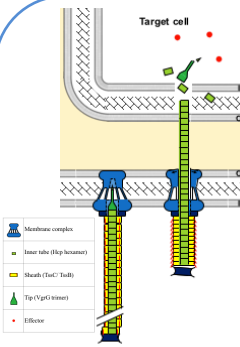


Figure 1 : Schematic representation of T6SS (Gallique et al., 2017).

Pseudomonas fluorescens MFE01 is an environmental strain, considered as a biocontrol agent that possess a type 6 secretion system (T6SS). T6SSs are contractile nanomachines involved in the secretion of effectors directly in other cells (bacteria or eukaryotic cells). T6SS is composed of a membrane complex, a baseplate and a tip that is expelled thanks to a contractile sheath (Figure 1). Effectors are also secreted with the proteins that compose the tip. Nowadays, the most studied effectors exhibit antibacterial activity, acting on peptidoglycan, lipid membrane, or DNA (Durand et al., 2014).

In *Pseudomonas fluorescens* MFE01, genes encoding T6SS membrane complex, baseplate and sheath are clustered. There are at least three different orphan genes encoding the tube (*hcp1*, *hcp2* and *hcp3*). It has already been demonstrated that T6SS of MFE01 protects potatoes tubers against the phytopathogen *Pectobacterium atrosepticum* (Decoin et al., 2014). Two different *hcp* genes (*hcp2* and *hcp3*) are involved in antibacterial activity, in contrary to 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).



We study the genetic environment and the interaction between three proteins, resulting of the expression of *hcp1*, *vgrG1* and *tec1* genes, in order to elucidate the structure of T6SS containing Hcp1. Deletion mutants were already constructed and phenotypical analysis are ongoing to understand the mechanisms of the motility decrease.

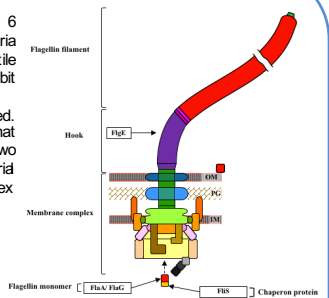


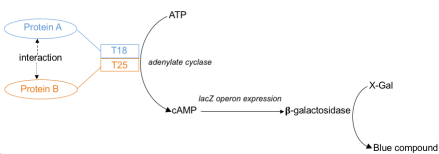
Figure 2 : Schematic representation of bacterial flagella.

Interactomic analysis : Bacterial Adenylate Cyclase Two Hybrid (BACTH)

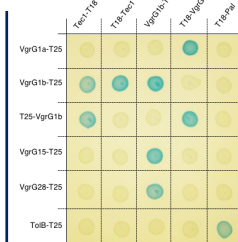
Principle

Translational fusions are realized between T18 or T25 domain, and interest proteins

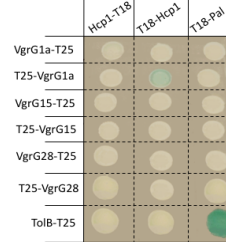
Both of these constructions are introduced in *E. coli* BTH101



Results

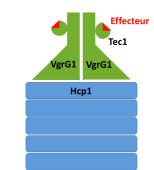


VgrG1 Cterm domain interacts with Tec1, VgrG1 Nterm domain and it-self.



VgrG1 Nterm domain interacts with Hcp1.

Hypothesis



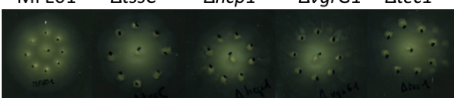
Interaction between Hcp1, VgrG1 and Tec1 (Type 6 effector chaperon).

One toxin is secreted by T6SS containing Hcp1 protein, and is involved in motility inhibition.

Motility inhibition

MFE01 and different deletion mutants were spotted around *Pseudomonas fluorescens* MFN1032 on a LB 0,3% agar plate. Δ tssC is a sheath mutant devoid of a functional T6SS.

MFE01 Δ tssC Δ hcp1 Δ vgrG1 Δ tec1



MFN1032 is sequestered by MFE01, but deletion mutants are unable to inhibit MFN1032 motility.

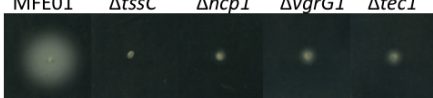
P. fluorescens MFE01 inhibits MFN1032 « swimming », by T6SS formed by Hcp1. VgrG1 and Tec1 are essential for this T6SS activity.

Phenotypical analysis

Swimming motility

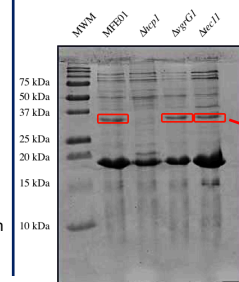
MFE01 and different deletion mutants were spotted on a LB 0,3% agar plate.

MFE01 Δ tssC Δ hcp1 Δ vgrG1 Δ tec1



Deletions of *tssC*, *hcp1*, *vgrG1* or *tec1* genes induce accumulation of a toxin that suppresses «swimming» ability.

Supernatant proteins



Secreted proteins of MFE01, and deletion mutants were separated by SDS-PAGE 18%, and identified by MALDI-TOF.

Contrary to what is observed in Δ hcp1, flagellin is secreted by *vgrG1* and *tec1* deletion mutants.

The toxin associated with Hcp1 impacts motility by suppressing flagellin secretion. An other effector, which is accumulated in Δ tec1 or Δ vgrG1 impacts flagellar activity, probably by acting on flagellum assembly.

Conclusion

In *P. fluorescens* MFE01, the T6SS formed by Hcp1 is surmounted by a VgrG1 trimer. Tec1 protein is a chaperon that permits the hanging of toxin on this T6SS. Our results show that the deletion of each component of this system leads to the loss of motility of MFE01 and decreases the inhibition of motility. Concerning the supernatant proteins analysis, we postulate that there are at least two toxins that are secreted, and both of them inhibit motility. The first toxin, associated with Hcp1, acts on flagellin secretion, probably by decreasing flagellin gene expression. The second toxin, associated with VgrG1, and indirectly with Tec1, seems act on flagellin filament assembly.

To identify the target of these effectors, transcriptomic analysis and overexpression of flagellar genes are ongoing. In order to pinpoint these effectors, co-immunoprecipitation assays will be done, and supernatant proteins analysis will be continue.

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Supported by



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