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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**

*Pseudomonas fluorescens* MFE01 is an environmental strain, considered as a biocontrol agent that possesses 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). Nowadays, the most studied T6SSs are involved in antibacterial activity, acting on peptidoglycan, lipopolysaccharide, or DNA (Durand et al., 2016).

In *Pseudomonas fluorescens* MFE01, genes encoding T6SS membrane complex, baseplate and sheath are clustered. There are at least three different orphan genes encoding the toxin (hcp1, hcp2 and hcp3). It has already been demonstrated that T6SS of MFE01 protects potatoes against the phytopathogen *Pseudomonas syringae* 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.

**Hypothesis**

Interaction between Hcp1, VgrG1 and Tec1 (Type VI effector chaperon). One toxin is secreted by T6SS containing Hcp1 protein, and is involved in motility inhibition.

**Results**

![Image](https://via.placeholder.com/150)

**Interactomic analysis : Bacterial Adenylate Cyclase Two Hybrid (BACTH)**

**Principle**

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

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Phenotypical analysis</th>
<th>Supernatant proteins</th>
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<tbody>
<tr>
<td>Motility inhibition</td>
<td>Swimming motility</td>
<td>Secreted proteins of MFE01, and deletion mutants were separated by SDS-PAGE 18%, and identified by MALDI-TOF.</td>
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<td>MFE01 and different deletion mutants were spotted around Pseudomonas fluorescens MFN0102 on a LB 0.3% agar plate.</td>
<td>The toxin associated with Hcp1 impacts motility by suppressing flagellar secretion. An other effector, which is accumulated in Tec1 or vgrG1 impacts flagellar activity, probably by acting on flagellum assembly.</td>
<td>Contrary to what is observed in Hcp1, flagelin is secreted by vgrG1 and tec1 deletion mutants.</td>
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<tr>
<td>MFE01 ΔvgrG1 Δtec1</td>
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<tr>
<td>MFE01 Δtec1</td>
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<td>MFE01 ΔvgrG1</td>
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<td>P. fluorescens MFE01 inhibits MN0232 (∆swimming) by T6SS formed by Hcp1. VgrG1 and Tec1 are essential for this T6SS activity.</td>
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**References**


