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# Non-coding RNAs control of phytobeneficial genes expression by *Pseudomonas brassicacearum*

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## Introduction

*Pseudomonas brassicacearum* is the major root-associated bacterium in the rhizosphere of *Arabidopsis thaliana*. This bacterium undergoes phenotypic switching *in vitro* as well as in the rhizosphere. This switch is caused by spontaneous mutations that occur at high frequency into *gacS/gacA* system. This system activates the transcription of several genes engaged in plant-bacteria molecular dialogue, especially thanks to non-coding RNAs (ncRNA) *rsmW*, *rsmX*, *rsmY* and *rsmZ*. The latter ncRNA have a positive control over plant-beneficial genes of *P. brassicacearum* (phytohormones, antifungal metabolites ...) [1, 2].

**Objectives :** monitoring and imaging of ncRNA expression *in planta*.

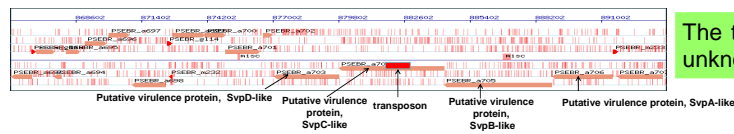
## Material and Methods

### Tagging bacteria with *rfp* :

*P. brassicacearum* labeling was made by triparental conjugation using PA1/04/03-RBSII *dsRed*-T0-T1 plasmid which has a mini-Tn5 transposon [3]

### Mapping of transposon insertion into genome :

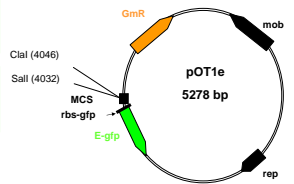
Genomic DNA extraction from *P. brassicacearum* mutants, digestion with a unique site digesting enzyme, digested fragment recycling with a T4 DNA ligase then PCR and nested PCR transposon flanking regions amplification and sequencing.



The transposon was localised inside an operon of unknown function [4].

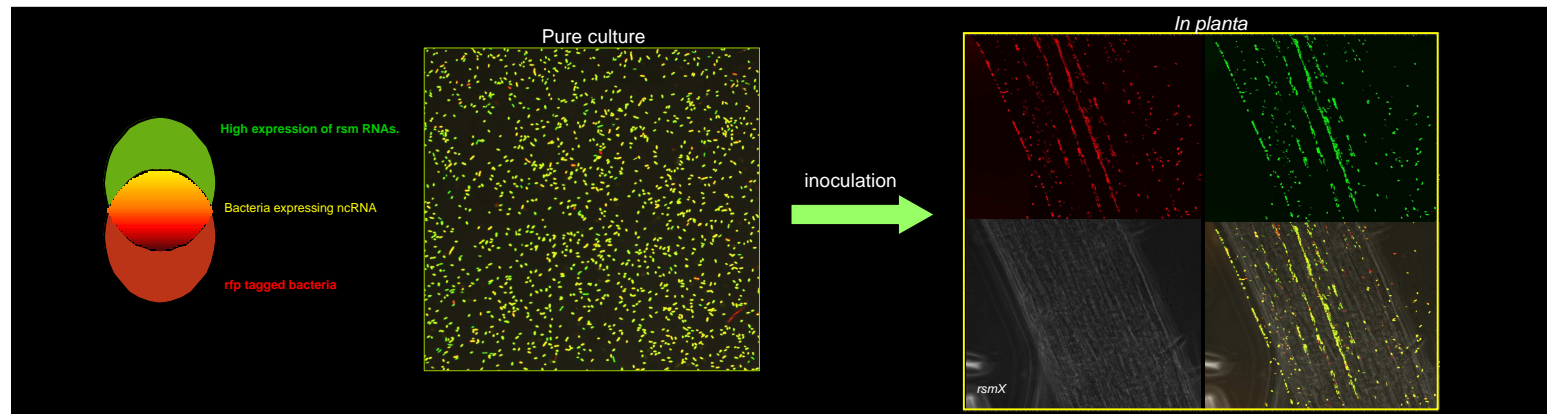
### Transcriptional fusion :

ncRNAs *rsmW*, *rsmX*, *rsmY*, *rsmZ* gene promoters are fused to E-gfp reporter gene in pOT1e plasmid.

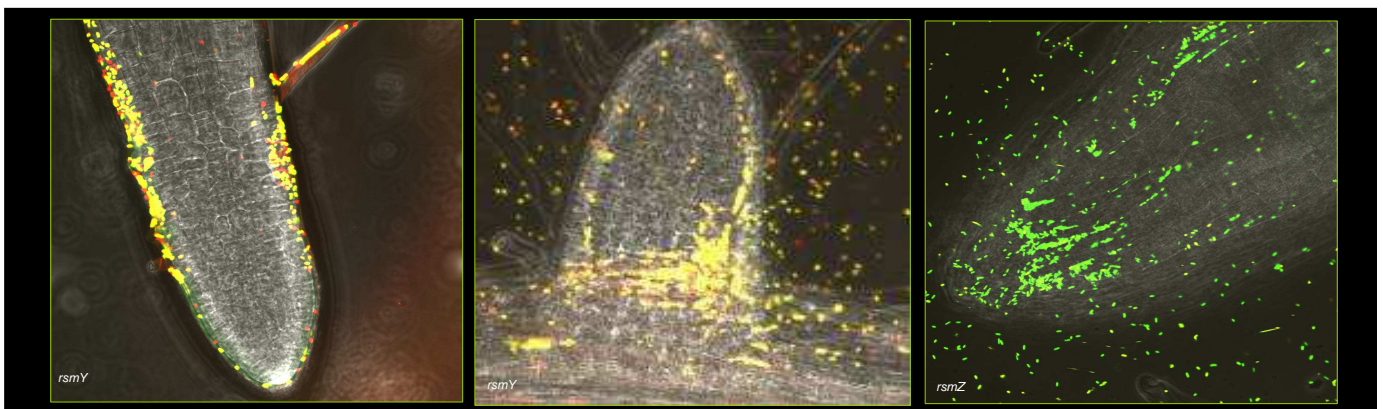


*P. brassicacearum* strain NFM421-I::*rfp* is transformed with transcriptional fusions. Seeds of *Arabidopsis thaliana* are inoculated with each construction and grown on Hoagland/2 phytigel medium. The roots are observed by laser scanning confocal microscopy.

## Results



Heterogeneity of expression within the same bacterial population. The "red" bacteria don't express ncRNA whereas the "green" ones show higher expression. The "yellow" bacteria display a moderate ncRNA expression.



Bacteria are mainly localized at the root tip and cracking zones of secondary roots and express ncRNAs .

## Conclusion and Perspectives

These tools allowed monitoring the spatiotemporal expression of ncRNA *rsmW*, *rsmX*, *rsmY* and *rsmZ* *in planta*, and will be used under natural conditions.

These constructs will be used to evidence phenotypic variants emergence *in planta* as they don't express ncRNA anymore (red bacteria). They should also allow us to evidence a possible reversion phenomenon by using these constructs with variants and looking for *gfp* producing-revertants. These tools are currently used for imaging the expression of other genes involved in plant beneficial features (*phlA*, *hcnA*,...), as well as other ncRNA (*prfF*, *crcZ*...).