HAL
open science

Non-coding RNAs control of phytobeneficial genes expression by pseudomonas brassicacearum

Marie Bertrand-Huleux, Sylvain Fochesato, Wafa Achouak

To cite this version:

Marie Bertrand-Huleux, Sylvain Fochesato, Wafa Achouak. Non-coding RNAs control of phytobeneficial genes expression by pseudomonas brassicacearum. 11ème rencontre plantes bactéries, 2014, AUSSOIS, France. hal-03154527

HAL Id: hal-03154527

https://hal.science/hal-03154527v1

Submitted on 1 Mar 2021

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.

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

Auteurs : Marie Bertrand, Sylvain Fochesato, Wafa Achouak

Laboratoire d'Ecologie Microbienne de la Rhizosphère et Environnements Extrêmes, UMR 7265 CNRS-CEA-AMU, CEA Cadarache 13108 St PAUL lez DURANCE

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*.

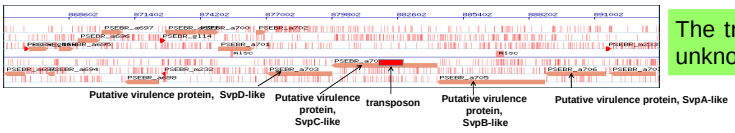
Material and Methods

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 :

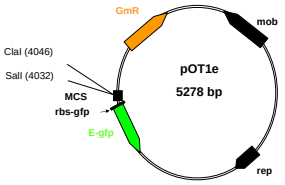
Genomic DNA extraction from *P. bracicacearum* mutants, digestion with a unique site digesting enzyme, digested fragment recycling with a T4 DNA ligase then PCR and nested PCR transposon flanking regions amplification and sequencing.



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

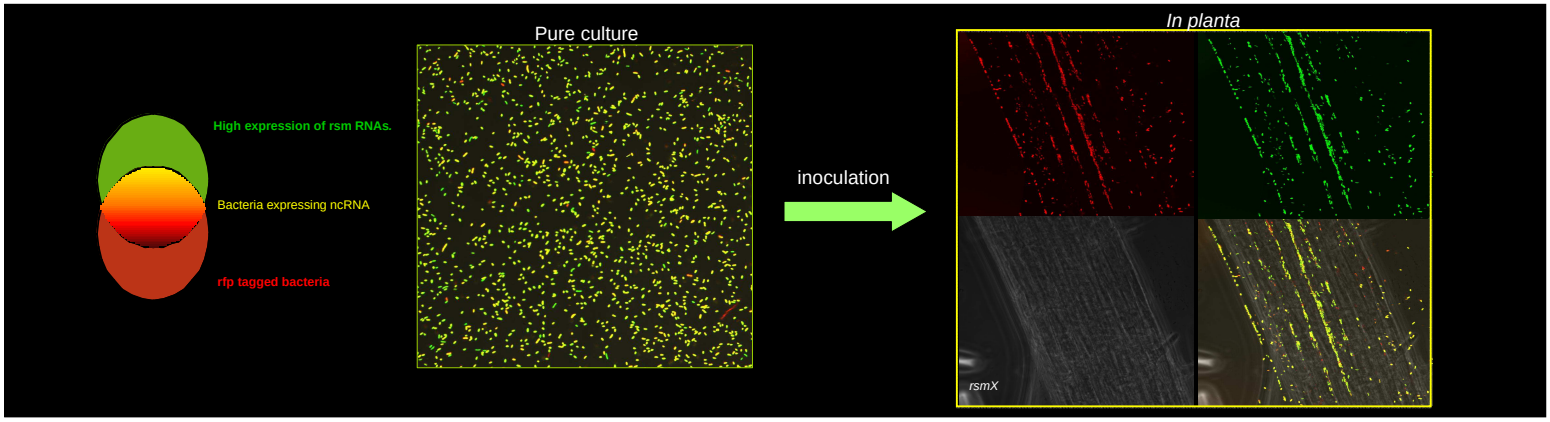
Transcriptional fusion :

ncRNAs *rsmW, rsmX, rsmY, rsmZ* gene promoters are fused to E-gfp reporter gene in poT1e plasmid.

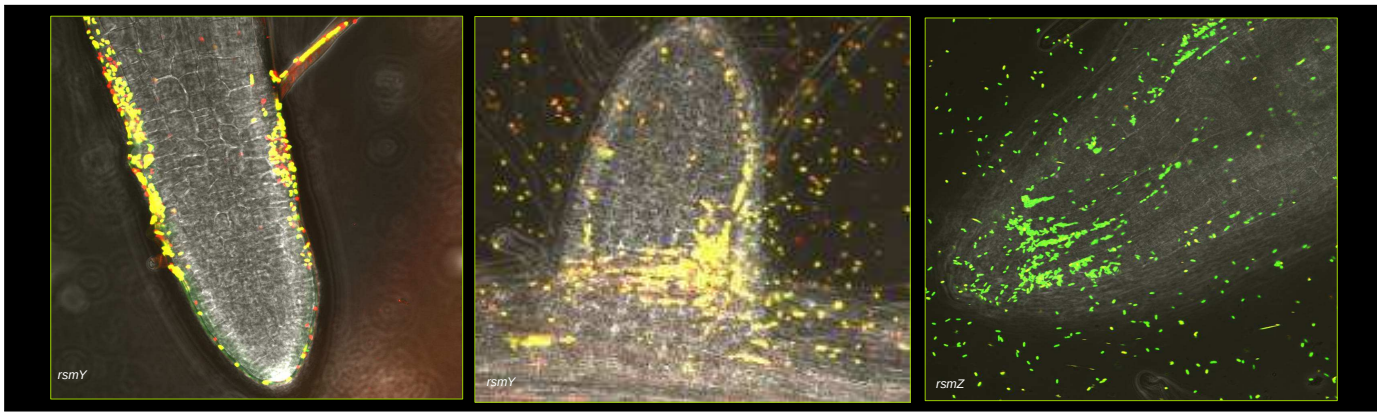


P. brassicacearum strain NFM421-I::*rfp* is transformed with transcriptional fusions. Seeds of *Arabidopsis thaliana* are inoculated with each construction and grown on Hoagland/2 phytagel medium. The roots are observed by laser scanning confocal microscopy.

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 (*prrF, crcZ…).*

References : [1] : **Lalaouna D. et al.** 2012. *Applied and Environmental Microbiology ; [2] :* **Lalaouna D. et al.** 2012. J. Bacterio l ; [3] : **Tolker-Nielsen et al**. 2000 ; [4] **Ortet P. et al.** 2011 J.bacteriol