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BOOK OF ABSTRACTS

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The biocontrol agent *Pseudomonas chlororaphis* subsp. aureofaciens M71 originates natural mutants impaired in the ability to control *Fusarium oxysporum* f. sp. *radicis-lycopersici* on tomato

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Highlights

- *Pseudomonas chlororaphis* subsp. *aureofaciens* M71 differentiated three natural mutants distinguishable for morphological traits
- *P. chlororaphis* subsp. *aureofaciens* M71 mutants were impaired in persisting on tomato roots and controlling tomato crown rot
- Mutants were characterised by a reduced ability in production of autoinducer signals and antibiotics

Introduction

Fluorescent pseudomonads are able to control plant diseases by effectively colonizing plant roots and releasing secondary metabolites toxic to phytopathogenic microorganisms. However, once applied on plant roots, mutants lacking these abilities may arise in biocontrol fluorescent pseudomonads impairing their success in controlling plant diseases (Chancey et al., 1999, 2002).

Pseudomonas chlororaphis subsp. *aureofaciens* M71 (M71) effectively controlled phytopathogenic fungi *in vivo* due to the production of phenazine-1- carboxylic acid (PCA; Puopolo et al., 2011; Raio et al., 2017). Three classes of M71 mutants, named M71a, M71b and M71c, were isolated from the rhizosphere of tomato plants treated with M71. The aim of this study was to evaluate how the occurrence of mutations may affect the M71 biocontrol performances and characterise the three mutants through a biochemical and microbiological approach.

Material and methods

The rhizosphere competence and antagonistic performance of M71 and its three mutants against *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Forl) were tested *in vivo* on tomato plants. Bacteria were re-isolated from roots of 20 days old tomato plantlets and colony phenotype was recorded. The stability of each strain phenotype was determined *in vitro* by treating tomato seeds with cell suspensions of rifampicin resistant marked strains. *In vitro* antagonistic activity of the four strains was tested against *F. o.* f. sp. *lycopersici*, Forl, *Pyrenochaeta lycopersici*, *Pythium ultimum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. The four strains were tested for the ability to produce PCA according to Raio et al. (2017) and the release of N-acyl homoserine lactones (AHLs) was assessed using the biosensor strain *Chromobacterium violaceum* CV026. M71 and its three mutants were tested for proteolytic activity on skim milk agar, while production of siderophore was tested on Chrome Azurol S medium.

Results and discussion

The three M71 mutants had a colony morphology different from M71 morphology (bright orange and mucoid). Indeed, M71a colony was translucent and mucoid; the M71b colony was pale orange and mucoid while M71c showed a bright orange and rough colony. M71 and M71c efficiently colonised tomato rhizosphere and determined a significant reduction in disease incidence caused by Forl (38 to 50% vs. 85% control) *in vivo*. In contrast, M71a and M71b were not able to effectively colonise tomato rhizosphere and, as a consequence, were not able to control Forl. In the *in vitro* test, 40% of the colonies originated from tomato plants treated with M71 showed the M71a phenotype. M71a and M71b did not originate different colony phenotypes, demonstrating that these may be considered stable mutations. In contrast, 42% of the colonies deriving from tomato plants treated with M71c showed wild-type phenotype suggesting that the mutation occurred in M71c is a reversible mutation. *In vitro* antagonistic activity of M71c against fungi was very similar to M71. Both were active against all fungal species tested and induced the highest reduction of mycelial growth. M71a was active against *P. lycopersici* and *P. ultimum* only. M71b was less active than M71 against Fol and *P. lycopersici* and had no effect against *R. solani*. M71 was the most active PCA producer while M71a and M71b produced the lowest amounts. The reduced PCA production was associated with a drastic reduction of AHL biosynthesis in M71a and M71b. Indeed, these two mutants were not able to restore violacein production in *C. violaceum* CV026. M71c was able to produce amounts of PCA and AHLs similar to the wild type strain. The three mutants showed a reduced proteolytic activity compared to M71 whereas siderophore production was significantly higher in M71a and M71b.

Results indicated that three classes of mutants might derive from M71 when this bacterial biocontrol agent is applied on tomato roots. One of these three (M71c) is probably due to a reversible mutation and it is not impaired in the colonisation of tomato rhizosphere and in controlling Forl since the characteristics of the wild type are restored during the experiments. In contrast, the M71a and M71b phenotypes are attributable to stable mutations that cause an increase in siderophore production and the loss of the ability to release proteases and produce PCA and AHLs. The outcome of these mutations is a significant impairment in the efficacy of M71 against Forl on tomato plants. In *P. chlororaphis* 30-84, these features are due to the lack of functional *gacA*/*gacS* genes encoding a two-component transcriptional system that positively controls AHL and PCA production (Chancey et al., 1999, 2002). Future works will be aimed to better identify the molecular mechanisms underlying the occurrence of these mutations in M71 to reduce the risk of not reliable results when applied in the field.

References

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