Role of antibiotic-producing pseudomonads in natural soil suppressiveness to Fusarium wilt

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ROLE OF ANTIBIOTIC-PRODUCING PSEUDOMONADS IN NATURAL SOIL SUPPRESSIVENESS TO FUSARIUM WILT

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INTRODUCTION

Some soils are known for their natural suppressiveness to soilborne diseases. Fusarium wilt suppressive soils from Châteaurenard (France) and Salinas Valley (California, USA) are among the best examples of long-standing suppressive soils. The suppressiveness was attributed in particular to the activity of non-pathogenic *F. oxysporum* and of fluorescent pseudomonads. Carbon competition and iron competition are involved in the soil suppressiveness to fusarium wilts. The role of antibiosis in fusarium wilt suppressive soils has not been studied to date, in spite of the fact that several antibiotic compounds produced by antagonistic bacteria have shown growth-inhibitory activity against pathogenic *F. oxysporum*. This has been demonstrated in particular for phenazine- and 2,4 phloroglucinol (DAPG)-producing fluorescent pseudomonads.

OBJECTIVE

Assess the role of antibiosis in the soil suppressiveness to fusarium wilt.

STRATEGY

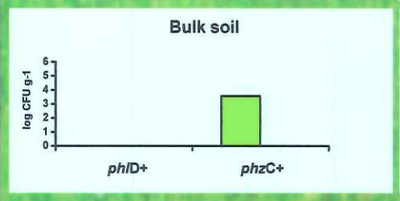
Compare DAPG- and phenazine-producing fluorescent pseudomonads isolated from a fusarium wilt suppressive soil (Châteaurenard, France) and from a conducive soil (Carquefou, France) for their:

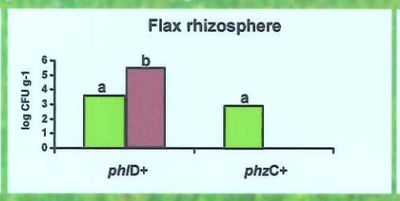
- density,
- diversity,
- ability to suppress fusarium wilt alone or in combination with non-pathogenic *F. oxysporum*.

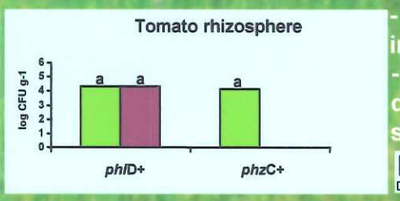
MATERIAL & METHODS

- Frequency of DAPG- and phenazine- producing fluorescent pseudomonads were determined by colony hybridization followed by PCR.
- Diversity of DAPG producers = *phl*D+ :
  - polymorphism of *phl*D (629bp fragment amplified using primers B2BF and BPR4 _McSpadden et al., *Phytopathology* 91:44-54_ and digested with *Hae*III, *Msp*I, and *Taq*I)
- Diversity of phenazine producers = *phz*C +:
  - polymorphism of *phz*C (522bp fragment amplified using primers PHZJR1 and PHZJR2 _Raaijmakers, unpublished_ and digested with *Fnu*4HI, *Nci*I, and *Nla*IV) + genotypic background (whole cell rep-PCR fingerprinting method using the BOXA1R primer)
- Ability to control flax fusarium wilt induced by *F. oxysporum* Foln3 was assessed in growth chamber for representatives of the genotypes described and for antibiotic deficient mutants, alone or in combination the with non-pathogenic *F. oxysporum* Fo47.

DENSITY of DAPG- and phenazine-producers





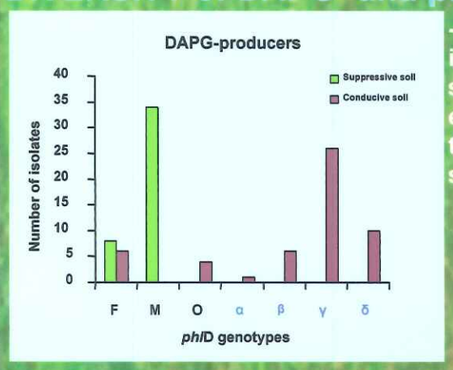


- DAPG-producers (*phl*D+) were not detected in any of the two bulk soils.
- Phenazine-producers (*phz*C+) were detected only in the Châteaurenard suppressive soil (bulk & rhizosphere).

Suppressive soil Conducive soil

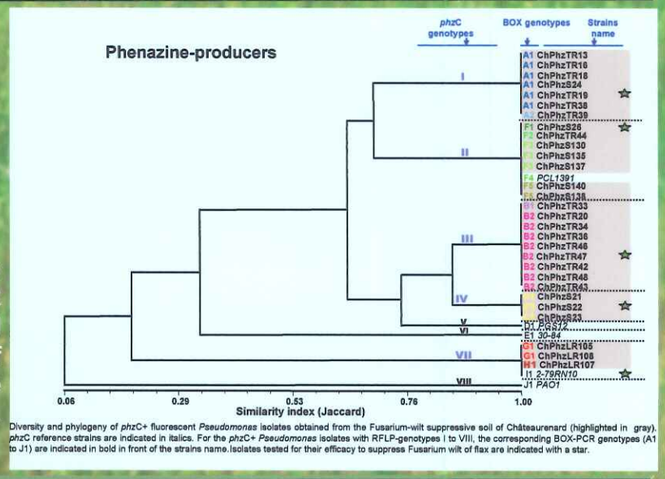
Detection limits: Bulk soil 3.3 X 10^1 CFU/g of soil; Rhizosphere 3.3 X 10^2 CFU/g of fresh root

DIVERSITY of DAPG- and phenazine-producers



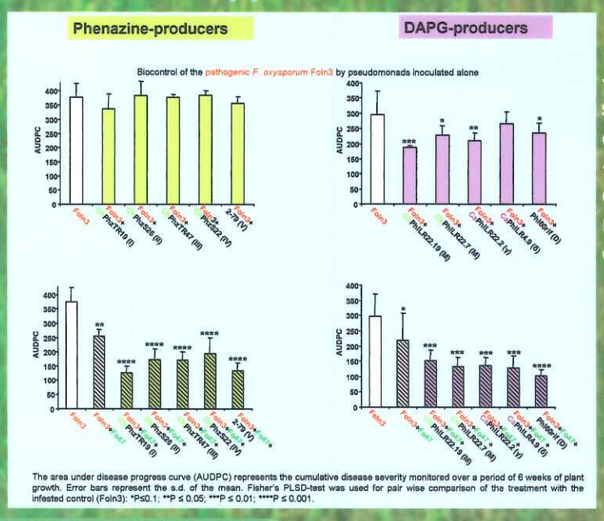
- *phl*D+ pseudomonads occurred both in the suppressive and conducive soils but their genotypic diversity, encompassing six *phl*D genotypes in the conducive soil and only two in the suppressive soil, differed.

- Five distinct *phz*C genotypes corresponding to 11 BOX-PCR genotypes were described among the *phz*C+ isolates of the Châteaurnard suppressive soil.



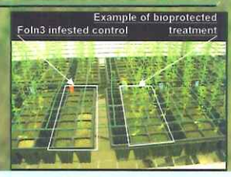
Diversity and phylogeny of *phz*C+ fluorescent *Pseudomonas* isolates obtained from the Fusarium-wilt suppressive soil of Châteaurenard (highlighted in gray). *phz*C reference strains are indicated in italics. For the *phz*C+ *Pseudomonas* isolates with RFLP-genotypes I to VIII, the corresponding BOX-PCR genotypes (A1 to J1) are indicated in bold in front of the strains name.Isolates tested for their efficacy to suppress Fusarium wilt of flax are indicated with a star.

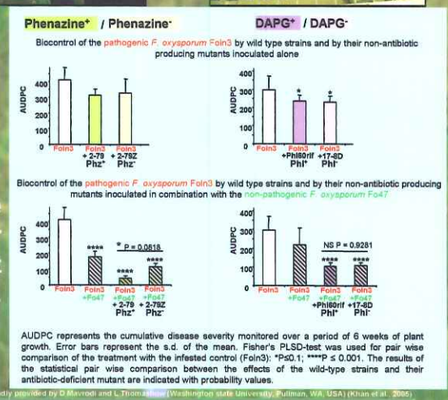
ABILITY to suppress flax fusarium wilt



The area under disease progress curve (AUDPC) represents the cumulative disease severity monitored over a period of 6 weeks of plant growth. Error bars represent the s.d. of the mean. Fisher's PLSD-test was used for pair wise comparison of the treatment with the infested control (Foln3): *P≤0.1; **P ≤ 0.05; ***P ≤ 0.01; ****P ≤ 0.001.

- DAPG-producing representative isolates suppressed efficiently Fusarium wilt independently of their genotypes and soil origin.
- All *phz*C+ and *phl*D+ representative isolates enhanced the biocontrol provided by the non-pathogenic *Fusarium* Fo47 when co-inoculated with this fungal strain.





- No significant difference in disease control between the DAPG-producer Phl60rif and its DAPG-deficient mutant 17.8D.
- Phenazine-producing strain 2-79 was more effective in combination with Fo47 than its phenazine-deficient mutant 2-79Z*.

AUDPC represents the cumulative disease severity monitored over a period of 6 weeks of plant growth. Error bars represent the s.d. of the mean. Fisher's PLSD-test was used for pair wise comparison of the treatment with the infested control (Foln3): *P≤0.1; ****P ≤ 0.001. The results of the statistical pair wise comparison between the effects of the wild-type strains and their antibiotic-deficient mutant are indicated with probability values.

*P. fluorescens 2-79 and its phenazine-deficient mutant 2-79Z were kindly provided by D.Mavrodi and L.Thomashow (Washington state University, Pullman, WA, USA) (Khan et al. 2006)

CONCLUSIONS

1. No quantitative difference was observed between populations of indigenous *phl*D+ pseudomonads in the Fusarium wilt suppressive and conducive soils. 2. The genotypic diversity of *phl*D+ pseudomonads was significantly different between the Fusarium wilt suppressive soil and the conducive soil but representatives of the different genotypic groups were equally effective in controlling Fusarium wilt of flax. 3. Phenazine producing pseudomonads were only detected in the Fusarium wilt suppressive soil and phenazine production was implied in the improvement of the protection achieved by the non-pathogenic Fusarium Fo47.

Collectively, these results suggest that phenazine-producing pseudomonads could provide a substantial contribution to the natural suppressiveness to Fusarium wilt diseases in combination with other microorganisms (e.g. non-pathogenic *Fusarium oxysporum*) that play a key role in Fusarium wilt suppressiveness.

Mazurier S., Corberand T., Lemanceau P. and Raaijmakers J.M. 2009. Phenazine antibiotics produced by fluorescent pseudomonads contribute to natural soil suppressiveness to Fusarium wilt. The ISME Journal (2009) 0, 000–000; doi:10.1038/ismej.2009.33. npg_ismej_ismej200933