Identification of phytotoxic secondary metabolites of *Pseudomonas fluorescens* Pf-5

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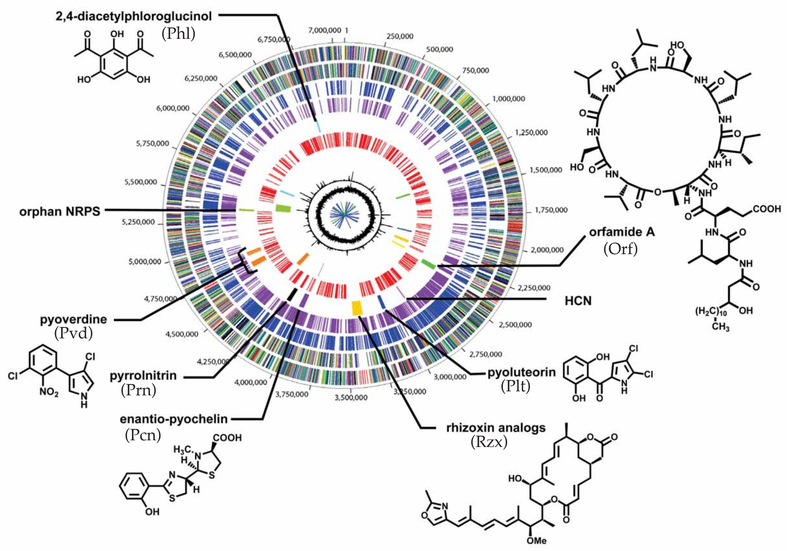
Introduction

- *Pseudomonas fluorescens* Pf-5 is a gram negative, rod shaped, rhizosphere bacterium.
- Pf-5 functions as a biocontrol agent that suppresses plant pathogens including take-all decline and seed-damping off disease caused by *Pythium ultimum*.
- Certain secondary metabolites associated with biocontrol are also phytotoxic.
- Which secondary metabolites produced by Pf-5 are phytotoxic?

Figure 1. Secondary metabolite production in the genome of *Pseudomonas fluorescens* Pf-5



Arrows show gene clusters that produce the secondary metabolites of Pf-5. Of these metabolites, 2,4-diacetylphloroglucinol and rhizoxin were shown to be phytotoxic.

Methods

Filter paper assays using Pf-5 and mutants were employed:

- Surface sterilization of Sugar Snap Peas
- Cultures washed and set to a uniform density
- Peas were inoculated with cultures and initial populations enumerated through serial dilutions.
- Peas were placed on wet filter paper in Petri plates and incubated
- Germination of peas checked at 6 days
- Serial dilutions of germinated seeds were performed to determine colonization and root lengths were measured at 6 days.

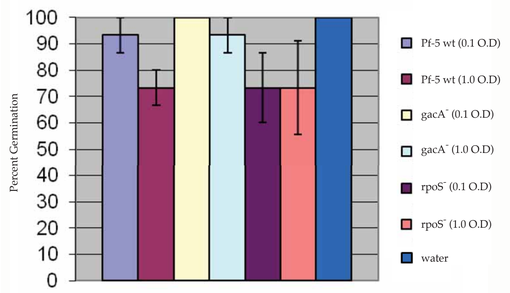
Table 1. Secondary metabolite production in knockout mutants of *Pseudomonas fluorescens* Pf-5

Strains	Secondary Metabolites					
	Pvd	Pcn	Prn	Phl	Plt	Rzx
Wild Type	+	+	+	+	+	+
gacA$^-$	+ +	+ +	-	-	-	-
rzxB$^-$	+	+	+	+	+	-
prnC$^-$	+	+	-	+	+	+
phlD$^-$	+	+	+	-	+	+
prnC$^-$, phlD$^-$	+	+	-	-	+	+
rzxB$^-$, prnC$^-$	+	+	-	+	+	-
rzxB$^-$, phlD$^-$	+	+	+	-	+	-
prnC$^-$, phlD$^-$, rzxB$^-$	+	+	-	-	+	-
rpoS$^-$	+	+	+	+ +	+	+

Illustrating the overproduction (++), wild type levels (+), or deficiency (-) of secondary metabolites.

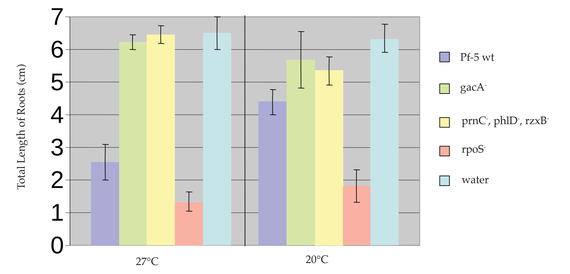
Results

Figure 2. Effect of initial concentration of Pf-5 and knockout mutants on germination of peas



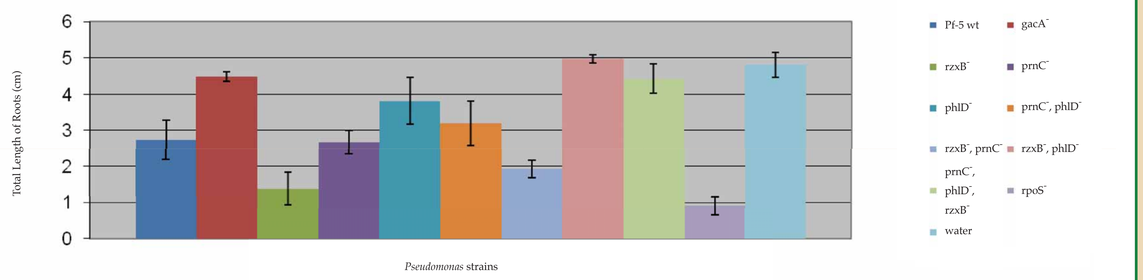
Two concentrations of inoculum (0.1 O.D. and 1.0 O.D.) were used with the Pf-5 wild type (postive control), gacA$^-$ (negative control), and rpoS$^-$ (2,4-DAPG overproducer) strains to determine the optimal concentration to be used for experiments. Higher germination with the low concentration determined the use of 0.1 O.D. for subsequent experiments.

Figure 3. Effect of temperature on root length inhibition by Pf-5 and knockout mutants on peas



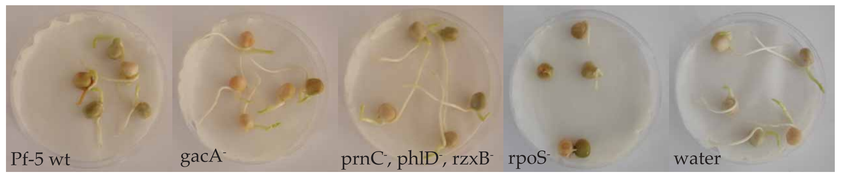
Peas were incoculated at 0.1 O.D. with the wild type (postive control), gacA$^-$ (negative control), rpoS$^-$ (2,4-DAPG overproducer), a triple knockout mutant deficient in prnC$^-$, phlD$^-$, and rzxB$^-$, and water at two temperatures (20°C and 27°C). Peas prefer the cooler 20°C while Pf-5 grows better at 27°C. Phytotoxic effects were greater at 27°C which determined the use of 27°C in subsequent experiments.

Figure 4. Effects of Pf-5 and knockout mutants on root lengths of peas



Assay performed with 0.1 O.D. inoculum at 27°C. In all cases of stunted growth, at least one if not both phenotypes for 2,4-DAPG and rhizoxin are present. Conversely, all cases of uninhibited growth in peas involve knockouts for phenotypes of 2,4-DAPG and rhizoxin. Overproduction of 2,4-DAPG leads to stunted growth as shown in rpoS$^-$ while knockouts of 2,4-DAPG showed no inhibition as seen in phlD$^-$. This illustrates the role of 2,4-DAPG as a phytotoxic secondary metabolite. Mutants with a knockout for 2,4-DAPG still show levels of phytotoxicity, indicating rhizoxin as a phytotoxic secondary metabolite. Pyrrolnitrin showed no phytotoxicity.

Figure 5. Phytotoxic effects of Pf-5 and knockout mutants on pea germination



Pictures were taken on day six of incubation. GacA$^-$, prnC$^-$, phlD$^-$, and rzxB$^-$ mutants, and water all show normal growth where no phytotoxic inhibition has taken place. In Pf-5 wild type and rpoS$^-$, where both 2,4-DAPG and rhizoxin are produced, phytotoxic effects of 2,4-DAPG and rhizoxin are shown.

Conclusion

- 2,4-diacetylphloroglucinol and rhizoxin were identified as phytotoxic secondary metabolites by using multiple mutants deficient in various secondary metabolites for assays.
- Phytotoxicity was reduced or elimated through use of mutants with knockouts of phenotypes for 2,4-diacetylphloroglucinol and rhizoxin.

Acknowledgements

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Future Work

- Assay on other plants such as cucumber.
- Perform pea assay in soil where the environment may have other factors that will affect pea germination, thus simulating a real world environment.