Genetic regulation of phenazine-1-carboxamide synthesis by Pseudomonas chlororaphis strain PCL1391

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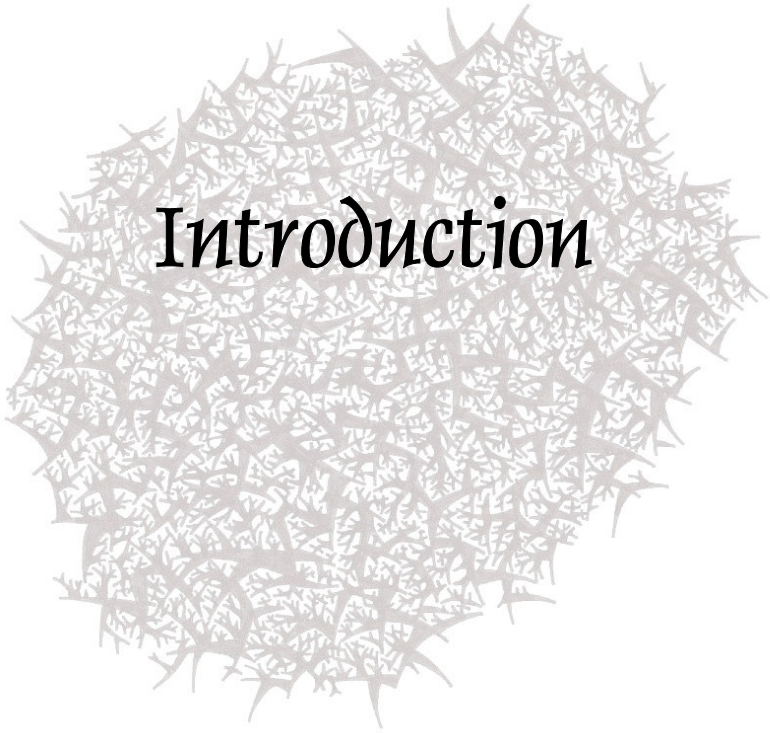

Introduction

Aims of this thesis

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Pseudomonas chlororaphis strain PCL1391 is a plant-beneficial rhizobacterium that protects tomato plants from tomato foot and root rot caused by the fungus *Fusarium oxysporum* f. sp. *radicis lycopersici*. Previously, it was shown that both microorganisms compete for colonization of the junctions between epidermic cells of tomato roots and that root colonization is required for the delivery of the antifungal metabolite phenazine-1-carboxamide (PCN) produced by strain PCL1391 (Chin-A-Woeng *et al.,* 2000; Bolwerk *et al.,* 2003) (Fig. 1). PCN is a secondary metabolite that inhibits the growth of *Fusarium oxysporum* and its synthesis is necessary for the biocontrol ability of PCL1391 (Chin-A-Woeng *et al.,* 1998).

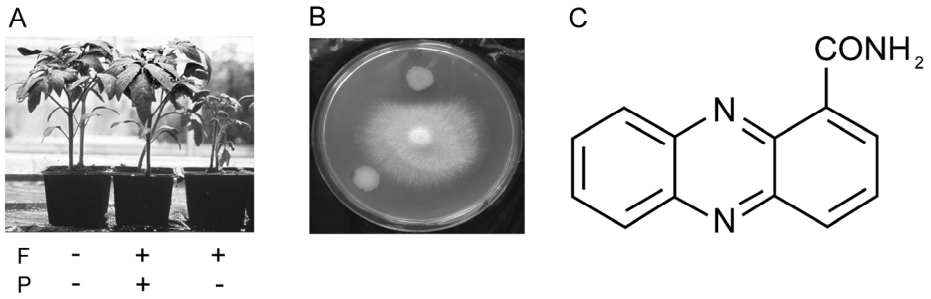

Figure 1. Biocontrol activity of *Pseudomonas chlororaphis* strain PCL1391.
Panel A. Presence of the fungus *Fusarium oxysporum* f. sp. *radicis lycopersici* (F) causes foot and root rot on the tomato plant. Coating of the tomato seeds with *P. chlororaphis* PCL1391 (P) protects the tomato plant against the fungus.
Panel B. On agar medium, strain PCL1391 (top) inhibits radial growth of *F. oxysporum* (center). However, the mutant strain PCL1119 (bottom left) derivative of strain PCL1391 is unable to inhibit the growth of the fungus. PCL1119 is mutated in *phzB*, the second gene of the *phzABCDEFGH* operon responsible for the biosynthesis of PCN by strain PCL1391 (Picture from T. van Rij).
Panel C. Structure of phenazine-1-carboxamide.

The major goal of the studies presented in this thesis is to elucidate the complex mechanisms regulating PCN production in *P. chlororaphis* strain PCL1391. So far three main genes or gene pairs have been isolated that regulate the PCN biosynthetic operon *phzABCDEFGH*. These include the two-component system *gacS*/*gacA*, the *tetR* homologue *psrA* and the quorum sensing system *phzI*/*phzR* (Chin-A-Woeng *et al.,* 2001b; Chin-A-Woeng *et al.,* 2005). Another regulator of phenazine synthesis, *ippA*, was recently characterized by van Rij *et al.* (van Rij et al., *in preparation*). Regulation of secondary metabolites synthesis in other bacterial

species indicated that additional genes could be involved in the regulation of PCN synthesis and that their interactions might be very complex.

A general overview of regulation of secondary metabolism in Pseudomonas species is given in Chapter 1. Several approaches were combined to identify novel genes involved in the regulation of PCN synthesis and to study their interactions with other regulators. Site-directed mutagenesis was used to test the hypothesis that *rpoS* is a regulatory gene of PCN synthesis (Chapter 2). To discover additional genes in the regulatory cascade, which already contains *psrA* and *rpoS*, a random DNA-fragment microarray of the PCL1391 genome was constructed and used for transcriptomics of the *psrA* and *rpoS* mutants (Chapter 3). A random mutagenesis approach resulted in the identification of *pip*, a novel gene that stimulates PCN production in PCL1391 (Chapter 4). Analyses on the role of Pip as a switch of PCN production depending on environmental conditions are described in Chapter 5. The results described in this thesis are summarized in Chapter 6, where in addition the regulatory network of PCN synthesis in *P. chlororaphis* PCL1391 is compared to regulatory networks of secondary metabolism in other *Pseudomonas* species.