



Universiteit
Leiden
The Netherlands

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

Girard, G.

Citation

Girard, G. (2006, June 6). *Genetic regulation of phenazine-1-carboxamide synthesis by Pseudomonas chlororaphis strain PCL1391*. Retrieved from <https://hdl.handle.net/1887/4406>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/4406>

Note: To cite this publication please use the final published version (if applicable).

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

Geneviève Girard

Cover: The picture on the front cover is used with the gracious authorization of Yann Arthus-Bertrand. Legend translated from his website (www.yannarthusbertrand.com): *“Tree of life”, Tsavo National Park, Kenya (2°11’ S, 38°25’ E). This acacia is a symbol of life in vast expanses of thorny savanna, where tracks of wild animals taking advantage of its leaves or its shade converge. Tsavo National Park in southeastern Kenya (...) is the country’s largest protected area (8,200 square miles of 21,000 km²) and was declared a national park in 1948. (...) The prohibition of international trading in ivory and rhino horns has enabled certain wild animal populations to increase in number. However, poaching and disappearing natural habitats remain disturbing threats. World trade in wild flora and fauna provides between \$12 billion and \$19 billion in revenues each year.*

This picture was chosen for two reasons. Firstly, it displays a complex network of animal tracks that can symbolize the complex networks present through the thesis (genetic networks regulating secondary metabolism or complex relationships between microorganisms in the rhizosphere). Secondly, the tree in the center of the picture strikingly resembles an aging colony of *Pseudomonas chlororaphis* PCL1391, which itself looks dark yellow and displays a green central area due to precipitation of phenazines. In analogy to the picture on the front cover, on the back cover a picture is shown of such two aging colonies of *P. chlororaphis* at different enlargements.

Cover designed by Peter Hock.

Similarly to the front cover illustration, the picture present on the first page of each chapter represents a complex plant network and can symbolize complex networks studied in the thesis. Drawing from Olivier Girard.

Printed by: Ridderprint, Ridderkerk, The Netherlands

ISBN-10: 90-9020621-3

ISBN-13: 978-90-9020621-9

Pour mes parents

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

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van de Rector Magnificus Dr. D. D. Breimer,
hoogleraar in de faculteit der Wiskunde en
Natuurwetenschappen en die der Geneeskunde,
volgens besluit van het College voor Promoties
te verdedigen op dinsdag 6 juni 2006
klokke 15.15 uur

door

Geneviève Anne Odile Girard
geboren te Les Lilas, Frankrijk, in 1979

Promotiecommissie

Promotor:	Prof. Dr. E. J. J. Lugtenberg
Co-promotor:	Dr. G. V. Bloembergen
Referent:	Dr. B. Kraal
Overige leden:	Prof. Dr. P. J. J. Hooykaas Prof. Dr. H. P. Spaink Prof. Dr. C. A. M. J. J. van den Hondel Prof. Dr. J. A. van Veen

J.E. Jurriaanse Foundation financially contributed to the print costs of this thesis.

TABLE OF CONTENTS

	List of abbreviations	9
Introduction	Aims of this thesis	13
Chapter 1	Regulatory mechanisms governing secondary metabolism of <i>Pseudomonas</i> species	17
Chapter 2	Regulatory roles of <i>psrA</i> and <i>rpoS</i> in phenazine-1-carboxamide synthesis by <i>Pseudomonas chlororaphis</i> PCL1391	45
Chapter 3	Development of a microarray of <i>Pseudomonas chlororaphis</i> PCL1391 and transcriptome analyses of its <i>psrA</i> and <i>rpoS</i> mutants	65
Chapter 4	The novel regulator Pip acts between PsrA/RpoS and quorum-sensing in the regulatory cascade of phenazine synthesis in <i>Pseudomonas chlororaphis</i> PCL1391	93
Chapter 5	The transcriptional regulator Pip is involved in the repression of phenazine synthesis under stress conditions in <i>Pseudomonas chlororaphis</i> PCL1391	117
Chapter 6	Summary and discussion	145
	References	157
	Résumé et samenvatting	175
	Curriculum vitae	189

LIST OF ABBREVIATIONS

- Acyl-ACP: acyl-acyl carrier protein
- Acyl-CoA: acyl-coenzyme A
- AcrAB: multidrug export pump in *E. coli*
- AcrR: TetR homologue, regulator of *acrAB* expression
- ANR: anaerobic regulator (see Table 1 in Chapter 1)
- AprA: secreted alkaline protease
- Bp: base pairs
- C₄-HSL: *N*-butanoyl-homoserine lactone, cognate *N*-AHL of RhII/RhIR
- C₆-HSL: *N*-hexanoyl-L-homoserine lactone, cognate *N*-AHL of PhzI/PhzR
- 3O-C₁₂-HSL: *N*-(3-oxododecanoyl)-homoserine lactone cognate *N*-AHL of LasI/LasR
- CF: cystic fibrosis
- CFU: colony forming units
- CsrA: small regulatory protein (carbon storage regulator) (see Table 1 in Chapter 1)
- CsrB: small regulatory RNA (see Table 1 in Chapter 1)
- FixJ: regulator of nitrogen fixation (see Table 1 in Chapter 1)
- Fur: ferric uptake regulator (see Table 1 in Chapter 1)
- GacS/GacA: two-component system including a sensor and a transcriptional activator (global antibiotic control) (see Table 1 in Chapter 1)
- galETK*: galactose operon regulated by Spot42
- glgC*: ADP-glucose pyrophosphorylase gene regulated by CsrA.
- Gm: gentamicin
- 4-HPPD: 4-hydroxyphenylpyruvate dioxygenase
- H₂O₂: hydrogen peroxide
- HCN: hydrogen cyanide
- Hfq: small regulatory protein (see Table 1 in Chapter 1)
- IppA: regulator of phenazine synthesis in *P. chlororaphis* PCL1391 (inducer of phenazine production)
- IsmB: regulator of phenazine synthesis and other traits of the secondary metabolism in *P. chlororaphis* PCL1391 (inducer of secondary metabolism)
- ISR: induced systemic resistance
- Kb: kilo base-pairs
- Km: kanamycin
- LasI/LasR: quorum-sensing system (see Table 1 in Chapter 1)
- LuxI/LuxR: quorum-sensing system (see Table 1 in Chapter 1)

List of abbreviations

Mb: mega base-pairs
MIC: minimum inhibitory concentration
N-AHL: *N*-acyl homoserine lactone
nlpD: lipoprotein gene
NMR: nucleic magnetic resonance
ORF: open reading frame
PCA: phenazine-1-carboxylic acid
PCN: phenazine-1-carboxamide
PGPR: plant-growth promoting rhizobacteria
PHA: polyhydroxyalkanoic acid
Phl: 2,4-diacetylphloroglucinol
phlACBD: biosynthetic operon for Phl
PhlF: TetR homologue, regulator of *phlABCD* (see Table 1 in Chapter 1)
PhlH: TetR homologue, regulator of *phlABCD* (see Table 1 in Chapter 1)
phzABCDEFGH: phenazine (*phz*) operon responsible for the synthesis of PCN
PhzI/PhzR: quorum-sensing system regulator of *phzABCDEFGH* (see Table 1 in Chapter 1)
Pip: TetR homologue indirectly regulating phenazine synthesis (phenazine inducing protein)
PsrA: TetR homologue regulating *rpoS* expression (*Pseudomonas* sigma regulator) (see Table 1 in Chapter 1)
ppGpp: guanosine tetraphosphate (see Table 1 in Chapter 1)
PQS: 2-heptyl-3-hydroxy-4-quinolone (*Pseudomonas* quinolone signal) (see Table 1 in Chapter 1)
PvdS: alternative sigma factor involved in the response to changes in iron concentrations in the environment
QscR: LuxR homologue (see Table 1 in Chapter 1)
RhlR/RhlI: quorum-sensing system (see Table 1 in Chapter 1)
RNAP: RNA polymerase
RpoS: alternative sigma factor (also called σ^S) that is recruited under stress conditions or during stationary phase of growth (see Table 1 in Chapter 1).
RsmA: small regulatory protein (regulator of secondary metabolites) (see Table 1 in Chapter 1)
RsmE: small regulatory protein (see Table 1 in Chapter 1)
RsmX: small regulatory RNA (see Table 1 in Chapter 1)
RsmY: small regulatory RNA (see Table 1 in Chapter 1)

RsmZ: small regulatory RNA (see Table 1 in Chapter 1)
RyhB: small regulatory RNA (see Table 1 in Chapter 1)
SAR: systemic acquired resistance
sdhCDAB: operon involved in iron metabolism, regulated by RyhB
Spot42: small regulatory RNA (see Table 1 in Chapter 1)
sRNA: small RNA
TetAB: pump exporting tetracycline
tetO: operator in the promoter of *tetAB* and *tetR*
TetR: transcriptional regulator of *tetAB* expression by binding to *tetO* operator in its promoter (tetracycline regulator)
VfR: cyclic AMP receptor protein (see Table 1 in Chapter 1)
VqsR: LuxR homologue (see Table 1 in Chapter 1)

