

## University of Groningen

### Genomic Wake-Up Call

Samol, Marta

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*  
2015

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Samol, M. (2015). Genomic Wake-Up Call: Activating Silent Biosynthetic Pathways for Novel Metabolites in *Penicillium chrysogenum* [Groningen]: University of Groningen

**Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

**Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

Genomic Wake-Up Call  
Activating Silent Biosynthetic Pathways  
for Novel Metabolites in *Penicillium chrysogenum*

Marta Maria Samol



university of  
groningen

Groningen Bio Institute PhD thesis.

ISSN: 1570-1530

ISBN: 978-90-367-7724-7 (printed version)

ISBN: 978-90-367-7723-0 (electronic version)

The research described in this thesis was carried out in the research group in Department of Molecular Microbiology in the Groningen Biotechnology and Biomolecular Sciences (GBB), University of Groningen in the Netherlands. (This work was financially supported by Integration of Biosynthesis and Organic Synthesis (IBOS) Program of Advanced Chemical Technologies for Sustainability (ACTS) (project no: IBOS 053.63.011).

Cover design: M.M. Samol & Off Page

Photo adapted for the cover, used with permission of EMLab P&K Laboratory, USA

Printed by: Off Page

© Copyright 2015 by M.M. Samol. All rights reserved. No part of this book may be reproduced or transmitted in any form or by any means, without prior permission of the author.

**Genomic Wake-Up Call**  
**Activating Silent Biosynthetic Pathways**  
**for Novel Metabolites in *Penicillium chrysogenum***

**PhD thesis**

to obtain the degree of PhD at the  
University of Groningen  
on the authority of the  
Rector Magnificus Prof. E. Sterken  
and in accordance with  
the decision by the College of Deans.

This thesis will be defended in public on

Friday 17 April 2015 at 14.30 hours

by

**Marta Maria Samol**

born on 20 February 1982  
in Bielsko-Biała, Poland

**Supervisor**

Prof. A.J.M. Driessen

**Assessment committee**

Prof. O.P. Kuipers

Prof. L. Dijkhuizen

Prof. H.A.B. Wösten

*dedicated to:*  
*Agata, Nikodem, Dominika*



# Contents

<b>1 General Introduction: Fungal Secondary Metabolites Gene Clusters: Strategies to Activate the Biosynthesis of Non-expressed and Novel Compounds . . . . .</b>	<b>1</b>
1.1 <i>Penicillium chrysogenum</i> . . . . .	3
1.2 Fungal Megasynthases . . . . .	3
1.2.1 NonRibosomal Peptide Synthetase (NRPS) . . . . .	3
1.2.2 Polyketide Synthetase (PKS) . . . . .	8
1.2.3 Polyketide Synthetase - Nonribosomal Peptide Synthetase Hybrids PKS-NRPS (HPN) . . . . .	10
1.3 Bioinformatic Analysis of Secondary Metabolite Genes in Fungal Genomes . . . . .	11
1.4 Molecular Methods for Secondary Metabolite Identification . . . . .	12
1.4.1 Canonical Molecular Techniques . . . . .	12
1.4.2 Heterologous Expression and Engineering of Megasynthetase Genes . . . . .	13
1.4.3 Gene Cluster- Specific Transcriptional Regulators . . . . .	14
1.5 Chromatin Landscape and Epigenetic Regulation . . . . .	15
1.5.1 Acetylation . . . . .	17
1.5.2 Histone Deacetylase Inhibitors . . . . .	18
1.5.3 Methylation . . . . .	18
1.5.4 Chromosomal Location . . . . .	21
1.5.5 LaeA - Global Regulator . . . . .	22
1.5.6 Sumoylation . . . . .	23
1.6 Environmental Simulation . . . . .	23
1.6.1 Co-Cultivation . . . . .	23
1.6.2 Physical-Chemical Cues . . . . .	24
1.7 Future Perspectives . . . . .	26
1.8 Scope of This Thesis . . . . .	26
References . . . . .	29

<b>2 Impact of the Regulator LaeA on Secondary Metabolite Production in Industrial Strains of <i>Penicillium chrysogenum</i></b>	<b>41</b>
2.1 Introduction	43
2.2 Materials and Methods	45
2.2.1 Fungal Stains, Media and Culture Conditions	45
2.2.2 Vectors and Cloning Procedures	46
2.2.3 Transformation of <i>P. chrysogenum laeA</i> Overexpression and Deletion Vector	46
2.2.4 Southern Blotting and DIG Detection	47
2.2.5 Expression Analyses by qPCR	47
2.2.6 Protein Analysis	47
2.2.7 HPLC-UV Analysis of PAA, POA, Penicillin V and G	48
2.2.8 Comparative Metabolite Profiling	48
2.3 Results	49
2.3.1 Sequencing and Expression of the <i>laeA</i> Gene in the High $\beta$ -lactam Yielding <i>P. chrysogenum</i> Strain DS17690	49
2.3.2 Overexpression and Inactivation of the <i>laeA</i> Gene in <i>P. chrysogenum</i>	50
2.3.3 Expression of Secondary Metabolite Genes in <i>P. chrysogenum</i> Strains Harboring Additional Copies of the <i>laeA</i> Gene	52
2.3.4 Overexpression of LaeA Affects Metabolites Formation	53
2.3.5 Effect of <i>laeA</i> Deletion and Overexpression on $\beta$ -lactam Formation in the High $\beta$ -lactam Yielding <i>P. chrysogenum</i> Strain	54
2.3.6 Expression Levels of CoA Ligases Genes upon LaeA Overexpression.	56
2.4 Discussion	57
2.4.1 Control Secondary Metabolites Formation by LaeA	57
2.4.2 Transcriptional Regulation of Penicillin Gene Cluster by LaeA.	58
2.5 Supplemental Data	60
References	65
<b>3 LaeA-Like Methyltransferases in <i>Penicillium chrysogenum</i></b>	<b>69</b>
3.1 Introduction	71
3.2 Material and Methods	72
3.2.1 Strains, Media, and Culture Conditions	72
3.2.2 Construction of Gene Deletion Mutants	72
3.2.3 Genomic DNA (gDNA) Extraction	72
3.2.4 Total RNA Extraction, cDNA Amplification and qPCR Analysis	73
3.2.5 HPLC-MS Analysis	73

3.3 Results . . . . .	73
3.3.1 Sequence Analysis of Putative LaeA-like Methyltransferases of <i>Penicillium chrysogenum</i> . . . . .	73
3.3.2 Array Profiling of <i>Pcllm</i> Genes in Industrial Strains of <i>P. chrysogenum</i> . . . . .	73
3.3.3 Expression of Secondary Metabolites Genes in <i>llm</i> Gene Deletion Mutants of <i>P. chrysogenum</i> . . . . .	76
3.3.4 Secondary Metabolite Formation by <i>llm</i> Gene Deletion Mutants of <i>P. chrysogenum</i> . . . . .	76
3.4 Discussion . . . . .	76
References . . . . .	81
<b>4 Polyketide Synthesized Pigment Regulated by Histone Deacetylase in <i>Penicillium chrysogenum</i> . . . . .</b>	<b>83</b>
4.1 Introduction . . . . .	85
4.2 Materials and Methods . . . . .	86
4.2.1 Media and Culture Conditions. . . . .	86
4.2.2 Plasmids Construction. . . . .	86
4.2.3 LC/MS Sample Preparation. . . . .	87
4.2.4 Genomic DNA Extraction. . . . .	87
4.2.5 Total RNA Extraction and cDNA Synthesis. . . . .	87
4.2.6 qPCR Analysis. . . . .	87
4.2.7 Southern Blot Analysis. . . . .	88
4.2.8 Secondary Metabolite Analysis. . . . .	88
4.2.9 Scanning Electron Microscopy. . . . .	89
4.2.10 Stress Assay. . . . .	89
4.3 Results . . . . .	90
4.3.1 Deletion of the <i>hdaA</i> Gene. . . . .	90
4.3.2 Effect of the <i>hdaA</i> Deletion on the Expression of Secondary Metabolite Genes. . . . .	90
4.3.3 Epigenetic Activation of a Gene Cluster Containing Two Polyketide Synthases. . . . .	91
4.3.4 HdaA Regulates the Transcription of the Chrysogine Biosynthetic Gene Cluster. . . . .	94
4.3.5 HdaA Regulates the DHN-melanin Gene Cluster Involved in Pigment Formation. . . . .	95
4.3.6 Role of <i>pks17</i> in Conidia Formation and Resistance. . . . .	97
4.4 Discussion . . . . .	99
4.5 Acknowledgements . . . . .	102
4.6 Supplemental Data . . . . .	103
References . . . . .	111

<b>5 Biosynthetic Pathways of Iron-Chelating Siderophores in Industrial Strains of <i>Penicillium chrysogenum</i></b>	<b>115</b>
5.1 Introduction . . . . .	117
5.2 Experimental Procedures . . . . .	119
5.2.1 Fungal Strains and Cultures Conditions . . . . .	119
5.2.2 Chemicals . . . . .	119
5.2.3 Strain Construction . . . . .	120
5.2.4 qPCR Analysis . . . . .	120
5.2.5 Siderophores Detection and Identification by LC -MS(/MS) . .	121
5.2.6 Data Processing . . . . .	122
5.3 Results . . . . .	123
5.3.1 Genomic Organization of Three Gene Clusters Putatively Involved in Siderophores Production . . . . .	123
5.3.2 Genes of <i>P. chrysogenum</i> Activated by Iron Deficiency . . . . .	125
5.3.3 Identification of Siderophores Produced under Iron Deficiency .	125
5.3.4 Strains with Deletion of <i>pss</i> Genes and Growth Phenotypes on Solid Media . . . . .	129
5.3.5 Metabolite Profiling of the Culture Broth of <i>pss</i> Deletion Strains	129
5.3.6 Origin of Siderophores Degradation Products . . . . .	130
5.3.7 Functional Analysis of <i>P. chrysogenum</i> Strains with a Deletion of N-acyltransferase Superfamily Encoding Genes . . . . .	130
5.4 Discussion . . . . .	134
5.5 Supplemental Data . . . . .	137
5.6 Acknowledgements . . . . .	145
References . . . . .	147
<b>6 Gene Deletion Analysis of Transporters of Siderophores in <i>Penicillium chrysogenum</i></b>	<b>151</b>
6.1 Introduction . . . . .	153
6.2 Materials . . . . .	153
6.3 Experimental Procedures . . . . .	154
6.4 Results - Transporters of Extracellular Siderophores . . . . .	155
6.5 Discussion . . . . .	155
References . . . . .	159
<b>7 Genetic Induction of Silent NRPS and NRPS-PKS (HPN) in <i>Penicillium chrysogenum</i></b>	<b>161</b>
7.1 Introduction . . . . .	163

7.2 Materials and Methods . . . . .	165
7.2.1 Strains, Media, and Growth Conditions . . . . .	165
7.2.2 Growth Curves . . . . .	165
7.2.3 Bioinformatics Tools . . . . .	166
7.2.4 Constructions of Plasmids . . . . .	166
7.2.5 Transformation and Integration into the Genome. . . . .	167
7.2.6 Gene Expression Assay. . . . .	170
7.2.7 Liquid Chromatography and Mass Spectrometry of Potential Products. . . . .	172
7.3 Results . . . . .	177
7.3.1 Expression of Silent <i>nrps</i> and <i>hpn</i> Genes under Control of the <i>PpcbC</i> Promoter . . . . .	179
7.3.2 Secondary Metabolite Formation . . . . .	181
7.4 Discussion . . . . .	182
References . . . . .	187
<b>A Relation Between Penicillin and Siderophores Production in <i>Penicillium chrysogenum</i> . . . . .</b>	<b>191</b>
A.1 Methodology . . . . .	191
A.1.1 Fungal Strains and Cultures Conditions . . . . .	191
A.1.2 qPCR Analysis and Gene Copy Number . . . . .	191
A.1.3 Extracellular Metabolite Levels . . . . .	191
A.2 Results - Penicillin Production under Iron Limited Conditions . . . . .	192
A.3 Discussion . . . . .	192
References . . . . .	197
<b>Summary . . . . .</b>	<b>199</b>
<b>Perspectives . . . . .</b>	<b>205</b>
<b>Samenvatting . . . . .</b>	<b>207</b>
<b>Streszczenie . . . . .</b>	<b>215</b>
<b>Acknowledgements/Podziękowania . . . . .</b>	<b>223</b>

