

CONTENTS

Contributors

xiii

Preface

xvii

Volumes in Series

xxiii

Section 1

Metabolites from Novel Sources

1. Methods for the Study of Endophytic Microorganisms from Traditional Chinese Medicine Plants

3

Li-Xing Zhao, Li-Hua Xu, and Cheng-Lin Jiang

1. Introduction

4

2. Experimental Methods

6

3. Novel Bioactive Compounds from Endophytic Actinobacteria

10

4. Conclusion

14

Acknowledgment

16

References

16

2. Cyanobacteria as a Source of Natural Products

23

Martin Welker, Elke Dittmann, and Hans von Döhren

1. Introduction

24

2. Cyanobacteria as Starting Material for Natural Products

25

3. Screening for New Compounds and Producers

31

4. Genomic Mining of Cyanobacterial Natural Products

36

5. Summary

41

References

41

3. Isolating Antifungals from Fungus-Growing Ant Symbionts Using a Genome-Guided Chemistry Approach

47

Ryan F. Seipke, Sabine Gruschow, Rebecca J. M. Goss,
and Matthew I. Hutchings

1. Introduction

48

2. Strain Isolation and Genome Sequencing

50

3. Identifying Antifungals made by *Pseudonocardia* P1

52

4. Identifying Antifungals made by *S. albus* S4

57

5. Summary and Perspectives

66

Acknowledgments	67
References	68

4. Gamma-Butyrolactone and Furan Signaling Systems in *Streptomyces* 71

John D. Sida and Christophe Corre

1. Introduction	71
2. AfsA-Like Enzymes and the Biosynthesis of Signaling Molecules	73
3. Heterologous Expression of an AfsA-Like-Containing Mini-Cluster Combined with Comparative Metabolic Profiling	80
4. Genetic Disruption of <i>mmfLHP</i> and Chemical Complementation Experiments	82
5. Conclusion	84
Acknowledgment	85
References	85

5. Old Meets New: Using Interspecies Interactions to Detect Secondary Metabolite Production in Actinomycetes 89

Mohammad R. Seyedsayamdost, Matthew F. Traxler, Jon Clardy, and Roberto Kolter

1. Introduction	90
2. Setting Up Binary Actinomycete Interactions	91
3. Activity-Guided Fractionation for Amychelin Isolation	98
4. Determining the Affinity of Amychelin for Fe	101
5. Identifying the Amychelin Biosynthetic Gene Cluster	103
6. Transcriptomic Analysis using Nanostring Technology	104
7. Summary	106
Acknowledgments	107
References	107

Section 2 Analyzing Gene Clusters

6. Finding and Analyzing Plant Metabolic Gene Clusters 113

Anne Osbourn, Kalliopi K. Papadopoulou, Xiaoquan Qi, Ben Field, and Eva Wegel

1. Introduction	114
2. Discovery of Plant Metabolic Gene Clusters Through Genetic Analysis	115
3. Mining Genomes for Secondary Metabolic Gene Clusters	119

4. Validating Candidate Metabolic Gene Clusters	123
5. Imaging Gene Clusters	129
6. Future Prospects	135
Acknowledgments	135
References	135
7. Genomic Approaches for Interrogating the Biochemistry of Medicinal Plant Species	139
Elsa Góngora-Castillo, Greg Fedewa, Yunsoo Yeo, Joe Chappell, Dean DellaPenna, and C. Robin Buell	
1. Introduction	140
2. Plant Material Selection and Quality	141
3. Isolation of RNA	142
4. Generation of Next-Generation whole Transcriptome Sequences	144
5. Assembly of a Reference Transcriptome	145
6. Assessment of Expression Abundances	154
7. Summary	157
8. Useful Links	157
Acknowledgments	158
References	158
8. Phylogenetic Approaches to Natural Product Structure Prediction	161
Nadine Ziemert and Paul R. Jensen	
1. Introduction	162
2. Working with Sequence Data	169
3. NaPDoS	176
4. Conclusions and Future Directions	177
Acknowledgments	178
References	178
Section 3	
Heterologous Expression of Pathways	
9. Using a Virus-Derived System to Manipulate Plant Natural Product Biosynthetic Pathways	185
Frank Sainsbury, Pooja Saxena, Katrin Geisler, Anne Osbourn, and George P. Lomonossoff	
1. Introduction	186
2. Deleted Vectors Based on Cowpea Mosaic Virus	186

3. Expression of Enzymes in Plants Using pEAQ Vectors	196
4. Conclusions and Perspectives	200
Acknowledgments	201
References	201
10. DNA Assembler: A Synthetic Biology Tool for Characterizing and Engineering Natural Product Gene Clusters	203
Zengyi Shao and Huimin Zhao	
1. Introduction	204
2. General Guidelines for Construct Design	205
3. Heterologous Expression and Fine Modification of Natural Product Gene Clusters	207
4. Characterization of Natural Product Gene Clusters	217
5. Construction of Artificial Gene Clusters	221
Acknowledgments	223
References	223
11. Reassembly of Functionally Intact Environmental DNA-Derived Biosynthetic Gene Clusters	225
Dimitris Kallifidas and Sean F. Brady	
1. Introduction	226
2. Protocol for Constructing, Arraying, and Screening Environmental Libraries	227
3. Use of TAR to Reassemble Overlapping Clones into Complete Biosynthetic Pathways	231
4. Downstream Analysis	236
5. Example	237
Acknowledgment	238
References	238
12. A Toolkit for Heterologous Expression of Metabolic Pathways in <i>Aspergillus oryzae</i>	241
Khomaizon A. K. Pahirulzaman, Katherine Williams, and Colin M. Lazarus	
1. Introduction	242
2. Fundamental Aspects of Vector Development	244
3. Heterologous Expression of a Fungal Natural Product Pathway in <i>A. oryzae</i>	246
4. Methods	250
5. Summary	256
Acknowledgments	257
References	257

13. De Novo Synthesis of High-Value Plant Sesquiterpenoids in Yeast **261**

Trinh-Don Nguyen, Gillian MacNevin, and Dae-Kyun Ro

1. Introduction	262
2. Yeast Culture and Metabolite Analysis	264
3. Expression Plasmid Construct and Transformation	268
4. De Novo Production of Sesquiterpenes	271
5. De Novo Production of Hydroxylated Sesquiterpene	272
6. General Discussion	274
Acknowledgments	276
References	276

14. Streptomyces coelicolor as an Expression Host for Heterologous Gene Clusters **279**

Juan Pablo Gomez-Escribano and Mervyn J. Bibb

1. Introduction	280
2. <i>S. coelicolor</i> Strains for Heterologous Expression	281
3. Cloning of Secondary Metabolite Gene Clusters	286
4. Introduction and Propagation of Cloned Gene Clusters in <i>S. coelicolor</i> Hosts	288
5. Preservation of Selected Clones	290
6. Growth in Liquid Culture for Metabolite Analysis	291
7. Comparative Metabolic Profiling	293
Acknowledgments	297
References	297

Section 4

Waking Up Silent Genes

15. Toward Awakening Cryptic Secondary Metabolite Gene Clusters in Filamentous Fungi **303**

Fang Yun Lim, James F. Sanchez, Clay C. Wang, and Nancy P. Keller

1. Introduction	304
2. Activation Handles of Fungal Secondary Metabolite Gene Clusters	305
3. Mining for Novel Compounds and Their Biosynthetic Genes	307
4. Genetic Manipulation of Biosynthetic and Regulatory Genes	309
5. Secondary Metabolite Analysis	316
Acknowledgments	322
References	322

16. Regulatory Cross Talk and Microbial Induction of Fungal Secondary Metabolite Gene Clusters	325
Hans-Wilhelm Nützmann, Volker Schroeckh, and Axel A. Brakhage	
1. Introduction	326
2. Production of Secondary Metabolites by Overexpression of Pathway-Specific Regulatory Genes	327
3. Genetic Manipulation of Signal Transduction Cascades	330
4. Microbial Induction of Fungal Secondary Metabolite Gene Clusters	333
Acknowledgments	338
References	338
17. Waking up <i>Streptomyces</i> Secondary Metabolism by Constitutive Expression of Activators or Genetic Disruption of Repressors	343
Bertrand Aigle and Christophe Corre	
1. Introduction	344
2. Awakening Cryptic Gene Clusters with Pathway-Specific Activators	347
3. Awakening Cryptic Gene Clusters Using Transcriptional Repressor Disruptions	355
4. Comparative Metabolic Profiling to Identify Novel Metabolites in the Engineered Strains	359
5. Conclusion/Perspectives	361
References	362
18. Use and Discovery of Chemical Elicitors That Stimulate Biosynthetic Gene Clusters in <i>Streptomyces</i> Bacteria	367
Jane M. Moore, Elizabeth Bradshaw, Ryan F. Seipke, Matthew I. Hutchings, and Michael McArthur	
1. Introduction	368
2. Role of HDAC-Like Acetyltransferases in Controlling the Expression of Biosynthetic Pathways	369
3. Use of HDAC Inhibitors to Stimulate Expression of Secondary Metabolic Genes	376
4. Screening for HDAC Inhibitors from <i>Streptomyces</i> Bacteria	381
5. Conclusion	383
References	383

19. Persister Eradication: Lessons from the World of Natural Products

387

Iris Keren, Lawrence R. Mulcahy, and Kim Lewis

1. Persisters and Infectious Disease	388
2. Mechanisms of Persister Formation	391
3. Persister Eradication	397
References	401

Author Index	407
---------------------	-----

Subject Index	437
----------------------	-----

Sean P. Brady

Laboratory of Chemical and Genetic Small Molecules, Howard Hughes Medical Institute, The Rockefeller University, New York, USA

Axel A. Brückhage

Department of Molecular and Applied Microbiology, Leibniz-Institute for Natural Product Research and Information Biology, Hans-Knoell-Institute, and Institute of Microbiology, Friedrich-Schiller-University, Jena, Germany

C. Robin Buell

Department of Plant Biology, Michigan State University, East Lansing, Michigan, USA

Joë Chappell

Department of Plant and Soil Science, University of Kentucky, Lexington, Kentucky, USA

Jon Clardy

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts, USA

Christophe Corré

Department of Chemistry, University of Warwick, Coventry, United Kingdom

Dean DeBoer

Department of Biochemistry & Molecular Biology, Michigan State University, East Lansing, Michigan, USA

Elke Dittmann

Department of Microbiology, Institute of Biochemistry and Biology, University of Potsdam, Potsdam, Germany

Greg Edwards

Department of Biochemistry & Molecular Biology, Michigan State University, East Lansing, Michigan, USA

Ben Field

Laboratoire de Chimie et de Biophysique des Plantes, Unité Mixte de Recherche 7263 Centre National de la Recherche Scientifique-Corseron et Université d'Orléans, 45071 Orléans Cedex 2, France