

CONTENTS

<i>Contributors</i>	xi
<i>Preface</i>	xv
<i>Volumes in Series</i>	xxi

Section 1 Terpenoids

1. Steady-State Kinetic Characterization of Sesquiterpene Synthases by Gas Chromatography–Mass Spectroscopy 3

Steven R. Garrett, Richard J. Morris, and Paul E. O'Maille

1. Introduction	4
2. Experimental Components and Considerations	7
3. Pilot Experiments	12
4. Steady-State Kinetic Experiments	15
5. Data Handling/Processing	16
6. Summary	17
References	18

2. Automating Gene Library Synthesis by Structure-Based Combinatorial Protein Engineering: Examples from Plant Sesquiterpene Synthases 21

Melissa Dokarry, Caroline Laurendon, and Paul E. O'Maille

1. Overview	22
2. Plasmid Library Synthesis	24
3. Optimization of SCOPE PCR Conditions	26
4. Generic SCOPE Method	29
5. Applications of SCOPE Synthesis	33
6. Troubleshooting	39
7. Conclusions	41
References	42

3. *In Planta* Transient Expression Analysis of Monoterpene Synthases 43

Sol A. Green, Xiuyin Chen, and Adam J. Match

1. Introduction	44
2. Plant Transformation Vector Construction	46

3. <i>Agrobacterium</i> -Mediated Transient Expression	49
4. <i>Agrobacterium</i> Preparation for High-Throughput Analysis	51
5. Product Identification	51
6. Transient Expression of a Kiwifruit Linalool Synthase	52
7. Conclusions	58
Acknowledgments	59
References	59
4. Natural Rubber Biosynthesis in Plants: Rubber Transferase	63
Katrina Cornish and Wenshuang Xie	
1. Introduction	64
2. Preparing Samples for Assaying Rubber Transferase Activity	64
3. The Rubber Transferase Assay	68
4. Identification and Purification of Rubber Transferase	71
5. Qualitative Protein Analysis	77
6. Protein Quantification	77
7. Summary	79
Acknowledgments	80
References	80
5. Discovery and Characterization of Terpenoid Biosynthetic Pathways of Fungi	83
Grayson T. Wawrzyn, Sarah E. Bloch, and Claudia Schmidt-Dannert	
1. Introduction	84
2. Identification, Cloning, and Characterization of Terpene Synthases	86
3. Structural Characterization and Engineering of Fungal Terpene Synthase Activities	90
4. Identification and Characterization of Biosynthetic Gene Clusters	92
5. Genome Mining for Sesquiterpene Synthases	98
6. Conclusions	101
Acknowledgments	102
References	102
6. Menaquinone Biosyntheses in Microorganisms	107
Tohru Dairi	
1. Introduction	108
2. Futasolone Pathway	109
Acknowledgments	121
References	121

7. Diversity and Analysis of Bacterial Terpene Synthases 123

Yuuki Yamada, David E. Cane, and Haruo Ikeda

1. Terpenoid Metabolites from Bacterial Cultures 124
2. Bacterial Terpene Synthases 124
3. Methods for the Study of Bacterial Terpene Synthases 129
- References 159

8. Platensimycin and Platencin Biosynthesis in *Streptomyces platensis*, Showcasing Discovery and Characterization of Novel Bacterial Diterpene Synthases 163

Michael J. Smanski, Ryan M. Peterson, and Ben Shen

1. Introduction 164
2. Methods 168
3. Conclusions 183
- Acknowledgment 183
- References 183

Section 2**Alkaloids and Glucosinolates****9. Strategies for Engineering Plant Natural Products: The Iridoid-Derived Monoterpene Indole Alkaloids of *Catharanthus roseus* 189**

Sarah E. O'Connor

1. Introduction 190
2. Metabolic Engineering Strategies 193
3. Conclusions and Future Directions 204
- References 204

10. Discovery and Functional Analysis of Monoterpenoid Indole Alkaloid Pathways in Plants 207

Vincenzo De Luca, Vonny Salim, Dylan Levac, Sayaka Masada Atsumi, and Fang Yu

1. Introduction 208
2. Enzyme Assays for MIA Pathway Steps 212
3. Methods for MIA Pathway Gene Discovery in *C. roseus* 214
4. Summary 226
- Acknowledgments 226
- References 227

11. Biochemical Genomics for Gene Discovery in Benzylisoquinoline Alkaloid Biosynthesis in Opium Poppy and Related Species	231
Thu Thuy T. Dang, Akpewwe Onoyowwi, Scott C. Farrow, and Peter J. Facchini	
1. Benzylisoquinoline Alkaloids	232
2. Transcriptomics	236
3. Proteomics	246
4. Metabolomics	250
5. Integrated Functional Genomics	255
Acknowledgments	262
References	263
12. Analysis and Modification of Ergot Alkaloid Profiles in Fungi	267
Daniel G. Panaccione, Katy L. Ryan, Christopher L. Schardl, and Simona Florea	
1. Introduction	268
2. Extraction of Ergot Alkaloids from Biological Sources	269
3. Analysis of Ergot Alkaloids by HPLC	272
4. Identification of Candidate Genes via Comparative Genomics	279
5. Modification of Ergot Alkaloid Pathways via Transformation	282
6. Summary	286
Acknowledgment	287
References	287
13. Engineering of Glucosinolate Biosynthesis: Candidate Gene Identification and Validation	291
Morten E. Møldrup, Bo Salomonsen, and Barbara A. Halkier	
1. Introduction	292
2. Pathway Elucidation and Candidate Identification <i>In Silico</i>	293
3. Metabolic Engineering of Glucosinolates in <i>N. benthamiana</i>	295
4. Metabolic Engineering of Glucosinolates in <i>S. cerevisiae</i>	303
5. Conclusion	310
Acknowledgments	311
References	311

Section 3

Type III PKSs

14. Structure–Function Analyses of Plant Type III Polyketide Synthases	317
Jing-Ke Weng and Joseph P. Noel	
1. Introduction	318
2. Bioinformatic Analyses of Type III PKSs	319
3. <i>In Vivo</i> Biochemical Function of Type III PKSs	322
4. Expression of Type III PKSs	324
5. Structural Analyses of Type III PKSs	327
6. Kinetic Analyses of Type III PKSs	329
7. Mutagenic Exploration of Type III PKS Function and Adaptive Evolution	331
Acknowledgments	332
References	332
15. Engineering of Plant Type III Polyketide Synthases	337
Toshiyuki Wakimoto, Hiroyuki Morita, and Ikuro Abe	
1. Introduction	337
2. Benzalacetone Synthase	339
3. Pentaketide Chromon Synthase	343
4. Octaketide Synthase	346
5. Polyketide Synthase 1	348
6. Practical Considerations	351
7. Summary and Future Prospects	354
Acknowledgments	355
References	355
16. Type III Polyketide Synthases in Microorganisms	359
Yohei Katsuyama and Yasuo Ohnishi	
1. Introduction	359
2. Methods of Study	363
References	374
Author Index	379
Subject Index	399