

CONTENTS

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

1. Characterization of the Recombinant Pyrophosphate-Dependent 6-Phosphofructokinases from <i>Methylobacterium alcaliphilum</i> 20Z and <i>Methylococcus capsulatus</i> Bath	1
Valentina N. Khmelenina, Olga N. Rozova, and Yuri A. Trotsenko	
1. Introduction	2
2. Methods	4
3. Brief Overview of Current Knowledge of PPI-PFK in Microorganisms	7
4. Properties of PPI-PFK from Methanotrophs	8
5. Conclusion	10
Acknowledgments	11
References	11
2. Genes and Enzymes of Ectoine Biosynthesis in Halotolerant Methanotrophs	15
Alexander S. Reshetnikov, Valentina N. Khmelenina, Ildar I. Mustakhimov, and Yuri A. Trotsenko	
1. Introduction	16
2. PCR-Based Approach for Identification of the Ectoine Biosynthesis Genes in Methanotrophs	17
3. Transcriptional Regulation of the Ectoine Biosynthesis Genes	22
4. Key Enzymes of Ectoine Biosynthesis	23
5. Conclusion	27
Acknowledgments	27
References	28
3. Facultative and Obligate Methanotrophs: How to Identify and Differentiate Them	31
Svetlana N. Dedysh and Peter F. Dunfield	
1. Introduction	32
2. Identification of Methanotrophic Capabilities in Novel Isolates	33

3. Substrate Utilization Tests	37
4. Tests for Culture Purity	38
References	43
4. Approaches for the Characterization and Description of Novel Methanotrophic Bacteria	45
John P. Bowman	
1. Introduction to Aerobic Methanotroph Characterization for Systematic Research and Other Purposes	46
2. Broad Level Systematic Diversity	46
3. Methodological Approaches	49
4. Concluding Remarks	56
References	59
5. <i>Methylococcus capsulatus</i> (Bath): From Genome to Protein Function, and Vice Versa	63
Odd A. Karlsen, Frode S. Berven, June I. Bagstevold, Øivind Larsen, and Harald B. Jensen	
1. Introduction	64
2. Mapping of the Outer Membrane Proteome of <i>M. capsulatus</i>	65
3. Inner Membrane Protein Complexes Studied with Blue-Native Polyacrylamide Gel Electrophoresis (BN-PAGE)	70
4. Visualization and Identification of <i>M. capsulatus</i> c-type Heme Proteins	74
Acknowledgments	77
References	77
6. Functional Metagenomics of Methylophiles	81
Marina G. Kalyuzhnaya, David A. C. Beck, and Ludmila Chistoserdova	
1. Introduction	82
2. Enrichment for Specific Functional Types Using SIP	83
3. Creating a Metagenomic Database and Linking It to Functionality	85
4. Ultrashort Read-Based Metatranscriptomics	90
5. Conclusions and Future Perspectives	95
Acknowledgments	96
References	96
7. Genetic Systems for Moderately Halo(alkali)philic Bacteria of the Genus <i>Methylobacterium</i>	99
David S. Ojala, David A. C. Beck, and Marina G. Kalyuzhnaya	
1. Introduction	100
2. Methods	101

3. Outlook	116
Acknowledgments	116
References	116
8. Development of a System for Genetic Manipulation of the Facultative Methanotroph <i>Methylocella silvestris</i> BL2	119
Andrew Crombie and J. Colin Murrell	
1. Introduction	120
2. Growth of <i>M. silvestris</i>	122
3. Introduction of Plasmid DNA by Conjugation and Electroporation	123
4. Gene Deletion by Electroporation of Linear DNA	123
5. Preparation of Competent Cells for Electroporation of <i>M. silvestris</i>	124
6. Construction of Linear DNA for Gene Deletion by Homologous Recombination	124
7. Electroporation	127
8. Efficiency of Gene Deletion	127
9. Case Study: Deletion of Isocitrate Lyase	128
10. Complementation	128
11. Phenotype of the Isocitrate Lyase Deletion Mutant	129
12. Conclusions	130
References	131
9. Mutagenesis of Soluble Methane Monooxygenase	135
Thomas J. Smith and J. Colin Murrell	
1. Introduction	136
2. Bacteriological Growth Media and Antibiotics	137
3. Mutagenesis and Subcloning	139
4. Expression Hosts and Conjugation	141
5. Analysis of Mutants	144
Acknowledgments	146
References	146
10. Single Cell Methods for Methane Oxidation Analysis	149
Michael C. Konopka, Sarah McQuaide, David S. Ojala, Marina G. Kalyuzhnaya, and Mary E. Lidstrom	
1. Introduction	150
2. Methods	151
Acknowledgment	165
References	165

11. Methanotroph Outer Membrane Preparation	167
Odd A. Karlsen, Frode S. Berven, Harald B. Jensen, and Anne Fjellbirkeland	
1. Introduction	168
2. Isolation of OMs	170
3. Enrichment of Integral and Tightly Associated OMPs	172
4. Extraction of Surface-Associated Proteins	172
5. Biotin Labeling of Surface-Exposed OMPs	173
References	175
12. Overexpression and Purification of the Particulate Methane Monooxygenase from <i>Methylococcus capsulatus</i> (Bath)	177
Sunney I. Chan, H.-Hoa T. Nguyen, Kelvin H.-C. Chen, and Steve S.-F. Yu	
1. Introduction	178
2. Overproduction of pMMO	179
3. Isolation and Purification of pMMO from the pMMO-Enriched Membranes	184
4. Characterization of the Purified pMMO-Detergent Complex	189
Acknowledgments	192
References	192
13. Metal Reconstitution of Particulate Methane Monooxygenase and Heterologous Expression of the pmoB Subunit	195
Stephen M. Smith, Ramakrishnan Balasubramanian, and Amy C. Rosenzweig	
1. Introduction	196
2. Preparation of apo pMMO Membranes and Metal Reconstitution	197
3. Soluble Domain Constructs of the pmoB Subunit	203
Acknowledgments	209
References	209
14. Particulate Methane Monooxygenase from <i>Methylosinus trichosporium</i> OB3b	211
Akimitsu Miyaji	
1. Introduction	212
2. Bacterial Cells Expressing pMMO	213
3. Membrane-Bound Form of pMMO	219
4. Purification of Detergent-Solubilized pMMO	221
5. Summary	224
Acknowledgments	224
References	224

15. Production, Isolation, Purification, and Functional Characterization of Methanobactins	227
David W. Graham and Hyung J. Kim	
1. Introduction: Copper, Siderophores, Chalkophores, and Methanobactin	228
2. Methanotroph Growth and Optimizing Methanobactin Production	231
3. Methanobactin Isolation and Purification	233
4. Studying Methanobactins in Pseudonatural Environments	239
Acknowledgments	243
References	243
16. A Simple Assay for Screening Microorganisms for Chalkophore Production	247
Sukhwan Yoon, Alan A. DiSpirito, Stephan M. Kraemer, and Jeremy D. Semrau	
1. Introduction	248
2. Cu-CAS Assay for Chalkophore Detection	249
3. Fe-CAS Assay for Detecting Nonspecific Binding of Copper from Cu-CAS by a Siderophore	252
4. Conclusions	256
Acknowledgments	256
References	257
17. Isolation of Methanobactin from the Spent Media of Methane-Oxidizing Bacteria	259
Nathan L. Bandow, Warren H. Gallagher, Lee Behling, Dong W. Choi, Jeremy D. Semrau, Scott C. Hartsel, Valerie S. Gilles, and Alan A. DiSpirito	
1. Introduction	260
2. Isolation of Methanobactin from the Spent Media of MOB	260
3. Purification of mb	265
4. Sample Variability	266
Acknowledgments	268
References	268

18. Measurements of Biosphere–Atmosphere Exchange of CH₄ in Terrestrial Ecosystems	271
Klaus Butterbach-Bahl, Ralf Kiese, and Chunyan Liu	
1. Introduction	272
2. Chamber Measurements of CH ₄ Exchange	274
3. Micrometeorological Measurements of CH ₄ Exchange	284
Acknowledgment	285
References	285
<i>Author Index</i>	289
<i>Subject Index</i>	303