

Contents

Preface — V

Contributing authors — XIII

Annette Summers Engel

- 1 Bringing Microbes into Focus for Speleology: An Introduction — 1**
 - 1.1 Introduction — 1
 - 1.2 Energy to Sustain Subsurface Ecosystems — 5
 - 1.3 Historical Framework of Cave Microbiology Research and Collaboration — 8
 - 1.3.1 Research following the advent of molecular genetics techniques — 9
 - 1.3.2 Sulfidic cave research — 11
 - 1.3.3 Other cave research – nonsulfidic cave systems — 13
 - 1.4 The Future of Cave Microbiology Research — 14

Daniel S. Jones

- 2 Methods for Characterizing Microbial Communities in Caves and Karst: A Review — 23**
 - 2.1 Introduction — 23
 - 2.2 Culture-based Analyses — 24
 - 2.3 Culture-independent Analyses Based on rRNA Genes — 25
 - 2.3.1 rRNA gene (rDNA) cloning — 25
 - 2.3.2 High-throughput rRNA amplicon sequencing — 29
 - 2.3.3 Terminal restriction fragment length polymorphism (T-RFLP) — 30
 - 2.3.4 Denaturing gradient gel electrophoresis (DGGE) — 31
 - 2.3.5 Fluorescence *in situ* hybridization (FISH) — 32
 - 2.4 PCR-Based Functional Gene Analysis — 33
 - 2.5 Other Methods — 34
 - 2.6 Metagenomics — 34
 - 2.7 RNA-Based Analyses and Other “-Omics” Approaches — 35
 - 2.8 Case Study: Sulfidic Cave Snottites — 35
 - 2.9 Conclusions — 39

Kathleen H. Lavoie

- 3 “A Grand, Gloomy, and Peculiar Place”: Microbiology in the Mammoth Cave Region — 47**
 - 3.1 Introduction to Mammoth Cave and the Region — 47
 - 3.1.1 The Mammoth Cave region — 48
 - 3.1.2 Mammoth Cave National Park — 49

3.2	Microorganisms in Caves — 49
3.2.1	Bacteria and Archaea — 50
3.2.2	Early microbiological studies from Mammoth Cave — 51
3.2.3	Recent microbiological studies from Mammoth Cave — 52
3.2.4	Actinobacteria — 52
3.3	Cave Ecosystem Energy — 54
3.3.1	Detrital-based ecosystems — 56
3.3.2	Phototrophy due to tourism — 57
3.3.3	Chemolithoautotrophically based cave ecosystems — 57
3.4	Geomicrobiology — 59
3.4.1	Saltpeter formation — 60
3.4.2	Ferromanganese deposits — 62
3.5	Eukaryotic Microorganisms — 63
3.5.1	Protozoa and algae — 63
3.5.2	Fungi — 64
3.6	Infections and Parasites — 67
3.6.1	Tuberculosis — 67
3.6.2	Parasites — 67
3.7	Human Impact — 68
3.8	Microbes and Cave Crickets — 69
3.8.1	Cricket crop microbes — 69
3.8.2	Crickets and fungi — 71
3.8.3	Cricket parasites — 72
3.9	Conservation of Microbes — 72
3.10	Conclusions — 73

Hazel A. Barton

4	Starving Artists: Bacterial Oligotrophic Heterotrophy in Caves — 79
4.1	Introduction — 79
4.1.1	Oligotrophy — 80
4.2	To Grow or Not to Grow — 80
4.3	The Culture-independent View of Heterotrophy in Caves — 81
4.4	Diversity of Oligotrophic Microbes in Caves — 82
4.4.1	Old friends: The Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes — 82
4.4.2	... and new: The Planctomycetes, Chloroflexi, Acidobacteria, and Verrucomicrobia — 85
4.5	Something Wicked This Way Comes – Understanding Carbon in Caves — 89
4.6	Whether 'Tis Nobler to Grow — 91

- 4.7 Out, Out, Brief Candle – Competition and Death in Cave Oligotrophs — 93
- 4.8 Heterotrophic Community Dynamics in Caves – If You Can Look into the Seeds of Time and Say Which Grain Will Grow and Which Will Not... — 95

Marianyoly Ortiz, Julia W. Neilson, Antje Legatzki, and Raina M. Maier

5 Bacterial and Archaeal Diversity on Cave Speleothem and Rock Surfaces: A Carbonate Cave Case Study from Kartchner Caverns — 105

- 5.1 Introduction — 105
- 5.2 Bacterial and Archaeal Diversity on Caves Surfaces — 106
- 5.3 Microbial Energy Dynamics in Caves — 107
- 5.4 Kartchner Caverns: An Epigenic Limestone Cave Case Study — 108
 - 5.4.1 Speleothem community diversity analysis — 109
 - 5.4.2 Cave functional dynamics – a metagenomic approach — 112
 - 5.4.3 The importance of culture-based characterizations — 113
- 5.5 Conclusions — 120

Sasha G. Tetu, Liam D. H. Elbourne, Andrew Cronan, Andrew J. Holmes, Michael R. Gillings, and Ian T. Paulsen

6 Microbial Slime Curtain Communities of the Nullarbor Caves — 125

- 6.1 Introduction — 125
 - 6.1.1 The Nullarbor Cave environment — 125
- 6.2 Microbial Slime Curtains — 126
 - 6.2.1 Microscopy and association of calcite crystals — 127
- 6.3 Community Membership — 129
- 6.4 Metabolism of Microbial Slime Communities — 132
 - 6.4.1 Weebubbie Cave nitrogen and carbon cycling — 132
- 6.5 Comparison of Metabolic Profiles from Other Habitats — 134
- 6.6 Conclusions — 134

Sarah K. Carmichael and Suzanna L. Bräuer

7 Microbial Diversity and Manganese Cycling: A Review of Manganese-oxidizing Microbial Cave Communities — 137

- 7.1 Introduction — 137
- 7.2 Manganese Oxides in Caves — 139
- 7.3 Functions and Mechanisms of Manganese Oxidation — 144
 - 7.3.1 Enzymes associated with manganese oxidation — 145
 - 7.3.2 Manganese-oxidizing bacteria and fungi — 147
 - 7.3.3 Mechanisms of microbial manganese oxidation in caves — 150
- 7.4 Remaining Questions about Manganese Oxidation in Caves — 152

Diana E. Northup and Kathleen H. Lavoie

8 Microbial Diversity and Ecology of Lava Caves — 161

- 8.1 Introduction — 161
- 8.2 Geology and Ecology of Lava Caves — 162
 - 8.2.1 Physical conditions — 164
- 8.3 Microbiological Studies in Lava Caves — 166
 - 8.3.1 Lava cave microorganisms — 169
- 8.4 Eukaryotic Microorganisms — 169
 - 8.4.1 Fungi — 169
 - 8.4.2 Protozoa and algae — 171
- 8.5 Bacteria and Archaea — 172
 - 8.5.1 Methods — 172
 - 8.5.2 Microbes in volcanic environments — 173
 - 8.5.3 Effects of mat color on microbial diversity — 174
 - 8.5.4 Impact of location on microbial diversity — 175
 - 8.5.5 Microbial endemism — 176
 - 8.5.6 Other lava cave microbiology — 177
 - 8.5.7 Nitrogen cycling — 177
- 8.6 Human Impacts and Conservation — 178
- 8.7 Microbial Morphologies — 178
 - 8.7.1 Microbial mats — 179
 - 8.7.2 Microbes masquerading as minerals — 180
- 8.8 Astrobiology — 184
- 8.8.1 Life detection strategies — 184
- 8.9 Conclusions and Future Opportunities — 186

Audrey Paterson and Annette Summers Engel

9 Predicting bacterial diversity in caves associated with sulfuric acid speleogenesis — 193

- 9.1 Introduction — 193
- 9.2 Compilation of Bacterial Diversity Data — 203
- 9.3 Controls on Bacterial Diversity in Sulfidic Karst — 206
- 9.4 Predicting the Distribution of Sulfur Bacteria in Sulfidic Karst — 208
- 9.5 Conclusions — 208

Deepak Kumaresan, Alexandra M. Hillebrand-Voiculescu, Daniela Wischer, Jason Stephenson, Yin Chen, and J. Colin Murrell

10 Microbial Life in Unusual Cave Ecosystems Sustained by Chemosynthetic Primary Production — 215

- 10.1 Introduction — 215
- 10.2 Cave Formation and Features — 216
 - 10.2.1 Mobile Cave — 216

- 10.2.2 Ayyalon Cave — 218
- 10.3 Microbial Life in a Chemolithoautotrophic Ecosystem — 219
- 10.3.1 Methanotrophy and methylotrophy — 219
- 10.3.2 Microbial metabolism of sulfur — 220
- 10.3.3 Nitrogen cycling — 222
- 10.4 Current Research and Future Perspectives — 223
- 10.4.1 Isolates and whole genome sequence analysis — 223
- 10.4.2 Microbial community composition analysis using metagenome sequences — 224
- 10.4.3 Archaeal communities — 224
- 10.5 Conclusions — 225

Cesareo Saiz-Jimenez

- 11 The Microbiology of Show Caves, Mines, Tunnels, and Tombs: Implications for Management and Conservation — 231**
- 11.1 Introduction — 231
- 11.2 Major Groups of Microorganisms — 233
- 11.2.1 Archaea — 234
- 11.2.2 Bacteria — 234
- 11.2.3 Fungi — 244
- 11.3 Consequences of Microbial Growth and Biogeochemical Cycling — 246
- 11.4 Cave Management — 249

Janez Mulec

- 12 The Diversity and Ecology of Microbes Associated with Lampenflora in Cave and Karst Settings — 263**
- 12.1 Introduction — 263
- 12.2 Photosynthesis and Artificial Lighting — 265
- 12.3 Species Composition — 267
- 12.4 Transport of Lampenflora Species and Their Relevance Underground — 268
- 12.5 Survival Strategies of Phototrophs — 270
- 12.6 Colonization of Solid Surfaces — 271
- 12.7 Biodeterioration and Remediation — 272
- 12.8 Conclusions — 274

Pedro M. Martin-Sanchez, Ana Z. Miller, and Cesareo Saiz-Jimenez

- 13 Lascaux Cave: An Example of Fragile Ecological Balance in Subterranean Environments — 279**
- 13.1 Introduction — 279
- 13.2 Review of Historical Events, Conservation Efforts, and Scientific Research — 282

13.2.1	Discovery and public exhibition —	282
13.2.2	First microbial crisis (1955–1970) —	282
13.2.3	Returning to the microbial balance (1970–2001) —	285
13.2.4	Second microbial crisis (2001–2006) —	285
13.2.5	Third microbial crisis (2006–Present) —	287
13.3	Recent Research on the Black Stains Outbreak (2009–2013) —	291
13.3.1	<i>Ochroconis</i> associated with the black stains —	292
13.3.2	Evaluation of biocide treatment of black stains on limestone —	293
13.3.3	Black stain fungal communities on clayey sediment —	294
13.3.4	Origin of the black stains on clayey sediments —	296
13.4	Conclusions —	298

Soledad Cuezva, Valme Jurado, Angel Fernandez-Cortes, Elena Garcia-Anton, Miguel Angel Rogerio-Candelera, Xavier Ariño, David Benavente, Juan Carlos Cañaveras, Cesareo Saiz-Jimenez, and Sergio Sanchez-Moral

14 Scientific Data Suggest Altamira Cave Should Remain Closed — 303

14.1	Introduction —	303
14.2	History —	305
14.3	Altamira Cave Environmental Conditions —	308
14.4	Altamira Cave Since 2009 —	310
14.4.1	Yellow colonies —	311
14.4.2	Gray colonies —	312
14.4.3	White colonies —	313
14.5	Fungi in Altamira Cave —	313
14.6	Why Should Altamira Cave Remain Closed? —	317
14.7	Final Remarks —	317

Index — 321