Thermophilic Microorganisms

Edited by

Fu-Li Li

Qingdao Institute of Bioenergy and Bioprocess Technology
Chinese Academy of Sciences
Qingdao
China
## Contents

**Contributors**

vi

**Preface**

ix

### 1. Ecology and Genetics of Deep-sea Thermophiles

Xuegong Li, Yu Zhang and Xiang Xiao

1

### 2. Diversity of Thermophilic Microorganisms and Their Roles in the Carbon Cycle

Shi-Qi Ji, Dong-Dong Meng, Kun-Di Zhang and Fu-Li Li

13

### 3. Biochemical Properties and Applications of Heat-active Biocatalysts

Christian Schäfers, Skander Elleuche and Garabed Antranikian

47

### 4. Lignocellulosic Biomass Deconstruction by the Extremely Thermophilic Genus *Caldicellulosiruptor*

Jonathan M. Conway, Jeffrey V. Zurawski, Laura L. Lee, Sara E. Blumer-Schuette and Robert M. Kelly

91

### 5. Cellulases from Thermophilic Fungi

Duochuan Li

121

### 6. Alcohol Dehydrogenases and Their Physiological Functions in Hyperthermophiles

Kesen Ma and Ching Tse

141

### 7. Roles of Polyamines in Thermophiles

Tairo Oshima

179

### 8. DNA Replication in Thermophilic Microorganisms

Sonoko Ishino and Yoshizumi Ishino

189

### 9. Metabolic Engineering of Thermophiles for Biofuel Production

Ya-Jun Liu and Qiu Cui

217

### 10. Thermophilic Viruses and Their Association with Thermophiles

Wakao Fukuda and Tadayuki Imanaka

237

**Index**

251
Thermophiles thrive in various environments in both marine and terrestrial habitats. The ability of microorganisms to proliferate under extreme conditions is of widespread importance in microbial physiology, biological evolution, the ecological cycle and industry biotechnology. The discovery of thermophilic microorganisms and their enzyme systems has created new opportunities for various industrial applications over the past decades. Temperature is one of the most important factors controlling the adaptation and evolution of organisms, and high-temperature environments are of special interest for scientists, as they reveal the extremes to which evolution has been pushed.

In this book, leading scientists in this field highlight the current achievements of the most updated topic areas. The diversity and ecological roles of thermophiles, biochemical properties of thermostable biocatalysts and their application, the role of polyamines and viruses in thermophiles, DNA replication and metabolic engineering of thermophiles are all covered. Extensive focus is given to industrial applications of thermostable catalysts including alcohol dehydrogenase, glycoside hydrolase, protease and lipases. In addition, authors discussed technical challenges and future development trends.

International experts in this field from Canada, China, Germany, Japan and the USA collaborated on this book. Thank you for all your valuable contribution. In addition, I give my thanks to Caister Academic Press. I hope and I do believe that the book will be useful to students, scientists and engineers who are interested in extreme microbial research.

Dr Fu-Li Li
Note: Where terms appear in tables, page references are in **bold**; where terms appear in figures, page references are in *italic*.

**A**
- Acetone–butanol–ethanol (ABE) 143
- Aeropyrum pernix 142, 151–152, 155
- Aigarchaeota 201
- Alcohol dehydrogenases (ADHs) 141–144
  - type I Zn-containing ADHs 147
  - type II ADHs 147
  - type III Fe-containing ADHs 150
- Alicyclobacillus acidocaldarius 50
- Alkaline Lost City Hydrothermal Field (LCHF) 6
- Alkaline serine proteases 71
- α-Glucosidases 54
- Aminopeptidase 70
- Amylases 49
  - anti-staling agents 57
- API 70
- Aqualysin I 67
- Aquifex aeolicus 192
- Aquitalea 15, 17
- Archaea 2, 4–6, 184, 190–192, 195
- Archaeal viruses 239
- Aspartic proteases 68–69
- ATP 204–205
- Auxotrophic selectable marker 8

**B**
- Bacillaceae 60
- Bacillus steathermophilus 157
- Bacillus thermoproteolyticus 69
- Bacterial immunoglobulin-like (Blg) 98
- Bacteriophage 246
- β-Elimination 97
- β-Glucosidase 130
- Bicaudaviridae 241
- Biocontrol 72, 75
- Biofuels 93, 161, 217–218, 225
- Biofungicides 72
- Butanol 228

**C**
- Caldicellulosiruptor 26, 27, 29, 91, 101–112
  - Caldicellulosiruptor saccharolyticus 225
  - Caldicellulosiruptor sp. F32 27, 31, 32
- Calditerricola saccharolyticus 181
- Carbohydrate active enzymes (CAZymes) 91, 94, 102–105, 110, 112
- Carbohydrate-binding modules (CBMs) 27, 30, 33, 34, 35, 38, 94, 96
  - type A surface binding 96
  - type B glycan chain binding 96
  - type C soluble sugar binding 96
- Carbohydrate esterase (CE) 97–98
- Carbon catabolite repression (CCR) 28, 32
- Carbon cycling 13, 14
- Carboxydothermus hydrogenoformans 158
- Catalytic residues 130
- Caudovirales 242
- CelA 103–106
- Cellobiohydrolase 126, 128
- Cellulase-binding domains (CBDs) 126–128, 134
- Cellulolytic community 24, 25
- Cellulolytic microorganisms 25, 26, 94, 99
- Cellulose 91, 121
- Cellulosomes 33, 34, 34, 100–101, 224
- Chemolithoautotrophic microorganisms 18
- Chiral chemicals 163
  - see also Racemates
- Chitin 72
- Chitinases 72–75
- Chitobiosidases 72
- Chromatography 144–145, 181
- Chymotrypsin 70
- Clavaviridae 241
- Clostridia 33, 226–228
- Clostridium 162
  - Clostridium acetobutylicum 162
  - Clostridium cellulosi 35
  - Clostridium clariflavum 37
<table>
<thead>
<tr>
<th>Protein/Enzyme</th>
<th>Page Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clostridium papyrosolvens</td>
<td>35</td>
</tr>
<tr>
<td>Clostridium thermocellum</td>
<td>33, 100, 109–111, 220–221, 223–224, 226–227</td>
</tr>
<tr>
<td>ClosTron</td>
<td>221, 230</td>
</tr>
<tr>
<td>Clusters, regularly interspaced short palindromic repeats (CRISPRs)</td>
<td>243–244</td>
</tr>
<tr>
<td>Coenzyme</td>
<td>150</td>
</tr>
<tr>
<td>Consolidated bioprocessing (CBP)</td>
<td>93, 109, 113, 218, 228</td>
</tr>
<tr>
<td>Crenarchaeota</td>
<td>239</td>
</tr>
<tr>
<td>Cyclodextrin glycosyltransferases (CGTases)</td>
<td>56</td>
</tr>
<tr>
<td>Cyclodextrins</td>
<td>55–57</td>
</tr>
<tr>
<td>Cysteine proteases</td>
<td>68</td>
</tr>
<tr>
<td>Pyrrolidone carboxyl peptidases (Pcp)</td>
<td>68</td>
</tr>
<tr>
<td>Decarboxylation</td>
<td>182</td>
</tr>
<tr>
<td>Deconstruction of lignocellulose</td>
<td>29</td>
</tr>
<tr>
<td>Defence mechanism</td>
<td>243</td>
</tr>
<tr>
<td>Deferribacteres</td>
<td>18, 19, 20, 21</td>
</tr>
<tr>
<td>Degumming</td>
<td>76</td>
</tr>
<tr>
<td>Desulfotomaculum</td>
<td>20, 22</td>
</tr>
<tr>
<td>Detergent</td>
<td>65, 71</td>
</tr>
<tr>
<td>Detoxification of aldehyde</td>
<td>160</td>
</tr>
<tr>
<td>Diastase</td>
<td>57</td>
</tr>
<tr>
<td>DNA depuration</td>
<td>186</td>
</tr>
<tr>
<td>DNA replication</td>
<td>189</td>
</tr>
<tr>
<td>DNA transformation</td>
<td>218</td>
</tr>
<tr>
<td>Dormant spores</td>
<td>19</td>
</tr>
<tr>
<td>East Pacific Rise (EPR)</td>
<td>6</td>
</tr>
<tr>
<td>Ecological global patterns</td>
<td>7</td>
</tr>
<tr>
<td>Electroporation</td>
<td>219</td>
</tr>
<tr>
<td>Endochitinase</td>
<td>75</td>
</tr>
<tr>
<td>Epsilonproteobacteria</td>
<td>5, 6, 15, 17, 18</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>161, 226</td>
</tr>
<tr>
<td>Esterases</td>
<td>61–63</td>
</tr>
<tr>
<td>Esterification</td>
<td>97</td>
</tr>
<tr>
<td>Ethanol production</td>
<td>162, 226</td>
</tr>
<tr>
<td>Euryarchaeota</td>
<td>239</td>
</tr>
<tr>
<td>Expansins</td>
<td>99</td>
</tr>
<tr>
<td>Extrachromosomal elements (ECEs)</td>
<td>238</td>
</tr>
<tr>
<td>FEN1</td>
<td>207</td>
</tr>
<tr>
<td>Feruloyl esterases</td>
<td>64</td>
</tr>
<tr>
<td>Fibronectin type III (FN3)</td>
<td>98, 107–110</td>
</tr>
<tr>
<td>Fimbriates</td>
<td>18, 19, 20, 21, 22, 23</td>
</tr>
<tr>
<td>Fungal cellulases</td>
<td>122–123, 132</td>
</tr>
<tr>
<td>Fuselloviridae</td>
<td>241</td>
</tr>
<tr>
<td>Genetic system</td>
<td>7, 8, 112–113</td>
</tr>
<tr>
<td>Genome, secretomes and transcriptome</td>
<td>32–35</td>
</tr>
<tr>
<td>Genomes</td>
<td>102, 240, 241</td>
</tr>
<tr>
<td>Geobacillus</td>
<td>162</td>
</tr>
<tr>
<td>GINS and Cdc6</td>
<td>194–198</td>
</tr>
<tr>
<td>Globuloviridae</td>
<td>241</td>
</tr>
<tr>
<td>Glucoamylases</td>
<td>53</td>
</tr>
<tr>
<td>Glucose repression</td>
<td>125</td>
</tr>
<tr>
<td>Glycoside hydrolases (GHs)</td>
<td>27, 29, 30, 50, 94–96</td>
</tr>
<tr>
<td>Free-acting GHs</td>
<td>35</td>
</tr>
<tr>
<td>Multidomain GHs</td>
<td>30, 30, 75, 101</td>
</tr>
<tr>
<td>Glycosylation</td>
<td>106, 126</td>
</tr>
<tr>
<td>Green fluorescent proteins (GFP)</td>
<td>219–220</td>
</tr>
<tr>
<td>Guaymas Basin</td>
<td>5</td>
</tr>
<tr>
<td>Guttaviridae</td>
<td>241</td>
</tr>
<tr>
<td>H</td>
<td></td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>14, 29–30, 93</td>
</tr>
<tr>
<td>Homologous recombination</td>
<td>221</td>
</tr>
<tr>
<td>Homology modelling</td>
<td>130</td>
</tr>
<tr>
<td>Horizontal gene transfer (HGT)</td>
<td>244</td>
</tr>
<tr>
<td>HPLC</td>
<td>181–182</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>225</td>
</tr>
<tr>
<td>Hydrogen-oxidizing</td>
<td>15, 23</td>
</tr>
<tr>
<td>Hydrothermal vent</td>
<td>1–4, 15, 16, 19, 21</td>
</tr>
<tr>
<td>See also Shallow hydrothermal vents</td>
<td></td>
</tr>
<tr>
<td>Hydrothermal vent community</td>
<td>4–7</td>
</tr>
<tr>
<td>Hyperthermophiles</td>
<td>7, 13, 26, 47</td>
</tr>
<tr>
<td>I</td>
<td></td>
</tr>
<tr>
<td>Immunoglobulin-like (Ig-like) proteins</td>
<td>98</td>
</tr>
<tr>
<td>Industrial application</td>
<td>57, 63, 71, 76, 143</td>
</tr>
<tr>
<td>Infection</td>
<td>242</td>
</tr>
<tr>
<td>In vitro</td>
<td>186</td>
</tr>
<tr>
<td>K</td>
<td></td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>162</td>
</tr>
<tr>
<td>Korarchaeota</td>
<td>201</td>
</tr>
<tr>
<td>L</td>
<td></td>
</tr>
<tr>
<td>Lig I protein</td>
<td>205</td>
</tr>
<tr>
<td>Lignocellulosic biomass</td>
<td>14, 25, 91–93, 217, 225</td>
</tr>
<tr>
<td>Pretreatment of lignocellulose</td>
<td>30</td>
</tr>
<tr>
<td>Lignocellulosic biomass deconstruction</td>
<td>91, 99</td>
</tr>
<tr>
<td>Linker regions</td>
<td>106</td>
</tr>
<tr>
<td>Lipases</td>
<td>59–60</td>
</tr>
<tr>
<td>Lipolytic enzymes</td>
<td>58–64</td>
</tr>
<tr>
<td>Lipothrixviridae</td>
<td>240</td>
</tr>
<tr>
<td>Low-temperature habitats</td>
<td>19</td>
</tr>
<tr>
<td>M</td>
<td></td>
</tr>
<tr>
<td>Macroalgae</td>
<td>14</td>
</tr>
<tr>
<td>Marine environments</td>
<td>72</td>
</tr>
<tr>
<td>See also Marine sediments</td>
<td></td>
</tr>
<tr>
<td>Marine Group I (MGI)</td>
<td>6</td>
</tr>
<tr>
<td>Marine microorganisms</td>
<td>14</td>
</tr>
<tr>
<td>Marine sediments</td>
<td>20, 22, 23</td>
</tr>
<tr>
<td>See also Marine environments</td>
<td></td>
</tr>
<tr>
<td>Maritimacin</td>
<td>70</td>
</tr>
<tr>
<td>Markerless disruption system</td>
<td>9</td>
</tr>
<tr>
<td>MCM (minichromosome maintenance)</td>
<td>190, 194–197</td>
</tr>
<tr>
<td>Meat tenderization</td>
<td>72</td>
</tr>
<tr>
<td>Metabolic engineering</td>
<td>218</td>
</tr>
<tr>
<td>Metabolic pathways</td>
<td>182</td>
</tr>
<tr>
<td>Metagenomics</td>
<td>112</td>
</tr>
<tr>
<td>Metal binding motifs</td>
<td>150</td>
</tr>
<tr>
<td>Metalloproteases</td>
<td>69–70</td>
</tr>
<tr>
<td>Microbial fuel cells (MFC)</td>
<td>20, 21</td>
</tr>
</tbody>
</table>
### N
- N-Acetyl-d-glycosaminidases 72
- NADP(H) 150
- NAD(P) + 159, 204
- Nascent strand synthesis 200

### O
- Okazaki fragment 204
- Omics technology 224
- Open reading frames (ORFs) 32, 35, 36, 123, 241
- Optimal pH 151
- oriC (origin of chromosome) 191–194, 196
- Oxidation of alcohols 159
- Oxidative enzymes 134
- Oxygen 18

### P
- PCNA (proliferating cell nuclear antigen) 190, 202–206
- Pectinases 75–77
- Phylogenetic analysis 131
- Phytoenes 77–78
- PolD 201
- Polyamines 179–181
- Polyalacturonases 75
- Polysaccharide lyase (PL) 97
- Polysaccharides 15, 24
- Pre-replication complex (pre-RC) 194
- Primer synthesis 199
- Processive DNA synthesis 202
- Proteolytic enzymes 64–71
- Proviruses regions 244–245
- Pullulanases 54, 55
- Putrescine 182
- Pyrococcus aerophilum 157
- Pyrococcus furiosus 26, 163, 191, 193
- Pyrococcus furiouos 146–149, 153, 156, 195, 225
- Pyrolysin 67

### R
- Racemates 63
- see also Chiral chemicals
- (R)-Benzoin 164
- Replication origin 190
- Replicative helicase complex 194
- Resistance selectable marker 7
- Reverse genetics 185
- RFC (replication factor C) 190, 202–204
- Rudriviridae 240

### S
- Saccharomyces cerevisiae 159, 161, 194
- S-Adenosylmethionine (SAM) 182
- Salterproviruses 242
- Separate enzymatic hydrolysis and fermentation (SHF) 218
- Serine proteases 65–67
- Shallow hydrothermal vents 18
- see also Hydrothermal vent
- Short-chain ADH 141, 155, 160, 163
- Simultaneous saccharification and fermentation (SSF) 93, 218, 226
- Single-stranded DNA (ssDNA) 194
- Site-directed mutagenesis 133–134
- S-layer homology (SLH) 107, 108–110
- S-layer protein (SLP) 99
- Spermine 179
- Sphaerolipoviridae 246
- Spiraviridae 241
- Spore-forming 18, 20, 22, 24
- Starch-degrading enzymes 49, 51–52
- Substrate-binding residues 130
- Substrate specificity 158
- Sulfate reduction rates (SRRs) 22
- Sulfolobus 155
- sulfur-oxidizing 18
- sulfur-reducing 5, 15, 18, 23
- Surface layer homology (SLH) 99

### T
- Taxonomy of viruses (ICTV) 239
- Textile processing 76
- Thaumarchaeota 201
- Thermacae 15, 17
- Thermoanaerobacter 162, 220, 227
- Thermoanaerobacter brockii 163
- Thermoanaerobacter ethanolicus 147, 153, 158
- Thermoanaerobacter tengcongensis 53
- Thermoanaerobacterium 220, 227
- Thermococcales 9–10, 242
- Thermococcus 156, 163
- Thermococcus kodakarensis 7–10, 150, 244–245
- Thermodesulfobacteria 15, 17
- Thermolyssin-like peptidases (TLP) 69
- Thermomicrobiurn 158
- Thermophilic fungi 121–125
- Thermophilic microorganisms 13, 15, 17, 60, 62, 218, 245–246
- see also Thermophiles
- Thermophilicity 132–133, 146
- Thermosyntrophina 60
- Thermotargetron 222
- Thermotoga 26, 157, 191
- Thermodomarina 164, 193
- Thermotogae 15, 17
- Thermostabilization 48–49
- Thermus thermophilus 157, 163, 191
- Thermus thermophilus 179–180, 182–184, 240
- Three-dimensional structures 128, 146–147
- Tn (thermal denaturation temperature) 186
- Transcription factors 125
- Transformation 7
- Turriviridae 242

### V
- Viruses 237
- see also Archaeal viruses
- Volatile fatty acids (VFA) 22

### Z
- Zymomonas mobilis 143, 161