Biofilms in Plant and Soil Health
Biofilms in Plant and Soil Health

Edited by

Iqbal Ahmad
Aligarh Muslim University, Aligarh, India

Fohad Mabood Husain
King Saud University, Saudi Arabia
## Contents

Preface xviii  
List of Contributors xx

1 Biofilms: An Overview of Their Significance in Plant and Soil Health 1  
   Iqbal Ahmad, Mohammad Shavez Khan, Mohd Musheer Altaf, Faizan Abul Qais,  
   Firoz Ahmad Ansari and Kendra P. Rumbaugh
1.1 Introduction 1  
1.2 Biofilm Associated with Plants 3  
1.3 Biofilm Formation Mechanisms: Recent Update on Key Factors 4  
1.4 Biofilm in Soil and Rhizospheres 7  
1.5 Genetic Exchange in Biofilms 7  
1.6 Diversity and Function of Soil Biofilms 8  
1.7 The Role of Biofilms in Competitive Colonization by PGPR 8  
1.8 Biofilm Synergy in Soil and Environmental Microbes 9  
1.9 Biofilms in Drought Stress Management 10  
1.10 Plant Health and Biofilm 10  
1.11 How Microbial Biofilms Influence Plant Health? 10  
1.12 Soil Health and Biofilms 12  
1.13 How to Assess Soil Health? 13  
1.14 Impact of Biofilms on Soil Health 14  
1.15 Biofilm EPS in Soil Health 14  
1.16 Conclusions and Future Directions 15  
References 15

2 Role of PGPR in Biofilm Formations and Its Importance in Plant Health 27  
   Govind Gupta, Sunil Kumar Snehi and Vinod Singh
2.1 Introduction 27  
2.2 Rhizosphere: A Unique Source of Microorganisms for Plant Growth  
   Promotion 27  
2.3 Plant Growth–Promoting Rhizobacteria 28  
2.3.1 Direct Impact of Plant Growth–Promoting Rhizobacteria on Plant  
   Nutrition 29  
2.3.1.1 Nitrogen Fixation 29  
2.3.1.2 Phosphorus Solubilization 30  
2.3.1.3 Potassium Solubilization 30
Contents

2.3.1.4 Siderophore Production 30
2.3.1.5 Phytohormone Production 31
2.3.1.6 Indole Acetic Acid (IAA) Production 31
2.3.1.7 Gibberellins and Cytokinins Production 31
2.3.2 In Direct Impact of Plant Growth–Promoting Rhizobacteria on Plant Nutrition 32
2.3.2.1 Antibiotic Production 32
2.3.2.2 Enzyme Production 32
2.3.2.3 Induced Systemic Resistance 32
2.3.2.4 Hydrogen Cyanide Production 33
2.3.2.5 Exopolysaccharides Production or Biofilm Formation 33
2.4 Biofilm Producing Plant Growth–Promoting Rhizobacteria 34
2.5 Role of PGPR in Biofilm Formations 35
2.6 Future Research and Development Strategies for Biofilm Producing Sustainable Technology 35
2.7 Conclusion 36
Acknowledgments 36
References 36

3 Concept of Mono and Mixed Biofilms and Their Role in Soil and in Plant Association 43
Janaina J. de V. Cavalcante, Alexander M. Cardoso and Vânia L. Muniz de Pádua
3.1 Introduction 43
3.2 Soil- and Plant-Associated Biofilms 45
3.3 Microbial Signaling, Regulation, and Quorum Sensing 46
3.4 Biotechnology 48
3.5 Outlook 49
Acknowledgments 49
References 49

4 Bacillus Biofilms and Their Role in Plant Health 55
Mohd Musheer Altaf, Iqbal Ahmad, Mohd Sajjad Ahmad Khan and Elisabeth Grohmann
4.1 Introduction 55
4.2 Interaction of Bacillus within Plant Rhizosphere and Biofilm Development 57
4.3 Multispecies Biofilms and Their Significance 59
4.4 Biofilm Detection and Characterization 60
4.5 Bacillus Biofilm and Plant Health Promotion 60
4.6 Conclusion and Future Prospects 62
References 63

5 Biofilm Formation by Pseudomonas spp. and Their Significance as a Biocontrol Agent 69
Zaki A. Siddiqui and Masudulla Khan
5.1 Introduction 69
5.2 Biofilms 79
5.3 Mechanisms of Biofilm Formation 81
5.3.1 Quorum Sensing 81
5.3.2 Regulation in Response to Phosphorus Starvation 82
5.3.3 Phase Variation 82
5.3.4 Motility and Chemotaxis 82
5.3.5 Surface Adhesins 83
5.3.6 Biofilm Matrix Components 83
5.4 Metabolites Affecting Biofilm Formation 84
5.4.1 Plant Defense Compounds 84
5.4.2 Phenazine 84
5.4.3 Surfactants 84
5.5 Biofilm Formation and Biological Control of Plant Diseases 84
5.6 Conclusion 85
References 86

6 Quorum Sensing Mechanisms in Rhizosphere Biofilms 99
Jorge Barriuso
6.1 Background 99
6.2 QS in Biofilms Formation 101
6.2.1 Positive Interactions 102
6.2.1.1 Plant Growth–Promoting Rhizobacteria (PGPR) 102
6.2.1.2 Rhizobia 104
6.2.2 Negative Interactions 105
6.2.3 Cross-Communication 105
6.3 Conclusions 106
References 107

7 Biofilm Formation and Quorum Sensing in Rhizosphere 111
Kusum Harjai and Neha Sabharwal
7.1 Introduction 111
7.2 Importance of Rhizosphere 111
7.3 Constituents of Rhizosphere 112
7.3.1 Physical/Chemical 112
7.3.2 Rhizosphere—A Hot Niche of Microbial Activity 112
7.3.2.1 Bacteria 112
7.3.2.2 Fungi 113
7.3.2.3 Actinomycetes and Protozoa 113
7.4 Communication in Rhizosphere 113
7.5 Quorum Sensing in Rhizobia 115
7.5.1 Quorum Sensing in Rhizobium 115
7.5.1.1 cinI and cinR 115
7.5.1.2 raiI and raiR 116
7.5.1.3 rhii and rhiR 116
7.5.1.4 traI and traR 116
7.5.2 Quorum Sensing in Sinorhizobium 117
7.5.2.1 sinI and sinR 117
7.5.2.2 expR 117
7.5.2.3 *trai, traR* and *mell* 118
7.5.3 Quorum Sensing in *Mesorhizobium* 118
7.6 Quorum Sensing in Pseudomonads 118
7.6.1 Quorum Sensing in *Pseudomonas aeruginosa* 118
7.6.1.1 *Las* System 118
7.6.1.2 *Rhl* System 118
7.6.1.3 PQS System 119
7.6.2 Quorum Sensing in Other Pseudomonads 120
7.7 Biofilm Formation in Rhizosphere 120
7.7.1 Beneficial Root Biofilm 121
7.7.2 Pathogenic Root Biofilm 123
7.7.3 Mixed-Species Biofilm 123
7.8 Conclusions 124
References 124

8 The Significance of Fungal Biofilms in Association with Plants and Soils 131
*Michael W. Harding, Lyriam L.R. Marques, Bryon Shore and G.C. Daniels*
8.1 Introduction 131
8.2 What Is a Biofilm? 132
8.3 Where Do We Find Filamentous Fungal Biofilms? 132
8.4 Fungal Biofilms: What Have We Learned from the Budding Yeasts? 133
8.5 What Does a Filamentous Fungal Biofilm Look Like? 134
8.6 Examples of Filamentous Fungal Biofilms 136
8.6.1 Ascomycete Biofilms 136
8.6.2 Zygomycete Biofilms 138
8.6.3 Basidiomycete Biofilms 138
8.6.4 Oomycete Biofilms 138
8.7 Examples of Fungal Biofilms in Soils and the Rhizosphere 139
8.7.1 Mycorrhizae 139
8.7.2 Ectomycorrhizae as a Biofilm 139
8.7.3 A Brief Look at Endomycorrhiza as a Biofilm 140
8.8 The Mycorhizosphere 141
8.9 A Biofilm Approach to Plant Disease Management 141
References 143

9 Chemical Nature of Biofilm Matrix and Its Significance 151
*Mohd Sajjad Ahmad Khan, Mohd Musheer Altaf and Iqbal Ahmad*
9.1 Introduction 151
9.2 Structural Composition of EPS 154
9.2.1 Exopolysaccharides of the Biofilm Matrix 154
9.2.1.1 Carbohydrate Content of Exopolysaccharides 155
9.2.1.2 Polysaccharides of Gram-Negative Bacteria 155
9.2.1.3 Polysaccharides and Related Compounds in Gram-Positive Bacteria 157
9.2.2 Proteins 158
9.2.3 eDNA 159
9.2.4 Surfactants and Lipids 159
9.2.5 Water 160
9.3 Properties of Matrices 160
9.4 Functions of the Extracellular Polymer Matrix: The Role of Matrix in Biofilm Biology 162
  9.4.1 Role of EPS in Biofilm Architecture 164
  9.4.2 Role of EPS in Mechanisms of Antimicrobial Resistance/Tolerance to Other Toxic Substances 165
9.5 Conclusion 168
Acknowledgments 168
References 169

10 Root Exudates: Composition and Impact on Plant–Microbe Interaction 179
Shamsul Hayat, Ahmad Faraz and Mohammad Faizan
10.1 Introduction 179
10.2 Chemical Composition of Root Exudates and Their Significance 180
10.3 Root Exudates in Mediating Plant–Microbe Interaction in Rhizosphere (Negative and Positive Interactions) 180
10.4 Direct and Indirect Effect of Root Exudates on PGPR, Root Colonization, and in Stress Tolerance 182
  10.4.1 Root Colonization 183
  10.4.2 Root Exudates and Stress Tolerance 184
10.5 Role of Root Exudates in Biofilm Formation by PGPR 185
10.6 Role of Root Exudates in Protecting Plants Pathogenic Biofilm, Quorum Sensing Inhibition 186
10.7 Isolation of Root Exudates 187
10.8 Conclusion 188
References 189

11 Biochemical and Molecular Mechanisms in Biofilm Formation of Plant-Associated Bacteria 195
Alwar Ramanujam Padmavathi, Dhamodharan Bakkiyaraj and Shunmugiah Karutha Pandian
11.1 Introduction 195
11.2 Plant-Associated Bacteria 196
11.3 Biofilms and Plant Pathogens 196
11.4 Molecular and Biochemical Mechanisms Involved in Biofilm Formation 197
  11.4.1 Pseudomonas 197
  11.4.2 Xanthomonas 199
  11.4.3 Erwinia 200
  11.4.4 Ralstonia 200
  11.4.5 Pectobacterium carotovorum 201
  11.4.6 Xylella fastidiosa 201
  11.4.7 Agrobacterium tumefaciens 202
  11.4.8 Dickeya 203
  11.4.9 Clavibacter michiganensis 204
### 12 Techniques in Studying Biofilms and Their Characterization: Microscopy to Advanced Imaging System in vitro and in situ

**Elisabeth Grohmann and Ankita Vaishampayan**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.1 Introduction</td>
<td>215</td>
</tr>
<tr>
<td>12.2 Classical Techniques to Study Biofilms</td>
<td>216</td>
</tr>
<tr>
<td>12.2.1 Nucleic Acid Stains and FISH (in Combination with Epifluorescence Microscopy)</td>
<td>216</td>
</tr>
<tr>
<td>12.2.2 FISH and Confocal Laser Scanning Microscopy (CLSM)</td>
<td>217</td>
</tr>
<tr>
<td>12.4 The Biofilm Flow Cell</td>
<td>218</td>
</tr>
<tr>
<td>12.5 Advanced Digital Analysis of Confocal Microscopy Images</td>
<td>221</td>
</tr>
<tr>
<td>12.6 Biofilm Studies at Different Scales</td>
<td>222</td>
</tr>
<tr>
<td>12.6.1 Microscale: LSM and Structural Fluorescent Sensors</td>
<td>223</td>
</tr>
<tr>
<td>12.6.2 Nanoscale: Structured Illumination Microscopy (SIM) and Stimulated Emission Depletion (STED) Microscopy</td>
<td>223</td>
</tr>
<tr>
<td>12.6.3 Mesoscale: Optical Coherence Tomography (OCT) and Scanning Laser Optical Tomography (SLOTy)</td>
<td>224</td>
</tr>
<tr>
<td>12.7 Conclusions and Perspectives</td>
<td>224</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>225</td>
</tr>
<tr>
<td>References</td>
<td>225</td>
</tr>
</tbody>
</table>

### 13 Gene Expression and Enhanced Antimicrobial Resistance in Biofilms

**Daniel Padilla-Chacón, Israel Castillo-Juárez, Naybi Muñoz-Cazares and Rodolfo García-Contreras**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.1 Introduction</td>
<td>231</td>
</tr>
<tr>
<td>13.2 Biofilms in the Plant–Microbe Relationship</td>
<td>232</td>
</tr>
<tr>
<td>13.2.1 Biofilm Formation in the Vascular System (Xylem)</td>
<td>232</td>
</tr>
<tr>
<td>13.2.2 Biofilm Formation in Rizosphere (Roots)</td>
<td>234</td>
</tr>
<tr>
<td>13.3 Stress Induces Biofilm Formation</td>
<td>236</td>
</tr>
<tr>
<td>13.4 Relevance for Bacterial-Associated Plants</td>
<td>237</td>
</tr>
<tr>
<td>13.5 Enhanced Antimicrobial Resistance in Biofilms Is Mediated by Biofilm Physicochemical Characteristics and Specific Changes in Gene Expression</td>
<td>237</td>
</tr>
<tr>
<td>13.6 Potential for Implementing Antibiofilm Strategies to Protect Crops</td>
<td>239</td>
</tr>
<tr>
<td>Conclusions</td>
<td>244</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>244</td>
</tr>
<tr>
<td>References</td>
<td>244</td>
</tr>
</tbody>
</table>

### 14 In Vitro Assessment of Biofilm Formation by Soil- and Plant-Associated Microorganisms

**Michael W. Harding and G.C. Daniels**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.1 Introduction</td>
<td>253</td>
</tr>
</tbody>
</table>
16.3.1 Rhizosphere Effect 293
16.3.2 Rhizosphere Competence 294
16.3.3 Involvement of Genes and Traits in Rhizosphere Colonization 294
16.4 Quorum Sensing as a Way of Interaction between Bacteria and Host Plant 295
16.5 Biofilms 296
16.5.1 Why Microorganisms Form Biofilms 297
16.5.2 Composition of Biofilms 297
16.5.2.1 Extrapolymeric Substance 297
16.5.2.2 Water 297
16.5.2.3 Biomolecules 297
16.5.3 Mechanism of Biofilm Formation 298
16.5.3.1 Surface Attachment of Bacteria 299
16.5.3.2 Microcolony Formation 299
16.5.3.3 Matured Biofilm and Dispersion 299
16.5.4 Dynamics of Biofilms 299
16.5.4.1 Nutritional Conditions 299
16.5.4.2 Surface Characteristics 300
16.5.4.3 Exopolysaccharides 300
16.5.4.4 Flagella and Motility 301
16.5.4.5 Quorum Sensing Signals 301
16.5.4.6 Gene Expression 301
16.5.4.7 Shear Stress 301
16.5.4.8 Phenazines 302
16.6 Effects of Stress on Plants 302
16.6.1 Abiotic Stress 302
16.6.1.1 Drought Stress in Plants 302
16.6.1.2 Salinity Stress in Plants 304
16.6.1.3 Flooding Stress in Plants 305
16.6.1.4 Heat Stress in Plants 305
16.6.1.5 Oxidative Stress in Plants 307
16.6.2 Biotic Stress in Plants 308
16.7 Stress Tolerance in Plants 309
16.7.1 Adaptation Mechanisms of Plants Toward Abiotic Stress 309
16.7.2 Management of Abiotic and Biotic Stresses in Plants 309
16.7.2.1 Phytohormone Production 310
16.7.2.2 Maintenance of Nutrient Content 310
16.7.2.3 Nitrogen Fixation 311
16.7.2.4 Phosphorous Solubilization 311
16.7.2.5 Siderophore Production 312
16.7.2.6 Exopolysaccharide (EPS) Production 312
16.7.2.7 ACC Deaminase Activity 312
16.7.2.8 Volatile Organic Compounds (VOCs) 312
16.7.2.9 PGPR as Biotic Elicitors 312
16.7.2.10 Induction of Systemic Disease Resistance 313
16.7.3 Management of Abiotic and Biotic Stress in Plants via Biofilm-Forming Rhizobacteria 313
16.7.3.1 Salt Stress Amelioration 313
16.7.3.2 Drought Stress Amelioration 313
16.7.3.3 Temperature 314
16.7.3.4 Metal Transformation 315
16.7.3.5 Biocontrol Activity 315
16.7.4 Stress Management via Quorum Sensing Signals Producing PGPR 315
16.8 Conclusion 316
16.9 Future Perspectives 317
Acknowledgments 317
List of Abbreviations 317
References 318

17 Developed Biofilm-Based Microbial Ameliorators for Remediating Degraded Agroecosystems and the Environment 327
G. Seneviratne, P.C. Wijepala and K.P.N.K. Chandrasiri
17.1 Introduction 327
17.2 Developed Microbial Communities as a Potential Tool to Regenerate Degraded Agroecosystems 328
17.3 Biochemistry of Fungal-Bacterial Biofilms 330
17.4 Endophytic Microbial Colonization with the Application of Fungal-Bacterial Biofilms 330
17.5 Biofilm Biofertilizers for Restoration of Conventional Agroecosystems 331
17.6 Developed Microbial Biofilms for Environmental Bioremediation 331
17.6.1 Fungal-Bacterial Biofilms for Heavy Metal Bioremediation in Soil–Plant Environment 332
17.6.2 Fungal-Bacterial Biofilms for Heavy Metal Bioremediation in Wastewater 332
17.7 Conclusion 333
References 333

18 Plant Root–Associated Biofilms in Bioremediation 337
Sadaf Kalam, Anirban Basu and Sravani Ankati
18.1 Introduction 337
18.2 Biofilms: Definition and Biochemical Composition 337
18.3 Bioremediation and Its Significance 338
18.4 Root-Associated Biofilms 340
18.4.1 Microbial Biofilm Associations on Plant Root Surface 340
18.4.2 Formation of Rhizospheric Biofilms by PGPR and Their Application 340
18.4.3 Role of Root Exudates in Triggering Biofilm Formation 342
18.4.4 Consequences of Root-Associated Biofilms on Plant Growth 342
18.5 Bioremediation of Contaminants in Rhizospheric Soils 344
18.5.1 Rhizosphere, Rhizodeposition, and Bioremediation 344
18.5.2 Bioremediation of Xenobiotics 344
18.5.3 Bioremediation of Heavy Metal(loid)s 344
18.5.4 Rhizobacteria Facilitating Bioremediation 345
18.5.5 Metal Accumulating Rhizobacteria 346
18.5.6 Role of Root Exudates in Heavy Metal Decontamination and Degradation of Organic Pollutants 346
18.6 Implications of Rhizospheric Biofilm Formation on Bioremediation 347
18.7 Conclusion and Future Prospects 348
Acknowledgments 349
References 349

19 Biofilms for Remediation of Xenobiotic Hydrocarbons—A Technical Review 357
John Pichtel
19.1 Introduction 357
19.1.1 Conventional Bioremediation Technologies 357
19.1.2 Composition and Properties of Biofilms 358
19.1.3 Unique Properties of Biofilms 358
19.1.4 Significance of Biofilms to Environmental Remediation 359
19.1.5 Objectives 359
19.2 Polycyclic Aromatic Hydrocarbons 359
19.2.1 Microbiology of PAH Degradation 360
19.2.2 Biofilm Processes and PAH Degradation 360
19.2.3 Microbial Production of Surfactant Molecules 361
19.2.4 Application of Surfactants 362
19.2.5 Degradation of PAHs in Biofilm Reactors 362
19.3 Chlorinated Ethanes, Ethenes, and Aromatics 364
19.3.1 Chlorinated Ethanes 364
19.3.1.1 Microbiology of Biodegradation of Chlorinated Ethanes 364
19.3.1.2 Degradation of Chlorinated Ethanes in Biofilm Reactors 365
19.3.2 Chlorinated Ethenes 366
19.3.3 Degradation of Chlorinated Ethenes in Biofilm Reactors 367
19.4 Chlorinated Aromatics 369
19.4.1 Degradation of Chlorinated Aromatics in Biofilm Reactors 369
19.4.2 Benefits of Activated Charcoal and Other Organic Matrixes for Biofilm Reactors 370
19.5 Polychlorinated Biphenyls (PCBs) 371
19.5.1 Microbiology of PCB Biodegradation 372
19.5.2 Biofilms and PCB Degradation 373
19.5.3 Degradation of PCBs in Biofilm Reactors 374
19.6 Polychlorinated Dibenzodioxins 374
19.7 Conclusions 375
References 375

20 Plant Pathogenic Bacteria: Role of Quorum Sensing and Biofilm in Disease Development 387
Deepak Dwivedi, Mayuri Khare, Himani Chaturvedi and Vinod Singh
20.1 Introduction 387
20.2 Mechanism of Biofilm Formation 388
20.2.1 Biofilm Formation in Vitro in Plants 389
20.2.1.1 Gram-Negative Bacteria 389
20.2.1.2 Gram-Positive Bacteria 390
20.3 Quorum Sensing Mechanism 391
20.3.1 Quorum Sensing Regulated Virulence Factors 392
20.3.1.1 Mechanisms in Gram-Negative Bacteria 392
20.3.1.2 Mechanisms in Gram-Positive Bacteria 393
20.3.2 Biofilm Formation in Candida 394
20.4 Plant Pathogenic Bacteria Diversity and Plant Diseases 395
20.5 Blocking Quorum Sensing and Virulence in Combating Phytopathogen 395
20.6 Conclusion 400
References 400

21 Biofilm Instigation of Plant Pathogenic Bacteria and Its Control Measures 409
A. Robert Antony, R. Janani and V. Rajesh Kannan
21.1 Introduction 409
21.2 Plant Pathogens 409
21.2.1 Importance and Impact of Plant Pathogenic Bacteria 410
21.2.2 Plant Pathology and Plant Bacteriology: Historical Background 411
21.2.3 Classification of Plant Pathogenic Bacteria 412
21.2.3.1 Rhizosphere Pathogen 412
21.3 Plant Physiological Alteration by Plant Pathogens 412
21.3.1 Photosynthesis 412
21.3.2 Vascular Function 412
21.3.3 Root Function 412
21.3.4 Respiration 413
21.3.5 Transpiration 413
21.4 Virulence Strategies of Plant Pathogenic Bacteria 413
21.5 Biofilm Formations 414
21.5.1 Mechanism of Biofilm Formation 415
21.5.2 Molecular Insights on Biofilm Formation 416
21.5.3 Structural and Functional Components Involved in Biofilm Formation 416
21.5.3.1 Surface Bacterial Factors 418
21.5.3.2 Extracellular Factors Involved in Bacterial Autoaggregation 418
21.5.4 Factors Favoring Biofilm Formation 419
21.6 Biofilm Controlling Strategies in Plant Pathogens 419
21.7 Main Targets and Some Potential Tools to Modify Biofilms 420
21.8 Physical Tools for Modifying Biofilms 421
21.8.1 Modification of Biofilm Surfaces 421
21.8.2 Hydrophobicity, Surface Roughness, and Surface Charge 422
21.8.3 Exopolysaccharides 422
21.8.4 Applications of Hydrolytic Enzymes 423
21.8.5 Applications of Surface Active Compounds and Natural Products 423
21.8.6 Quorum Quenching 423
21.8.6.1 Compound Interfering Systems of AHLs 424
21.8.6.2 Compound Interfering with Regulation Molecules 425
21.8.6.3 Action of 3-Indolyl Acetyl Nitrile 425
21.9 Chemical Methods 425
21.9.1 Inhibitors of Nucleotide Biosynthesis and DNA Replication as Antibiofilm Agents 425
21.9.2 Effect of Salicylic Acid on Biofilms 426
21.9.3 N-acetyl Cysteine Effects on Biofilm 426
21.10 Biological Methods 426
21.10.1 Biosurfactants as Antibiofilm Agents 426
21.10.2 Phage Mediated Biocontrol as Antibiofilm Agents 428
21.11 Future Prospects of Antibiofilm 429
21.12 Conclusion 430
References 430

22 Applications of Biofilm and Quorum Sensing Inhibitors in Food Protection and Safety 439
Ashraf A. Khan, John B. Sutherland, Mohammad Shavez Khan, Abdullah S. Althubiani and Iqbal Ahmad
22.1 Introduction 439
22.2 Biofilm Formation by Foodborne Pathogens 439
22.3 Significance of Biofilms in Food and Food Environments 440
22.4 Biofilm Control Strategies in the Food Industry 441
22.5 Natural Products as Antibiofilm Agents and Their Potential Applications 446
22.6 Role of QS Inhibitors in Biofilm Control 449
22.7 Conclusions 451
Acknowledgments 451
References 451

23 Biofilm Inhibition by Natural Products of Marine Origin and Their Environmental Applications 465
Alwar Ramanujam Padmavathi, Dhamodharan Bakkiyaraj and Shunmugiah Karutha Pandian
23.1 Introduction 465
23.2 Unity Is Strength: Benefits of Biofilm Formers 466
23.3 Transition of Slimy Film to Persistent Biofilm 467
23.4 Biofilm-Related Infections in Plants 467
23.5 Need for Antibiofilm Agents 467
23.6 Natural Products of Marine Origin as Antibiofilm Agents 469
23.7 Semi-synthetic Antibiofilm Agents Inspired by Marine Natural Products 469
23.8 Environmental Applications of Antibiofilm Agents 469
23.9 Conclusion 472
References 472
24  Plant-Associated Biofilms Formed by Enteric Bacterial Pathogens and Their Significance 479
Meenu Maheshwari, Mohammad Shavez Khan, Iqbal Ahmad, Ashraf A. Khan, John B. Sutherland and Abdullah S. Althubiani

24.1  Introduction 479
24.2  Enteric Pathogens in the Plant Environment 480
24.3  Colonization and Biofilm Formation by Enteric Bacteria on Plant Surfaces 483
24.4  Biofilm Regulation in Enteric Bacteria 484
24.5  Influence of Plant Defense on Survival and Biofilm Formation by Enteropathogens 485
24.6  Plant-Associated Enteric Bacteria in Food Safety and Human Health 486
24.7  Conclusions 487
References 487

25  Anti-QS/Anti-Biofilm Agents in Controlling Bacterial Disease: An in silico Approach 497
K. Ahmad, M.H. Baig, Fohad Mabood Husain, Iqbal Ahmad, M.E. Khan, M. Oves, Inho Choi and Nasser Abdulatif Al-Shabib

25.1  Introduction 497
25.2  Biofilm and Its Significance 498
25.3  Bioinformatics Approaches in Drug Target Identification and Drug Discovery 500
25.4  Target Identification Using in silico Technologies 500
25.5  Data Resources for Drug Target Identification 501
25.6  Homology Modeling 501
25.7  Docking 502
25.8  Virtual Screening 503
25.9  Application of Bioinformatics in Development of Anti-QS/anti-biofilm Agents 503
25.10 Virtual Screening for Identification of QS Inhibitors 505
25.11 Conclusion 507
References 507

Index 513
Microbes are well known for their diverse metabolic activity and unique survival strategies under various natural ecological niches. The lifestyles of microbial community in diverse environments are now being increasingly explored. Throughout the biological world, microbes thrive predominantly in surface-attached, matrix-enclosed, multicellular communities or biofilms, as opposed to isolated planktonic mode.

Our understanding of microbial interaction with biotic and abiotic surfaces in biofilm growth has been extensively investigated, and its relevance in microbial survival under continuously fluctuating environments is recognized. The physiology, molecular mechanism of biofilm formation, and its regulation have been observed in many bacteria and yeasts. The role of biofilm in medical settings, the food industry, and the environment has also been studied. Recently, the functions of biofilm under more complex habitats associated with soil and plants have been investigated. The nature of these biofilms may be synergistic, associative or pathogenic.

Bacteria causing plant diseases are relatively well understood with respect to the role of biofilm in pathogenesis. However, complex environments like plant root, rhizosphere, and bulk soil have been less explored. Several researchers across the globe are attempting to understand the biofilm in these niches, explore polymicrobial biofilm in these conditions, and exploit these interactions for sustainable agriculture through maintenance of plant and soil health. Developing methods for sustaining crop production and environmental health are of prime importance in feeding global populations on a sustainable basis. Using improved ultrastructure techniques to research molecular biology and biofilm has revolutionized the study on biofilm in complex ecosystem.

Recently, many books on biofilm have been published—mainly on medical, food, and environmental aspects, including bioremediation. However, this is probably the first book that takes a holistic view on biofilms and their significance in plant and soil health.

This book addresses current literature and issues in four sub areas: (i) fundamental significance of biofilm in plant and soil health, and the concept of mono and mixed biofilms by PGPR and fungal biofilms; (ii) biochemical and molecular mechanism in biofilm studies in plant associated bacteria, techniques in studying biofilms and their characterization, gene expression and enhanced antimicrobial resistance in biofilms, and biotic and abiotic factors affecting biofilm in vitro; (iii) the ecological significance of soil-associated biofilms and stress management and bioremediation of contaminated soils and degraded ecosystem; and (iv) pathogenic biofilm associated with plants and food and its control measures.
The book is essential for everyone interested in biofilms and their application in agriculture, plant and soil health, and bioremediation, as well as public health concern with environmental pathogenic biofilms. It is recommended to students and researchers of all disciplines of microbiology, biotechnology, and plant and soil sciences and agriculture and environmental biotechnology industry.

With great pleasure, we extend our sincere thanks to all the learned contributors for their timely response, excellent contributions, and consistent support and cooperation.

We would like to sincerely acknowledge the support for scientific evaluation of chapters from learned professors/senior scientists, especially Professor John Pichtel (Ball State University, USA), Dr. Mahipal Singh (Buffalo University USA), Dr. Ashraf A. Khan and Dr. John B. Sutherland (NCTR, USA), Prof. Rumbaugh Kendra (Texas University, USA), and Prof. Elisabeth Grohmann (Germany).

We are grateful to Lt. Gen. (retd.) Zameer Uddin Shah (Vice-Chancellor, AMU), Dr. Bakri bin Matouk Bakri Assas (Rector, UQU, Makkah), Prof. Mohammad Iqbal A. Khan, Dr. Waleed Jameel Altaf, Dr. H. H. Abulees, and Dr. Abdullah Safar Althubiani (UQU, Makkah, KSA), Prof. Shamim Ahmad (JNMC, AMU, Aligarh), and Dr. Gurbachan Singh (Director, ASRB, ICAR, New Delhi, India), and Dr. S. Farooq, Director, Himalaya Drug Co., for their encouragement and moral support.

We express our deep sense of gratitude to all our respected teachers, scientific collaborators, biofilm scientific community, and friends for their guidance, support, and healthy criticism. The cooperation received from doctoral research students in the preparation of this book is gratefully acknowledged. The names of a few require special mention: Mohd Musheer Altaf, Mohd Shavez Khan, Menu Maheshwari, and Faizan Abul Qais.

The technical assistance and support rendered from the dynamic Wiley book publishing team is most appreciated and acknowledged.

Many thanks to the members of our families for all the support they have provided. Finally, we acknowledge Almighty God, who provided all the inspirations, insights, positive thoughts, and channels to complete this book project.

We hope that the readers will find the book interesting and informative. We have strived to provide current research trends on this rapidly increasing field, both for instruction and as a motivation for further investigation.

We welcome suggestions and comments by readers for future improvement.

Iqbal Ahmad,
Aligarh, India
Fohad Mabood Husain,
Reyadh, KSA
List of Contributors

Hussein H. Abulreesh  
Department of Biology  
Faculty of Applied Science  
Umm Al-Qura University  
Makkah, Saudi Arabia

Iqbal Ahmad  
Department of Agricultural Microbiology  
Faculty of Agricultural Sciences  
Aligarh Muslim University  
Aligarh, India

K. Ahmad  
Department of Biosciences  
Integral University  
Lucknow, India

Nasser Abudalatif Al-Shabib  
Department of Food Science and Nutrition  
College of Food and Agriculture  
King Saud University  
Riyadh, Saudi Arabia

Mohd Musheer Altaf  
Plant Biofilm Research Group  
Department of Agricultural Microbiology  
Aligarh Muslim University  
Aligarh, India

Abdullah Safar Althubiani  
Department of Biology  
Faculty of Applied Science  
Umm Al-Qura University  
Makkah, Saudi Arabia

Sravani Ankati  
Molecular Plant-Microbe Interaction Lab  
Department of Plant Sciences  
School of Life Sciences  
University of Hyderabad  
Hyderabad, India

Firoz Ahmad Ansari  
Department of Agricultural Microbiology  
Aligarh Muslim University  
Aligarh, India

A. Robert Antony  
Rhizosphere Biology Laboratory  
Department of Microbiology  
Bharathidasan University  
Tiruchirappalli, India

M. H. Baig  
Department of Medical Biotechnology  
Yeungnam University  
Republic of Korea

Dhamodharan Bakkiyaraj  
Department of Biotechnology  
Alagappa University  
Karaikudi, India

and

Department of Microbiology  
Faculty of Science  
Prince of Songkla University  
Hat Yai, Songkhla, Thailand
**Jorge Barriuso**  
Department of Environmental Biology (Lab 245)  
Centro de Investigaciones Biológicas (CIB-CSIC)  
Consejo Superior de Investigaciones Científicas  
Madrid, Spain

**Anirban Basu**  
Molecular Plant-Microbe Interaction Lab  
Department of Plant Sciences  
School of Life Sciences  
University of Hyderabad  
Hyderabad, India

**Israel Castillo-Juárez**  
Colegio de Postgraduados  
Campus Montecillo  
Posgrado de Botánica  
Montecillo, Mexico City, Mexico

**Janaina Japiassu de Vasconcelos Cavalcante**  
Environmental Biotechnology Laboratory  
West Zone State University – Uezo  
RJ, Brazil

**Naybi Muñoz-Cazares**  
Colegio de Postgraduados  
Campus Montecillo  
Posgrado de Botánica  
Montecillo, Mexico City, Mexico

**K.P.N.K. Chandrasiri**  
Microbial Biotechnology Unit  
National Institute of Fundamental Studies (NIFS)  
Kandy, Sri Lanka

**Himani Chaturvedi**  
Department of Microbiology  
Barkatullah University  
Bhopal, India

**Inho Choi**  
Department of Medical Biotechnology  
Yeungnam University  
Republic of Korea

**Puneet Singh Chauhan**  
Division of Plant Microbe Interactions  
CSIR-National Botanical Research Institute  
Lucknow, India

**G.C. Daniels**  
Alberta Agriculture and Forestry  
Crop Diversification Centre South  
Brooks, Canada

**Deepak Dwivedi**  
Department of Microbiology  
Barkatullah University  
Bhopal, India

**Mohammad Faizan**  
Department of Botany  
Aligarh Muslim University  
Aligarh, India

**Ahmad Faraz**  
Department of Botany  
Aligarh Muslim University  
Aligarh, India

**Rodolfo García-Contreras**  
Universidad Nacional Autónoma de México  
Department of Microbiology and Parasitology  
Faculty of Medicine  
Mexico City, Mexico

**Elisabeth Grohmann**  
Department of Life Sciences and Technology  
Beuth University of Applied Sciences  
Berlin  
Berlin, Germany
Govind Gupta  
Department of Microbiology  
Barkatullah University  
Bhopal, India

Michael W. Harding  
Alberta Agriculture and Forestry  
Crop Diversification Centre South  
Brooks, Canada

Kusum Harjai  
Department of Microbiology  
Panjab University  
Chandigarh, India

Shamsul Hayat  
Department of Botany  
Aligarh Muslim University  
Aligarh, India

Fohad Mabood Husain  
Department of Food Science and Nutrition  
College of Food and Agriculture Sciences  
King Saud University  
Riyadh, Saudi Arabia

Huma Jafri  
Department of Agricultural Microbiology  
Aligarh Muslim University  
Aligarh, India

Rajendran Janani  
Rhizosphere Biology Laboratory  
Department of Microbiology  
Bharathidasan University  
Tiruchirappalli, India

Sadaf Kalam  
Molecular Plant-Microbe Interaction Lab  
Department of Plant Sciences  
School of Life Sciences  
University of Hyderabad  
Hyderabad, India

Ashraf A. Khan  
Division of Microbiology (HFT-250)  
National Center for Toxicological Research  
U.S. Food and Drug Administration  
Jefferson, Arkansas, USA

Masudulla Khan  
Division of Plant pathology and Biocontrol  
Department of Botany  
Aligarh Muslim University  
Aligarh, India

M.E. Khan  
School of Chemical Engineering  
Yeungnam University  
Republic of Korea

Mohammad Shavez Khan  
Department of Agricultural Microbiology  
Faculty of Agricultural Sciences  
Aligarh Muslim University  
Aligarh, India

Mohd Sajjad Ahmad Khan  
Department of Biology  
College of Medicine  
Imam Abdulrahman Bin Faisal University, Dammam  
Saudi Arabia

Mayuri Khare  
Department of Microbiology  
Barkatullah University  
Bhopal, India

Meenu Mahaeshewari  
Department of Agricultural Microbiology  
Faculty of Agricultural Sciences  
Aligarh Muslim University  
Aligarh, India
Lyriam L.R. Marques
MicroBio SMARTS
Calgary, Canada

Alexander Cardoso Machado
Environmental Biotechnology Laboratory
West Zone State University – Uezo
RJ, Brazil

Mohd. Oves
Faulty of Science
Centre of Excellence in Environmental Studies
King Abdul Aziz University
Jeddah, Saudi Arabia

Daniel Padilla-Chacón
Colegio de Postgraduados
Campus Montecillo
Posgrado de Botánica
Montecillo, Mexico City, Mexico

Alwar Ramanujam Padmavathi
Department of Biotechnology
Alagappa University
Karaikudi, India

and

Nanotec-PSU Center of Excellence on Drug Delivery System and Department of Pharmaceutical Technology
Faculty of Pharmaceutical Sciences
Prince of Songkla University
Hat Yai, Songkhla, Thailand

Shunmugiah Karutha Pandian
Department of Biotechnology
Alagappa University
Karaikudi, India

Vânia Lúcia Muniz de Pádua
Environmental Biotechnology Laboratory
West Zone State University – Uezo
RJ, Brazil

John Pichtel
Ball State University
Natural Resources and Environmental Management, Muncie, USA

Faizan Abul Qais
Department of Agricultural Microbiology
Aligarh Muslim University
Aligarh, India

Velu Rajesh Kannan
Rhizosphere Biology Laboratory
Department of Microbiology
Bharathidasan University
Tiruchirappalli, India

Kendra P. Rumbaugh
Department of Surgery
Texas Tech University Health Sciences Center
Lubbock, Texas, USA

Neha Sabharwal
Department of Microbiology
Panjab University
Chandigarh, India

Gamini Seneviratne
Microbial Biotechnology Unit
National Institute of Fundamental Studies (NIFS)
Kandy, Sri Lanka

Byron Shore
Nautilus Environmental
Calgary, Canada

Zaki Anwar Siddiqui
Division of Plant Pathology and Biocontrol,
Department of Botany
Aligarh Muslim University
Aligarh, India
**Arpita Singh**  
Division of Plant Microbe Interactions  
CSIR-National Botanical Research Institute  
Lucknow, India

**Vinod Singh**  
Department of Microbiology  
Barkatullah University  
Bhopal, India

**Sunil Kumar Snehi**  
Department of Microbiology  
Barkatullah University  
Bhopal, India

**John B. Sutherland**  
Division of Microbiology (HFT-250)  
National Center for Toxicological Research  
U.S. Food and Drug Administration  
Jefferson, Arkansas, USA

**Ankita Vaishampayan**  
Beuth University of Applied Sciences  
Berlin  
Department of Life Sciences and Technology  
Berlin, Germany

**P.C. Wijepala**  
Microbial Biotechnology Unit  
National Institute of Fundamental Studies (NIFS)  
Kandy, Sri Lanka
1

Biofilms: An Overview of Their Significance in Plant and Soil Health

Iqbal Ahmad¹, Mohammad Shavez Khan¹, Mohd Musheer Altaf¹, Faizan Abul Qais¹, Firoz Ahmad Ansari¹ and Kendra P. Rumbaugh²

¹ Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh, India
² Department of Surgery, Texas Tech University Health Sciences Center, Lubbock, Texas, USA

1.1 Introduction

The green revolution has enhanced agricultural productivity to a great extent with the increased use of high-yielding crop varieties, heavy farm equipment, synthetic fertilizers, pesticide applications, improved irrigation, better soil management, and massive conversion of forest to agricultural lands [1, 2]. But there is a growing concern that intensive agricultural practices promote large-scale ecosystem degradation and loss of productivity. Adverse environmental effects include deforestation, soil degradation, large-scale greenhouse gas emissions, accumulation of pesticides and chemical fertilizers, pollution of groundwater, and decreased water table due to excessive irrigation [1, 3].

The world population is currently around 7 billion and is projected to approximately 8 billion by the year 2025 and 9 billion by 2050. Considering this population growth and the environmental damage due to ever-increasing industrialization, it is clear that feeding the world’s population will be a daunting task over the next 50 years. Therefore, there is a need for new strategies and approaches to improve agricultural productivity in a sustainable and environmentally friendly manner [4]. The effective use of beneficial microorganisms in agriculture in an integrated manner is an attractive technology to address these problems. The role of soil microorganisms in agriculture to improve the availability of plant nutrients and plant health is well known [5]. However, the ability of root-associated microbes to improve nutrient supply and plant protection has yet to be fully exploited [6].

The colonization of the adjacent volume of soil under the plant root is known as rhizosphere colonization. Rhizosphere colonization not only works as a fundamental step in the pathogenesis of soil microbes but also plays an important role in the employment of microorganisms for beneficial purposes [7]. Beneficial rhizobacteria normally promote plant growth by establishing themselves on plant roots and suppressing the colonization or eliminating the presence of pathogenic microorganisms [8]. The competitive exclusion of deleterious rhizosphere organisms is directly linked to the ability to successfully colonize a root surface. However, disease suppressive mechanisms were
shown by plant growth–promoting rhizobacteria (PGPR) to be of no use until these microbes successfully colonized and established themselves on root surfaces [9, 10].

Bacterial root colonization is primarily influenced by the presence of the specific character of bacteria necessary for adherence and subsequent colonization. Moreover, several biotic and abiotic factors also play significant roles in bacterial-plant root interactions and colonization. When an organism colonizes a root, factors like water content, temperature, pH, soil characteristics, composition of root exudates, mineral contents, and other microorganisms may influence the process of root colonization. However, plants are the major determinant of microbial diversity [11]. Recent studies on the root-microbe interaction have indicated that rhizobacteria can colonize the root zone and form biofilm and biofilm-like structures. This phenomenon is considered to be a survival strategy by the rhizobacteria, which provides protection to the plant under stress conditions [12].

Traditionally, microbes have been characterized as freely suspended (planktonic) cells; although, many pioneering microbiologists recognized the surface-associated growth of microorganisms on tooth surfaces, aquatic environments, and other biotic and abiotic surfaces. However, a detailed examination of biofilms only became possible after observation under the electron microscope [13, 14]. Based on the observation of dental plaque and other sessile communities, in 1987 Costerton et al. put forth a theory on biofilms that explained the mechanisms of microbial adherence to living and nonliving material, and the benefits associated with this lifestyle. Since then, studies on biofilms in environmental, industrial, and ecological settings relevant to public health have increased significantly [15]. Much of the work on biofilms in the last few decades has demonstrated tremendous growth and understanding through the utilization of scanning electron microscopy, scanning confocal laser microscopy, and both standard microbiology cultural techniques and molecular-based investigation. The ultrastructures of biofilm, roles of various adhesins, genes, and regulatory pathways have all been explored in model organisms [16]. Our understanding of biofilms in natural settings has also substantially improved as new methods allow us to better distinguish different microbial species within complex communities [17–19].

According to Costerton, “the father of biofilm,” a biofilm is defined as “a structural community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface” [20]. However, this definition was later modified to include other characteristics of biofilm such as irreversible cell attachment, altered phenotype with respect to growth rate, and characteristic changes in gene transcription [21]. The composition of the self-produced polymeric material is mainly exopolysaccharide, protein, lipid, and DNA [19]. (Chapter 9 provides details of EPS composition.)

Biofilm formation is a complex process involving various steps such as initial adsorption or reversible attachment, irreversible attachment and the formation of a microbial monolayer on the substrate, early development of microcolonies, maturation of the biofilm structure, including the formation of characteristic architectural features, and lastly, the dispersion (or shedding) of planktonic cells from the biofilm [22]. Each of these stages is very distinct in their morphology and regulation [23]. The sessile growth of microorganisms has distinct phenotypes compared to planktonic cells and exhibits enhanced resistance to antimicrobial compounds and alterations in nutrient uptake [24].

Biofilms provide an important and fundamental strategy for adaptation and survival in the environment, as well as in the pathogenesis of various bacterial pathogens
associated with humans, animals and plants [25]. Other applications of biofilms, which have been subsequently studied and are under active investigation, relate to the environmental sciences and food industry. However, in this chapter we will only address the roles of biofilm in plant and soil health, as well as briefly touch on their public health perspective.

1.2 Biofilm Associated with Plants

Biofilms are assemblages of microorganisms adhered to each other and/or to a surface and embedded in a matrix of exopolymers [26]. Biofilms are microniches, which are entirely different from their surrounding environment, and which allow microbes to work as a functional unit, accomplishing tasks not possible in their planktonic state or outside biofilms. The list of the possible effects of biofilms on bacterial ecology and biology, such as protection from desiccation, salinity, UV exposures, acid exposures, metal toxicity, predation and bactericides, and enhancement of genetic exchange and of synergistic interactions is impressive [22, 26]. Biofilms might also foster the expression of density-dependent phenotypes. Induction of the expression of certain bacterial genes, in a density-dependent manner, is known to require the accumulation of diffusible molecules such as acyl homoserine lactones, via a process called quorum sensing (QS) [27].

Research on microbial biofilms is proceeding extensively on many fronts in the medical, environmental, and food industries [22, 28]. Biofilm formation by bacteria on various biotic and abiotic surfaces such as mineral crystals, corrosion particles, clay, silt particles, living cells/tissues of human, animals, and plants has been extensively demonstrated. However, our understanding of plant-associated biofilms is still limited. This is probably due to the complexity of microbes in the soil-root association and difficulties in studying the mixed biofilm under natural/ simulated models [29]. However, over the last decade, many researchers have explored the beneficial association of biofilm with plants [26, 30], which can be exploited to enhance plant protection and promote growth even under stress conditions [31].

Plant-associated microbes can be distinguished as commensal, mutualistic, and pathogenic, and can interact with different parts of the plant such as leaves, stems, roots, seeds, and the vascular system. A number of well-studied, pathogenic, plant bacteria that form biofilms on leaves, vascular system and other plant parts are described in detail by various investigators, as well as in this book (see Chapters 20 and 21). For example, pathogenic bacteria such as *Pseudomonas syringae* colonize leaves and cause brown spot disease. Various studies have demonstrated the importance of surface colonization and aggregation for bacterial survival and competition on aerial plant surfaces [32, 33]. Similarly, vascular pathogens colonizing xylem are prevalent and of great economic importance. *Xylella fastidiosa* is an endophyte and cause of Pierce’s disease on grapevines and citreous variegated chlorosis [34]. The gene expression profile of *Xylella fastidiosa* growing as a biofilm indicates the role of several genes likely involved in attachment, such as fimbrial proteins and surface proteins. Elevated expression of plasmid HGT has also been reported in biofilms [35]. In addition, *Xanthomonas campestris*, *Pantoea stewart*, and *Ralstonia solanacearum*, among others, have been studied in recent years for their capacity to form biofilm during the infection process [36–38].
Conversely, other bacterial species form mutualistic or beneficial biofilms within rhizo­spheres and on root surfaces. These relationships have been the subject of recent investigations, and some excellent review articles have been published [16, 39–41]. This topic is further discussed in Chapters 2 and 3.

The attachment and surface colonization of PGPR has been widely studied in agriculture and horticulture. Rhizobacteria was found to be effective in root colonization and plant growth promotion after inoculation into wheat or rice and other seedlings [42]. Competitive root colonization by PGPR is considered to be one of the major prerequisites for sustained crop productivity. In many cases, attachment by bacteria leading to root surface colonization also results in biofilm formation [43]. Studies conducted on various PGPR, such as *Bacillus*, *Pseudomonas*, *Rhizobium*, and *Azotobacter*, have demonstrated successful biofilm formation and root colonization [44–47], although both are also influenced by biotic and abiotic factors [48]. Therefore, it is now considered that biofilm-forming PGPR will be more effectively colonized on the plant roots when inoculated and will thus be able to sufficiently withstand the fluctuating conditions of the soil environment to perform its plant growth–promotion activity.

Another aspect of biofilm research that has received increased attention is the association of human pathogens with plants [49]. Biofilm on seeds and sprouts and salad crops for human consumption are a potential health concern, as they may harbor pathogenic or opportunistic pathogens. Plant-associated pathogens such as *Salmonella*, *E. coli*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* [50–53] may form biofilms, with maximum thicknesses ranging from 5 to 12 µm [54] on plants. Such bacterial species may come into contact with humans, causing sickness, as well as poor food quality and other hygiene concerns. Details on this aspect are also discussed in Chapter 23 of this book.

### 1.3 Biofilm Formation Mechanisms: Recent Update on Key Factors

Biofilm formation by several human, animal, and plant pathogenic bacteria has been well studied and reported. A general layout of the biofilm formation process and key regulatory factors is depicted in Figure 1.1.

The mechanism of biofilm formation is a highly regulated process. Each species responds to its own set of environmental conditions via distinct molecular mechanisms. However, several stimuli are generally important for plant-associated biofilms. Recently, new insights on the molecular regulation of biofilm formation in plant-associated bacteria have been described by Castiblanco and Sundin [55]. The authors also review progress in understanding the role of cyclic diGMP, cyclic GMP, and small RNAs during the regulation of biofilm formation by plant pathogens such as *Erwina amylovora*, *Agrobacterium tumefaciens*, and *Xanthomonas* spp. [55]. This topic is also discussed in Chapters 20 and 21 of this book.

However, the exact mechanisms of regulation have yet to be discovered and understood in many plant-associated biofilms, especially in regard to rhizospheres and mixed-species biofilms. We are just beginning to understand how various components of complex soil impacts biofilm establishment in the rhizosphere and functions under natural conditions. Novel mechanisms of genetic regulation in biofilms by pathogenic