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

Iqbal Ahmad[1], *Mohammad Shavez Khan*[1], *Mohd Musheer Altaf*[1], *Faizan Abul Qais*[1], *Firoz Ahmad Ansari*[1] *and Kendra P. Rumbaugh*[2]

[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

Biofilms in Plant and Soil Health, First Edition. Edited by Iqbal Ahmad and Fohad Mabood Husain.

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. *Xyllela fastidosa* is an endophyte and cause of Pierce's disease on grapevines and citreous variegated chlorosis [34]. The gene expression profile of *Xyllela fastidosa* 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

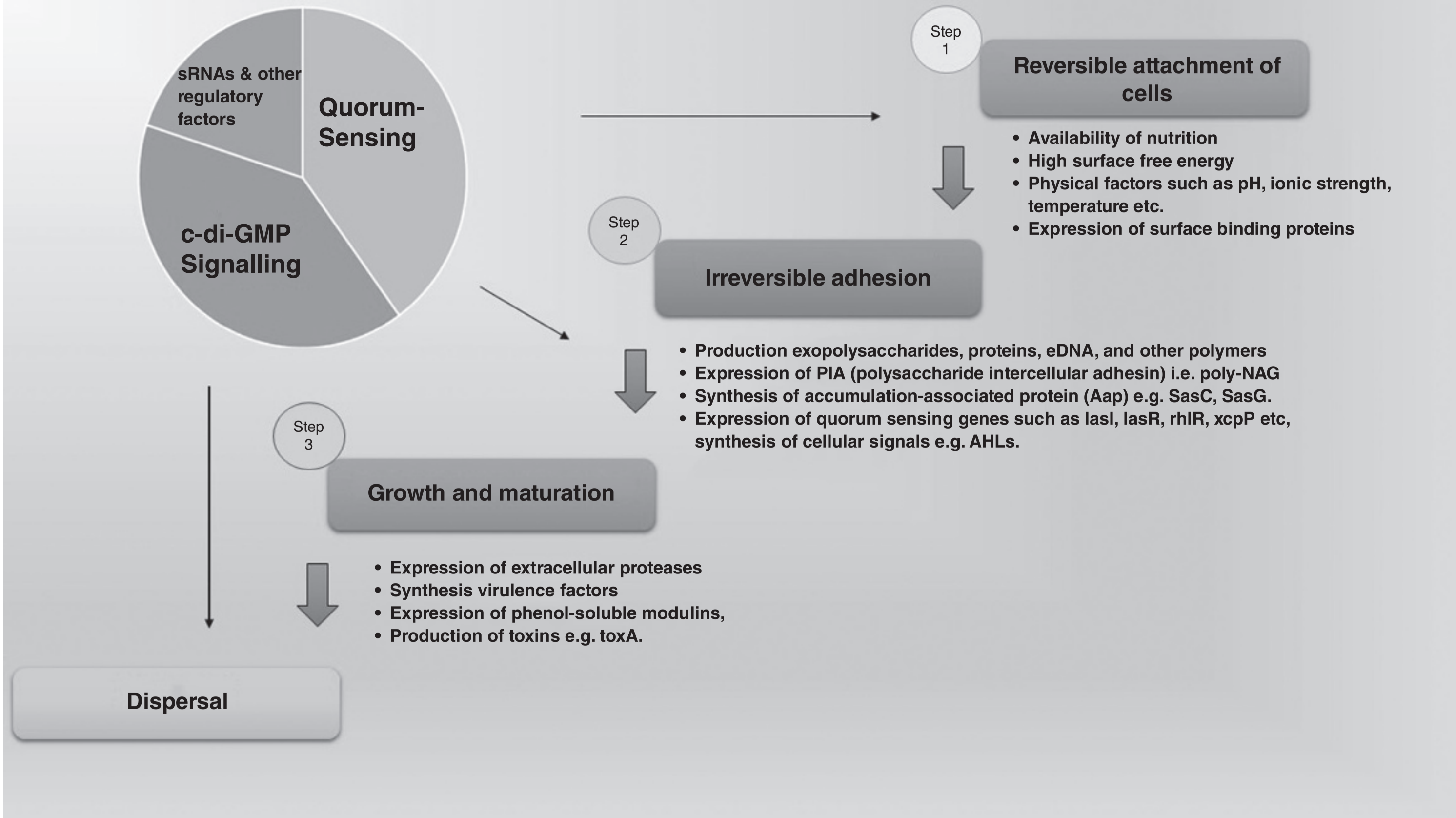

Figure 1.1 Key steps involved in biofilm formation and the role of regulatory factors.

plant bacteria include the secondary messenger molecule cyclic diGMP in *E. amylovora* [56], *A. tumefaciens* [57], and *P. syrengie* [58]. Similarly, the role of cyclic GMP in biofilm formation was also reported by An et al. [59]. Small RNA and RNA-binding proteins in pathogenic plant bacteria have also been shown to activate biofilm formation *in vitro* by other investigators [60–62].

Excellent review articles on the molecular mechanisms and genes involved in biofilm formation by various bacteria have been published recently [26]. A number of regulatory pathways that control biofilm formation are explained, for example, in *Bacillus subtilis*. SpoOA is a central transcriptional regulator that controls more than 100 genes, including those necessary for biofilm matrix gene expression and sporulation [63, 64]. Similarly, SlrR/SlrA controls the initiation of biofilm formation in *Bacillus subtilis* [65, 66]. The molecular mechanisms involved in biofilm formation by various Gram-negative bacteria, especially in *Pseudomonas* spp., have been well documented [67, 68].

The role of plant exudates in attracting bacteria toward point of release from root surfaces and providing optimal nutrient availability is well known. Some bacteria like *A. brasilense* may use aerotaxics to identify an optimal O_2 concentration that will allow, but not inhibit, nitrogen fixation [69]. These processes are helpful in root colonization. Motility is also involved in the biofilm formation of several bacteria, although its importance is often conditional. Many reports are contradictory because the importance of motility in biofilm formation seems to differ from strain to strain and in different conditions. Bacterial fimbrial and afimbrial adhesins are widely known for their role in bacterial–host and solid-surface adhesions [70]. Various types of fimbrial adhesins are also known, and play important roles in adhesion and biofilm formation on solid surfaces, including the type I fimbrial structure and type IV pili (T4P) proteins in pathogenic, plant bacteria [71]. Afimbrial adhesins in Gram-negative bacteria are secreted by type V secretion systems (T5SS) or autotransporters. These adhesins are surface proteins and are used by bacteria for adhesion [72].

Newly described attachment strategies by plant pathogenic bacteria in biofilm formation include other secretion systems, which play an important role the secretion of different types of proteins and nucleic acids to the extracellular environment or direct translocation into adjacent eukaryotic or prokaryotic cells [73]. The type III secretion system (T3SS) is the most widely studied, and its role in biofilm formation has been described. Another secretion system described includes the type VI (T6SS) [74]. These secretion systems appear to play important roles in virulence and pathogenicity of the plant pathogenic bacteria and biofilm formation, as some of the proteins they secrete are essential for the attachment process. Other attachment strategies have been reported in plant-bacterial interactions and biofilm formation for example, *Bacillus amyloliquifaciens* FZB42, a PGPR, utilizes a collagen-like protein (CLPs), which was found to be an ECM component of biofilms present in the root of *A. thaliana* [75].

A number of signals and/or conditions have been identified that trigger biofilm formation by plant pathogenic bacteria on plant surfaces, including: (i) nutritional status of the plant; (ii) plant tissue; (iii) QS signals; and (iv) O_2 concentration and iron availability [76]. The role of calcium [77] has been explored in *X. fastidiosa*. Similarly, other metals (e.g., Cu, Zn, Mn) also differently influence biofilm and cell aggregation or inhibition. Recently, Nagar and Schwarz [78] published an interesting review on self-inhibition of biofilm development and highlighted that the transition between the planktonic and biofilm modes of growth is a highly regulated developmental shift that has a significant impact on cell fate. There are three mechanisms involved in the self-inhibition process:

1) The process is regulated by secreted small molecules and decrease in biofilm development.
2) Physiochemical properties of the substratum are modified by extracellular polysaccharides.
3) eDNA masks an adhesive structure.

1.4 Biofilm in Soil and Rhizospheres

Bacteria are not evenly distributed in the soil environment. Microbial populations are found in higher density near/in a rhizosphere, or in decaying organic matter compared to bulk soil. The microcolonies of bacteria, which are found in bulk soil, are often composed of different bacterial species [79, 80]. However, in the presence of a nutrient source, these microcolonies can develop into a multispecies biofilm. It is thought that due to dramatic changes in physical and chemical conditions in soil, bacteria periodically adapt the biofilm mode of growth for self-protection. For example, production of EPS by soil bacteria provides protection to water stress [81, 82]. Another stress condition is caused by the antibiotics produced naturally by microorganisms in the soil. Similarly, heavy metal contamination or soil pollution with organic compounds can result in a stressful environment. Bacteria present in biofilms become more tolerant to antibiotics and other toxic pollutants [83]. As these soil bacterial biofilms also consist of a variety of species interacting metabolically and socially, they can also convey selective advantages to their inhabitants [84].

1.5 Genetic Exchange in Biofilms

Genetic exchange by bacteria under *in vitro* and *in situ* conditions has been widely studied since the discovery of transferable plasmids in Japan [85]. Similarly, biofilms have long been a subject of interest in environmental, medical, and industrial microbiology. The interconnection between biofilm formation and horizontal gene transfer (HGT) has been the topic of recent attention. There is an enhanced rate of horizontal gene transfer due to high density of bacteria in biofilms [86]. Thus, the chances of acquiring new genes relevant to tolerance and adaptability are increased and therefore the environmental or ecological fitness of bacteria is increased [87]. The roles of QS signals in this process have also been demonstrated by several investigators [88, 89]. An excellent review on the literature of the above areas by Madsen et al. [90] reached the following conclusions:

1) HGT rates are typically higher in biofilm communities compared to planktonic cells.
2) Biofilms promote plasmid stability and may improve host range.
3) Plasmids are well-suited to promote the evolution of social traits such as biofilm formation.
4) This exchange may result in overall interconnectedness between HGT, mobile genetic elements, and social evolution of bacteria.

1.6 Diversity and Function of Soil Biofilms

Succession of microbial populations on any surface, but especially in soil or rhizospheres, is a complex process and influenced by a number of physical, chemical and biological factors. Most of the biofilms in nature are mainly composed of bacteria attached to the surface of soil or water environments. But other microorganisms like fungi, algae, and protozoa also play important roles in the establishment of bacterial/polymicrobial biofilms [91].

Biofilms play a significant role in the degradation of organic material in the soil, since this degradation is chiefly dependent on the extracellular enzymes elaborated by soil microbes. The bacteria attached to soil or present near degradable sources such as roots and litters have advantages compared to planktonic bacteria for nutrient availability [82]. Various enzymes elaborated chiefly by heterotrophic populations like fungi and heterotrophic bacteria, degrade a variety of organic matter. Lignolytic and cellulolytic microorganisms also play a specific role [92, 93]. Such processes in biofilms are enhanced due to altered growth rate and physiological capabilities [94]. Thus nutrient turnover, mineralization, and soil fertility are directly under the control of microbial activity in biofilms. Another important function of biofilms in soil is their ability to bioremediate metal and organic pollutants, and the bioremediation potential of mixed microbial consortiums or multispecies soil biofilms has been evaluated by several investigators. For example, VonCanstein et al. [95] demonstrated the role of biofilm in bioremediation of mercury in different environmental conditions. This topic is also addressed in Chapter 18 of this book.

The degradation of various organic pollutants, such as polyaromatic hydrocarbon (PAH), fenamiphos, and toluene, by soil bacteria have been demonstrated to be more efficient when they are in the biofilm mode of growth [96–98]. Similarly, microorganisms pathogenic to humans, animals, and plants, may survive in the soil for extended periods of time and become active pathogens once they reach a susceptible host. Many opportunistic pathogens such as *Pseudomonas aeruginosa*, species of *Salmonella, E. coli, Listeria,* and *Campylobacter* are naturally occurring in the soil environment [99–101]. Soil conditions may select for biofilm formation and attachment, which may also enhance their protection, survival, and pathogenicity [94].

1.7 The Role of Biofilms in Competitive Colonization by PGPR

Studies of soil biofilms are complex and require relevant models closely resembling natural soil environments. However, common techniques based on biofilm formation on glass surfaces or in flow models, and monitored through confocal and SEM analysis, are used more frequently. Thus, further progress in our understanding of complex microbial interactions in soil and rhizosphere environments awaits improvements in soil biofilm models.

Competitive root/rhizosphere colonization by plant pathogenic and root symbiotic or PGPR bacteria has been investigated, and their role is established. However, biofilm formation as a means of biocontrol has been studied for *Pseudomonas fluorescens* and wheat, *Bacillus subtilis* and several plants, and *Penibacillus polymyxa* and peanut plants

[102–106]. The role of QS in biofilm formation and the release of antifungal compounds, such as phenazine, has been well documented for wheat rhizospheres. In addition, surface chemistry on roots, and the production of surfactin was also found to influence biofilm formation and root colonization. Despite these advances, further investigation is needed to elucidate the exact mechanisms of interaction and the contribution of various microbial factors in rhizosphere competence [107].

1.8 Biofilm Synergy in Soil and Environmental Microbes

Under natural conditions, microbial biofilms are found on essentially any moist living or nonliving substrate, including plants and soil. Microbial communities within the biofilm and neighboring cells can influence the outcome of this interaction, which may alter community productivity. Interactions may be antagonistic such as competition, parasitism, predation, or social cheating, which can adversely affect community productivity. On the other hand, positive interactions among species such as cooperation, synergistic metabolism, construction of new niches, and can increase productivity. In a well-defined ecological succession, long-term evolved biofilm communities display synergistic interactions among the species within the biofilm [108]. For example, Poltak and Cooper [109] demonstrated long-term ecological succession experimentally by evaluating *Burkholderia cenocepacia* biofilms over 1,500 generations and concluded:

1) There is a successive adaptive diversification.
2) Mixed population is more productive due to complementary interactions.
3) Spatial partitioning and cross feeding generate community synergy.

Soil is typically a reservoir habitat for almost all types of microbes with varying metabolic capabilities. Soil provides a good setting for multispecies biofilm formation [110]. These microbes can interact positively or negatively through various microbial interaction mechanisms [29, 111]. *In vitro* interactions between two strains of the same species and interspecific interactions in biofilm formation have been reported in the literature [112]. Members of different species may also interact negatively through resource competition or by producing inhibitory compounds [113]. For example, Burmolle et al. [89] demonstrated a strong synergy among four epiphytic isolates from marine origin. Similar, synergistic interactions between *Candida albicans* and *S. aureus* or Streptococci have also been observed on abiotic surfaces and an oral mucosal analog [114]. Additionally, many investigators have reported the inability of single strains to form biofilms independently but can promote the formation of mixed-species biofilm [115–117].

The role of multispecies biofilms is evident in maintaining ecological balance in soil [40]. Many benefits are associated with the biofilm mode of growth, compared to the planktonic mode such as (i) protection from desiccation; (ii) protection from protozoan predation; (iii) increased resistance to antibacterial compounds; and (iv) an enhanced rate of genetic exchange [118, 119].

For example, it was recently demonstrated that there was a synergistic effect among seven different isolates co-cultured in combinations of four species, which included *Stenotrophomonas rhizophila, Xanthomonas retroflexes, Paenibacillus amylolyticus* and *Microbacterium oxydans*. The findings concluded that a high prevalence of synergy in multispecies biofilms indicated interspecific cooperation under natural conditions [120].

1.9 Biofilms in Drought Stress Management

Global climate change is considered to be one of the most serious threats to agricultural productivity worldwide in recent years. Sustainability in agricultural production implies high yield that can be maintained, even in the face of climate change. It is expected that global water shortage will be the key challenge for food security in the near future [121]. Rhizobacteria can alleviate plant drought stress. In this direction, the first report on drought tolerance enriched by PGPR was published by investigators in Sweden [122] and then followed by those in Canada [123].

Plants cope with drought through drought escape, dehydration avoidance, and dehydration tolerance. Tolerance mechanisms such as osmoprotection, detoxification, ion transport, or chaperone functions take over when tissues are no longer protected by avoidance mechanisms [121]. Drought tolerance enhancement by PGPR through 1-aminocyclopropane 1-carboxylate deaminase (ACC) can provide significant protection from drought and heat. The role of ACC deaminase in providing a plant tolerance mechanism is widely acknowledged [124, 125].

1.10 Plant Health and Biofilm

The health of green plants is of pivotal importance to everyone [126]. The term *plant health* is frequently used in two overlapping contexts: (i) the scientific and regulatory framework of checking plant imports for the presence of potential pathogens and pests [127, 128], and (ii) a less specific concept that touches on all areas of plant protection and is sometimes referred to as *plant health protection*.

For agricultural crops and the production and cultivation of medicinal plants, the major focus of plant health is centered on their protection from pests and pathogens, while enhancing productivity. Another term loosely associated with plant health is *plant growth promotion*. Although plant health cannot be directly compared with human and animal health, there are four similarities between human and plant health issues [129]:

1) Health variations between individuals, such as those due to age differences.
2) Health or disease is a dynamic process.
3) Health is subject to the geographical occurrences of pathogens.
4) Pathogens can develop resistance to treatment.

In the following section, our aim is to address the role of microbial biofilms in plant health. Many microbes deteriorate plant health and cause disease; however, several other microbes protect and promote plant health and control pathogens and pests.

1.11 How Microbial Biofilms Influence Plant Health?

Plant-associated biofilms above and below the ground can interact positively or negatively with plants, depending on the microorganisms involved. Various phytopathogenic bacteria form biofilms that cause plant diseases. This topic is the focus of Chapter 21. On the other hand, positively interacting rhizobacteria, known as PGPR, as well as certain bacterial biocontrol agents, also colonize plant roots, and this involves microcolonies and biofilm formation. Extensive literature is available on *Bacillus*, fluorescent *Pseudomonas,* and other PGPR, as discussed in Chapters 4 and 5. The overall interaction is depicted in Figure 1.2.

As our understanding about the microbial worlds associated with plant, human, animal and environments increases, we are beginning to see that the role of the microbiome on influencing human health is enormous, acting both positively and, in some cases, negatively [130]. Similarly, the rhizosphere microbiome has a direct impact on plant health. The rhizosphere microbiome exists mainly in biofilm mode. The collective genome of the rhizosphere microbial community is much larger than that of the plant and is often referred to as the plant's second genome [131].

In humans, the role of the intestinal microflora on health is now more understood, and similar functions can be ascribed to the human gut as to the plant rhizosphere. Root microbiomes are now under scrutiny for their exact role in plant health. The root microbiome can (i) influence disease suppressive soil and (ii) modulate the host immune system by beneficial microbes in the rhizosphere [131]. Plants actively shape their root microbiome through the secretion of active compounds that modulate bacterial QS, and influence the recruitment of beneficial microbes. The details of these mechanisms may be obtained in several published review articles [131–134].

Understanding the complex interactions between plants and microbes in the rhizosphere is still in its infancy [83]. Recent metagenomic studies on root microbes still provide very limited information. Nevertheless, complex interactions among various groups of microorganisms inhabiting rhizospheres, and their role in plant–microbe interactions in

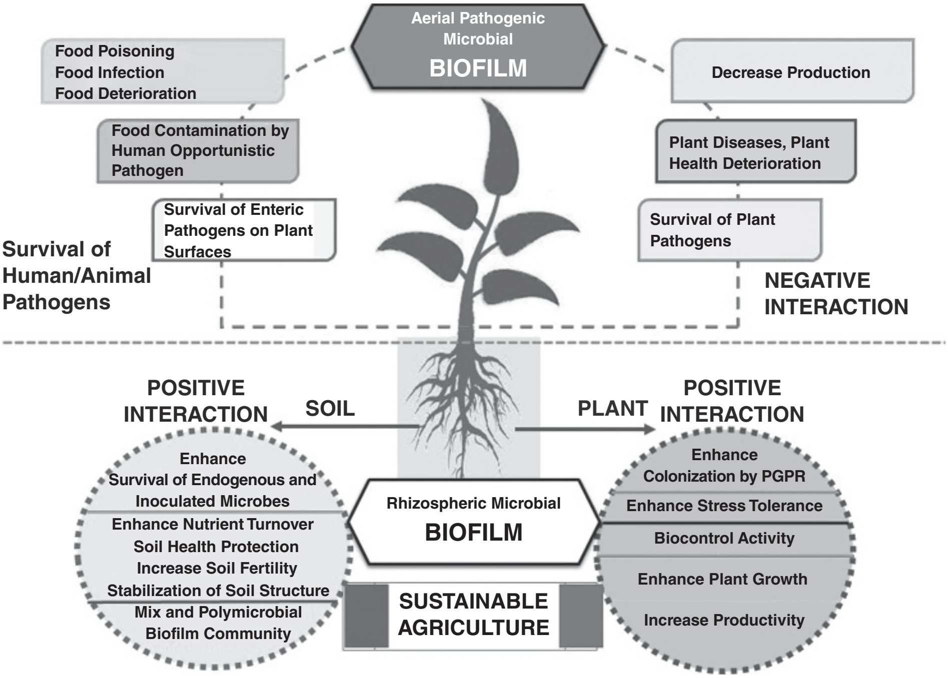

Figure 1.2 Biofilm interactions with plant and soil, and their significance.

biofilms must be explored to gain a better understanding, and for improved ecological niche engineering of plant and soil health in sustainable agriculture.

1.12 Soil Health and Biofilms

Biofilms grown on or around plant surfaces, tissues, soil, rhizosphere, and other habitats interact synergistically or antagonistically, both *in vitro* and in natural environments (Figure 1.2). Extensive research on biofilm stages, phenotypic, physiological, and molecular mechanisms has led to a better understanding of biofilms and application strategies. However, research on understanding and exploiting stable mixed biofilms for crop productivity, bioremediation, and the improvement of plant health (crop protection and fertilization) and for soil fertility and health is just beginning to gain momentum. New technologies for analyzing plant and soil-associated biofilms and determining their role in root colonization, plant growth promotion, and soil health improvement are needed. Some of the issues concerning the importance of microbes and microbial biofilms in maintaining soil health, and therefore sustainable environments and agriculture, are described next.

Soil has been defined by various scientists in different ways, depending on their purpose of study. Biologists, such as agricultural scientists, define the soil as the uppermost layer of the earth's crust, which supports the growth of plants and directly influences plant productivity [135]. Various management practices have long been known and used by farmers with the available resources to maintain soil fertility. Various human industrial and agricultural activities such as agrochemical discharge, industrial pollution, and extensive exploitation of soil resources in intensive agricultural practices, has resulted in a huge deficit of plant nutrient availability in soil versus the amount of nutrients taken up by plants. This has been realized in several parts of the world by the decreased productivity of soil. Therefore, scientists have promoted the concepts of the integrated plant nutrient supply system (IPNS), integrated plant nutrient management (IPNM), or the concept of organic farming for sustainable practices [136]. The term *soil health* is widely used in the discussion of sustainable agriculture pertaining to overall conditions or the quality of soil resources. Major factors known to cause soil quality degradation include erosion, decrease in organic matter content, and increased salinity [137].

Soil is a very complex system consisting of both abiotic and biotic components. Several physical, chemical, biochemical, and biological components of soil interact and give rise to the characteristics of a particular soil [93]. In a review published in *Philosophical Transaction of the Royal Society,* Kibblewhite et al. [138] define "the soil health as an integrative property that reflects the capacity of soil to respond to agricultural intervention or continue to support agricultural production and other ecosystem services." Nevertheless, Doran and Jones [139] defined soil health as "the continued capacity of soil to function as vital living system, within ecosystem and land use boundaries, to sustain biological productivity, maintain the quality of air, water environments and promote plant, animal and human health." Although there is no rigid definition of soil health, it constitutes the "soil quality," aspects that are based on the measurement of various parameters specific to soil properties such as physical, chemical, and biological.

The terms *soil quality* and *soil health* are sometimes used interchangeably. Warkentin and Fletcher [140] were probably the first to introduce the concept of soil quality as an approach to improve land use planning. Soil quality is simply defined as “the capacity of soil to function” [141, 142]. However, soil quality describes quantitative soil properties and linkages between properties and functions. Many prefer *soil health*, a term that clearly conveys the idea of a living thing.

There are various biochemical indicators, such as the production of soil enzymes, that are used to evaluate soil health, and several excellent review articles have been published describing soil quality [143, 144]. Another relevant alternative approach to studying soil health is based on an integrated approach that assumes the health of soil is more likely to be influenced by the interaction between different processes and properties of soil components [145]. It has been proposed that soil health is dependent on the maintenance of four functions [138]:

1) Carbon transformation
2) Nutrient cycling
3) Soil structure
4) Regulation of pests and diseases

Each of the above functions is controlled and regulated through multicomponent and multifunctional systems—an array of biological processes provided by diverse interacting living organisms under the influence of the abiotic soil environment. The ecosystem services provided by the soil are driven by biological process. Soil constitutes an important habitat for organisms and their reactions. The major components of soil include soil, air, water, pH and nutrients, and organic matter. Soil pores and gasses contribute significantly by providing a suitable habitat for a variety of organisms and their interactions. Various factors are known to have roles in controlling soil health, including (i) soil type; (ii) organisms; and (iii) nutrients. Therefore, in order to maintain good soil health for sustainable agriculture productivity, various integrated approaches have been adopted such as integrated plant nutrient management, integrated pest and disease management, integrated water management, and integrated water and land use management under the concept of organic farming.

1.13 How to Assess Soil Health?

Considering the complexity of soil and agricultural systems in different climates, it is difficult to develop common guidelines to assess soil health for all agricultural systems. It is evident that a single indicator of soil health is not appropriate, and it will be not be possible to measure all parameters to assess soil health. Several national and international proposals for soil assessment are linked to legal frameworks for the protection of soil [146–148]. Various soil quality/health assessment criteria have been developed which are based on the soil’s physical, chemical and biological characterization. Frequently used sensitive indicators recommended to determine soil quality are (i) Soil organic matter; (ii) major plant nutrients and other physiochemical characteristics of soil; (iii) top soil depth; (iv) filtration rate [149, 150]; and (v) levels of soil enzymes such as β-glucosidase [151].

1.14 Impact of Biofilms on Soil Health

Soil health is dependent on the maintenance of carbon transformation, nutrient cycling, soil structure, and the regulation of pests and diseases. Soil microorganisms, both indigenous and those specifically introduced into soil, are widely known for their overall contribution in the processes just discussed. The role of autotrophic organisms is to provide a carbon source, and soil algae, blue green algae Cyanobacteria, and other chemoautotrophic bacteria have been documented to serve this purpose [152]. Additionally, a major contribution to the degradation of organic matter comes from the combined activities of extracellular enzymes produced by heterotrophic bacteria and fungi such as cellulose, hemicellulose, pectinase, and ligninolytic enzymes. Nutrient cycling of C, N, S, P, Zn, and Mn, for example, is regulated by this diverse microbial population [153]. The formation of mixed-species biofilms by diverse microbes in soil and on plant roots and other related surfaces, significantly helps in the survival of these microbes, improving soil modification [134]. Thus, intentional inoculation of soil and/or plants with probiotic biofilms can also improve field conditions [154].

1.15 Biofilm EPS in Soil Health

The majority of soil microbes exist in biofilms [155]. The major components of the biofilm matrix are polysaccharides, glycoconjugates, and protein. Measurements of microbial biomass, levels of ATP, and other enzymatic processes can be used to study soil microbial communities and their functions. As the EPS constitutes approximately 80 percent of a soil biofilm's dry mass, the extraction and estimation of biofilm EPS is now considered to be an important indicator for soil function [156].

Competitive advantages for microbial life are known to be influenced by EPS production, which provides advantages to biofilm communities such as (i) improved QS, (ii) colony adhesion, (iii) syntrophy, (iv) tolerance to heavy metals and desiccation [157], (v) improved stability of soil enzymes [158], (vi) bacterial gliding, and (vii) soil health [159]. The EPS is also reported to contribute to the process of soil aggregation [160], tolerance of wheat seedlings to saline [161], and drought tolerance of sunflowers inoculated with EPS-producing *Pseudomonas* [162]. Fully understanding the role of the EPS to mixed-species biofilms is difficult due to the complex nature of soil. However, Redmile-Gordon et al. [163] recently described a new method of EPS extraction from soil biofilms, which may help expand our knowledge.

Understanding multispecies biofilms under natural conditions, especially in soil, is complex. It is not yet well established that biofilms are associated with some properties that increase microbial fitness under specific selective pressure. In natural environments, the microbial communities are heterogeneous and are composed of multiple bacterial species. A recent study demonstrated that bacteria that coexist in natural environments facilitate interspecific biofilm formations [164].

This study also reported a positive relation between biofilm induction and phylogenetic history, suggesting that an increase in biofilm formation is a common adaptive response to long-term coexistence. Understanding multispecies biofilms under *in vitro* conditions is much more commonplace, and various model systems have been adopted.

However, attention now must be directed toward the study of these biofilms in their natural environments or controlled microcosms [112].

Based on the previous discussion, biofilm interaction with plant surfaces and soil and their significance in plant, soil, and environment health through various activities and processes are presented in Figure 1.2.

1.16 Conclusions and Future Directions

An in-depth understanding of microbial biofilm communities in plant and soil is still elusive. What is known is that biofilms are associated with a number of properties that increase the ecological fitness of bacteria by various operative mechanisms, including increased antimicrobial tolerance and protection from host defense systems, evasion of protozoan grazing, better utilization of secreted compounds, and increased horizontal gene transfer. However, in natural environments, biofilms are heterogeneous. Coexistence facilitates interspecific biofilm formation, and Madsen et al. have recently demonstrated complex microbial communities [164]. However, difficulties in investigating interactions in polymicrobial biofilms under natural environmental conditions, and in the succession of microbial communities in response to environmental conditions, have sustained a poor understanding of the soil environment. Biofilms associated with plants are better understood in the context of pathogenesis rather than symbiosis or mutualism, with few exceptions. Thus, while the significance of biofilms has been well documented in environmental, food, and medical aspects, our understanding of soil and rhizosphere-associated biofilms is still weak and woefully incomplete.

Recent advances and progress has been made on the metagenomic analysis of soil through various microbial surveys, such as the Earth Microbiome Project (EMP) [165], Terra Genome [166], and China Soil Microbiome Initiate. These are all excellent resources to explore taxonomic and functional diversity, as described by Nesme et al. [167], but it is likely that the future will hold even more questions regarding the genomic diversity of polymicrobial biofilms in natural environments, especially in soil and rhizospheres. One certainty, based on current knowledge, is that biofilm research will certainly expand our understanding of microbe–microbe and microbe–plant interactions, and will help in the development of new strategies to solve problems related to plant productivity and protection, soil health, and sustainability of agriculture.

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