Chapter 7
Nature's Protectors: A Biofilm Perspective on Bacterial Disease Control in Plants

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Abstract Bacterial diseases significantly threaten agricultural productivity, necessitating innovative and sustainable approaches to disease management. Above- and belowground biofilms are essential in mitigating plant bacterial diseases. We examine aboveground biofilms, primarily located on leaf surfaces, for their ability to form protective barriers and produce antimicrobial compounds, thereby impeding the establishment of pathogenic bacteria. Concurrently, we explore belowground biofilms in the rhizosphere for their contributions to nutrient cycling, enhanced nutrient uptake, and the deployment of biocontrol agents against soilborne bacterial pathogens. Here, we employ a multidisciplinary approach by integrating molecular, microbiological, and ecological analyses to unravel the mechanisms underlying the formation and function of these beneficial biofilms. We investigate quorum sensing, microbial communication, and the intricate interplay between plant hosts and beneficial microbes to elucidate the complex networks orchestrating disease resistance. We consider environmental factors such as surface structure, temperature, and nutrient availability. We acknowledge their impact on biofilm-mediated disease management strategies relevant for controlling plant disease. Furthermore, we emphasize the importance of microbial diversity within biofilms for its role in enhancing plant resilience to bacterial diseases. Overall, this chapter provides a foundation for developing targeted and sustainable strategies in bacterial disease management, underpinning the critical role of above- and belowground beneficial biofilms.

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Insights gained from beneficial biofilms contribute to the fundamental understanding of synergistic relationships in the plant microbiome environment and their implications for sustainable agriculture

Keywords Biofilm · Quorum Sensing · Amyloids · Biocontrol · Phyllosphere · Rhizosphere

7.1 Introduction

Feeding the fast-growing human population, which will reach approximately 9.5 billion by the year 2050 (OECD 2012), will pose challenges for food and agricultural scientists in the coming decades. Global crop production faces alarming threats from both abiotic and biotic stresses, which are increasingly exacerbated by the effects of climate change (Teshome et al. 2020). Regarding biotic factors, plants are under constant attack from diverse organisms, including fungi, bacteria, oomycetes, nematodes, and herbivores. It is essential to mitigate plant disease to produce nutritionally improved food in sufficient quantities for the rapidly growing global population. Several disease control approaches are helpful in the management of plant diseases, including crop rotation, resistance breeding, and the use of biological control substances. Regarding the latter, commonly used are chemical fertilizers and pesticides to improve crop quality and quantity. However, these methods carry disadvantages, including disruption of ecosystems and downstream contamination (Aktar et al. 2009).

Plant disease occurs due to the complex interaction of a pathogen and its host plant. Within the plant-associated microbial diversity, only a fraction of the microbes are considered pathogenic, while the majority of them are beneficial and promote host plant growth and its fitness (van Elsas et al. 2012; Mendes et al. 2013; Philippot et al. 2013). Detrimental microbes have adapted to infect plants as specialized pathogens, causing numerous diseases and severely damaging crop plants. These are a serious concern to agriculture and crop productivity as they are directly responsible for crop losses (Mansfield et al. 2012). Therefore, the understanding of plant–pathogen interactions paves the way for disease management.

The percentage of bacterial phytopathogens causing plant diseases can vary significantly based on numerous factors, including geographical region, crop type, agricultural practices, and environmental conditions. Phytopathogens of bacterial origin are responsible for a notable portion of plant diseases; generally, it is estimated that bacterial phytopathogens contribute to roughly 10–20% of plant diseases worldwide. Factors such as the pervasiveness of specific pathogens, crop susceptibility, and climate conditions can influence the prevalence of bacterial diseases in plants. Bacterial phytopathogens pose a significant threat to agricultural production as plant diseases that trace their etiology to bacteria amount to a large percentage of all crop losses (Savary et al. 2019). Amid these challenges, innovative approaches such as the harnessing of biofilms have emerged as sustainable means to protect crops from bacterial pathogens.

7.2 Plant–Microbe Interactions: Mechanisms of Pathogen Success and Host Defense

Plants frequently encounter pathogenic microbes that challenge their growth and development throughout their life cycle. The intricate innate immune system in plants safeguards against the proliferation of harmful microbes, employing two interconnected defense mechanisms rooted in pathogen detection. These defense strategies encompass microbe-associated molecular pattern (MAMP)-triggered immunity and microbial effector-triggered immunity (ETI). Both MAMP and ETI responses activate multiple defense mechanisms to restrict pathogen invasion, safeguard the plant against pathogens, and curtail their growth (Thrall et al. 2007; Pieterse et al. 2014; Rodriguez et al. 2019). After exposure to pathogens, plants often develop immunological memory, leading to systemic acquired resistance. In contrast to their interactions with harmful microbes, plants establish and promote symbiotic relationships with beneficial microbes to fortify their immune system. The success of bacterial phytopathogens can be attributed to several factors, including their ability to colonize the plant, evade its defense mechanisms, and obtain essential nutrients for growth and reproduction. Bacterial pathogens also exhibit less specific physiological adaptations to ensure survival outside the host plant, which include metabolic responses to abiotic stress. Some of these are based on the production of exopolysaccharides, which can protect against desiccation and cold, and pigments, which protect against ultraviolet (UV) radiation.

Bacterial pathogens cause disease symptoms, including leaf spots, blights, cankers, and wilts. Notable bacterial pathogens, including species of *Xanthomonas*, *Pseudomonas*, *Agrobacterium*, *Ralstonia*, *Erwinia*, and others, are known to cause diseases in a wide range of crops, affecting agriculture globally (Mansfield et al. 2012). Bacterial diseases impact crops significantly, leading to yield losses and affecting food security. Bacterial phytopathogens have adapted in response to developments in plant resistance, resulting in an evolutionary arms race (Wang et al. 2021). This has led to several strategies for successful infection, including the expansion of effectors released through the type III secretion system in Gram-negative bacteria (Tang et al. 2006). The latter encompass some of the most important groups of bacterial phytopathogens, the *Pseudomonas syringae*'s pathovars (pv.) (Chen et al. 2022).

The entry of bacterial phytopathogens into plants determines their fitness and disease development. Pathogens typically penetrate plants through natural openings, such as stomata and hydathodes, wounds, or by using specialized mechanisms such as the secretion of enzymes or toxins that facilitate their entry into plant tissues (van der Wolf and De Boer 2014). Some pathogens can swim or move along moisture gradients to reach these openings. Wounds on plant tissues, caused by mechanical damage from herbivore feeding, also act as entry points for bacteria to infect plants. Some bacterial phytopathogens secrete enzymes such as cellulases and pectinases that break down the structural barriers and degrade plant cell walls, allowing them to penetrate plant tissues. Some bacterial phytopathogens have evolved mechanisms to bypass plant defenses inside plant tissues (Abramovitch and Martin 2004).

Advancements in understanding these pathogens, improved disease management practices, and the development of resistant crop varieties are essential for minimizing the impact of bacterial phytopathogens on plant health and agricultural productivity. In addition, the plant microbiome plays a key role in controlling and regulating the virulence of these phytopathogens (Gao et al. 2021). Plants have been shown to actively recruit bacteria from their environment to overcome and prevent disease (Liu et al. 2021). Similarly, dysbiosis in the phyllosphere, which refers to the lack of stability in the microbiota composition, has been associated with a higher likelihood of infection (Gong and Xin 2021). The mechanisms by which microbes can control pathogen-prone bacteria in the plant include direct inhibition of these through, for example, the release of antimicrobials or indirectly boosting plant defenses (Legein et al. 2020).

7.3 Microbial Adaptation: From Planktonic Cells to Biofilm Communities

Microbes can remarkably transform and adapt to their environments by converting key nutrients and energy into accessible forms for survival. Well-adapted microbes participate in many microbe–microbe and microbe–environment interactions. In nature, microbial cells exist as solitary planktonic cells or are assembled into structured codependent communities known as biofilms. This transition from planktonic to biofilm is a widespread phenomenon observed in the environment and in bacteria associated with hosts such as humans, animals, and plants (Jefferson 2004). Biofilms consist of either single- or multispecies communities that join forces to promote their survival. Biofilms are a dominant form of microbial lifestyle that can persist for many generations wherein microbial communities are shaped by common evolutionary pressures (Steenackers et al. 2016). The community performs different tasks through stable co-occurrence, intercellular interactions, and communication, which can hardly be attained by free-living microbes (Moons et al. 2009). Biofilms impact different fields of study, from clinical and plant infection to beneficial colonization in humans and plants. Compared to monospecies biofilms, multispecies biofilms with a complex mix of prokaryotes and eukaryotes are predominant and, in general, exhibit higher stability (Costerton et al. 1987; León-Romero et al. 2015; Flemming and Wuertz 2019).

Biofilm formation is a complex and dynamic process where microbial communities are embedded in a self-produced matrix with cell densities ranging between 10^8–10^{11} cells/gram wet weight. Matrix components contribute > 90% of dry weight, whereas microbial dry weight accounts for < 10% (Flemming et al. 2016). This matrix forms during the attachment stage, stabilizing the interacting microbes within the biofilm (Jamal et al. 2018). The properties of biofilm microbial communities are distinct from those of free-living microbes due to their presence in a matrix (Berlanga and Guerrero 2016). Biofilms in natural settings develop viable but nonculturable subpopulation phenotypes that are successful in situ but sometimes fail in culture-based isolations. Predicting the conditions under which microbial biofilm communities are successful or benefit the host remains challenging in microbiome research.

7.4 Key Factors Governing Biofilm Formation

The formation of beneficial biofilms is a complex process mainly divided into four stages: (1) initial reversible attachment: adhesion of planktonic cells to the surface; (2) irreversible attachment: formation of microcolonies; (3) maturation stage: macrocolonies formation and development of matured biofilm; and (4) dispersal stage: detachment of microbial cells. Different factors influence these steps, and their understanding is crucial for both applied and fundamental aspects of microbial research (Fig. 7.1).

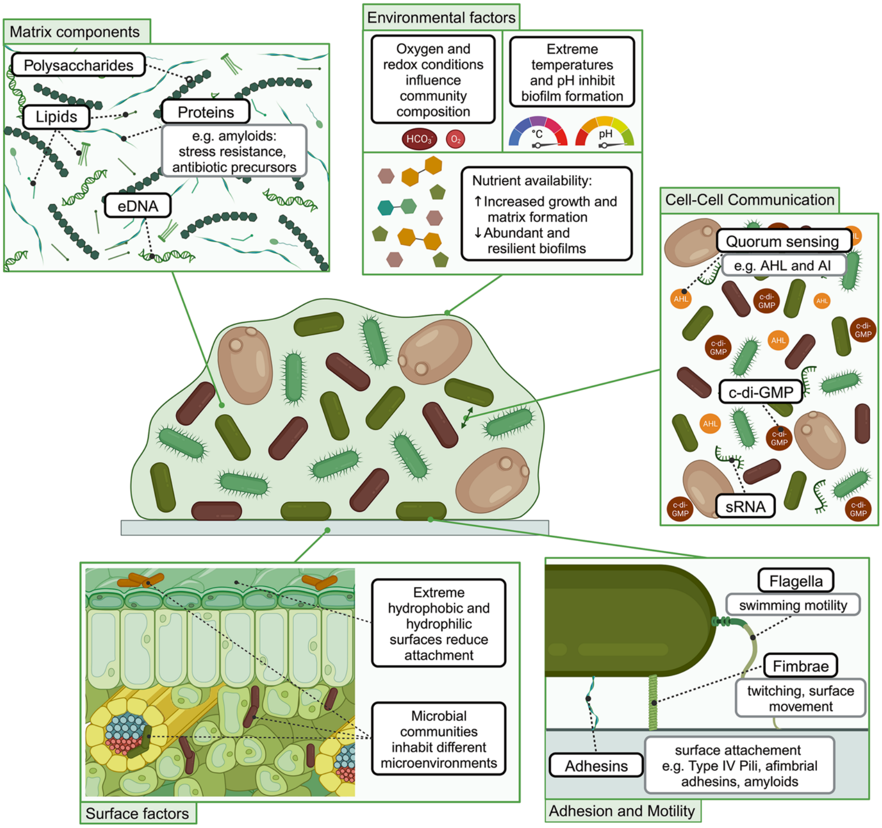

Fig. 7.1 Overview of the key factors influencing biofilm formation. Biofilm formation, whether in plants or on other surfaces, is a complex process, influenced by many biotic and abiotic factors. Biofilm community composition, attachment, and persistence are shaped by environmental conditions and surface structure. Biofilm structure is, furthermore, determined by the producing microbes, their associated matrix, communication and attachment genes. *eDNA* extracellular DNA, *AHL* acyl-homoserine lactone, *AI* autoinducer (e.g., autoinducing Peptides AIP, AI-2), *c-di-GMP* cyclic diguanylate, *sRNA* small RNA. (Graphic created with BioRender.com)

7.5 Bacterial Mobility and Adhesins

Bacterial mobility is considered an important factor for biofilm formation though its mechanism differs between strains and it is mostly required for the earlier stages of biofilm formation such as the initial attachment (Ahmad et al. 2017a). Bacterial mobility is mostly facilitated by two types of proteins: flagella and fimbriae. Flagella are long, spiral structures on bacterial cell surfaces that facilitate movement in liquid media. They act like propellers, allowing bacteria to swim and navigate the liquid environment (Nakamura and Minamino 2019). On the other hand, fimbriae are short, straight projections on bacterial cytoplasmic membranes that enable limited, twitching motility of bacteria on surfaces. They also facilitate bacterial attachment and can be involved in the crawling or gliding motion on solid surfaces (Jin and Marshall 2020). Both flagella and fimbriae play crucial roles in bacterial mobility, allowing bacteria to adapt and respond to different environments. Bacterial cells first establish contact with a substrate surface through adhesins, which are specific cell membrane proteins that play a crucial role in the initial attachment. Once the initial interaction forms, adhesins help maintain a stable connection between the bacterial cell and the substrate surface. Adhesins or adhesin-like structures can be of a fimbrial nature, like type IV pili, which aid in the adhesion of several Gram-positive bacteria (Piepenbrink and Sundberg 2016). Afimbrial adhesins are often secreted by Gram-negative bacteria via the type V secretion system. Type VIII secretion systems are used by *Escherichia coli* to secrete curli, amyloid-like adhesins (Leo et al. 2012).

7.6 Surface Characteristics

Plant surfaces available for biofilm colonization consist of a variable range of different microenvironments. Each type of plant tissue has a different surface structure, and bacteria must adapt their biofilm-forming strategies to the unique conditions and characteristics. The nature of the surface to which bacteria attach shapes biofilm development. In plants, surfaces colonized by bacterial biofilms include the leaves, stems, roots, seeds, and vasculature (Ramey et al. 2004). Bacteria may prefer specific surface characteristics based on hydrophobicity, charge, and roughness. In general, bacteria tend to form biofilms on moderately hydrophobic or hydrophilic surfaces, avoiding extremes, and prefer relatively rougher surfaces for increased attachment (Ahmad et al. 2017b; Zheng et al. 2021).

7.7 Cell–Cell Communication and Regulation

Formation of biofilms requires many individual microbial cells to work together toward the same goal. Microbes interact with each other through specialized communication systems, known broadly as quorum sensing (QS). Bacteria use QS molecules to communicate and coordinate with each other in monospecies and multispecies communities. QS allows bacteria to sense their population density and regulate gene expression accordingly (Miller and Bassler 2001). One of the best characterized QS systems consists in the response against *N*-acyl-homoserine lactone molecules (AHLs). It is used, among others, by symbiotic rhizobia and during biofilm formation in plant-associated pseudomonads (Whitehead et al. 2001; Loh et al. 2002). Shrestha et al. (2020) showed that long-chain AHLs induce expression of several plant defense genes and also resistance to the bacterial pathogen *P. syringae* (Shrestha et al. 2020). In addition to AHLs, Gram-negative and Gram-positive bacteria engage in interspecific communication through autoinducer-2 (AI-2) molecules (Li and Nair 2012).

Apart from QS, other regulatory factors that govern biofilm formation include cyclic diguanosine-5′-monophosphate (c-di-GMP) signaling and small non-coding RNA (sRNA) release (Chambers and Sauer 2013; Fazli et al. 2014). These both bind and interact with a large number of effector molecules and gene targets, modifying the expression of many genes related to motility and biofilm formation. Ultimately, sRNAs and c-di-GMP, together with AI-2- or AHL-based QS, determine the balance between planktonic and biofilm stages in bacteria.

7.8 Extracellular Polymeric Substance Production

Bacteria in biofilms produce extracellular polymeric substances (EPS), which consist of an intricate mix of lipids, polysaccharides, extracellular DNA (eDNA), and proteins (Yin et al. 2019). The composition of EPS can vary between species and influences the organization of the biofilm and its protective effect on plants (Fong and Yildiz 2015; Heredia-Ponce et al. 2021a, b). The EPS enables the adhesion and aggregation of cells, as well as retention of nutrients and water within the biofilm matrix (Flemming and Wingender 2010).

A major part of the proteinaceous component of the EPS is composed of proteins of amyloidogenic nature, meaning that they spontaneously form molecular amyloid structures. Amyloids play key roles in the integrity and functionality of biofilms, including those that are found in plants (Garcia et al. 2011; Gómez-Pérez et al. 2021). The main function of amyloids is the establishment of a scaffold whereupon the rest of the EPS components and cells can be assembled. This is due to secreted amyloidogenic monomers aggregating and forming robust amyloid fibrils. Fibrils provide stability and resilience against environmental pressures since the mature filaments are highly resistant to chemical and thermal denaturation, as well as mechanical stress (Taglialegna et al. 2016). Furthermore, the monomeric state of some amyloids exhibits toxic properties. The amyloid fibrils in the biofilm can, therefore, also act as reservoirs for antimicrobial peptides, which may be liberated if needed (Syed and Boles 2014). This can be triggered by changes in the surrounding areas such as pH shifts, potentially caused by the arrival of bacterial pathogens. Although most components of the EPS are variable, amyloids are a ubiquitous central part of biofilm formation for many microbes, most recently described for cyanobacterial biofilms (Erskine et al. 2018; Frenkel et al. 2023). The synthesis of amyloids often entails the development of dedicated systems for their production since they carry the risk of cytoplasmic polymerization, leading to cell toxicity (Taglialegna et al. 2016).

Apart from playing several roles as part of the biofilm structure, such as nucleation of amyloids and binding to hydrophobic surfaces, eDNA also contributes to the exchange of genetic information (Heijstra et al. 2009; Peterson et al. 2013; Gallo et al. 2015). The release of eDNA, similar to other aspects of the formation of biofilms, is promoted by QS molecule signaling (Spoering and Gilmore 2006). The ability of cells within a biofilm to integrate eDNA enables genetic material exchange related to, for example, resistance to a particular antibiotic, allowing certain phenotypes to quickly spread throughout the biofilm population (Bender et al. 2022). Finally, other components of the EPS such as polysaccharides and lipids are also essential for ensuring the structural integrity and cohesion of the matrix (Karygianni et al. 2020).

7.9 Environmental Conditions

Various abiotic factors influence biofilm formation, including nutrient availability and environmental conditions. Nutrient-rich conditions may promote biofilm development as bacteria utilize resources for growth and matrix production. However, in general, biofilms formed under nutrient-scarce conditions, such as in soil, are more prevalent and resilient than those formed under abundance conditions, likely as a way to promote survival in adverse conditions (Petrova and Sauer 2012).

Temperature and pH are both critical environmental factors affecting biofilm formation. The optimal temperature for the production of biofilms varies per strain, but it is typically within the range of 25–37 °C. A lower temperature can decrease bacterial motility, reducing the first attachment phase and biofilm dispersal. The pH of the environment can affect the charge of surfaces, potentially reducing attachment, particularly in the initial phases of biofilm formation (Palmer et al. 2007). Generally, bacterial biofilm production is optimal under near-neutral pH. Extreme conditions relating to temperature or pH can both negatively affect biofilm production.

Relating to the microenvironmental conditions within the biofilm, oxygen and redox states can vary, influencing the diversity and abundance of bacterial species. Different bacteria may thrive in specific microenvironments within the biofilm structure, which is particularly relevant concerning multispecies biofilms (Mitri et al. 2011). In turn, biofilms have been shown to also directly impact their own and their host's environmental conditions. For example, in a grass leaf, bacteria within biofilms have been shown to increase O_2 levels and pH, decreasing the photosynthetic potential of the plant (Zhang et al. 2022).

7.10 Beneficial Biofilms

The significance of beneficial biofilms associated with a plant host lies in their protective role for the contained bacteria against environmental stresses, including UV radiation, pH fluctuation, osmotic stress, and dehydration. The adaptive capability of biofilms enhances the resilience of bacterial communities, allowing them to thrive in diverse ecological niches. Though not just the microbes but also the host plant profit from the biofilm formation. The role of aboveground and belowground plant-associated biofilms in bacterial disease management is complex and has been the subject of increased research interest in recent years (Fig. 7.2). To study plant biofilms, it is often convenient to separately look at the microbial communities associated with the aboveground (phyllosphere) and belowground (rhizosphere) parts of the plant (Fig. 7.3). In both habitats, a number of beneficial biofilm-forming bacteria have been identified (Table 7.1).

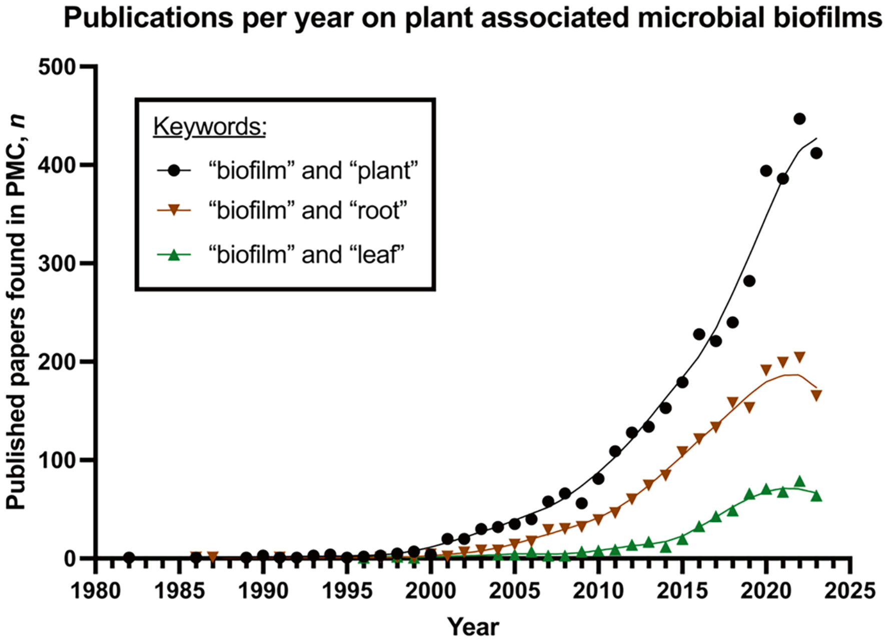

Fig. 7.2 Publications per year relating to plant-associated biofilms. The plots depict the number of publications found in the PMC database with the keywords "biofilm" together with either "plant," "root," or "leaf" in the title and abstract per year (as of December 4th, 2023). The number of publications (n) is represented by each dot of the scatter plot and trend lines were drawn with local regression based on the LOWESS method

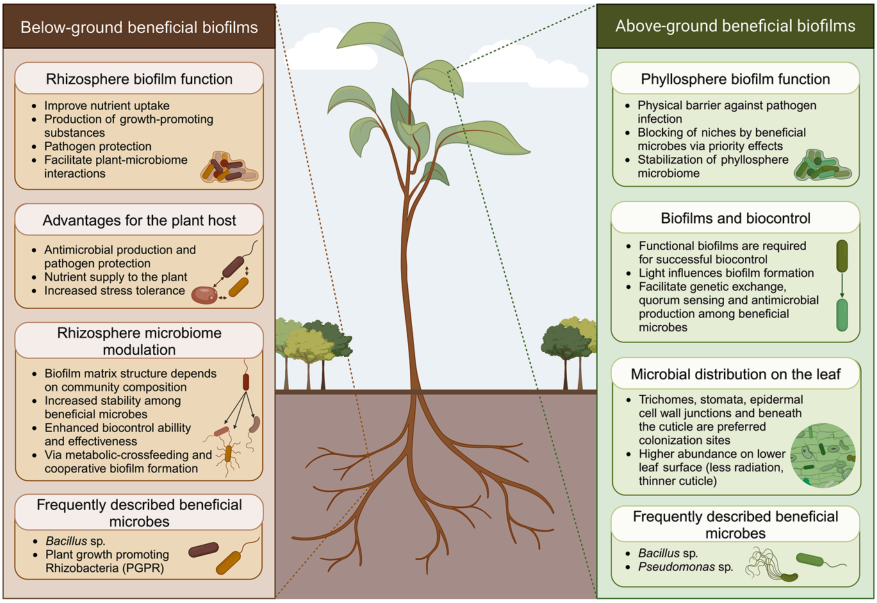

Fig. 7.3 Comparison of belowground and aboveground beneficial biofilms in plants. In both environments, beneficial biofilms are important for plant health and plant growth. Aboveground and belowground biofilms both offer pathogen protection and stabilize their respective microbial communities. While belowground biofilms have primarily been reported to enhance nutrient uptake, aboveground biofilms are known to adapt their benefits to a varying environment. (Graphic created with BioRender.com)

Table 7.1 Summary of important biofilm-forming plant-beneficial microbes

Microbe	Host plant	Colonization site	Reference
Azospirillum sp.	Cereals	Root	Burdman et al. (2000)
Bacillus amyloliquefaciens	Cucumber, maize, tomato, barley	Rhizosphere, root, seed, soil	Liu et al. (2014), Kasim et al. (2016), Wang et al. (2019), Hazarika et al. (2021)
Bacillus aryabhattai	Tomato	Root	Haque et al. (2020)
Bacillus subtilis	Tomato, *Arabidopsis*, lettuce, rice	Root, rhizosphere, seed	Luo et al. (2015), Arnaouteli et al. (2021), Sarti et al. (2023)
Bacillus pumilus	Masson pine	Roots	Zhu et al. (2020)
Bacillus velezensis	Tomato	Root, leaf	Stoll et al. (2021)
Bacillus sp.	Maize	Leaf	Fessia et al. (2022b)
Paenibacillus polymyxa	Peanut	Seed	Haggag and Timmusk (2008)
Pseudomonas aeruginosa	Tomato	Root	Ghadamgahi et al. (2022)
Pseudomonas chlororaphis	Avocado	Root	Heredia-Ponce et al. (2021a, b)

(continued)

Table 7.1 (continued)

Microbe	Host plant	Colonization site	Reference
Pseudomonas putida	Maize, chickpea, tomato	Rhizosphere, root	Espinosa-Urgel et al. (2002), Ramos-González et al. (2005), Srivastava et al. (2008), Sun et al. (2017)
Pseudomonas synxantha, *Pseudomonas brassicacearum*	*Arabidopsis*	Root	Harting et al. (2021)
Pseudomonas sp.	Tea	Root	Hazarika et al. (2021)
Rhizobium leguminosarum	Clover	Root nodulation	Janczarek et al. (2010)

7.11 Belowground Beneficial Biofilms

The rhizosphere is the region of soil influenced by root secretions and comprises a vast diversity of microorganisms. Being a microbially dense and diverse habitat, constant competition for niches and nutrients among root- and rhizosphere-associated beneficial microbes and pathogens is essential for the mechanisms behind protection (Lugtenberg and Kamilova 2009). It is a critical zone for beneficial biofilms that can improve nutrient uptake, produce growth-promoting substances, and compete with pathogenic organisms for resources. Root biofilms are a complex and coexisting community of microorganisms that can form a protective layer on the root surface that interacts directly with the plant (Pandit et al. 2020). The biofilm-structured microbial communities attach to the plant root surface forming a three-dimensional matrix (Castiblanco and Sundin 2016). Microbe–microbe interactions are essential for the stability and functional potential of root biofilms. These interactions can help maintain the structural integrity of the biofilm and regulate the diversity or evenness of the community of microbes that can carry out various functions (Ajijah et al. 2023). For instance, some microbes focus on producing EPS that help bind the biofilm together. Besides, others produce antimicrobials that inhibit the growth of competing or "cheater" microorganisms. In addition, some microorganisms form symbiotic relationships with plant roots, providing the plant with essential nutrients and helping to promote plant growth. This specialization of bacterial cells within biofilms is known as division of labor and is a staple of biofilms produced by many different bacteria (van Gestel et al. 2015). Root biofilms contribute to plant health by, for example, providing a protective barrier against pathogens, improving water and nutrient uptake, and facilitating the exchange of signaling molecules between the plant and the microbiome (Bogino et al. 2013). Finally, the root biofilm can also influence the plant's tolerance to stress (Timmusk and Nevo 2011).

The successful root colonization and community assemblage by rhizosphere and root-associated microbial communities depend on critical steps, including uptake of plant metabolites, chemotaxis, cell motility, and biofilm formation (Trivedi et al.

2020). The colonized beneficial microbes on the root surface are crucial to ward off soilborne pathogens from invading the root (Soni and Keharia 2021). These microbes in the rhizosphere can directly inhibit pathogenic organisms by producing antibiotics or competing for space and nutrients. *Bacillus* is the most studied beneficial microbial genus and usually colonizes plant roots as cell aggregates enclosed in a self-produced extracellular matrix, a biofilm (Arnaouteli et al. 2021). Multiple studies using fluorescence reporter and microscopy techniques have shown that biofilm formation on roots by *Bacillus* strains involved in competition with plant pathogens leads to disease suppression (Chen et al. 2013; Pandin et al. 2017; Molina-Santiago et al. 2019; Xu et al. 2019; Berlanga-Clavero et al. 2022).

Competition for utilizing root and rhizosphere nutrients also significantly confers pathogen suppression (Xia et al. 2022). One of the essential micronutrients is iron, which is highly abundant in soils but has low solubility and is unsuitable for microbial uptake, particularly in acidic environments (Lugtenberg and Kamilova 2009). Therefore, iron bioavailability is a limiting factor and promotes nutrient competition among living microbes. Some microorganisms synthesize specialized iron-chelating compounds, siderophores, that restrict the available iron to pathogens and, hence, their growth (Dimopoulou et al. 2021; Ghazy and El-Nahrawy 2021; Lahlali et al. 2022). Members of *Bacillus* species synthesize iron-chelating compounds, such as the bacillibactin siderophore (Yu et al. 2011).

Some *Bacillus* strains interact with plant roots, elicit induced systemic resistance (ISR), activate host defense responses, and induce the resistance of the entire plant against pathogenic bacteria and other pathogens (Kloepper et al. 2004). Examples of molecules from *Bacillus* that function as "elicitors" belong to lipopeptides, polyketides, exopolysaccharides, and volatile organic compounds. These activate salicylic acid, jasmonic acid (JA), or ethylene (ET) signaling pathways involved in priming the JA/ET-dependent defense-related genes, callose deposition, and stomatal closure, and help the plant's resistance against pathogen infection (Niu et al. 2012; Gowtham et al. 2018; Vanthana et al. 2019). Root inoculation of *B. velezensis* SQR9 activated plant systemic resistance in *Arabidopsis* and reduced the disease incidence of the foliar pathogens *P. syringae* pv. tomato (Pst DC3000) and *B. cinerea* (Wu et al. 2018a, b).

Plant growth-promoting rhizobacteria (PGPR) associations in the rhizosphere and phyllosphere protect plants from pathogens and promote plant growth by facilitating robust biofilm formation and surfactin production (Bais et al. 2004; Hashem et al. 2019). By colonizing the root surface, some bacteria trigger ISR in aerial portions of the plant (Kloepper et al. 2004). Research showed that pathogens like Pst DC3000 can induce root secretion in *Arabidopsis thaliana,* recruiting beneficial biofilm-formers such as *Bacillus subtilis*, which can then aid the plant in controlling the pathogen (Rudrappa et al. 2008). *P. syringae* infection in *A. thaliana* leaves imparts chemical signals belowground through malic acid as root exudate secretions. Further, *P. syringae* stimulates the root secretions of the tricarboxylic acid cycle pathway intermediate, L-malic acid, which selectively attracts beneficial rhizobacterium *B. subtilis* FB17 by promoting its root colonization and biofilm formation, which is dependent on the sensor histidine kinase KinD, specifically on the extracellular CACHE domain (Chen et al. 2012).

Another well-studied biofilm-forming genus that has been associated with a beneficial effect for the plant is *Pseudomonas*. Strain KT2440 of *P. putida* forms biofilms on maize roots, resulting in plant growth promotion and pathogen protection (Espinosa-Urgel et al. 2002). Another strain of *P. putida*, A1, has been shown to decrease bacterial wilt disease in tomato seedlings and to produce biofilms in vitro (Sun et al. 2017). *P. aeruginosa* strain FG106, isolated from the tomato rhizosphere, forms biofilms and exhibits broad-spectrum pathogen control, including antagonism against fungal diseases caused by *Alternaria alternata* and *B. cinerea* (Ghadamgahi et al. 2022).

Biofilm formation as monoassociation and in cooperative interaction of specific taxa can enhance the stability and effectiveness of these beneficial microbes. For example, a biofilm-deficient mutant strain of *Bacillus pumilus* was shown to have decreased biocontrol capacity against damping-off disease in pine when compared to the same strain with the capacity to form biofilms (Zhu et al. 2020). Some studies have shown that *Bacillus* species can modulate the native rhizosphere microbiome, which involves the stimulation of specific taxa exhibiting direct biocontrol activity and cooperative interactions with other beneficial microbes. Collectively, these modulations improve plant disease suppression (Tao et al. 2020; Moreira et al. 2023). One such example is the cooperation by biofilm formation of beneficial *Bacillus* and *Pseudomonas* strains through metabolic cross-feeding where *Bacillus* assists *Pseudomonas* by providing valeric acid and levulinic acid and *Pseudomonas* in return provides branched-chain amino acids (Sun et al. 2021). In another study, cocultivation of *B. velezensis* with helper bacteria *Flavobacterium* showed enhanced biofilm formation under in vitro assay. Further, the study revealed that *Flavobacterium* improved *B. velezensis* biocontrol efficiency against *Ralstonia solanacearum*, causing tomato bacterial wilt to decrease, reducing the colonization of *R. solanacearum* in the rhizosphere, and increasing transcription of plant defense gene PR1α in tomato (Wang et al. 2023).

7.12 Aboveground Beneficial Biofilms

Compared to the rhizosphere, microbes exhibit a nonuniform distribution in the phyllosphere. In particular, on leaf surfaces, bacterial cells preferentially attach to specific locations, such as the base of trichomes, stomata, epidermal cell wall junctions, grooves along veins, depressions, and beneath the cuticle (Beattie and Lindow 1999; Lindow and Brandl 2003; Chaudhry et al. 2020). Bacteria are more abundant on the lower leaf surface, potentially due to reduced radiation exposure, a higher density of stomata or trichomes, and a thinner cuticular layer, creating favorable conditions for their survival and growth, increasing their likelihood of persistence compared to upper leaf parts (Karamanoli et al. 2012). There is a general lack of experimental evidence regarding resistance against biotic stresses, particularly related to in vivo studies as outlined in (Fessia et al. 2022a). However, phyllosphere biofilms, analogously to rhizosphere biofilms, could act as a protective mechanism for plant growth and increased productivity. The main mechanism by which this could take place is by acting as a physical barrier that blocks biological niches or seals plant surface injuries potentially sought after by a pathogen to start an infection. This is related to the priority effect in ecology, which postulates that early-arriving organisms have a fitness advantage that, after the community has stabilized, is hard for newcomers to disrupt (Debray et al. 2022). Consistent with the latter, the higher resistance to infection of stable microbial communities (as opposed to a dysbiosis state) is known for many host organisms, including crop plants (Shade et al. 2012; Fu et al. 2019).

Biofilms originating from bacteria in the phyllosphere have the potential to act as a biocontrol agent, as shown for some *Bacillus* strains in maize (Fessia et al. 2022b). For a *Bacillus subtilis* strain, it was shown that the biocontrol activity depends on the formation of a functional biofilm to effectively antagonize bacterial pathogens in the phyllosphere (Zeriouh et al. 2014). Similar observations were made for *Pseudomonas piscium* in the wheat head microbiome, where biofilm formation was necessary to induce the production of antimicrobial substances (Chen et al. 2018). An effective establishment on the plant is key for the formation of viable biofilms composed of bacteria with biocontrol activity. For this, the influence of abiotic factors should not be underestimated. One essential difference between the phyllosphere and soil or rhizosphere is the exposure to light. It has been shown that non-phototrophic, biocontrol-associated bacteria in the phyllosphere respond to access and quality of light. Certain wavelengths, particularly in the UV range, can promote the biofilm formation of these bacteria in the phyllosphere (Karlsson et al. 2023). Factors like light are relatively easy to control during certain kinds of agricultural production as light conditions in greenhouses could be adjusted to promote biofilm formation of desired microbes and thereby enhance biocontrol activity.

Additionally, biofilms promote genetic exchange among their members, enabling the faster spread of resistance genes among the population through horizontal gene transfer, increasing their chance of fending off invaders (Madsen et al. 2012). The latter is enhanced by QS within the members of the biofilm, which promotes a distribution of roles, allowing a subset of specialized cells to focus on the production of antimicrobials, ultimately increasing their biocontrol potential (van Gestel et al. 2015). However, drawbacks related to the lack of dispersal of biofilm-prone biocontrol agents could limit their effectiveness in practice (Wei et al. 2016). Nevertheless, looking forward, the elucidation of the dynamics of these biocontrol mechanisms remains a promising avenue for future research, offering insights into their practical applications in agriculture and plant protection.

7.13 Challenges and Considerations: Harnessing Biofilms for Plant Health

Despite progress in recent years, harnessing beneficial biofilms for effective disease management and general plant health promotion presents a multifaceted landscape, marked by challenges and exciting prospects (Dewi et al. 2023). A critical challenge is the need for a comprehensive understanding of the mechanisms underlying biofilm-mediated biocontrol. The intricate interplay between the biofilm-forming microbes and pathogenic bacteria within the plant microbiome demands in-depth studies to unravel the specific biochemical and molecular processes involved in pathogen inhibition (Patil et al. 2022). Furthermore, application-oriented studies are needed to ensure biofilm treatments are successful in the field with minimal disruption of the native ecosystem.

7.14 Biofilm Stability and Specificity

As described earlier, environmental conditions such as soil type, temperature, pH, and nutrient availability influence biofilm stability (Ansari et al. 2017). Understanding the impact of these factors on biofilm formation and persistence is necessary for successful disease management. For example, pathogenic bacteria may also form biofilms on plant surfaces and compete with beneficial biofilms (Carezzano et al. 2023). For the persistence of beneficial biofilms and the prevalence of their producing microbes, these environmental conditions should be kept within optimal ranges to prevent disruption and displacement by pathogens.

Identifying and selecting microbial strains with beneficial traits and pathogen-suppressing properties is another crucial point. Ensuring that the biofilm-forming microbes have a higher affinity and dominance in the habitat than target pathogens can enhance the efficacy of disease suppression (Afridi et al. 2022). For example, this can be achieved through a combination of compatible biocontrol agents and certain chemical pesticides (Ons et al. 2020; Djaenuddin et al. 2021). In this case, the pesticides carve out a niche for the biocontrol agents to colonize and prevent loss of beneficial bacteria.

A complex multispecies biofilm, including beneficial bacteria and fungi, could contribute to a more robust defense against pathogens. Consideration must be given to the host plant's compatibility with the biofilm-forming microbes to avoid negative effects. Most bacteria have a narrow host range and only colonize certain areas within their host (Tovi et al. 2019). To ensure successful colonization of beneficial microbes, treatment strategies have to be adjusted to the individual plant species.

7.15 Biofilm Application

Determining the optimal timing for biofilm application is essential for effective disease management. Factors such as plant growth and disease development stages should be taken into consideration. This challenge overlaps with the optimization of environmental conditions as these also determine application success (Lopes et al. 2021). Developing effective formulations for delivering biofilm-forming microbes to plant surfaces is challenging. Inoculation success depends strongly on the individual microbe, as well as on the plant host (Lopes et al. 2021). Antagonistic interactions within the inoculation members must be taken into account to ensure effectiveness (Xu et al. 2011). Additionally, formulations should ideally also have a long microbial shelf life and ensure host adherence and biofilm formation in natural settings.

Developing reliable methods for monitoring the establishment and effectiveness of beneficial biofilms in the field is essential for the success of the treatment. This includes nondestructive techniques for assessing biofilm coverage and activity (Romano et al. 2020). Most methods in use have their downsides, hence the appropriate method has to be chosen based on individual needs. Current methods include culture isolation (Morris et al. 1998) microscopy (Velmourougane et al. 2017), quantitative PCR (Stets et al. 2015), and next-generation or whole-genome sequencing (Guzmán et al. 2020). Continuous monitoring of plant health and incidence (Roper et al. 2021) is necessary to evaluate the long-term impact of biofilm applications on disease suppression and plant productivity. Modern techniques suggest using “phytosensor” plants, which can immediately alter their phenotype upon pathogen recognition (Mazarei et al. 2008). Other simple and noninvasive methods include imaging (Mahlein 2016) and spectroscopy (Altangerel et al. 2017). Additionally, approaches have been described based on measuring the electrical conductivity of nutrient solutions (Jeon et al. 2017).

Biofilm-forming microbes should be cautiously introduced to avoid disrupting the natural microbial balance in the ecosystem (Cook 1996). Risks include gene transfer and uncontrolled spread of, for example, antibiotic resistances among other microbes native to an ecosystem. Other concerns may relate to the production of unintended secondary metabolites, causing unwanted antagonism among the microbial community and leading to elicitation of plant defenses (Woo and Lorito 2007). Assessing the potential environmental impact and long-term consequences of biofilm applications is essential as well as challenging in itself, and is an area of ongoing research and legal regulation (Liu et al. 2022; Vassileva et al. 2022).

7.16 Conclusions and Future Perspective

Biofilm research has grown substantially over the past few decades. Initially centered on preventive and destructive strategies against the detrimental effects, mainly in the medical domain, the focus has shifted toward harnessing biofilms for biotic and abiotic stress protection. This presents a promising avenue for sustainable agriculture. The diverse outcomes of these biofilms, ranging from disease suppression to enhanced nutrient uptake, underscore their potential to revolutionize plant health management. As research advances, it is evident that understanding the intricate microbial interactions within biofilms and their influence on plant hosts is pivotal. Notably, research has expanded to explore monospecies biofilms and artificial synthetic communities that combine diverse metabolic activities. Future directions in this field should emphasize the exploration of microbial diversity within biofilms and the integration of biofilm engineering to tailor these microbial communities for optimal plant–microbe interactions. Additionally, they should not neglect the development of monitoring techniques and investigation into climate-resilient bacterial biofilms. Global collaborative initiatives will further accelerate progress, facilitating the commercialization and widespread adoption of beneficial biofilm technologies in agriculture. The convergence of these future directions holds the key to unlocking the full potential of beneficial biofilms, offering sustainable solutions for plant protection, and contributing to the resilience of agricultural systems in the face of a growing global demand for food.

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