Bacterial Biofilm Formation in Phytopathogens: Mechanisms, Environmental Drivers, and Implications for Disease Management

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ABSTRACT

Bacterial diseases are among the leading plant diseases that pose significant challenges to crops, fruits, and vegetable production worldwide, leading to yield losses, reduced quality and economic loss and more than 200 bacterial pathogens cause severe diseases in economically important crops worldwide. Management of plant pathogenic bacteria is crucial for optimum yield of crops that engender food production and the food security. The use of antibiotics and chemicals are currently the major treatment for bacterial diseases for quick resolution especially in an overwhelming situations in a large scale fields. However, biofilms, being a barrier that exists around bacterial cells, reduces the susceptibility of bacteria to antibiotics and causes persistent infections and chemical control options usually have side effect on human health and the environment as some bactericides are toxic to living organisms and non-biodegradable. Moreover, these tactics are continuously challenged by the ever-evolving resistant bacterial population midwifed by bacteria biofilms formation and it has been shown that bacteria in a biofilm increase their resistance against antibiotics by about 1000-fold. The aim of the study was to Review and elucidate Bacterial Biofilm Formation in Phytopathogens: Mechanisms, Environmental Drivers, and Implications for Disease Management. Effective management methods should be targeting quorum sensing, a strategy that interferes with the cell-to-cell communication systems of bacteria, is a promising approach for the development of novel anti-biofilm therapeutics and ultimately resolved virtually most of bacterial diseases incidence. Quorum sensing inhibitors (QSIs) have been extensively evaluated for their efficacy in clinically relevant bacterial biofilms using in vitro and in vivo models, specifically, the development of an autoinducing peptide inhibitor which can effectively reduce biofilm formation in both biological and non-biological surfaces.

Keywords: *Bacterial biofilms, Phytopathogenic bacteria, Quorum sensing, Antibiotic resistance, Plant diseases management*

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INTRODUCTION

Food production and food security for the world's growing population is facing daunting challenge due to increase of pests and diseases exacerbated by climate change (Calero Preciado et al., 2022; Carezzano et al., 2023**;** Hamim et al., 2024; Tkaczyk, 2025).). Bacterial diseases are among the leading plant diseases that pose significant challenges to crops, fruits, and vegetable production worldwide, leading to yield losses, reduced quality and economic loss (Tancos and Cox 2017; Savary *et al*., 2019). It has been reported that plant diseases caused by bacterial pathogens are responsible for ~ 10% loss in global food production (Buttimer *et al*. 2017). According to the reports, more than 200 bacterial pathogens cause severe diseases in economically important crops worldwide (Buttimer *et al.,* 2017). Bacterial diseases like spots/blights reduce the photosynthetic ability of the plant, and fruit rots or spots affect the quality of fruits and selling price for the farmer. Majority of bacteria pathogens that causes significant damage to crops belong to the genera *Ralstonia*, *Pseudomonas, Xanthomonas*, *Agrobacterium*, *Erwinia,* *Xyllela*, and *Dickeya,* (Buttimer *et al*. 2017). The management of bacterial diseases is predicated on the application of antibiotics, antibacterial chemicals, bio-control agents, and resistant varieties (Tancos and Cox 2017; Bain *et al.,* 2020; Ailing *et al.,* 2023). Chemical control options usually have side effect on human health and the environment as some pesticides are toxic to living organisms and non-biodegradable (Tancos and Cox 2017). Moreover, these tactics are continuously challenged by the ever-evolving resistant bacterial population midwifed by bacteria biofilms formation (Bain *et al*., 2020; Miller *et al*., 2022). Most of the bacteria that cause us problems are sessile attached to a surface and they live in biofilm. Most researchers study extensively on planktonic cells (Free moving cell), while the actual problems involve biofilm (Zhou *et al*., 2020; Calero Preciado *et al*., 2022). The management of bacterial diseases is predicated on the application of antibiotics, antibacterial chemicals, bio-control agents, and resistant varieties (Tancos and Cox 2017; Bain *et al.,* 2020; Ailing *et al.,* 2023).The use of antibiotics and chemicals are currently the major treatment for bacterial diseases for quick resolution especially in an overwhelming situations in a large scale fields. However, biofilms, being a barrier that exists around bacterial cells, reduces the susceptibility of bacteria to antibiotics and causes persistent infections and chemical control options usually have side effect on human health and the environment as some bactericides are toxic to living organisms and non-biodegradable (Tancos and Cox 2017; Bain *et al*., 2020; Miller *et al*., 2022; Calero Preciado *et al*., 2022; Hamim et al., 2024). Moreover, these tactics are continuously challenged by the ever-evolving resistant bacterial population midwifed by bacteria biofilms formation and it has been shown that bacteria in a biofilm increase their resistance against antibiotics by about 1000-fold (Hamim et al., 2024; Tkaczyk, 2025).The aim of the study was to elucidate Bacterial biofilm formation in phytopathogens, Mechanisms, Environmental drivers, and Implications for Disease Management. Management strategies are based on a better understanding of how bacteria attach, develop biofilms and detach (spread) are urgently needed so as to develop new and affective control strategies. Biofilms are wide spread and play critical roles in diverse biogeochemical processes, natural and anthropogenic environments (Flemming and Wuertz 2019; Ailing *et al*., 2023).

LITERATURE REVIEW

Bacterial Biofilm Formation in Phytopathogens

Biofilm is a complex aggregation of microorganisms growing on a solid substrate. Microbial biofilms are not simply structured assemblages of cells that are attached to surfaces or/and to each other but is a dynamic complex biological system that evolves by a tightly controlled process (Zhou *et al.,* 2020; Ailing et al., 2023). Bacteria stick to environmental surfaces in multi-cellular assemblies during pathogenesis, symbiosis and in commensally relationships (Penesyan *et al*., 2021). Formation of biofilm commences with the attachment of non-binding moving microorganisms to a surface through weak, reversible Van der waals forces. If the colonies are not immediately separated from the surface, they can anchor themselves more permanently using adhesion molecules such as pilli and polysarcharides and surface proteins (Ailing *et al*., 2023). The attached cells lay foundation for the arrival of other cells by offering more diverse adhesion spaces and beginning to form the matrix that holds the biofilm together. The building blocks of biofilms are mainly related to the extracellular polymeric substances (EPS) contained in the biofilm matrix (Seviour *et al*., 2019). Although larger parts of biofilm matrix component is water (up to 97%), plus multiple microbial biopolymers including proteins, exo-polysaccharides, nucleic acids (RNA and extracellular DNA), and lipids, including non-regular minerals such as quartz, basalt, clay minerals Penesyan *et al*., 2021). EPS forms a thick, muddy, hydrated matrix in which cohesion is based on multiple electrostatic forces, hydrogen bonds, and ionic interactions, which produce weak or strong networks (Fleming et al., 2025).

MECHANISMS

Biofilm develops when bacteria adhere to surfaces in aqueous environments and start to produce a slimy, glue-like substance that can anchor them to any available materials. Although biofilm can be composed of a single bacteria species, but more often biofilms consist of many species of bacteria, as well as fungi, algae, protozoa, and debris (Fathollahi *et al*., 2021; Ailing et al., 2023). Biofilm-producing phytopathogenic bacteria are difficult to manage and therefore, in-depth comprehension of the mechanisms involved in their survival is essential to develop new strategies to manage plant disease. Phyto-pathogenic bacteria colonize the intercellular spaces and the vascular system of the host plants and cause a wide range of symptoms such as necrosis, wilting, leaf spots, blight, soft rot, and hyperplasia (Carezzano et al., 2023).

Quorum-Sensing

Formation of biofilm and the resulting intimate interactions with plants often require cell-cell communication between colonizing bacteria called "quorum sensing (Costa *et al*., 2018; Galdiero *et al*., 2019). Most bacteria communities interact among themselves using secreted chemical molecules to coordinate the behavior of the groups (Costa et al., 2018; Galdiero *et al.*, 2019). Gram-negative bacteria use quorum sensing communication circuits to regulate series of physiological activities such as symbiosis, virulence, competence, conjugation, antibiotic production, mobility, sporulation and biofilm formation. Bacterial cells communicate by secreting and detecting extracellular signals, particularly through specific small signaling molecules known as auto inducers. These quorum-sensing signals are fundamental in all steps of biofilm formation such as initial adhesion, maturation, and dispersion that triggers gene expression that either results in bacterial virulence factors, stimulates immune responses in host tissues, or contributes to antibiotic resistance development. The regulatory mechanisms of QS depend on AHL system, AIP system. The AHL system exists in Gram-negative bacteria and the signaling molecules employed in this system are *N*-acyl homoserine lactones (AHLs) (Kim et al., 2020). While, autoinducing peptides (AIPs) are employed in the interspecies signal exchange. These two systems have been detected to be present in both Gram-positive and Gram-negative bacterial species and to contribute in interspecies signal exchange (Kim et al., 2020). Quorum sensing systems, cyclic-di-GMP signaling pathways, and transcriptional regulatory networks regulate transitions between planktonic and sessile states, determining bacterial population behaviors in response to environmental cues and host interactions (Portaccio et al., 2025). In general, Gram-negative bacteria use acylated homoserine lactose as autoinducers or to communicate, and Gram-positive bacteria use processed oligopeptides to communicate (Costa *et al*., 2018; Galdiero *et al*., 2019). Although the nature of chemical signals, the signal relay mechanisms, and the target genes controlled by bacterial quorum sensing systems differ, in every case the ability to communicate with one another allows bacteria to coordinate the gene expression, and therefore the behavior of the entire community (Castro et al., 2023). Presumably, this process bestows upon bacteria some of the qualities of higher organisms (Costa *et al*., 2018; Galdiero *et al*., 2019). Biofilm are common in nature, as bacteria commonly have mechanisms in which they can adhere to surfaces and to each other. Observation of bacteria associated with plants increasingly reveals biofilm-type structures that vary from small clusters of cells to extensive biofilm. The surface properties of plant tissue, nutrient and water availability, and the proclivities of the colonizing bacteria strongly influence the resulting biofilm structure (Ailing *et al.,* 2023). A typical bacterial biofilm may composed of a single microorganism or a mixture of bacteria, fungi, archaea, protozoa, and yeasts. When stimulated by the harsh environment, the exo-polysaccharides, fibrins and lipoproteins secreted by bacteria adhere to the surface of inert objects and form microbial substances (Schilcher and Horswill, 2020; Chiba *et al*., 2022).

Biofilms in Pathogenicity

Pathogenesis is the steps pathogen takes to manifest symptoms on host (diseases). The many ways bacteria interact with host is paramount to determine the nature of the outcomes. These interactions ranges from mutualistic/symbiotic relationships that benefit both parties to explosive infection in which the pathogens rapidly kill the host (Park *et al.,* 2022). Perennation of plant pathogenic bacteria in nature occurs most commonly in biofilms attached to plant debris left on the soil surface, in and on seeds, in soil, and in association with perennial hosts (Vidaver & Lambrecht, 2004). Information of bacteria perennation in ecosystem usually is prerequisite for effective disease management. Steps in pathogenesis are pathogen inoculation, pathogen incubation and pathogen produces diseases in the host. Increasing evidence suggests that biofilm mode of growth may play a key role in both of these adaptations (Fathollahi *et al*., 2021). Biofilms are simply a perennation mechanism of bacteria cells with individual bacterium comes together as a whole in order to become stronger (Park *et al*.,2022). As the cliché goes, there is strength in numbers, with bacteria being no exceptions. The overriding benefits of biofilms to phyto-pathogenic bacteria is that biofilms enhance the feeding capacity of bacteria pathogen and increase their fecundity. The fact is that the feeding activities of bacteria pathogens is what cause diseases in plants. The virulence factors of most plant pathogenic bacteria are categorize as; a) Toxin production b) Cell wall degrading enzymes, c) Production of Extra-cellular polysaccharides (EPS) d) Hormone inducing (Schilcher and Horswill, 2020; Chiba *et al*., 2022; Ailing *et al.,* 2023).. Bacteria pathogens in a bid to feed and multiply engages in one or more of these factors. Planktonic pathogen cannot carry out any of these activities except when in biofilms environment (Schilcher and Horswill, 2020; Chiba *et al.*, 2022). Attachment to host is made possible by the hair like structure on the surface of the bacterial body is called fimbriae or pili. These hairs are able to attach themselves to certain sites of the host, in such a way the bacteria cannot be washed away. Additionally, some bacteria can adhere to the host by means of matrix of extracellular polymeric substance (EPS) and successful attachment is usually a necessary prerequisite for virulence and even infection (Schilcher and Horswill, 2020; Chiba *et al.*, 2022). Many phytopathogenic bacteria produce biofilm virtually on many parts of the host plant such as the roots, stems, fruits, and flower and on the leaf surface and internal vasculature which represent the various type of diseases caused by bacteria pathogen (Fatholahi *et al.,* 2021). Each tissue type has unique chemical and physical properties that represent challenges and opportunities for microbial colonists (Parrilli *et al*., 2022). Most of plant pathogenic species such as *Pseudomonas. syringae pv. syringeae*, can survive drought by living within biofilms on leaves (Yang et al., 2023). Other Pseudomonas spp., such as P. aeruginosa on Arabidopsis taliana and P. syringae pv. syringae B728a, form biofilms on trichomes. Biofilms structures retain water **and contain** nutrients, two key components that ensure persistence and endurance of the 3-D matrix (Tkacyk, 2025). Biofilms confer longevity of bacteria cells on leaf and bark surfaces as well as within vascular tissues, underscore the basis for persistent infections that are difficult to control. The recently described *L. populi* exemplifies a bark-colonising, biofilm-producing tree pathogen whose EPS exudates mediate both protection and vector attraction, highlighting novel ecological dimensions of biofilm function in forest systems (Tkaczyk, 2025). . Recent microscopic and biochemical analyses confirm that the EPS of *L. populi* acts as a key virulence factor and supports adhesion to xylem parenchyma, impedes host phenolic diffusion, and mediates cell-to-cell communication through quorum sensing signals analogous to AHL-type autoinducers (Tkaczyk, 2025). Notably, the biofilm exudate appears to attract insect vectors, promoting bacterial dissemination between trees a rare ecological linkage between microbial virulence and insect-mediated spread in forest systems (Tkaczyk, 2025)**.** This role of biofilms as diversity incubators where the dynamic process of generating genotypic and phenotypic variants occurs makes it the most successful strategy to colonize extreme environments and probably the only possible one. This is clear example of how the *physiological heterogeneity* of biofilm is a key element to survive in a hostile environment (Parrilli *et al.*, 2022).

Biofilms on Leaves and Shoots

In forest ecosystems, *P. syringae* displays a broad host range among both deciduous and coniferous trees, indicating a significant phytopathogen in temperate climate zones. *P. syringae* is a typical epiphytic pathogen whose life cycle involves prolonged residence on leaf and shoot surfaces before initiating infection (Yang et al., 2023). Many strains can form stable, multilayered biofilms on the leaf cuticle, where the EPS matrix interacts with adhesin proteins and type IV pili, strengthening attachment and microcolony formation (Yang et al., 2023)

Biofilm Formation in Vascular Tissues

In *E. amylovora*, biofilm formation plays important role in colonizing vascular tissues. Biofilm structures within the xylem and adjacent mesophyll spaces facilitate the establishment of persistent infection foci, while three main polysaccharides amylovoran, levan, and cellulose—have been seen as key EPS components, determining the architecture and viscosity of the matrix and Mutants lacking one or more of these components show reduced biofilm-forming ability and diminished virulence in planta (Yang et al., 2023).

Biofilm Detection Methods

Evidence that bacterial diseases is caused by biofilms: (a) the infecting bacteria are adherent to some certain part of the host (b) direct examination of infected tissue shows bacteria living in cell clusters or in micro colonies, encased in an extra-cellular matrix (c) the infection is generally confined to a particular location, although dissemination may occur. (d) The infection is difficult or impossible to eradicate with antibiics despite the fact that the responsible organisms are susceptible to killing in the planktonic state (Park *et al*., 2022). There are limitations to these criteria; however, they provide general characteristics with which to consider the role of biofilm in plant diseases. Practically challenging, visualising biofilms inside wood, especially within xylem vessels due to lignification, tissue heterogeneity, and limited access for light or fluorescent dyes. However, methods such as confocal laser scanning microscopy (CLSM) or FISH must be adapted or replaced by imaging approaches such as microtomography. For example, Castro et al. (2023) demonstrated that β-1, 4-endoglucanase affects EPS polymer length and biofilm dynamics, but these experiments were conducted primarily in vitro, highlighting the gap in studies under natural tree conditions. The cycle commences with microbial attachment to an interface followed by micro-colonies formation, biofilm maturation and cells dispersion, which is necessary for microorganism spreading and serve as inoculum for new biofilm initiation and the concept is recently known as single-cell centric (Penesyan et al. 2021; Ailing *et al*., 2023).

Classical Methods

In this method, the biofilm layer formed on a synthetic surface can be examined visually using dyes such as crystal violet and safranin. Spectrophotometric measurement of biofilms formed in microplates instead of test tubes is a new method resulting from adaptation and development of method (Arciola et al., 2018; Gamalero, and Glick, 2022). Slime-producing strains are differentiated using this method. When adhesion is examined visually, it can be scored from 1+ to 4+ although not an exact numeric measurement, visual grading of the slime layer is an accepted method in the literature. A fully numeric comparison is possible when measuring the biofilm (Hoiby et al., 2015).

Imaging Methods Used in the Diagnosis of Biofilm

Evaluating the adherence of microorganisms to cells by counting is a commonly used method and the light microscope offers a two-dimensional image, but does not allow precise measurement of the biofilm. In addition to classical imaging methods, new systems that have been recently developed can be used to visualize the biofilm in vivo (Abdelhamid and Yousef, 2023). Electron microscopy offers a three dimensional, quantifiable image and has been long used to demonstrate the biofilm mass. The surface of a microcolony can be visualized using scanning electron microscopy (SEM), while the inner structure of the biofilm mass can be visualized using transmission electron microscopy (Abdelhamid and Yousef, 2023).

The Role of Molecular Methods in Biofilm Diagnosis

Molecular methods can be used for two purposes in the diagnosis of biofilm and the first is to identify the microorganisms in the biofilm via molecular target genes. The moment the microorganism is known, the next step is to identify the genes responsible for biofilm formation, adhesion, and slime production. Molecular method may be an amplification-based method with a single target, or a DNA array system that targets gene under- and overexpression in the biofilm. Demonstrating DNA requires RNA isolation from the biofilm and a transcription-based molecular method (Xu et al., 2019; Abdelhamid and Yousef, 2023).

Identification of Biofilm-related Infection at the Infection Site

Biofilm can be visualized and measured with the prosthesis while in place or after removing it and this procedure can be used to evaluate biofilm in situ (Xu et al., 2019). Without identifying the causative microorganism, the mass can be approached by visual and quantitative methods. A biofilm can be identified on a prosthesis by direct inoculation of the prosthesis to a liquid or solid media, obtaining samples from the prosthesis after sonication or rinsing, and performing inoculation using fluorescent microscopy and fluorescent in situ hybridization, confocal LSM, SEM, or DNA isolation and analysis from the prosthesis (Abdelhamid and Yousef, 2023)

Antimicrobial Resistance

The primary reason bacteria forming biofilms is to raise protection of cells from hostile environmental conditions such as ultraviolet radiation, extreme pH, high salinity, extreme temperature, high pressure (Yin *et al*. 2019), and tolerance to desiccation (Flemming and Wingender 2010; Ailing *et al*., 2023). The persistence of infections associated with biofilms remains a considerable unresolved (Hamim et al, 2024). Bacteria naturally live in colony and when the colony advances with many structure and forms it become biofilms or bacteria home or cities and within these cities are groups of bacteria consisting multiple species (Park *et al*., 2022). Individual bacteria coalesce by linking extracellular polysaccharides on their cell walls while polysaccharide chains exhibit chemical properties that make them polar and thus very "sticky" and the polarity is what leads to surface adhesion and cell cohesion (Park et al., 2022). Additionally, biofilms is a source of protection against host defence mechanisms and equally reduce the penetration of toxic compounds released by the plant (e.g., defensins, reactive oxygen species, phenolics) as well as degradative enzymes (Parrilli *et al*., 2022). Bacteria home is a place of protection from environmental hazards especially if biofilm is composed of aerobic (requiring oxygen) and anaerobic (not requiring oxygen) bacteria, oxygen requiring bacteria actually form a layer over the non-oxygen requiring bacteria. Thus the anaerobic bacteria exist in a low-oxygen microclimate while the aerobic bacteria have a neighbor that's not competing for the oxygen they require. Meanwhile, they both benefit from the protective nature of a biofilm (Schilcher and Horswill, 2020; Chiba *et al*., 2022). The EPS matrix mitigates the effect of antibiotics by neutralizing antimicrobial agents or limiting diffusion using extracellular polysaccharides. Indeed, biofilm matrix confers a high tolerance to desiccation, bacteria in the biofilm actively respond to desiccation by the production of EPS molecules (Anderson and Hughes, 2010; Weaver *et al*. 2015), which, due to the high amount of hydrated polymers in the EPS matrix, *protects* the biofilm from dryness by acting as a hydrogel that holds water (Flemming et al., 2025). EPS mediates intercellular communication, protects cells from chemical damage, provides oxygen diffusion, releases extracellular enzymes for nutrition and, in turn, stimulates the spread of bacteria within the biofilm (Costa *et al*., 2018; Galdiero *et al*., 2019). Biofilms confer resistance on many bacteria pathogens to many antibiotics (Parrilli *et al.,* 2022). In some cases, antibiotics resistance can be increased a thousand fold (Nicole *et al*. 2016). Biofilms are notoriously resistant to washing and other common antibacterial treatments and may make organisms less conspicuous to the immune system of the host. Also, biofilms are much more resistance than planktonic cells to antimicrobial agents. Gottel et al., (2024) reported that both *Escherichia coli* and *Salmonella* population on alfafa sprouts required treatments much harsher than simple treatment to reduce the numbers of adherent microns, and full removal was never achieved. Biofilms have been found to be involved over 80 % of all infections (Park *et a*l., 2022; Abdelhamid and Yousef 2023) Biofilms are ubiquitous just as bacteria and are found both on the surface and inside plants. Sometime this community serve the needs of the plant as in the case of nitrogen-fixing Rhizobium on roots, exist symbiotically with plant (Andersen-nsissen *et al*; 2007; Parrilli *et al*., 2022). These clusters of individual bacterium frequently inhabit both inert and living surfaces in a non-pathological manner. Biofilms engage involve chelation by the complex formation and/or enzymatic degradation of antimicrobials substances (Jha and Anand, 2023).). Finally, slow growth within sections of the biofilm and the generation of dormant persister cells further increase the survival of cells to antibiotic actions (Stewart 2015; Ailing *et al*., 2023).

IMPLICATIONS FOR DISEASE MANAGEMENT

The impact of phytopathogenic biofilms on agriculture cannot be understated as they are formed on leaves (mesophyll, parenchyma), in the rhizosphere, and/or in vascular bundles, they reduce crop yield and quality and reduce the safety of agricultural products intended for human consumption and animal feeds (Rafigue et al., 2015; Mina et al., 2019). The consequences are felt as food security is undermined at a time when booming populations around the world require high productivity rates, as expected by the UN (Lomovatkaya and Romanenko 2020). The fact that biofilms can be constantly remodeled and restructured also makes them versa-tile systems in the face of changing environments in terms of UV radiation, desiccation, and nutrient availability, among many other factors. This enhanced survival in the plant (whether in or on leaves, roots, conductive vessels, etc.) as well as in the environment makes phytopathogens all the more harmful to crop yield, health, and quality (Lomovatkaya and Romanenko 2020). Biofilm that are formed in vascular system of plants have higher virulence and pathogenicity factors, and they can resist an- timicrobials synthesized by the host and overcome other plant immune responses (Mina et al., 2019). It is therefore pertinent to note that meaningful and effective management practices of bacteria disease can be achieved following some simple understanding of how to disrupt quorum sensing, a significant condition for biofilm formation Abdelhamid and Yousef, 2023)

Quorum Sensing Inhibiting Peptides

Effective management methods should be targeting quorum sensing a strategy that interferes with the cell-to-cell communication systems of bacteria, is a promising approach for the development of novel anti-biofilm therapeutics and ultimately resolved virtually most of bacterial diseases incidence. Quorum sensing inhibitors (QSIs) have been extensively evaluated for their efficacy in clinically relevant bacterial biofilms using in vitro and in vivo models, specifically, the development of an autoinducing peptide inhibitor which can effectively reduce subcutaneous biofilm formation during transplantation in a mouse model (Shang et al., 2021). The use of an RNAIII-inhibiting peptide reduced MRSA biofilms in a mouse wound model (Fernicola et al., 2015; Abdelhamid and Yousef, 2023). Tryptophan-containing peptides interfered with the *Pseudomonas* quorum sensing system and inhibited virulence factor production, biofilm formation, and EPS accumulation in invitro experimentation (Shang et al., 2021). Any action aim at preventing bacterial adhesion to surfaces, impeding bacterial aggregation in viscous mucus layers, degrading the extracellular polymeric matrix, and developing nanoparticle-based antimicrobial complexes which target persistent cells within the biofilm core are inevitable steps towards effective bacteria control Abdelhamid and Yousef, 2023). It is important to acknowledge, however, that the use of anti-biofilm agents faces obstacles, such as limited effectiveness in vivo, potential cytotoxicity to host cells, and propensity to elicit resistance in targeted biofilm-forming microbes. Emerging next generation anti-biofilm strategies, which rely on multipronged approaches, are needed, and these will benefit from current advances in nanotechnology, synthetic biology, and antimicrobial pesticides discovery (Shang et al., 2021). The seriousness of health risks associated with biofilm-related infections drives the ongoing endeavors to create innovative anti-biofilm agents that target extracellular matrix formation, promote biofilm dispersal, or act against the resilient cells within the biofilm core (Abdelhamid and Yousef 2023). The following are samples of anti-biofilm formation in the literatures.

Small Molecule Inhibitors

Few examples of biofilm-related small molecule inhibitors include those targeting the synthesis of the intracellular signaling molecules (second messengers) often found in bacteria, such as cyclic-di-guanosine monophosphate (c-di-GMP), which regulates EPS-producing enzymes in Gram-positive (e.g., *S. mutans*) and Gram-negative (e.g., *P. aeruginosa*) bacteria Abdelhamid and Yousef, 2023). Hence, interrupting the production of c-di-GMP, using small molecule inhibitors, could be implemented as a strategy in combating biofilms and associated infections. Small molecule inhibitors (e.g., catechol-containing sulfonohydrazide compounds) of di-guanylate or di-adenylyl cyclase were identified as potent antibiofilm agents in in vitro biofilm models, but their efficacy against biofilms in vivo requires further validation (Fernicola et al., 2015; Abdelhamid and Yousef, 2023)

Enzymes Degrading Extracellular Polymeric Substances

Depending on their chemical structures, degrading EPSs can be a useful strategy in combating bacterial biofilms. Divers methods include the use of exopolysaccharide-degrading enzymes, such as dispersin B, to disrupt the matrix of pathogenic oral biofilms, and glucan hydrolases, which have been used to degrade the EPSs associated with dental biofilms (Nett et al., 2016). In another approach, purified serine protease, Esp, is used to inhibit *S. aureus* biofilm formation and eradicate pre-existing biofilms *in vitro*, enhancing the susceptibility of biofilm-forming cells to antimicrobial β-defensin 2, and reducing *S. aureus* nasal colonization in humans (Nett et al., 2016; Abdelhamid and Yousef 2023).

Biofilm Dispersion-Based Strategies

The biofilm dispersal process represents a novel approach to disrupting biofilms, targeting the metabolic pathway of c-di-GMP, which plays a key role in the biofilm life cycle of both Gram-positive and Gram-negative bacteria, and a veritable strategy, although the complexity of c-di-GMP regulation makes it challenging to control in a general term (Shang et al., 2021; Abdelhamid and Yousef, 2023)

Antibodies and Nucleic-Acid-Binding Proteins

Extra cellular PolySacharride -targeting antibodies and nucleic-acid-binding proteins may be used to resolve biofilm infection (Zhao et al., 2023). Although the use of vaccines faces considerable challenges due to the antigenic variability in biofilm-forming clinical isolates, the use of monoclonal antibodies against specific EPS components has shown potential option. Psl-specific antibodies increased the opsonophagocytic killing of *P. aeruginosa*, inhibited pathogens' adherence to lung epithelial cells, and showed prophylactic protection in several animal models against *P. aeruginosa* infection (Xu et al., 2019). The current strategies to control biofilms include pesticides and antibiotics, but they are not efficient enough and they pose their own risks (Zhao et al., 2023). Infections usually reappear and the overuse of chemical products causes water and soil contamination. If new management strategies are to emerge, it will be contingent on the thorough knowledge of how phytopathogenic biofilm is produced and regulated (Lv et al., 2022). An understanding of the nature and role of biofilm in pathogenesis is critically needed in order to evolve effective management strategies to control and/or minimize diseases caused by biofilm-producing plant pathogenic bacteria (Zhao et al., 2023). Currently, much is known about biofilm structure, EPS composition, signaling mechanisms, plant penetration and colonization, and symptoms of disease. These variables depend mainly on the microbial species, its metabolic activity, nutrient availability, the ecosystem, and the plant's growth stage as well as sophisticated mechanisms that regulate plant-host associations (Zhao et al., 2023).

ENVIRONMENTAL DRIVERS

Biofilms formation are affected by climate change, temperature, Oxygen, nutrient, nature of surface, and time.

Climate Change

The overwhelming effect of climate change on both the visible and microscopic life has far-reaching consequences on our overall ecosystem. Climate change is projected to induce environmental alterations that will have profound effect on virtually all life activities (Grilo *et al*., 2021). The key element of climate change such as rising temperature, extreme weather activities and shift in seasons influence cell multiplication, growth and development (Ugya, 2022). These abiotic changes will impact ecosystem dynamics and promote disruptions in species equilibrium. Biofilms formation which leads to antimicrobial resistance, virulence traits, as well as their growth, may vary with changing environmental conditions (Schneider *et al*., 2022). Different species of bacteria respond differently in terms of biofilms formation in variable temperature and PH of the culture. Climate change has been implicated as a factor involved in increasing levels of antimicrobial resistance among different bacterial species over a period of time. Greenhouse gas emissions (GHGE) and climate change causes alteration of temperature, acidification and increase in de-oxygenation in culture environment (Schneider *et al*., 2022). Climate change influence the survival, spread (geographical and within the system, virulence nature of bacterial pathogens. The virulence factors of most bacterial pathogens are toxin, Extracellular polysacharride system (EPS), Enzyme and induced hormone production which are the survival mechanisms and are function of substrate (Schilcher and Horswill, 2020; Chiba *et al*., 2022). The nature, quality and quantity of substrate available for the pathogens is regulated by the element of climate change (Schneider *et al.,* 2022). Since the entrance of virulent pathogen into the susceptible host does not automatically leads to diseases development but depend on optimum environmental factors makes the important of climate change on bacterial biofilms not overemphasized (Schneider *et a*l., 2022). Climate change has literally affected the existence, abundance and distribution of biological organisms both on land, air and Sea and bacterial organisms are not exceptional (Ugya, 2022).

Temperature

Temperature is of particular concern, as it is a variable outside our control, its changing is due to climate change, and impacts many other important environmental factors. Temperature increase had a significant effect on planktonic bacterial communities, although less marked than in biofilm communities. This lower effect is probably because planktonic cells are less protected than biofilms against disinfectant action, and thus, they are effectively controlled in chlorinated DWDS (Schwering *et al*., 2013; Prest *et al*., 2016). Therefore, although fungi are more resistant to environmental changes (Denham et al., 2019), the temperature can affect the abundance of certain genera including some that can produce mycotoxins including Aspergillus, Alternaria, Penicillium, Fusarium, Cladosporium, Malassezia, and/or Trichoderma, which have been found in in both biofilm and water samples. An increase in mean temperature leads to accelerated bacteria cell consumption, development, and movement, fecundity, survival, generation time, population size, and geographic range distribution, and these effects depend on the species' ecology, feeding mode and degree of specialization (Ugya, 2022;Parrilli *et al.,* 2022). In another development, the results from spread plate technique, shows that the biofilm grown at 25 °C recorded the highest number of viable cells (7.8×10^{10} CFU mL^{-1}) but the biofilms incubated at 35 °C contained a 0.2-log lower number of cells (Fathollahi and Coupe 2021). The minimum growth of bacterial cells was associated with bioreactors with temperature of 45 °C with 0.8-log lower number of viable cells in comparison to those incubated at 35 °C. Several studies have reported 25 °C as the optimum temperature for bacterial biofilms (Mizan *et al*., 2018)

Oxygen

The effect of oxygen on the development of biofilms varies from one microbial species to the other and the conditions of exposure. Certain Oxygen levels can increase biofilm growth in some microorganisms under particular environmental conditions while some species show no effect or even the opposite effect in response to higher oxygen content in their environment. Oxygen level is a essential aspect that contributes significantly to biofilm formation. Studies have shown that aerobic environments can facilitate the rate at which biofilms are formed in certain bacteria, for example Campylobacter NCTC 11168 biofilms grew more rapidly under aerobic (20% oxygen) compared to microaerobic (5% oxygen, 10% carbon dioxide) conditions, although the level of biofilm formation was the same after three days (Pokhrel et al., 2022; Reuter et al., 2021). The actual level of oxygen required for the formation of biofilms varies considerably and often depend on the bacterial species and the environment in which it resides in plant (Reuter et al., 2021).

Nutrient

The type and amount of nutrients in the bioreactor/medium has a significant effect on the formation of bacterial biofilms (Haney *et al*., 2018; Salgar-Chaparro *et al*., 2020; Kilic, 2025; (Shabana et al., 2026). In the substrate containing 25 g L^{-1} of phosphate, the growth of the biofilm increased after 21days of incubation. In general, with the increase of phosphate content in the bioreactor, the growth of the biofilm increased. Similarly, by increasing the glucose content to 20 and 40 g L^{-1} the number of bacterial cells within the biofilm decreased by 0.5- and 0.7-log, respectively (Fathollahi and Coupe 2021). In an experiment to determine the role of nutrient on *Xanthomonas axonopodis pv vignicola* (*Xav*) biofilms formation, it has been established that medium/media that are rich in nutrients support the production of biofilms than the control medium/media that lack nutrients and that many bacteria utilize many nutrient types to ensure that some functions are only expressed when a particular population density has been reached (Ugya, 2022). Nutrient such as Ife brown extract + 0.5 g glucose induced the highest biofilms formation followed by millet extract, followed by sorghum extract, and maize extract (Amodu *et al*., 2018). Although nutrient support biofilms formation but the magnitude differs and this shows that bacteria growth/biofilm formation is dependent on external source of carbon and nitrogen provided by the host plant or the surrounding environment (Shabana et al., 2026). In the case of amino acid effect on biofilms formation, increasing the content of amino acid from 0 to 1 gL^{-1} resulted in a 3-log increase in the viable cells while further, increases of the amino acid to 5, 25 and 50 g L^{-1} showed a 2-, 4- and 6-log decrease in bacterial cells within the 21-days incubated biofilm (Warraich *et al*. 2020; Fathollahi and Coupe, 2021). Furthermore, the number of viable cells did not change significantly by increasing the nitrate content of the bioreactor from 0 to 0.2 g L^{-1}. However, the nitrate content of 0.5 and 1.5 g L^{-1} showed a 2-log increase in the number of viable cells on day 21 of biofilm growth. Dixon *et al.* (2018) reported an increase in biofilm growth with increasing the calcium ions to 0.5 M, higher concentrations of calcium were reported to inhibit biofilm growth (Fathollahi and Coupe 2021; (Shabana et al., 2026).

Effect of pH

The results from spread plates, mass analysis and FTIR microscopy of the mixed-species biofilms incubated in pH 4, 5, 6, 7, 8 and 9 shows that the number of viable cells, maximum planktonic and biofilm growth was observed at pH 7. By increasing the pH to 9, a 1.8-log decrease in the number of viable cells was detected. Acidic environments showed the same effect on the biofilm growth with a 3-log decrease in bacterial cell number at pH 4 (Papakonstantinou and Efthimiou, 2019; Carezzano et al., 2023; Kilic, 2025).

Effect of Nature of Surface

Though bacteria attachment to surfaces appear to be nonspecific (Kilic, 2025), particular bacteria pathogens are known to have an apparent preference for certain tissues over other. For example, *Salmonella mutan* is abundant in dental plaque but does not occur on epithelial surfaces of the tongue; the reverse is true for *Salmonella salivariu*s which is attached in high numbers to epithelial cells of the tongue but is absent in dental plague (Carezzano et al., 2023; Kilic, 2025). The nature of the surface influence the attachment of the pathogen to the surface while rough and fleshy surface attract adhesion, the stony and leathery surface was not suitable for attachment (Amodu *et al.,* 2018). In an experiment to determine the influence of different surface properties on (*Xav*) biofilms formation shows that biofilms formed vary considerably with the surface properties of seeds though after time of inoculation, all the seeds had *Xav* attached to it. Bacterial adhesion is the initial step in colonization and biofilm formation and it begins with the attachment of free floating bacteria to a surface through weak, reversible vander waal forces (Shabana et al., 2026). The 100 % of infection on cowpea seeds at 2 h after inoculation could be partly due to high inbibition of cowpea seeds that draw the bacteria to the seeds and tissue tropism (Kilic, 2025). The surface properties of seeds used in this work vary considerably. After 4 h of inoculation, all the seeds had *Xav* attached to it. Physico-chemical properties such as surface's roughness, hydrophobicity, film modulation, and surface charge can influence the efficiency of planktonic bacterial colonization and biofilm formation.

Time

In an experiment to determine the role of time on (*Xav*) biofilms formation shows that the rate at which the biofilm grows beyond the initial attachments is influenced by time, and the amount of nutrient within the aqueous medium (Amodu *et al*., 2018; Kilic, 2025). Observation of bacteria associated with plants increasingly reveals biofilm-type structures that vary from small clusters of cells to extensive biofilm. The five stages of biofilm development are: initial attachment (planktonic cell attachment), irreversible attachment, maturation I, maturation II and dispersal which are dependent on time. The biofilm formation did not significantly increase with time except Ife brown extract + 0.5 g glucose where biofilm formed at 96 h was higher than that formed 72 h but did not increase at 120 h. Non reversible attachment and biofilm formation are quorum sensing regulated (Huber *et al*., 2003). Adhesion is a widely conserved virulence mechanism among gram-negative bacteria, no matter whether they are human, animal or plant pathogens, since attachment to the host tissue is one of the key early steps of the bacterial infections process (Amodu *et al.,* 2018).

CONCLUSION

Bacterial diseases are among the leading plant diseases that pose significant challenges to crops, fruits, and vegetable production worldwide, leading to yield losses, reduced quality and economic loss and more than 200 bacterial pathogens cause severe diseases in economically important crops worldwide. The management of bacterial diseases is predicated on the application of antibiotics, antibacterial chemicals, biocontrol agents, and resistant varieties. Moreover, these tactics are continuously challenged by the ever-evolving resistant bacterial population midwifed by bacteria biofilms formation and it has been shown that bacteria in a biofilm increase their resistance against antibiotics by about 1000-fold. The infection is difficult or impossible to eradicate with antibiotics despite the fact that the responsible organisms are susceptible to killing in the planktonic state. Additionally, biofilms is a source of protection against host defence mechanisms and equally reduce the penetration of toxic compounds released by the plant (e.g., defensins, reactive oxygen species, phenolics) as well as degradative enzymes. Effective management methods should be targeting quorum sensing, a strategy that interferes with the cell-to-cell communication systems of bacteria, is a promising approach for the development of novel anti-biofilm therapeutics and ultimately resolved virtually most of bacterial diseases incidence. Quorum sensing inhibitors (QSIs) have been extensively evaluated for their efficacy in clinically relevant bacterial biofilms using in vitro and in vivo models, specifically, the development of an autoinducing peptide inhibitor which can effectively reduce biofilm formation in both biological and non-biological surfaces. Infections usually reappear and the overuse of chemical products causes water and soil contamination. If new management strategies are to emerge, it will be contingent on the thorough knowledge of mechanism of phytopathogenic biofilm production and regulation. Biofilms formation are affected by many factors but not limited to the following climate change, temperature, Oxygen, nutrient, nature of surface, and time. Climate change influence the survival, spread (geographical and within the system, virulence nature of bacterial pathogens. An optimum level Oxygen and temperature leads to accelerated bacteria cell consumption, development, and movement, fecundity, survival, generation time, population size, and geographic range distribution, and these effects depend on the species' ecology, feeding mode and degree of specialization. The type and amount of nutrients available in the bacteria culture/host has a significant effect on the formation of bacterial biofilms. Bacteria biofilms formation are the survival strategy of phyto-pathogenic bacteria to subsist between off seasons and are the reason for ubiquitous nature of bacteria. It is important to acknowledge, however, that the use of anti-biofilm agents faces obstacles, such as limited effectiveness in vivo, potential cytotoxicity to host cells, and propensity to elicit resistance in targeted biofilm-forming microbes. Emerging next generation anti-biofilm strategies, which rely on multipronged approaches, are needed, and these will benefit from current advances in nanotechnology, synthetic biology, and antimicrobial pesticides discovery. The seriousness of health risks associated with biofilm-related infections drives the ongoing endeavors to create innovative anti-biofilm agents that target extracellular matrix formation, promote biofilm dispersal, or act against the resilient cells within the biofilm core.

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