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Factors Affecting Biofilm Formation in *in vitro* and in the Rhizosphere

Firoz Ahmad Ansari[1], Huma Jafri[1], Iqbal Ahmad[1] and Hussein H Abulreesh[2]

[1] *Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh, India*
[2] *Department of Biology, Faculty of Applied Science, Umm Al-Qura, Makkah, Saudi Arabia*

15.1 Introduction

Biofilms are bacterial communities enclosed within self-produced extracellular polymeric substances (EPS). In nature, biofilms constitute a protected growth modality allowing bacteria to survive in hostile environments [1]. This cellular colonization confirms the nutrient utilization, expression of surface molecules, and virulence factors, and furnishes bacteria with an arsenal of properties that facilitate their survival in unfavorable conditions. It has been now well understood that bacterial ability to grow adhered to almost every surface-forming biofilm, although the mechanisms involved in the process of biofilm formation differ depending on characteristics of bacterial strain and environmental conditions [2]. Various organisms are studied extensively in this regards, such as *Pseudomonas aeruginosa*, *Bacillus subtilis, Staphylococcus aeureus, Escherichia coli,* and other environmental bacteria as reviewed by Lopez et al. [3]. Similarly, biofilm formation under natural setting such as plant and associated soil are now the subject of intense investigation.

One of the major hotspot for biofilm interaction is at the interface between plant roots and soil, rhizosphere and rhizoplane. Rhizosphere microorganisms are known to have significant impact on plant health through various type of interactions with plants, as well as within other soil microflora. Considering the complex and fluctuating conditions in soil and presence of an overwhelming number of micro and microorganisms, it is much more challenging to study biofilm in rhizosphere [4]. Rhizosphere is the soil area around the plant roots where convoluted biological and ecological processes take place, and it forms an environment suited for biofilm formation, including sufficient moisture and inventory of nutrients, which are mainly contributed by the plant. Biofilm formation on plant surface can take place in a response of associative, symbiotic, or pathogenic association (negative interaction).

It is still a mystery how plants regulate the microbial association. The main function of biofilm is to provide a resistant structure against stress factors such as antibiosis, UV radiation, desiccation, and predation [5].

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In the rhizosphere, roots liberate large amount of metabolites from root hairs or fibrous root systems in the form of root exudates. These metabolites act as chemical signals to provide motility to the bacteria toward the root surfaces but also the main nutrient sources to support the growth and endurance in the rhizosphere. Some microbes that occupy plant rhizosphere are bacteria that are adequate to colonize very efficiently on the root surface or the rhizospheric soil [6]. These bacteria are attributed as plant growth–promoting rhizobacteria (PGPR). PGPR fulfill dominant responsibility for plant growth promotion and plant health protection by disparate manners. Direct plant growth promotion may results either from upgraded nutrient acquisition and/or from hormonal stimulation. Disparate mechanisms are involved in the defeat of plant pathogens, which is often incidentally linked with plant growth promotion [7].

In nature, interaction among different types of microorganism may result in multispecies biofilm. Mixed biofilm is the interaction between the different types of microorganisms that critically influence the development and shape of the community. Generally, interspecies interactions involve communication, commonly through quorum sensing, and metabolic cooperation or competition. Some interactions such as antagonistic, synergistic, or competition for the nutrients and growth inhibition take place among different bacterial species within the biofilm. These comprise the encouragement of biofilm formation through co-aggregation and metabolic cooperation where one species utilizes a metabolite produced by a neighboring species. This beneficial cooperation in mixed biofilm has important environmental, clinical, and industrial implications [8]. The bacterial colony in biofilm shows comparatively more resistance against various kinds of stress than the planktonic cells. The biofilm-associated protection is explained by several factors, often operating in concert, including structural changes and reductions in the diffusion rates of compounds in the biofilm matrix, changes in gene expression patterns, and low rates of growth of the biofilm cells [9].

In this chapter, we emphasize the process of biofilm formation briefly and provide an overview of how different factors—both biotic and abiotic—might influence the biofilm and its functions under rhizospheric conditions.

15.2 Process of Biofilm Formation

Biofilm formation process is multistep process involving attachment, maturation of biofilm, and detachment and return to the planktonic growth [10]. These processes of biofilm formation on the surfaces are briefly summarized here.

15.2.1 Attachment

Microbial cell attachment to both abiotic and biotic surfaces is the first interaction that may be reversible or irreversible. It is turning point from planktonic life to the biofilm mode. Initial reversible attachment is mediated by cell structure such as flagella, pili, and fimbriae.

During the attachment stage, the aggregation of rhizobacteria undergoes physiological changes that lead to EPS production and fix the cells to root surface. The cells then divide and form microcolonies by clonal propagation. Bacterial cells following maturation of biofilm consist of reversible and irreversible processes and involve diverse

conserved and/or species-specific factors. Initially, bacterial cells are introduced to a surface, driven by Brownian movement and gravitational forces and simultaneously influenced by surrounding hydrodynamic forces [11]. Within a niche, bacteria encounter attractive or repelling forces that vary, depending on nutrient levels, pH, ionic strength, and temperature. Medium properties, along with bacterial cell-surface composition, affect velocity and direction toward or away from the contact surface [12]. Motile bacteria have a competitive advantage, utilizing flagella to overcome hydrodynamic and repulsive forces. The flagellar motility are important for initial attachment, as has been chronicled for many bacteria [12]. Chemotaxis also plays a role in directing attachment in response to nutrient composition in some bacterial species [13]. Attachment of microbial cells to biotic surfaces involve more cooperative / complex interaction with plant root [14]. It has been demonstrated that exopolysaccharides production is a key factor in determining optimal cell-to-cell and cell-to-surface interaction and biofilm formation by *Pseudomonas putida.* Similarly, rhizobial adhesion protein RapA1 was found to play a specific role in colonization of biotic surfaces [15]. Similarly, the role of root exudates in stimulation of attachment of rhizobia through production of acid EPS and arabinogalactans proteins from both legumes and non-legumes has been evident [16]. The role of secondary messenger is well described, and the concentrations of cAMP and cyclic diGMP are controlled by various environmental factors, such as carbon and oxygen, and thus regulate surface attachment [17].

15.2.2 Maturation of the Biofilm

Biofilm maturation requires two factors: (i) QS signal and (ii) EPS accumulation through continued cell division. Differential gene expression between the two bacterial growth states that is planktonic / sessile is related to adhesive needs of the population during surface colonization. For example, production of surface appendages is inhibited in sessile forms because motility is no longer necessary. Expression of genes involved in production of cell surface proteins and excretion products increases concomitantly. Transport of extracellular products in the cell is facilitated by surface proteins (porins) such as *Opr*C and *Opr*E, whereas transport of excretion products out of the cell is facilitated by certain polysaccharides [18]. Extracellular matrix composition has been more extensively investigated in *P. aeruginosa*, and has been shown to vary, depending on the environmental conditions [19]. As the biofilm matures, eDNA amounts increase through lysis of a bacterial subpopulation in response to the *P. aeruginosa* quinolone signal (Pqs) quorum sensing system [19]. Harmensen et al. [19] demonstrated that eDNA is organized in distinct patterns and localizes in the stalk portion of the mushroom-shaped biofilms. This localization may act as a scaffold for the formation of the mushroom structure, as type IV pili show high eDNA binding affinity, inducing the accumulation of migrating bacteria toward the areas of high eDNA concentration [20].

15.2.3 Detachment and Return to the Planktonic Growth Mode

Within the mature biofilm there is a bustling community that actively exchanges and shares products that play a pivotal role in maintaining biofilm architecture and providing a favorable living environment for the resident bacteria. However, as biofilm matures, dispersal becomes an option. Besides passive dispersal, brought about by shear

stresses, bacteria have evolved ways to perceive environmental changes and gauge whether it is still beneficial to reside within the biofilm or whether it is time to resume a planktonic lifestyle. Biofilm dispersal can be the result of several cues, such as alterations in nutrient availability, oxygen fluctuations, and increase in the toxic products or other stress-inducing conditions [20]. Several sensory systems monitor the levels of small molecules, as a proxy to environmental changes, and alter gene expression accordingly, promoting dispersal [21]. Among other signals, the universal c-di-GMP has been extensively implicated in the shift between sessility and motility in bacteria; typically, an increase in c-di-GMP favors sessility, whereas reduced c-di-GMP leads to upregulation of motility [22]. EPS-degrading enzymes, such as alginate lyase in *P. aeruginosa*, also contribute to bacterial detachment from the matrix [23], although a controlled rhamnolipid production take place that contributes to channel formation within mature *P. aeruginosa* biofilm. An increase in the level of rhamnolipid aids bacterial dispersal [24].

15.3 Factor Influencing Biofilm Formation

Various factors in *in vitro* and in soil are known to influence the growth, survival, root colonization, and activities of microorganisms. Such factors are contributed by microbial cell structure and physiology, synthesis of exopolysaccharides, quorum sensing interference, interaction with other microorganism, influence of plant root and root exudates, as well as physico-chemical characteristics of soil and organic matter in soil [25]. In the rhizosphere, plant roots cope with both pathogenic and beneficial bacterial interactions. The exometabolite production in certain bacterial species regulates root growth and other root–microbe interactions in the rhizosphere [26].

Various microbial products can also influence the process of biofilm formation. The role of cyanide production in pseudomonad virulence affecting plant root growth and other rhizospheric processes has been demonstrated by Rudrappa et al. [27]. They used model plant *Arabidopsis thaliana* Col-0 seedlings and treated with both direct (with KCN) and indirect forms of cyanide from different pseudomonad strains. The treatment causes significant inhibition of primary root growth due to suppression of an auxin responsive gene, specifically at the root tip region by *pseudomonad cyanogenesis*. Additionally, *Pseudomonad cyanogenesis* also affected other beneficial rhizospheric processes such as *Bacillus subtilis* colonization by biofilm formation on *A. thaliana* Col-0 roots. The effect of cyanogenesis on *B. subtilis* biofilm formation was further established by the downregulation of important *B. subtilis* biofilm operons epsA and yqxM. The authors demonstrated the functional significance of pseudomonad cyanogenesis in regulating the multitrophic rhizospheric interactions [27].

An important advantage of the biofilm lifestyle for soil bacteria (rhizobacteria) is the protection against water deprivation (desiccation or osmotic effect) [28]. The composition and functions of bacterial biofilms in soil microniches are poorly understood. In one study, multibacterial communities established as biofilm-like structures in the rhizosphere of *Medicago sativa* (alfalfa) exposed to triple experimental conditions of water limitation. It was observed that the whole biofilm-forming ability (WBFA) for rhizospheric communities exposed to desiccation is higher than that of communities exposed to saline or nonstressful conditions [29].

The effect of various factors on biofilm formation/disruption is briefly summarized in the following subsections and also presented in Table 15.1 and Figure 15.1.

15.3.1 Surfaces

Under a rhizospheric environment, various interacting factors contributed by soil environment, microbial characteristics, and plant surfaces are influencing some of the attachment leading to biofilm formation. For the sake of convenience, we briefly describe the individual factors known to influence microbial adherence on abiotic or biotic surfaces as follows.

Biofilm formation is dependent on the surrounding environmental conditions and substratum parameters. Cell adhesion to a surface is a prerequisite for colonization. Physicochemical parameters are known to affect initial attachment of cell [29]. Once the cells attach, the surface chemistry will influence cell adhesion, while topographic features allow maximum cell-surface binding, enhancing strength of attachment and thus retention. In an aqueous environment (liquid–solid), bacterial attachment to a surface such as material surface, plant surface including root and shoot, animal tissues, and soil occurs rapidly, over a few seconds to a few minutes. Moreover, the binding of microorganisms to a surface can confer advantages to cell survival—for example, the attachment of cells to solid surfaces has been reported to immediately upregulate alginate synthesis in a strain of *Pseudomonas aeruginosa* [30]. The metal surfaces are susceptible for the microbial attack and hence for biofilm formation. The attachment on the metal surfaces crucially depends on growth medium, characteristics of cell surface, and substratum [31]. The microbial cell attachment and thus biofilm formation can occur on metal surfaces also, including aluminium [32], stainless steel [33], and copper as well. However, some metals, such as aluminium or copper, are considered toxic to bacteria [34]. It has been suggested that microbial resistance to some metals (e.g., lead acetate) can be attributed to the high lead content of disinfectants and antiseptics, while resistance to copper sulphate may be due to its use as an algicide [35].

15.3.2 Temperature and Moisture Content

Terrestrial bacterial communities are exposed to various environmental stress factors, of which limited water availability is typically the most critical factor to exhibit the greatest effect on survival and activity of these communities [36]. The availability of water in soils (water potential, ψ) depends on dissolved solutes (osmotic potential) and characteristics of the matrix environment (matric potential; water retention force on the ground). These two potentials represent different types of water deprivation that may affect bacterial physiology in different ways.

Understanding the role of temperature and water stress in protocooperation between the plants and beneficial rhizobacteria may enhance the efficacy of biocontrol agents in reducing plant diseases. The influence of low or high temperature, combined with a normal and reduced water regime on the interaction between *Bacillus amyloliquefaciens* strain S499 and plants, results in the induction of systemic resistance (ISR). A reduction in ISR level was observed when plants were subjected to stress before bacterization; however, root treatment with S499 prior to stress exposure attenuated this negative effect. Further investigation revealed that relative production of surfactin by

Table 15.1 Factors Influencing Rhizobacterial Biofim Formation and Root Colonization in the Rhizosphere.

Name of Bacteria	Phenotype	Relevant Characteristics	Factors Affecting Biofilm	Reference
Pseudomonas chlororaphis	Root colonization of wheat	PGPB, bioremediation	Microbial products (Phenazine)	[72]
Bacillus subtilis	Root colonization of *Arabidopsis thaliana*	Biocontrol	Microbial products (Enzymes)	[73]
Bacillus spp.	Root colonization of wheat	PGPR, biocontrol	Microbial products (EPS)	[74]
Paenibacillus polymyxa	Colonization and biofilm formation on pea nut	PGPR, biocontrol	Microbial products (EPS)	[75]
Bacillus cereus and Bacillus Pumilus	Root colonization of wild barley found in the Evolution Canyon, Israel	Salt, heat, and desiccation tolerance	Salinity, temperature, and desiccation	[76]
Bacillus sp.	Root colonization of wheat	PGPB, salt tolerance	Salinity (>100M)	[82]
Azospirillum brasilense, Sp7	Biofilm formation	PGPB, desiccation and osmotic pressure	Starvation, salinity, and osmotic pressure	[83]
Bacillus amyloliquefaciens SQR9	Colonization on cucumber	PGPR	Root exudates (organic acids)	[84]
Stenotrophomonas rhizophila	Enhanced mixed-species biofilm formation with *Rhizobium, Azotobacter*	Various PGPB activities	Root exudates (organic acids)	[85]
Pseudomonas putida	Root colonization of maize and *Arabidopsis thaliana*	Bioremediation and desiccation tolerance	High temperature (≤45°C)	[80, 13]
Pantoea agglomerans	Root colonization of chickpea and wheat	PGPR and moisture control	High salinity (≥150 M)	[79, 80]
Bacillus amyloliquefaciens strain S499	Biocontrol	Temperature tolerance	High temperature (≤45°C)	[36]
Agrobacterium sp.	Biofilm formation on *alfalfa*	Drought tolerance	High temperature (≥40°C)	[28]
Rhizobium alamii	Root colonization of *Arabidopsis thaliana* and rapeseed	Heavy metal tolerance	Microbial products (exopolysaccharides)	[78]
Staphylococcus saprophyticus	Biofilm on *Lens esculenta*	Salt tolerance	Salinity (≥200 M)	[40]
Bacillus amyloliquefaciens NJN-6	Colonization on banana root	PGPR, biocontrol	Root exudates (organic acids)	[77]

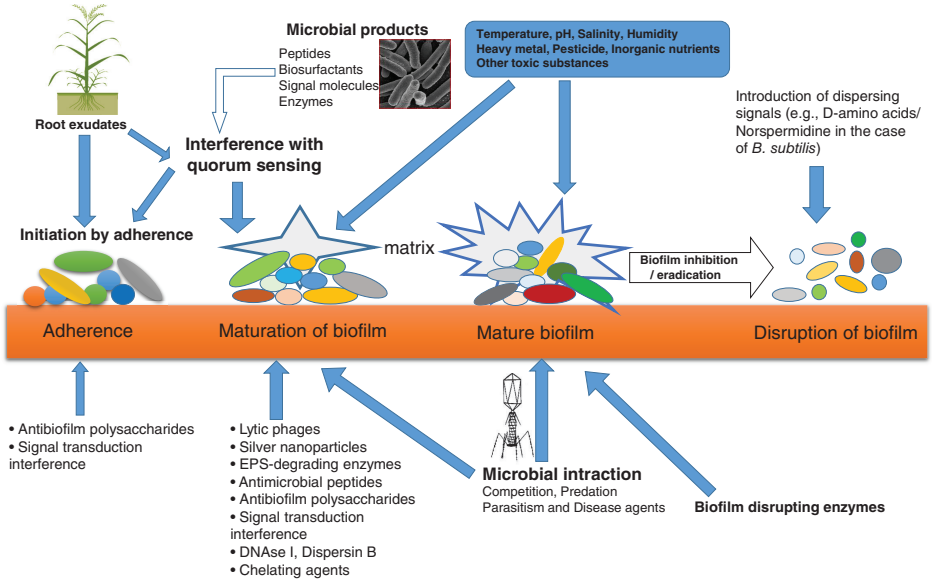

Figure 15.1 Effect of various factors on biofilm formation/disruption *in vitro* and in the rhizosphere.

S499 was clearly enhanced at low temperatures, making it possible to counter-balance the negative effect on the traits associated with rhizosphere fitness (colonization, motility, and biofilm formation) observed *in vitro* in cold conditions [37]. In anaerobic bacteria like *clostridium perfringens*, biofilm formation is drastically affected by temperature. The morphology, thickness, and cell density reflect the temperature-dependent regulation of EPS production [38].

15.3.3 Salinity

One-third of the world's arable land resources are affected by salinity [39]. Salt tolerance in plants depends mainly on the capability of roots for (i) restricted or controlled uptake of Na^+ and Cl^-, and (ii) continued uptake of essential elements, particularly K+ and NO3-. Consequently, the preferential uptake of K^+ over Na^+ has generally been considered as an important trait contributing to salt tolerance in various halophytes and nonhalophytes. Considering the potential of bacterial exopolysaccharides (EPSs) to bind cations including Na^+ [40], it may be envisaged that increasing the population density of EPS-producing bacteria in the root zone would decrease the content of Na^+ available for plant uptake, and thus help alleviating salt stress in plants growing in saline environments.

Establishment of biofilm, production of exopolysacharides (EPS), and accumulation of endogenous osmolytes under varying stress conditions are significant strategies adopted by bacterial strains for their successful survival in plant rhizosphere [41]. A study was conducted on determining the osmoadaptation strategies used by two native salt-tolerant strains *Oceanobacillus profundus* (Pmt2) and *Staphylococcus saprophyticus* (ST1) and their plant growth–promoting abilities. The ability of these strains to be used as inoculants for *Lens esculenta Var.* masoor 93 under salt stress was tested in the laboratory. Unlike the bacterial growth, biofilm formation, exopolysaccharide production, and endogenous osmolytes (proline, glycine, and betaine) accumulation increased at higher salt stress. Biofilm formation and endogenous osmolytes accumulation increased with increasing salt concentrations. The maximum increase in EPS accumulation was observed at maximum NaCl stress for ST1. Bacterial inoculation improved growth parameters and endogenous osmolytes accumulation of plants under salt stress compared to noninoculated control plants. The ST1 strain efficiently produced biofilm and exopolysacharide and accumulated osmolytes in response to NaCl stress. It is suggested that these strategies reverse the detrimental effects of high osmolarity in soil and are helpful for improving crop under salt stress [42].

15.3.4 Nutrient Availability

Nutrient availability is one of the major factor influencing growth and activities of microorganisms in soil and other habitats. Under rhizosphere conditions, nutrient availability is more compared to bulk soil due to nutrients release in root exudates by plants. However, there is tough competition between various microbial communities of soil. The effect of various nutrients and their influence on biofilm formation under natural conditions has been poorly exposed [43]. Biofilm bacteria acquire nutrients by concentrating trace organics on surfaces by the extracellular polymers, using the waste products from their neighbors and secondary colonizers, and by using different enzymes to break down food supplies. Biofilm matrix is often negatively charged; many nutrients

(particulary cations) are attracted to the biofilm surface. Besides, nutrients with negative charge can exchange with ions on the surface. This provides bacterial cells within the biofilm with plenty of food compared to the surrounding [44]. Various factors, including carbon source, amount of nitrate, phosphate, calcium, and magnesium as well as the effects of osmolarity and pH, have been investigated on biofilm on *Sinorhizobium meliloti in vitro.* Nutrients such as sucrose, phosphate, and calcium enhance biofilm formation as their concentrations increase, whereas extreme temperatures and pH negatively affect biofilm formation of rhizobia. Similarly, in case of *B. subtilis,* growth was not limited in any of the conditions that did not result in pellicle formation. Similarly, galactose, arabinose, fucose, xylose, and glucuronic acid added at a 0.5 percent concentration and did not induce pellicle formation, which further demonstrated that the effect of plant polysaccharides as an environmental cue inducing biofilm formation is not due to increased growth attributed to the presence of additional sugars [45].

15.3.5 Microbial Products

Various exometabolites of microorganisms are known to influence biofilm formation. Some of the well-known examples are briefly mentioned here.

15.3.5.1 QS Signal Molecules in Biofilm Formation

Quorum sensing plays an important role in the communication between neighboring bacterial cells via signal molecules. It is a social behavior that enables interactions within the mono and mixed bacterial communities. Quorum sensing depends on the release and production of signal molecules, called *autoinducers,* and it increases in concentration of cell density while physiological conditions may also play significant role [46]. The quorum sensing system assists bacteria to express specific genes in a hormonize fashion [47]. Quorum sensing plays an important role in the development of biofilms in terms of induction vs. repression of biofilm formation, which varies depending on the bacterial species and environmental conditions [48]. In Gram-positive bacteria, the autoinducers are often peptides.

Production of several extracellular proteases involved in dispersal of biofilm is regulated by Agr QS system in *S. aureus* [49]. Similarly, in *B. subtilis* the production and secretion of QS molecule and surfactin is important for biofilm formation [50]. Specifically, the role of QS signals that is acylhomoserinelactone (AHL) produced by Gram-negative bacteria and their role in the induction of biofilm have been demonstrated through regulation of eDNA and production of PEL polysaccharides [51]. In QS the AI-2 system is studied globally and can mediate interspecies communication [52]. This system was identified in several Gram-negative and Gram-positive bacterial species. Many bacterial species are found in intimate association with one another in natural environmental condition [53].

In addition to QS signal molecules, other microbial products act as non-QS signal molecules that influence biofilm formation. Microbial products of this group include secondary metabolites like antibiotics, pigments, and siderophores.

Antibiotics are naturally produced by soil microorganisms and have a regulatory role in soil bacterial population. Major producers of such compounds include actinomycetes, fungi, and bacteria such as *Bacillus, Pseudomonas* [54]. *In vitro* experiment biofilm modulation as well QS interference have been documented concerning

various antibiotics at sub-MICs of Imipenem, aminoglycoside, tobramycin induced biofilm in *Pseudomonas aeuroginosa,* and *E. coli*. Many QS inhibitory molecules such as furanoses may favor biofilm formation in *S. aeureus*. However, antibiotics such as Doxycyclin and Azithromycin at sub-MIC inhibit QS as well as biofilm formation at sub-MIC [55].

Within the biofilms, the phenazine pyocyanin functions in extracellular electron transfer to generate energy for growth. Small amounts of diffusible molecules shuttling electrons in a biofilm where the diffusion solubility may be limited is beneficial for the community [56]. Phenazines in *P. aeruginosa* also function as signaling molecules in biofilm formation, as a mutant unable to produce phenazines produced dramatically more wrinkled colony morphology than a wild-type strain [57].

15.3.5.2 Antimicrobial Peptides

Soil rhizosphere contains several habitats with functional microbial communities, where some microbial communities defend themselves from others by producing antimicrobial metabolites. These antimicrobial compounds produced by bacteria are found in all major bacterial lineages [58] and are produced by both Gram-negative and Gram-positive bacteria [59]. The antimicrobial peptides are the cystine-rich low-molecular-weight compounds, also called *host defense peptides*. The lytic peptides are assessed for their effects on biofilm formation. Lytic peptides bind the LPS (lipopolysaccharide) moieties of the bacterial cell membrane and disrupting membrane stability. Studies on *Staphylococcus aureus* have shown that the lytic peptide PTP-7 prevented *in vitro* biofilm formation and was also capable of diffusing into the deep layer of preformed biofilm that results killing of 99.9 percent of biofilm-forming bacteria. This peptide retained activity under highly acidic environments and in the presence of excess of metals, conditions that mimic the *S. aureus* biofilm environment [60].

15.3.5.3 Exopolysaccarides

The synthesis of exopolysaccharides by bacteria is well known and researchers have documented the relationship between exopolysaccharides production and biofilm formation [61]. Exopolysaccharides mediate cell-to-surface and cell-to-cell interactions that are critical for biofilm formation and stabilization. Mutants that are typically deficient in adherence and biofilm formation could not synthesize or export such polysaccharides and thus are highly sensitive to killing by antibiotics and host immune defenses [62]. However, recent evidences indicate that some bacterial exopolysaccharides inhibit or destabilize biofilm formation by other species [63]. Polysaccharides with nonbiocidal antibiofilm properties have also been isolated from cell-free biofilm extracts of several species. Their antibiofilm properties are believed to depend on their ability to (i) alter the physical characteristics of bacterial cells or abiotic surfaces; (ii) act as signaling molecules that impact the gene expression patterns of susceptible bacteria; or (iii) competitively inhibit multivalent carbohydrate–protein interactions, thereby interfering with adhesion [64]. Polymers are basically high molecular mass compounds formed by joining together a large number of repeating units of simple molecules, called monomers. On the basis of their origin, they may be natural polymers or synthetic polymers. Synthetic polymers may contain many additive chemicals, such as antioxidants, light stabilizers, lubricants, pigments, and plasticizers, added to improve the desired physical and chemical properties of the material [65]. However, these additives may leach into

the surrounding environment and provide nutrients for microorganisms present. Phosphorus has been shown to increase the formation of biofilms on polyvinyl chloride in phosphorus-limited water [66]. Several studies have shown that plastic materials can support the growth of biofilms, but it has been suggested that growth in plastic pipes is usually comparable with that on iron, steel, or cement [67]. However, Bachmann et al. [68] used *Aquabacterium commune* cells under continuous cultivation with stainless steel and medium density polyethylene (MDPE) surfaces and found that biofilm cell density on MDPE slides was four times greater than on stainless steel.

15.3.6 Soil Enzymes

In the soil ecosystem, there are several extracellular enzymes mainly contributed by microorganism as well as plant roots. These enzymes are also abundant in aquatic and terrestrial ecosystems and may be present in significant amounts in soil and water biofilm. Complexes between enzymes and humic matter from soil have been reported to be extremely resistant to thermal denaturation, dehydration, and proteolysis [69]. N-acetyl-D-glucosamine-1-phosphate acetyltransferase (GlmU), which is involved in the biosynthesis of activated UDP-GlcNAc, an essential peptidoglycan and lipopolysaccharide (LPS) precursor in Gram-positive and Gram-negative bacteria, respectively, is among the enzymes targeted for matrix disruption. The effects of GlmU inhibitors, including N-ethyl maleimide (NEM), and its analogs showed antibiofilm activity against *E. coli, P. aeruginosa, K. pneumoniae,* and *E. faecalis* [70]. The enzymes DNase-I and Dispersin-B have also recently gained attention as potential antibiofilm agents, particularly against Gram-positive bacteria. The effects of DNase-I are linked with its ability to digest the eDNA found within the biofilm structure [71].

15.4 Conclusions and Future Direction

Biofilm formation by bacteria on various surfaces, both living and nonliving, is a common phenomenon, provided that conditions are suitable. The majority of bacteria are known to form biofilm under natural conditions / habitats. The ability of bacteria to form biofilm largely depends on the characteristics of bacterial strains and environmental factors. Many bacteria of soil and environments such as *Bacillus subtilis* and *Pseudomonas spp.* have been widely studied as model organisms for biofilm studies. Typical characteristics of bacteria responsible for biofilm formation includes presence of specific adhesins, ability to express various gene products such as EPS, quorum sensing, and regulation and switching ability from planktonic to sessile and vice versa. However other major factors include environmental factors such as temperature, moisture, host surface chemistry, nutrient availability, as well as interfering agents, both of biotic and abiotic nature. Under soil–plant systems, these factors are more complex, and various interacting factors affect biofilm. Mixed biofilm formed under natural conditions is least understood. Further new insight on the molecular basis of gene expression under suitable biofilm model is needed to study the impact of fluctuating environmental conditions on biofilm. Further contribution of plant genotype and its role in recruiting root microbes specifically in biofilm state have to be explored to understand the mechanism recruitment of microorganisms by plant.

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