American Journal of
Biomedical Science & Research

www.biomedgrid.com
ISSN: 2642-1747

Review Article

Factors Influencing Bacterial Biofilm Formation and Development

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To Cite This Article: *Ghazay F Alotaibi, Mamdouh A Bukhari. Factors Influencing Bacterial Biofilm Formation and Development. Am J Biomed Sci & Res. 2021 - 12(6). AJBSR.MS.ID.001820.* DOI: 10.34297/AJBSR.2021.12.001820.

Received: September 30, 2020; **Published:** May 21, 2021

Abstract

Bacterial communities attached to surfaces and established a protected mode of growth within extracellular matrix substances are defined as a bacterial biofilm. Formation of the biofilm occurs naturally as a result of balancing between a variety of chemical, physical and biological processes. Biofilms form on different surfaces, such as inert materials and living tissues or cells after the surface bacterial adhesion followed by the growth and production of extracellular polymeric substances (EPS). In this article, biofilm formation from the initial attachment of bacterial cells to the substratum, physiological changes within the microbe, multiplication of adhered cells to form microcolonies, and finally biofilm maturation is reviewed. The review article will also highlight factors involved in biofilm formation. Hopefully, this article will serve as a supportive document for further research into the molecular nature of biofilms.

Keywords: Biofilm Formation, Hydrodynamic Conditions, Adhesion, Extracellular Polymeric Substances.

Introduction

Bacterial biofilms are aggregations of cells attached to surfaces and surrounded by a matrix of extracellular polymeric substances (EPS) [1-4]. On Earth, over 99% of bacteria are thought to live in structured biofilm communities [5,6]. The mostly self-produced extracellular polymeric matrix that encases the microorganisms promotes survival in hostile environments, including tolerance to antibiotics and provides structure to the biofilm [7]. Biofilms can exist in both natural and anthropogenic environments [8]. Biofilms may also form on a wide variety of surfaces, including inert or living materials, such as tissues or cells [9,10].

Establishment and development of bacterial biofilms are known to be dynamic and complex processes regulated by intrinsic biological properties and also by many environmental factors, since changes in the environment often trigger the formation of biofilm [11-13]. Biofilm-associated bacteria demonstrate distinct features from their free-living planktonic counterparts.

i. Intercellular signalling systems, such as QS, in which cells produce signalling molecules that regulate the development of the biofilm.

ii. Cyclic nucleotide second messengers, such as the bacterial second messenger c-di-GMP, which regulates biofilm formation and dispersal by controlling flagellar motility, attachment and extracellular polysaccharide production.

iii. Biofilm-associated proteins, which form a scaffold and builds the biofilm matrix [14].

The composition of biofilms depends on environmental factors, such as temperature, pH and nutrient availability [14-17]. Although the composition may not be identical, the major components are typically water, bacterial cells and their secreted EPS [18,19]. Biofilms are complex highly hydrated structures since they incorporate large amounts of water by hydrogen bonding [9,20,21]. Hence, within the biofilm matrix, water is the primary component up to 97% of the mass and a biofilm is considered as an absorbent and porous structure that has water channels and pores

[22,23]. The water channels allow the distribution of nutrients, oxygen and even microorganisms through fluid circulation [24,25]. Extracellular polymeric substances constitute about 1-2% while microbial cells account for about 2-5% of the biofilm matrix mass [26].

Biofilm Formation Process

Biofilm formation is the net result of several physical, chemical and biological processes [5,27]. Biofilm formation and development can be viewed as a multi-step process [28,29]. These steps include an initial reversible attachment of planktonic microorganisms to a pre-conditioned surface, a transition from reversible to irreversible attachment during the formation of biofilm through the production of EPS [10]. The development of microcolonies into a mature biofilm then occurs followed by cells dispersion from the biofilm into the surrounding environment (Figure 1).

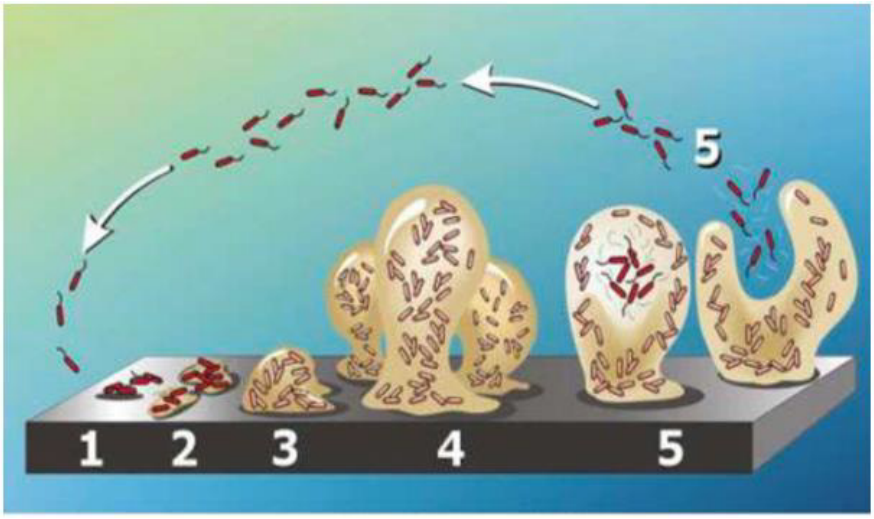

Figure 1: The five stages of biofilm development. Stage 1: Free-floating (planktonic) bacteria adhere to the surface. Stage 2: Bacterial cells aggregate form microcolonies, secrete extracellular polymeric substances (EPS) and the attachment becomes irreversible. Stage 3: A biofilm is formed and matures, and cells form multi-layered clusters. Stage 4: The growth of three-dimensional and further maturation of the biofilm, providing protection against the external environment. Stage 5: The biofilm reaches a critical mass, and planktonic bacteria disperse to colonize other surfaces. Image from [30].

Initial Reversible Attachment

Biofilm formation begins with the adhesion of single cells to material surfaces that are exposed to an aqueous medium and formation of a conditioning layer [21]. Adhesion is known to be a critical step in biofilm formation; once the bacteria attach to the surface, the chances of further transport of other free-floating microbes increases resulting in coaggregation and the creation of multiple layers [31]. The conditioning layer is an organic monolayer which forms on surfaces and acts as a docking place for the first reversibly attached cells; however, the strength of the biofilm is dependent upon the cohesiveness of the conditioning film [5,31]. This layer can be formed within minutes of exposure to an aqueous medium (e.g., water) and then proceed to grow for several hours [21].

The conditioning film plays a significant role in cellular or microbial adhesion and can be dependent on the concentration of organic molecules in the medium that is in contact with the surface [5]. Surface physical properties, such as charge and roughness play a role in the adhesion of organic molecules [5,32,33]. Increased surface roughness has been found to efficiently promote initial biofilm development and microbial colonization due to the larger surface area [21,34]. After the establishment of the conditioning layer, planktonic microorganisms in the aqueous medium attach themselves to this layer [25,35]. The attachment depends on the motility of microbes or the moving of the planktonic (free-floating) cells through gravity, diffusion or the forces of the fluid dynamic forces from the surrounding liquid phase [35-37].

Thus, the existence of external appendages on the bacteria, such as flagella, pili and fimbriae can overcome the repulsive physical forces and reach the bulk lattice of the conditioning layer stimulating chemical reactions and consolidating the bacteria–surface bond [5,38]. Adhesion can also be affected by the availability of nutrients in the surrounding medium [35]. After the initial interaction has developed between the bacterial cells and the substratum, many interaction forces can influence this reversible adhesion, such as van der Waals attraction forces, electrostatic forces and hydrophobic interactions, and fluid shear forces can often easily remove the bacterial cells at this stage [35]. Electrostatic forces tend to favor repulsion since many bacteria and inert surfaces are negatively charged [9].

Irreversible Attachment

In this stage, microorganisms are irreversibly attached to the surface and synthesize EPS [35,39]. Secretion of EPS by bacteria reaches a certain level, forming a strong interaction between the microbe and the surface [14]. During this stage, planktonic microorganisms can stick to each other or different species of surface-bound organisms, forming aggregates on the substratum and the adhesion becomes irreversible in the absence of physical or chemical intervention, thus the bacterial cells become attached firmly to the surface [9].

Biofilm Architecture Development

After an initial lag phase, a rapid increase (the exponential growth phase) in population can be observed which depends on the nature of the environment, both physically and chemically [40]. The irreversibly attached cells start growing and dividing using the nutrients in the conditioning film and in the surrounding fluid to form microcolonies and produce the further polymer (EPS) which helps anchor the cells to the surface and stabilize the colony from environmental fluctuations [35]. In this stage, both physical and chemical processes contribute to the initial adhesion ends, biological processes start to dominate, production of polysaccharide intercellular adhesion (PIA) polymers and the presence of divalent cations interact to form stabler bonding [40]. Besides, surface appendages production is inhibited in sessile cells since motility is restricted and no longer necessary; however, the expression of genes that are responsible for cell surface proteins production and secreted products increases concurrently [5].

Maturation

During this stage, the attached small colonies grow into a mature biofilm, with the characteristic three-dimensional biofilm structure, through reproduction and by accumulating debris and new planktonic bacteria from the surrounding environment [14,41]. In this stage, cell to cell and cell to substratum attachment depends on the EPS [14]. At high cell density, cell signalling mechanisms that use a range of different signal types known as quorum sensing are used by the biofilm; however, QS is important for biofilm maturation processes since bacteria monitor cell density and regulate collective behaviour [5,14,42].

Although quorum sensing is typically thought to mediate intraspecies communication, there is evidence that interspecies interaction also occurs [43]. Gram-positive and gram-negative bacteria use different types of QS systems that involve the production, detection, and response to extracellular signalling molecules called autoinducers, including specific peptides for gram-positive bacteria and acylated homoserine lactones for gram-negative bacteria (Figure 2) [44]. The population of bacteria regulate their gene expression by producing and responding to these autoinducers [42]. Once mature, the biofilm has three layers: a joining film binding the biofilm to the surface; a base film composed of a dense layer of bacteria; and a surface film from which free-floating bacteria can arise and spread [45].

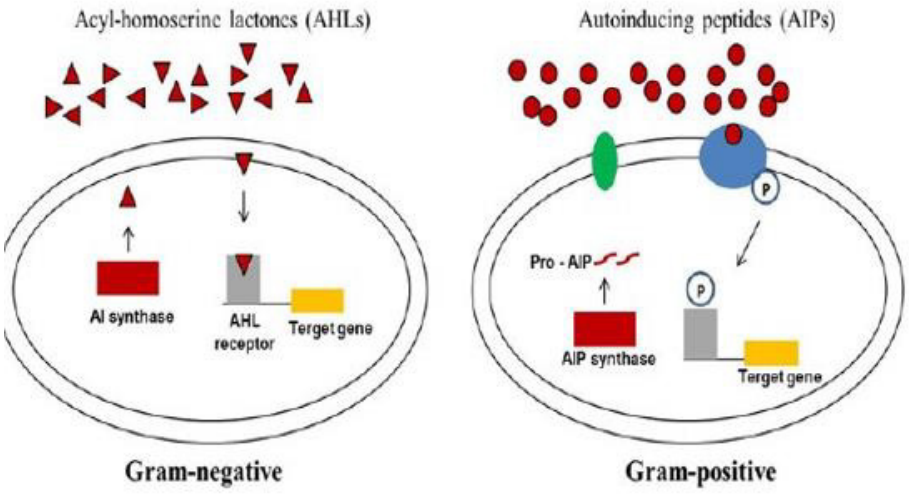

Figure 2: Quorum sensing systems in bacteria.
Gram-negative bacteria produce acylated homoserine lactones (AHLs) that upon reaching threshold concentrations enter the cells and activate the cognate AHL receptor and induce the expression of QS-regulated genes. Gram-positive bacteria secrete mature autoinducing peptides (AIPs) that interact with a transmembrane histidine kinase receptor activating target gene expression through autophosphorylation of the cognate transcriptional regulator. Image from [46].

Dispersal

Dispersal is the final stage of the biofilm process where attached cells detach and disperse to colonize a new niche [47]. Biofilm cells can be dispersed either by shedding of daughter cells from actively growing cells or detachment can arise due to various factors, such as nutrient limitation, fluid dynamics and shear effects of the bulk fluid, secretory proteins and catabolite repression [14,47]. The detachment stage consists of sloughing, erosion and abrasion [21]. Erosion refers to the continuous removal of single cells or small biofilm fragments [48]. Sloughing is the loss of large particles of biofilm biomass (Figure 3) [48]. This loss is due to nutrient and dissolved oxygen depletion at the base of the biofilm or to a sudden increase in nutrient concentration in the bulk liquid [21].

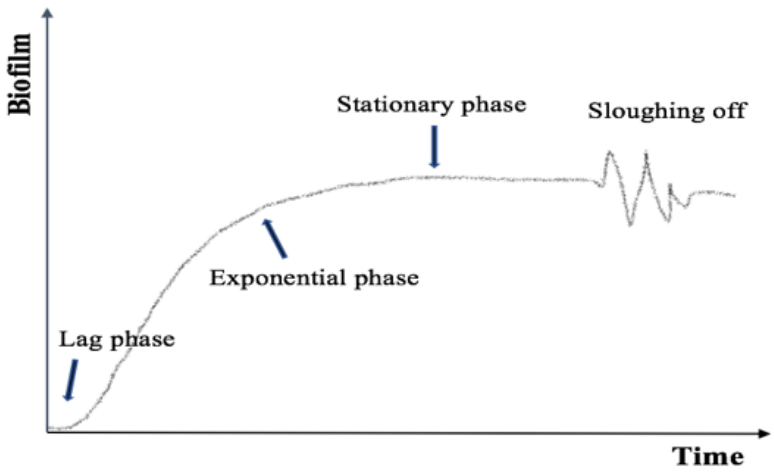

Figure 3: Biofilm accumulation through time.

After an initial lag phase, the growth of bacterial population increased rapidly, otherwise described as the exponential growth phase. The biofilm tends to reach a maximum thickness that may vary from a few microns to some millimeters. In the stationary phase, the rate of bacterial cell growth is equal to the rate of bacterial cell death. Shear stress is actively releasing surface bacteria for colonization of new substrates. Figure based on [49].

Abrasion is a loss of biofilm by suspended particles [48]. Any released cells may be transported to new locations and then restart the process of biofilm formation [47]. Detachment from biofilms is thought to be a key reason for the spread of pathogens [14].

Factors Involved in Biofilm Formation

The formation of biofilm is a dynamic and complex process which includes initial attachment of bacterial cells to the substratum, physiological changes within the microbe, multiplication of adhered cells to form microcolonies and finally biofilm maturation [50]. Biofilm-associated bacteria demonstrate distinct features from their free-living planktonic counterparts, such as different physiology and high resistance to immune system and antibiotics that render biofilm a source of chronic and persistent infections [51]. It is known that the change of phenotype from planktonic to the sessile form occurs in response to changes in environmental conditions [52]. These environmental factors, such as nutrient level, temperature, pH, ionic strength can influence biofilm formation [53].

Various factors can influence bacterial adhesion; cell surface properties, such as hydrophobicity, flagellation, and motility, surface properties, such as hydrophobicity and roughness and environmental factors, such as temperature, pH, availability of nutrients and hydrodynamic conditions (Table 1) [54]. Cell surface properties, specifically the presence of extracellular appendages, such as fimbriae, flagella, the interactions involved in cell-to-cell communication and EPS production, such as surface-associated polysaccharides or proteins possibly provide a competitive advantage for one organism in a mixed microbial community [21,55].

Table 1: Important variables in bacterial cell attachment and biofilm formation.

Properties of The Substratum	Properties of The Bulk Fluid	Properties of The Cell
Hydrophobicity	Temperature	Cell surface hydrophobicity
Conditioning film	pH	Extracellular appendages, such as fimbriae and flagella
Texture or roughness	Flow velocity and nutrient availability	Extracellular polymeric substances

Information based on [21].

Bacteria with hydrophobic properties are more likely to attach to surfaces than hydrophilic bacteria; however, the attachment of biofilm will occur readily on surfaces which are rough, hydrophobic, and coated by surface conditioning films [17,21]. The physicochemical properties of the substratum, such as texture (rough or smooth), hydrophobicity and charge can also be modified by environmental conditions, such as pH, temperature, and nutrient levels [17]. In aquatic environments, the rate of microbial attachment can be increased with increasing the velocity of the flow, water temperature or nutrient concentration, providing these factors do not exceed critical levels [56].

Cyclic-di-GMP

The bacterial second messenger c-di-GMP plays a central role in the formation of biofilm [57]. The molecule was originally identified as an allosteric activator of cellulose synthesis in *Gluconacetobacter xylinus* [58]. Since then, it has emerged as an important molecule that controls the switch from a motile, planktonic lifestyle to a sessile, biofilm-associated existence [59]. Its role in regulating the transition from a motile to a settled state has been observed in several bacteria, including but not limited to *P. aeruginosa, Salmonella enterica* and *Vibrio cholerae* [43]. Many bacteria can produce c-di-GMP, which has been shown to regulate a wide range of functions, including bacterial adhesion and biofilm formation, EPS production, bacterial motility and control of virulence [60,61]. Cyclic-di-GMP was also found to affect a wide array of other fundamental bacterial behaviors, such as cell cycle proliferation, development, fimbrial synthesis, type III secretion, RNA modulation and stress response [43].

The intracellular levels of c-di-GMP are regulated by the antagonistic activity of diguanylate cyclases (DGCs) and *phosphodiesterases* (PDEs) enzymes that catalyze the synthesis and hydrolysis of this molecule [62]. Cyclic di-GMP is synthesized from two molecules of GTP by DGCs and degraded into 5′-phosphoguanylyl-(3′-5′)-guanosine (pGpG) or to two molecules of GMP by PDEs [63]. Diguanylate cyclases share a conserved domain containing the amino acid motif GGDEF, whereas PDEs include one of two conserved domain families: one defined by the EAL motif and the other by an HD-GYP motif [64]. High levels of c-di-GMP are found to be associated with the adhesion to surfaces, production of EPS, and formation of bacterial biofilm or a sessile lifestyle, whereas low levels can increase bacterial motility, promote biofilm disassembly and lead to the activation of virulence pathways [62,63,65].

GGDEF and EAL domains may be found individually or together as hybrid proteins that harbor both domains; however, hybrid proteins usually have either PDE or DGC activity only, although in some cases both functions can be present [62]. Proteins containing GGDEF and EAL domains or HD-GYPs are generally modular in nature, with the enzymatic domain associated with various amino-terminal sensory domains; these sensory domains respond to environmental or host-derived signals to regulate the downstream enzymatic activity [43].

In the biofilm formation process, the intracellular levels of c-di-GMP play a role in the regulation of multiple stages [57]. For example, in *P. aeruginosa*, reversible attachment, the first step of biofilm development, is regulated by flagellar movement which is regulated by FleQ, a transcriptional regulator of flagellar gene expression; however, binding of c-di-GMP to FleQ results in a conformational change which reduces the bacterial swimming motility [63]. The production of extracellular polysaccharides, such as Pel and Psl in *P. aeruginosa* and other biofilm matrix biopolymers during biofilm maturation were also found to be regulated by c-di-GMP [57]. Cyclic di-GMP can also be involved in controlling biofilm dispersion [63]. For example, exposure to nitric oxide (NO)-releasing compounds in *P. aeruginosa* biofilms can lead to dispersal since the exposure increases PDE activity, resulting in decreased c-di-GMP levels in the exposed biofilms [57].

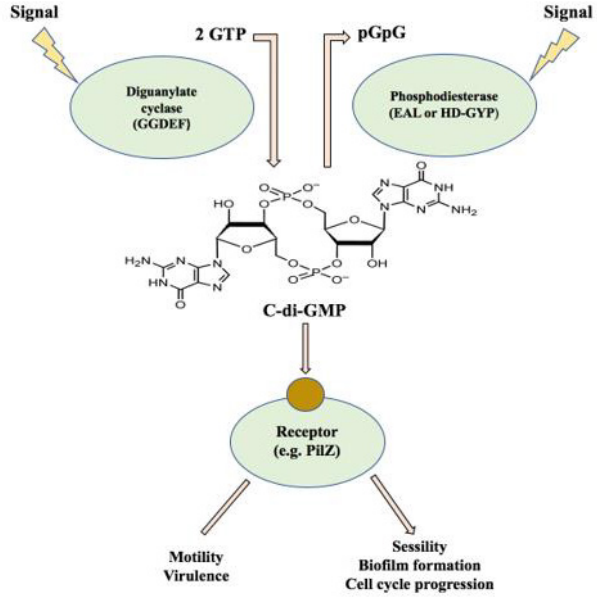

Figure 4: The c-di-GMP signalling module.

Intracellular c-di-GMP is generated from two GTP molecules by diguanylate cyclases, containing GGDEF motifs, and is degraded to linear diguanylate by phosphodiesterases containing either EAL or HD-GYP domains. Cyclic di-GMP is afterwards bound by a variety of effectors, including PilZ domain- containing proteins, and act on targets affecting motility, virulence, and biofilm formation. Figure based on [107].

Cyclic di-GMP signalling consists of enzymes needed for the synthesis and degradation of this molecule, effector proteins associated with binding c-di-GMP, and target elements that are eventually the output of the control module (Figure 4). Thus, DGCs and PDEs sense internal or external signals and translate them

into c-di-GMP levels, which then regulate the function of c-di-GMP binding molecules, resulting in a change of the physiology and behaviour of the cell [64].

Hydrodynamic Conditions

Biofilms in different environments are exposed to a variety of hydrodynamic conditions that can have an effect on biofilm matrix [66]. These conditions affect biofilm formation by changing nutrient and oxygen supply and by applying shear forces, which can affect the cells' attachment to surfaces [12]. The transport rate of the bacterial cells, nutrients and oxygen from the bulk fluid to the biofilm were also found to be determined by fluid hydrodynamics [67]. Besides, these conditions can influence the physical properties of biofilm, such as the density and strength, which in turn might affect the diffusion of nutrients and signals within the biofilm [68].

There is considerable evidence that shear stress has effects on the growth rate, biofilm structure, EPS production, mass transfer and metabolic/genetic behaviour of biofilm [54,69,70]. For example, analysis of microbial populations that developed under a given wall shear force has shown that shear stress slows down *P. putida* biofilm maturation [71]. Quorum-sensing in *P. aeruginosa* biofilm was also found to be influenced by hydrodynamic conditions [72]. In *P. aeruginosa* biofilm, high shear stress caused by high flow velocity was found to induce cell detachment [73].

Detachment usually occurs when external shear forces are higher than the inner strength of the matrix that joins the biofilm together [74]. Two mechanisms can lead to cell detachment from the biofilm, either increase of the external shear forces or decrease of the internal strength (e.g., through hydrolysis of the polymeric biofilm matrix) [75]. High flow rate is known to cause two phenomena of opposite nature: it supports the transport of nutrients to the surface, contributing to the growth of the cell in the microbial layer and exopolymers production; on the other hand, with increasing flow velocity the shear rates also rise which cause additional erosion and detachment of biofilm parts, and then decrease in the amount of biomass attached to the solid support [12]. However, the detachment of the biofilm under hydrodynamic forces leads to viable biomass reduction, which also decreases EPS secretion; thus, higher shear stresses can result in denser and thinner biofilm [76,77]. Stronger adhesion and lower detachment rates have been observed for cells that are grown under high shear conditions [78].

Environmental Conditions

In aquatic habitats, such as a river, the structure and function of biofilms can be affected by various environmental factors that control these ecosystems; physical (light penetration, temperature and water current), chemical (pH, nutrient availability and toxicant effects), as well as biological factors, including community composition (bacteria, algae and fungi), relative contribution of autotrophs and heterotrophs biomass thickness and grazing [79,80]. Environmental conditions can influence both bacterial properties (mediated by changes in gene regulation and/or cell surface physicochemical properties) and surface properties (mainly through physicochemical changes) [17]. They can also control the concentration of the second messenger c-di-GMP which regulates biofilm-related factors, such as cell appendages, surface proteins, EPS, and cell motility [61].

The environmental pH can have a major effect on the formation of biofilms [53]. Microbial adhesion to surfaces, which is the first step in biofilm formation, has been shown to be influenced by this factor [54,81]. In bacteria, such as *Staphylococcus epidermidis*, pH is considered an important determinant in the primary adhesion to surfaces [82]. Besides, the production of bacterial biofilm slime has been shown to be dependent on the pH of the medium, which can affect the activity of enzymes, since each enzyme has an optimal pH [83]. For example, *S. aureus* biofilm formation was lower at highly acidic (pH 3) and alkaline (pH 12) pH compared to pH 7 [84].

It is known that the optimum pH for polysaccharide secretion depends on the individual species; however, it is around pH 7 for most bacteria [85]. Exopolysaccharide production plays a role in biofilm protection against environmental stress factors, such as pH [18]. Thus, bacterial cells within the biofilm can withstand pH changes compared to the free-floating cells [86]. For example, under highly acidic conditions, the bacterial biofilm's gel-like structure can help in reducing the rapid diffusion of ions and allows for the development of a pH gradient within the extracellular matrix [108]. However, under alkaline conditions, poorly structured and very thin biofilm, as a result of impairment of biofilm maturation, have also been observed, along with adhesion inhibition for some bacteria, such as *S. aureus* and *S. epidermidis* [82].

The formation of bacterial biofilm can also be affected by temperature [53,87]. The optimal temperature for bacterial growth is associated with a raise in the intake of the nutrient [88]. It is known that nutrient metabolism depends on the presence and reaction rates of enzymes that regulate the development of various physiological and biochemical systems in bacteria and as a result, the optimum temperature enhances bacterial growth, resulting in a rapid formation of biofilm [85,88]. In contrast, when the temperature is removed from the optimum, bacterial growth can be decreased, due to a decline in reaction rates, and as a consequence, the biofilm development might be affected [5]. In addition to enzymes, environmental temperature can affect the physical properties of the compounds within and surrounding the cells [5].

For bacteria, such as *S. aureus*, increasing the growth temperature from 20 to 37°C was found to increase their hydrophobicity and adhesion to surfaces was enhanced [89]. Also,

the presence of bacterial cell surface appendages, such as flagella, pili and fimbriae that help the bacteria to adhere to surfaces has been shown to be dependent on temperature [5]. For example, a decrease in temperature reduces the adhesive properties of an aquatic *Pseudomonad* due to a reduction in bacterial surface polymers [5].

Bacterial EPS properties, such as the viscosity of the polysaccharides can also be influenced by temperature [17]. It was found that the increase in EPS temperature creates a gel-like substance which gradually increases in strength until a critical point after which the gel forms a solution [17]. Thus, lower temperatures can lead to more uniform properties of the polysaccharides that often increase the possibility of bacterial biofilm adhesion [5]. On the other hand, in some microbes, high temperatures were found to increase the adherent nature of the biofilm to the surface [90].

Bacterial attachment to submerged surfaces and subsequent biofilm development can be dependent on oxygen accessibility [91]. Oxygen availability can also determine bacterial energy production with a possible influence on biofilm formation; for example, bacterial biofilm metabolic activity can be decreased as a result of poor supply of oxygen reduction [17]. Thus, oxygen supply is considered a key environmental factor which can have an impact on biofilm composition and development [92,93]. Lower oxygen availability usually triggers active dispersal, which is critical for the biofilm life cycle [61]. For example, at the base of a biofilm, bacterial cells were found to receive limited oxygen compared to those at the surface enhancing detachment from the deeper layers of a biofilm [35]. Also, sloughing was observed within biofilm grown under oxygen limitation [94]. In some microbes, such as *E. coli*, the presence of oxygen is required for the formation of biofilm, since lack of oxygen can be a detachment signal [95]. For other bacteria, such as *P. aeruginosa*, biofilm has been shown to form when grown anaerobically [96].

The transition between planktonic and sessile bacterial lifestyles can be affected by nutrients since the bacterial response to form a biofilm or to remain in suspension depends on the nutritional status [85]. Availability of nutrient in the surrounding medium has been found to influence the bacterial attachment to the surfaces; thus, an increase in nutrient concentration increases the microbial attachment rate [35,56]. Besides, biofilm development and dispersal of cells from the biofilm can be affected by nutrient levels [14,47]. Also, changes in the essential nutrient availability have been shown to have an impact on bacterial physiology in growing biofilms [97].

Several studies have addressed the effect of nutrient levels on the formation of bacterial biofilm. For example, in drinking water distribution systems, high nutrient concentration increased cell numbers within biofilms [98,99]. In a paper mill water stream the rate and quantity of *P. putida* biofilm increased with increasing nutrient levels [100]. In some microbes, the addition of glucose as carbon source to the medium has been found to enhance the formation of biofilm, such as *E coli* [97] *P. putida* [100]. However, the addition of glucose to different media was found to hinder biofilm formation in several species of *Enterobacteriaceae* family, such as *K. pneumoniae, Citrobacter freundii*, and *Salmonella enterica* [101].

There is some information about the effect of glucose levels on biofilm formation, but little is known about the impact of changing nitrogen concentrations in the same process. Rochex and Lebeault, (2007) have shown that rate and extent of *P. putida* biofilm accumulation increased with nitrogen concentration from carbon/nitrogen=90 to carbon/nitrogen=20. In contrast, depletion of nitrogen led to the active detachment of *Pseudomonas fluorescens* biofilm, similar to that observed under glucose limitation [102]. However, variation in peptone and yeast extract concentration, which are good nitrogen sources had no significant impact on *E. coli* biofilm formation [12].

In most natural environments, bacteria–surface association leads to biofilm formation, which is the prevailing microbial lifestyle [103]. Bacteria in these environments are exposed to a variety of abiotic stresses, such as osmolarity; however, planktonic and biofilm bacteria, by inducing stress response genes, might become more tolerant to these environmental stresses [23]. In bacteria, such as *Lactobacillus rhamnosus*, *Listeria monocytogenes* and *Shigella boydii* formation of biofilm has been found to be associated with high osmolarity [14]. For other microbes, an increase in NaCl concentrations inhibited biofilm formation, such as *Salmonella species* [1], *Sinorhizobium meliloti* [104], *S. aureus* [105], *Enterococcus faecalis* and *P. aeruginosa* [106].

Conclusion

Bacteria have the ability to grow in both a free form (planktonic lifestyle) or as biofilms attached to various surfaces. Biofilms are complex communities of microorganisms attached to surfaces and enclosed in a matrix of extracellular polysaccharide matrix (EPS). Thus, biofilms seem to play an essential role in bacterial survival under natural and harsh conditions and protect the bacterial cells from antimicrobial agents and toxic compounds. The quantity of EPS varies depending on the age of biofilms, the type of microbes present, and the environmental conditions. As the biofilm ages, the number of EPS tends to increase. Bacterial biofilms can form on all kinds of surfaces, including glass, plastic, wood, metal, soil particles, medical implant materials, tissues, and food products. The attachment and bacterial biofilm-forming abilities depend on numerous factors, such as inherent biological characteristics and environmental factors. Biofilm-associated bacteria are known to differ significantly from their free-living planktonic counterparts.

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