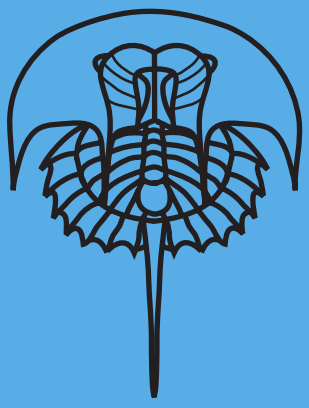

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PART B, VOLUME 2, CHAPTER 2
BIOFILMS

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INTRODUCTION

Most microorganisms found in natural, clinical, and industrial environments prevail associated with surfaces rather than as free-living (planktonic) organisms (Costerton & others, 1995; Flemming & Wuertz, 2019). These communities can develop as biofilms in a diverse range of environments (e.g. living tissues, indwelling medical devices, water distribution systems, natural aquatic and sediment systems, rocks, surfaces of buildings, stromatolites, etc.). Biofilms are "aggregates of microorganisms in which cells that are frequently embedded within a self-produced matrix of extracellular polymeric substances (EPS) adhere to each other and/or to a surface" (Vert & others, 2012, p. 383). The aggregation of cells can result in highly structured microbial communities that allow for cell-to-cell contact. This proximity of the cells, the intra- and intercellular interactions within the microbial community, and the properties of the EPS matrix can confer distinct emergent properties upon the biofilm substantially different from planktonic communities (Flemming & others, 2016). Biofilms are characterized by their unique: 1) physicochemical and biological heterogeneity, which provides habitat diversity; 2) services provided by the EPS matrix, which provides architecture and stability to the biofilm and acts as a protective barrier; 3) physical and social interactions, which in conjunction determine the survival strategies for the community, such as quorum sensing, gene exchange, EPS production, or coordination of metabolic action; and 4) increased tolerance and/or resistance to survive environmental stress (Costerton, Stewart, & Greenberg, 1999; Flemming & others, 2016). The biological and physicochemical characteristics of biofilms (e.g., structure, EPS production, and cell biomass) are the result of the environment, the nutritional and physical conditions in which the biofilm develops (Nielsen, Jahn, & Palmgren, 1997).

The significance of biofilms in the geological record of life was recently recognized (Noffke, 2010). Examples of the manifestation of biofilms in the geological record include microbially induced sedimentary structures (MISS) and stromatolites (Astafieva, 2013; Noffke, 2010). These structures suggest that biofilms have existed throughout the geological record of life (Costerton & Stoodley, 2003; Noffke, 2010). Considering that cells within a biofilm can exhibit different phenotypes and change their metabolic activities compared to their planktonic counterparts, it is possible that biofilms induce distinct characteristics (e.g., structures, textures, chemical signatures) in the consolidated rock record. Thus, a better understanding of the biofilm way of life can aid in reconstructing the evolution of prokaryotes throughout Earth history.

BIOFILM FORMATION AND DEVELOPMENT

Biofilm formation follows a number of progressive steps including initial microbial

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attachment to a surface, microcolony formation, development of a three-dimensional community structure, maturation, and detachment.

ATTACHMENT OF MICROORGANISMS

The first step in biofilm formation is microbial attachment, which includes planktonic cells being able to find, interact with, and adhere to a surface. Microbial attachment is influenced by several factors, including the type of substratum (e.g. an inert surface or living tissue), hydrodynamics of the aqueous medium, physicochemical characteristics of the medium (e.g., pH, nutrient levels, temperature), and properties of the cell surface and cell motility (Bouwer & others, 2000; Donlan, 2002; Palmer, Flint, & Brooks, 2007). Attachment is more likely to occur on surfaces that are rough, hydrophobic, and coated by conditioning films (i.e., surfaces in nature and industry are often at least partially coated by compounds—including polymers—from the liquid medium) (Donlan, 2002; Palmer, Flint, & Brooks, 2007).

FORMATION OF MICROCOLONIES

With the initial attachment of cells, microbial association to the surface (substratum) begins and—given appropriate growth conditions—becomes suitable for microcolony formation. During this stage of biofilm development, microbial cells undergo growth, which is usually accompanied by the excretion of EPS, resulting in the formation of aggregates or microcolonies. EPS production aids in promoting the irreversible attachment of cells to a substratum (Flemming & Wingender, 2010). Microbial aggregation also occurs as a result of the interaction of already attached cells and the recruitment of planktonic cells from the surrounding medium (McLean & others, 1997). Initial EPS production can be a response to attachment and environmental conditions such as osmotic pressure, pH, temperature, starvation and likely other factors (Flemming & others, 2016).

FORMATION OF THREE-DIMENSIONAL STRUCTURE AND MATURATION

Given suitable growth conditions, microcolonies develop into an organized structure over time and differentiate into true biofilms. Mature biofilms are typically comprised of multilayered microcolonies encased in EPS and separated by interspersed water channels. The EPS matrix has an active role in microbial attachment to surfaces, acts as a glue that keeps cells together, and allows for the development of a three-dimensional structure (Flemming & Wingender, 2010).

DETACHMENT

As the biofilm matures, detachment or dispersal occurs, which is crucial to the biofilm life cycle. Detachment of microbial cells occurs due to multiple factors including the lack of nutrients, competition, hydrodynamic stresses, among others (Stewart, 1993). The release and dispersion of microbial cells can lead to the formation of new biofilms (Stewart, 1993). Detachment can occur as a rapid, extensive loss of parts of the biofilm known as sloughing, or as continuous loss of single cells (small fractions of the biofilm) known as erosion (Bryers, 1988; Stewart, 1993). Detachment can influence the competition in biofilms (Morgenroth & Wilderer, 2000) and the biofilm morphology (Picioreanu, van Loosdrecht, & Heijnen, 2001). For instance, erosion can result in smoother biofilms, whereas sloughing usually increases the morphological heterogeneity of the biofilm (Picioreanu, van Loosdrecht, & Heijnen, 2001).

THE BIOFILM MATRIX

The biofilm matrix is a conglomeration of different extracellular biopolymers in which the biofilm cells are embedded. The microbial extracellular material, known as extracellular polymeric substances or EPS, typically accounts for ~90% of the biofilm,

and the rest corresponds to biomass as well as minor components such as particulates, gas bubbles, etc. (Flemming & Wingender, 2010). EPS are comprised mostly of water (up to ~97%) (Zhang, Bishop, & Kupferle, 1998) and are usually a mixture of polysaccharides, proteins, lipids, nucleic acids, and other organic compounds (Flemming & Wingender, 2010; More & others, 2014). The EPS composition within a biofilm can vary greatly; it can be strain-dependent but can also be affected by the nutritional and physical conditions in which the biofilm develops (Nielsen, Jahn, & Palmgren, 1997). It has also been suggested that the presence of microenvironments within biofilms may lead to the production of various mixtures of polysaccharides by specific subpopulations (Sutherland, 2001).

The presence of EPS does not seem to be key for the initial attachment of microbial cells to surfaces (Gaylarde & Gaylarde, 2005). However, EPS production is essential for the development of the architecture of any biofilm (Flemming & Wingender, 2010; Sutherland, 2001). EPS production appears to begin after the initial attachment of the microbial cells and the formation of the first microcolonies; production of EPS is often associated with the so-called irreversible attachment of cells (Flemming & Wingender, 2010).

Although the production of EPS can also occur during planktonic growth (e.g., microbial aggregates) (More & others, 2014), EPS provide biofilms with many of their unique physical characteristics. The EPS matrix has different functions in biofilms, including: 1) adhesion, cohesion, and aggregation of microbial cells—the EPS immobilize cells and keep them close allowing for cell-cell communication; 2) architecture and stability of the biofilm—formation of the structural support of the biofilm is a continuous and dynamic process that results in the spatial organization of biofilms; 3) protective barrier for cells and retention of water to prevent desiccation, which increases the tolerance and/or resistance to antimicrobials and other stressors; 4) resource capture (nutrients, organic compounds and inorganic ions) by sorption; 5) enzyme retention, which provides digestive capabilities; 6) exchange of genetic information; 7) function as electron donor or acceptor; 8) export of cell components; 9) sink for excess energy; and 10) binding of enzymes (Flemming & Wingender, 2010; Flemming & others, 2016). For excellent reviews summarizing the possible services the EPS matrix can provide to biofilms, see Flemming and Wingender, 2010; More and others, 2014; and Sutherland, 2001.

CHARACTERISTICS OF BIOFILMS

HETEROGENEITY

Biofilms are comprised of dense clusters of microbial cells (microcolonies) held together by the EPS matrix with fluid channels formed within the biofilm through which nutrients circulate. This structural organization leads to the formation of numerous microenvironments within the biofilm with different microbial composition, activity, cell density, pH, EPS production, water content, presence of channels, and solute concentrations (Stewart & Franklin, 2008). As a result, biofilms are physically, chemically, and biologically heterogeneous.

Mature biofilms are characterized by the presence of concentration gradients of metabolic substrates and products, resulting in chemical heterogeneity within the biofilm matrix. Specific patterns of chemical heterogeneity can be observed within biofilms due to reaction-diffusion interactions for metabolic substrates, metabolic products, and metabolic intermediates (Stewart & Franklin, 2008) (Fig. 1). As biofilms grow, the microbial cell density often increases, leading to an increase in the demand of nutrients (metabolic substrate). In general, cells located closest to the substratum are more limited for nutrients, whereas cells closest to the surrounding environment

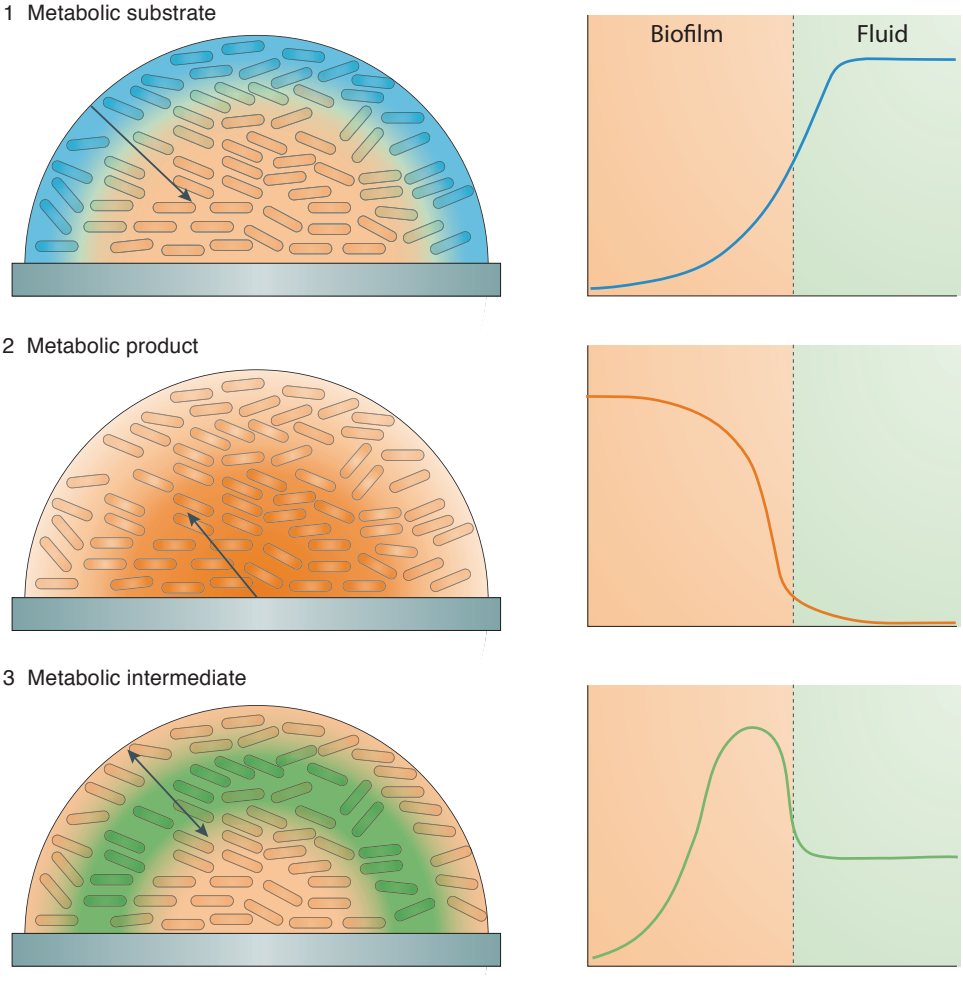

FIG. 1. Chemical heterogeneity in biofilms. Three qualitatively distinct patterns of chemical heterogeneity arise in biofilms owing to reaction-diffusion interactions for a metabolic substrate, *blue* (*1*); a metabolic product, *orange* (*2*); and a metabolic intermediate, *green* (*3*). *1,* The concentration of a substrate that is consumed inside the biofilm decreases with depth into the biofilm and distance away from the bulk fluid. *2,* Conversely, a metabolic product is more concentrated inside the biofilm. *3,* A metabolic intermediate that is both consumed and produced within the biofilm can exhibit concentration profiles that have local maxima (reprinted by permission from Springer Nature Customer Service Center, Nature Reviews Microbiology, Stewart & Franklin, 2008, fig. 2).

(e.g., farthest from the substratum) have higher availability of nutrients (STEWART & FRANKLIN, 2008). Opposite to nutrients, metabolic products are usually present at higher concentrations inside the biofilm with decreasing concentrations in the outer layers. Metabolic intermediates can be produced and consumed in the biofilms, leading to concentration profiles with maxima somewhere within the biofilm; for instance, in a multi-species biofilm, the waste product of one species can serve as substrate for another species (Fig. 1) (STEWART & FRANKLIN, 2008).

Under well-mixed conditions, planktonic microorganisms show fairly uniform physiological activity, whereas the chemical gradients within biofilms are commonly accompanied by physiological heterogeneity (GU & others, 2013; JENSEN & others, 2017). Due to limitations in metabolic

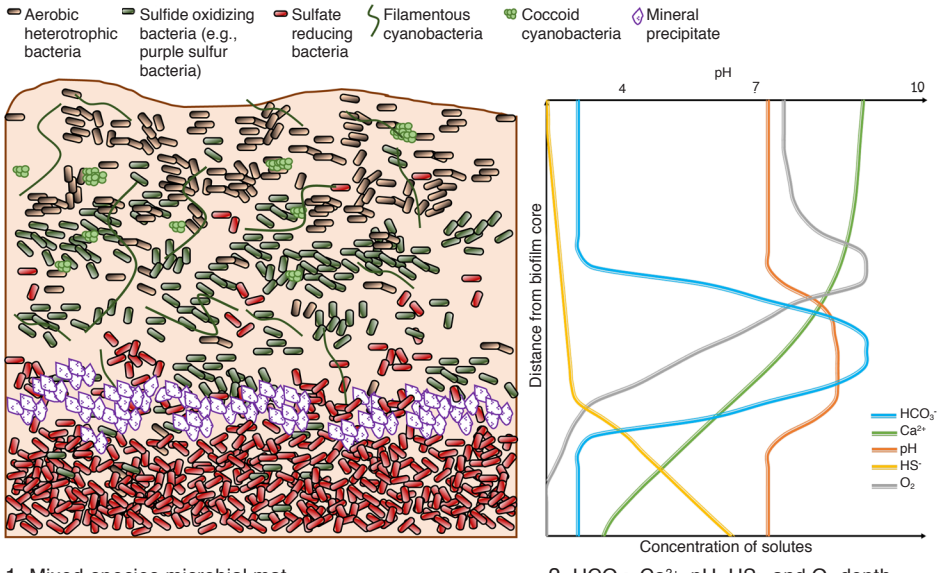

FIG. 2. Microbial diversity in biofilms. *1,* Conceptual representation of the microbial diversity observed in a mat similar to the one in the superficial layer of the stromatolite in the Cayo Coco Lagoonal Network described by Pace and others (2018); various groups of microorganisms are distributed within the mat and are located based on their physiological preferences, including photosynthetic microorganisms (filamentous and coccoid cyanobacteria), aerobic heterotrophic bacteria, and sulfate-reducing and sulfide-oxidizing bacteria. *2,* Sketch of chemical micro-environments developing within the mat indicated by the HCO_3^-, Ca^{2+}, pH, HS^-, and O_2 depth profiles. Mineral precipitation is observed in the oxygenic-anoxygenic photosynthetic interface as a result of a pH maximum induced by the microbial activity. (adapted from Pace & others, 2018, fig. 3 and fig. 8).

substrates and oxygen (or other electron acceptor) availability, there are usually regions of slow microbial growth and activity within a biofilm. Furthermore, as a response to microenvironments inside a biofilm, microorganisms can modify gene expression patterns and physiological activities, favor the growth of particular microbial species, and select for fitter strains that can adapt to and survive in particular conditions (STEWART & FRANKLIN, 2008).

As an illustration of the various biogeochemical gradients that can be found in a biofilm, consider a mixed-species microbial mat, which may be viewed as complex biofilms (STOLZ, 2000) growing in (and producing) a lithifying stromatolite (Fig. 2) (PACE & others, 2018). Stromatolite growth can be the result of dynamic and successive cycles of sedimentation and microbial lithification in which the metabolism of microbial mats plays a key role (REID & others, 2000). Early studies reported the formation of chemical micro-gradients within microbial mats due to the metabolic activity of various microbial groups (VISSCHER & VAN GEMERDEN, 1993; STAL, GEMERDEN, & KRUMBEIN, 1985; JØRGENSEN, REVSBECH, & COHEN, 1983; JØRGENSEN & REVSBECH, 1983). PACE and others (2018) collected an actively growing microbial mat from a lithifying stromatolite in the hypersaline Cayo Coco Lagoonal Network (Fig. 2.1–2.2). Based on confocal laser scanning microscopy, microbial community analysis, dissolved oxygen (O_2), sulfide (H_2S/HS-/S^{2-}) concentration, and pH profiles, various chemical microenvironments were observed along a vertical profile in the stromatolite (Fig. 2b). Microbial activity in the upper layers of the stromatolite is indicated by the O_2 and bicarbonate profiles (Fig.

2.2); and within the first few millimeters from the surface, O_2 concentration peak and bicarbonate concentrations are low due to oxygenic photosynthesis by, most likely, cyanobacteria. Below ~3 mm depth, bicarbonate concentrations increase and O_2 decreases rapidly due to reduced photosynthetic activity and increased net-aerobic respiration creating an oxic-anoxic interface at about 5 mm depth. Sulfide appears below the oxic-anoxic interface.

TOLERANCE AND RESISTANCE TO ENVIRONMENTAL STRESS

One of the unique properties of biofilm-grown cells is their enhanced tolerance and/or resistance to antimicrobials (e.g. disinfectants, toxic compounds, antibiotics) and stresses compared to their planktonic counterparts. Flemming and others (2016) described biofilms as fortresses due to the ability of biofilm-grown cells to survive exposure to antimicrobials as well as desiccation. We refer here to resistance as the inherited ability of microorganisms to survive exposure to concentrations of antimicrobials that can be lethal (Sholar & Pratt, 2000) and that remains even when cells in the biofilm are dispersed. The term tolerance is described as the ability of the cells to survive transient exposure to compounds or stresses that could be lethal (Kester & Fortune, 2014), a phenomenon that is uniquely observed when cells grow as biofilms (Olsen, 2015).

Tolerance in biofilms is often attributed to the role of the EPS matrix acting as a protective barrier as well as to the development of regions with low metabolic activity created as a result of the intrinsically heterogeneous nature of biofilms. The EPS matrix acts as a protective barrier by: 1) quenching the activity of antimicrobials that diffuse through the biofilms via diffusion-reaction inhibition (Daddi Oubekka & others, 2012); this could involve the binding of the antimicrobials to components of the biofilm matrix or to microbial membranes (Chiang & others, 2013) as well as degradation of antimicrobials by enzymes contained in the EPS (Høiby & others, 2010), and 2) acting as a hydrogel that holds water protecting the organisms from desiccation (Flemming & Wingender, 2010). The intrinsic heterogeneity of the biofilms promotes the creation of zones of low metabolic activity and dormancy, which can decrease the susceptibility of the biofilm to harmful substances and increase the resistance of the biofilm to changing environmental conditions (Brown, Allison, & Gilbert, 1988; Stewart & Franklin, 2008). Cells in these zones of low metabolic activity and dormancy have reduced susceptibility to antimicrobials that depend on the microbial metabolism for their activities (Amato & others, 2014). Furthermore, biofilms can contain inactive microbial subpopulations (up to 1%) known as persisters that appear to exhibit unique phenotypic traits that make them more tolerant to antimicrobials (Wood, Knabel, & Kwan, 2013).

Microbial diversity within biofilms is a factor that can further increase the tolerance of biofilm-grown cells. Biofilms comprised of multiple species are affected by cross-species interactions, which can influence the development and structure of the microbial species within the biofilms and, in turn, provide an increased tolerance to stresses compared to their single-species biofilms (Lee & others, 2014; Moons, Michiels, & Aertsen, 2009). More information about the social behavior and the interspecies interactions within mixed-biofilms is presented below in *Biofilms as Complex Microbial Communities*, p. 7.

DIVISION OF LABOR

Biofilm-grown cells can demonstrate division of labor (Armbruster & others, 2019; Dragoš & others, 2018; van Gestel, Vlamakis, & Kolter, 2015; Vlamakis & others, 2008), which refers to the specialization of subpopulations of cells to perform different tasks within a microbial community. Division of labor appears to be based on

three conditions: 1) development of different microbial phenotypes (task allocation); 2) associated microorganisms having a cooperative interaction; and 3) all partners involved in the interactions gaining inclusive fitness benefits (West & Cooper, 2016).

An example of division of labor can be found in *Bacillus subtilis* biofilms, which have subpopulations that are genetically similar but are able to perform different specialized activities including motility, matrix production, and sporulation, which in conjunction are key for the successful development of the biofilm (Dragoš & others, 2018; van Gestel, Vlamakis, & Kolter, 2015; Vlamakis & others, 2008). In *B. subtilis* biofilms, flagellum-independent migration is achieved by two different cell types: surfactin-producing cells that aid lubricating the substratum and matrix-producing cells, which agglomerate as bundles (van Gogh bundles) that are able to move away from the colony; these bundles can migrate greater distances compared to what would be possible without the division of labor (van Gestel, Vlamakis, & Kolter, 2015).

BIOFILMS AS COMPLEX MICROBIAL COMMUNITIES

Biofilms in the environment typically consist of complex microbial communities that host multiple species. Subaerial biofilms, biofilms that grow on solid mineral surfaces exposed to the atmosphere (e.g., rocks, surface of buildings, stromatolites), are perfect examples of complex communities with different cross-species interactions. A diverse community of microorganisms is usually present in subaerial biofilms, including algae, bacteria, fungi, protozoa, and even microscopic animals such as mites and insects (Gaylarde & Gaylarde, 2005; Gorbushina & Petersen, 2000). Interactions among different microbial species in mixed-biofilm communities seem to influence the development, structure, and functions of these communities (Moons, Michiels, & Aertsen, 2009). Cross-species interactions in mixed biofilms can range from synergistic (cooperative) to antagonistic (competitive) (Elias & Banin, 2012), and they can lead to a number of microbial adaptations by promoting horizontal gene transfer events, cell-cell communication (quorum-sensing abilities) (Davies & others, 1998; Parsek & Greenberg, 2005), and can induce protein secretion systems resulting in phenotypic changes that can affect the survival, dynamics, spatial distribution, and coexistence of the microbial communities (Elias & Banin, 2012).

Cross-species interactions can influence the development and structure of microbial species within the biofilms, which can provide an increased resistance to stresses compared to their single-species biofilms (Lee & others, 2014; Moons, Michiels, & Aertsen, 2009). Lee and others (2014) tested the response of mixed-species biofilms, comprised of *Pseudomonas aeruginosa, Pseudomonas protegens,* and *Klebsella pneumoniae* to their exposure to two antimicrobials—sodium dodecyl sulfate and tobramycin. Compared to single-species biofilms, the mixed-species biofilm was more adept at maximizing and optimizing the use of nutrients to enhance their growth and persistence, which made it more resilient to these antimicrobials. Furthermore, the increased tolerance observed in the mixed-species biofilm was suggested to be a result of a cross protection effect provided by the resistant species to all other members of the microbial community, rather than selecting for the least sensitive species in the biofilm (Lee & others, 2014). The way microorganisms interact within biofilms can indeed influence the spatial organization of the biofilm (see Fig. 3). Liu and others (2016), for instance, described that 1) species exhibiting strong cooperation appear to develop intermixed distributions or layered structures without patchy patterning; 2) in the absence of nutrient or space limitation, species with weak interdependence tend to interspecifically segregate; 3) exploitation by

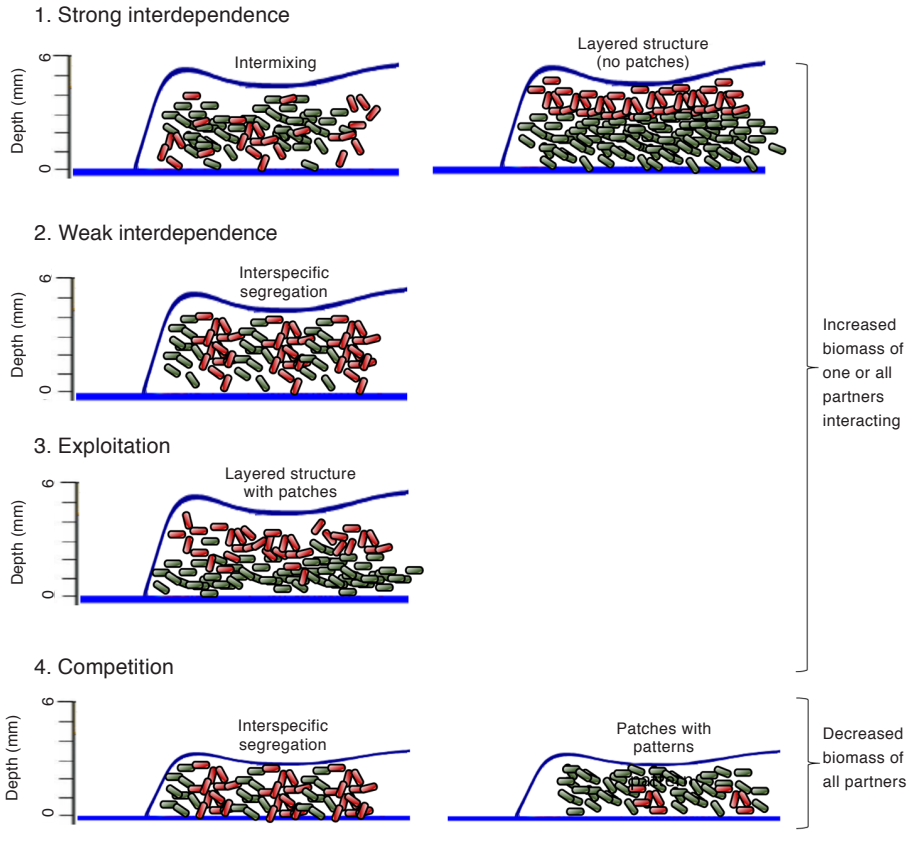

FIG. 3. Cross-species interactions influence the spatial organization of mixed-species biofilms. *1,* Strong interdependence (cooperation) leads to the formation of intermixing or layered structures. *2,* Weak interdependence results in interspecific segregation. *3,* Exploitation results in layered structures with patches. *4,* Competition can lead to species segregation and the formation of patches with patterns. Negative interactions (competition) can result in the overall decrease of biomass (new; based on information in Liu & others, 2016).

one of the species can result in the formation of layered structures with patchy patterning; and 4) competition appears to lead to an overall decrease in biomass with patchy patterning or interspecific segregation (Fig. 3.1–3.4) (LIU & others, 2016).

Whereas biofilms in the environment can be dominated by a particular species, other secondary species are almost always present. Dominance by one species in a biofilm is determined by: 1) the particular location within the biofilm; 2) the environmental conditions; and 3) the specific stage in the development of the biofilm. In the example of the microbial mat studied from the lithifying stromatolite in the hypersaline Cayo Coco Lagoonal Network, dominance of a particular species varied according to the specific location within the biofilm. The green lamina of the stromatolite (top layer of the biofilm) was dominated by cyanobacteria, whereas deeper layers (mineralized lamina) were dominated by purple sulfur (sulfide-oxidizing) bacteria (PACE & others, 2018) (see Fig. 2.1). The development of freshwater phototrophic biofilms can also be influenced by environmental conditions, such as the presence of light (ROESELERS, VAN LOOSDRECHT, & MUYZER, 2007). For instance, under high light conditions, initial colonizers can predominantly consist of green algae, whereas under low light

intensities, heterotrophic bacteria tend to colonize. Moreover, over time, as the biofilm matures, filamentous cyanobacteria can become predominant in these phototrophic biofilms (Roeselers, van Loosdrecht, & Muyzer, 2007).

BIOFILMS AND MINERAL PRECIPITATION

Microbially induced precipitation of minerals (biomineralization) is a relevant process in various biological, geological, medical, and engineered systems (Phillips & others, 2013). Of importance for the study of the evolution of prokaryotes throughout Earth history, is the understanding of carbonate biomineralization. The formation of carbonate sediments in different environments (e.g., marine reefs, fluviatile tufas, hot springs, travertines, etc.) seems to be influenced by microbial mineralization.

Various microbial metabolic processes, including photosynthesis, sulfate reduction, urea hydrolysis, ammonification, denitrification, and methane oxidation, affect the solution chemistry of the surrounding environment (e.g., increase carbonate alkalinity, pH values, or dissolved inorganic carbon), which in turn can induce carbonate or other mineral precipitation (Dupraz & others, 2009; Zhu & Dittrich, 2016).

Biomineralization is a common event in microbial mats or biofilms (Braissant & others, 2003; Handley & others, 2008; Shiraishi & others, 2008). Chemical heterogeneity in biofilms can lead to the formation of microenvironments that create gradients of alkalinity and/or supersaturation, which can facilitate mineral precipitation within the biofilm. Furthermore, the presence of EPS in biofilms can influence the biomineralization process by providing nucleation sites for mineral precipitation, regulating the patterns of mineralization and the types of minerals produced (Braissant & others, 2003; Decho, 2010). Certain functional groups in the EPS can inhibit carbonate precipitation: negatively charged groups can bind with mineral ions such as Ca^{2+} and Mg^{2+}, thus, a high binding capacity of the EPS can potentially inhibit carbonate precipitation (Flemming, 1995). Release of cations from the EPS can occur due to EPS degradation or after release from the binding sites through an external trigger (e.g., change in ionic strength, salinity), which can lead to carbonate and other mineral precipitation (Decho, 2010; Dupraz & others, 2009).

As an illustration of the various metabolic processes that can promote mineral precipitation, consider again the example of the stromatolite in the Cayo Coco Lagoonal Network described by Pace and others (2018) (see Fig. 2). As mentioned earlier, stromatolites result from successive cycles of microbial mineralization triggered by the metabolism of biofilm forming microbiota. Pace and others (2018) suggested that mat formation starts with the development of biofilms comprised of coccoid and filamentous cyanobacteria-fixing CO_2, leading to the formation of biomass and the production of O_2 through oxygenic photosynthesis. Oxygenic photosynthesis also consumes CO_2 and increases the pH, which can result in the precipitation of (calcium) carbonates. In the top layer of the microbial mat, aerobic heterotrophs consume O_2; in the anoxic depths, sulfate-reducing bacteria produce HS^- from sulfate. Sulfate reduction can increase carbonate alkalinity (in the form of bicarbonate, HCO_3^-). In an intermediate zone, both sulfide and O_2 are present. Purple sulfur (sulfide-oxidizing) bacteria are involved in recycling the sulfide back to sulfate, and other microbes are involved in this process as well. The microbial activity of cyanobacteria, sulfate-reducing and sulfide-oxidizing bacteria creates a daytime pH maximum, which promotes the precipitation of magnesium calcite from dissolved ions in the lagoon. Mineral precipitates are mostly located at the oxygenic-anoxygenic photosynthetic interface (see Fig. 2.2). Figure 2.2 shows the different chemical profiles in the microbial mat that can be created due

to the different microbial activities. The repetition of these series of physicochemical and biological steps along with the upward growth of the biofilm leads to the formation of stromatolites in the studied lagoon (Pace & others, 2018).

Pace and colleagues suggested a role of the EPS in the different mineralization steps, hypothesizing that cyanobacterial EPS acts as a binding agent for calcium, thus inhibiting carbonate precipitation in the green lamina of the stromatolite (upper layer of the mat). EPS in the oxic-anoxic zone appears to have a decreased cation-binding capacity, which would make Ca^{2+} more available for carbonate precipitation or indicate that the EPS in these layers is saturated with multivalent cations.

SUMMARY

Most microorganisms persist associated with surfaces in the natural environment, most likely in the form of biofilms. Biofilms are complex microbial communities attached to surfaces and embedded in a matrix of extracellular polymeric substances (EPS). The presence of EPS provides architecture, stability, and protection to the microbial communities within the biofilm. Furthermore, these microbial communities typically contain multiple species that interact with each other and with the environment.

Due to the spatial arrangement of the microbial communities, biofilms develop microenvironments, which result in highly physically, chemically, and biologically heterogeneous arrangements. Biofilm-grown cells can exhibit different phenotypes and change their metabolic activities compared to their planktonic counterparts.

Considering that biofilm-grown cells exhibit characteristics distinct from their corresponding planktonic communities, it is possible that biofilms produce specific marks in the consolidated rock record. Thus, a better understanding of the biofilm way of life can aid in the reconstruction of the evolution of prokaryotes throughout Earth history.

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