



Biofilm as an adaptation strategy to extreme conditions

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Abstract

Biofilm is the most successful and widely distributed form of life on earth, it is not simply structured collections of cells attached to surfaces but is a dynamic complex biological system able to respond to environmental changes. The biofilm characteristics make it unique and central to microbial evolution and adaptation. The ability to establish biofilms is a key trait for microorganisms growing in extreme environments like extreme temperature, high radiation, acidic or alkaline pH values, heavy metal pollution, and high salinity. In this article, we report the main features of biofilm and how these characteristics make biofilms a successful survival strategy in extreme conditions. All aspects examined in this article help to explain why biofilms are a successful survival strategy in extreme conditions and why the ability to establish biofilms is a key trait for microorganisms growing in extreme environments.

Keywords Biofilm · Extremophiles · Microbial adaptation · Microbial evolution

1 Introduction to microbial biofilm

Biofilm is the oldest most successful and widely distributed form of life on earth (Westall et al. 2001), in fact, current estimates suggest that up to 80% of bacterial and archaeal cells reside in biofilms (Flemming and Wuertz 2019). There are several evidence of biofilm formation in the fossil samples, putative biofilm microcolonies were discovered by morphology in the 3.3–3.4-billion-year-old South African Kornberg formation (Westall et al. 2001) and filamentous biofilms were found in the 3.2-billion-year-old deep-sea hydrothermal rocks of the Pilbara Craton, Australia. These data suggest that the ability to form biofilms is an ancient feature of prokaryotes. It is likely that biofilms provided homeostasis in the face of the unstable and harsh conditions of the primitive earth (pH and exposure to ultraviolet (UV) light, extreme temperatures) helping the development of complex interactions between individual cells. Hall-Stoodley and co-authors hypothesize that the catalytic and protective conditions offered by biofilm might have led to the concurrent development of both sessile and planktonic forms in biofilm cellular communities. These considerations suggest

that biofilm characteristics have been acquired through billions of years of evolutionary adaptation.

Biofilms are ubiquitous and play critical roles in all global biogeochemical processes and natural and anthropogenic environments (Flemming and Wuertz 2019). Indeed, biofilm communities are important for ecosystem functioning and nutrient cycling. The definition of biofilm as a microbial community is not limited to microbial films on surfaces but applies to multicellular microbial aggregates in general. It consists of a wide range of forms including, flocs, pellicles, slimes, microbial mats, microorganisms in soils, endolithic microbial populations (Flemming et al. 2021). Interestingly, biofilms growing in diverse environments are remarkably similar, indicating there are crucial convergent survival strategies that are partially given by structural properties; For example, biofilms growing in fast-moving water, like freshwater rivers or hydrothermal hot springs, tend to form filamentous biofilms regardless of whether they occur in hydrothermal photosynthetic mats, in the drainage run-off from acid mines or in rivers, while in quiescent waters biofilm tends to have a mushroom structure characterized by no clear indication of flow direction.

The structural properties of biofilms are mainly related to the extracellular polymeric substances (EPS) contained in the biofilm matrix (Seviour et al. 2019). Although the main biofilm matrix component is water (up to 97%), it contains several microbial biopolymers including proteins,

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exopolysaccharides, nucleic acids (RNA and extracellular DNA), and lipids, it may also contain minerals such as quartz, basalt, clay minerals. EPS forms a thick, muddy, hydrated matrix in which cohesion is based on multiple electrostatic forces, hydrogen bonds, and ionic interactions, which produce weak or strong networks (Flemming and Wingender 2010). The characteristics and composition of EPS generally establish the mechanical properties of biofilms, which can range from rigid solids to viscous liquids, it sometimes has features of both solids and liquids and its actual mechanical response to forces is similar to that of a viscoelastic fluid. The properties of the EPS matrix let a good balance between structural stability and flexibility/adaptability that allows the development of structured microbial communities with emergent properties that are distinct from planktonic single cells (Flemming et al. 2016).

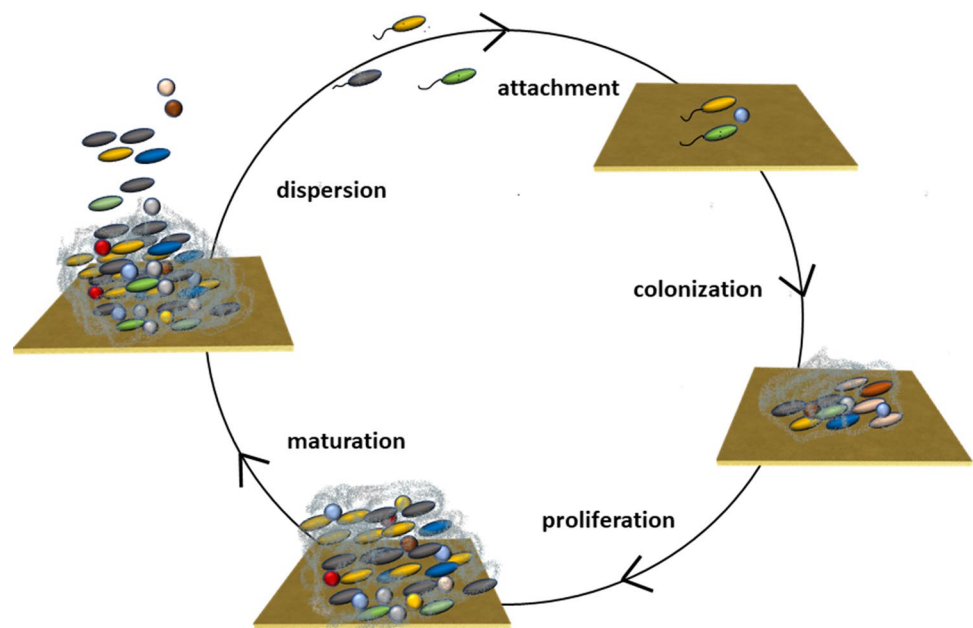
Microbial biofilms are not simply structured assemblages of cells that are attached to surfaces or/and to each other but is a dynamic complex biological system that evolves by a tightly controlled process (Zhou et al. 2020). The evolution of the biofilm is an endless cycle that numerous studies have summarized in several stages (Fig. 1). The first is characterized by a loose/transient association (via weak interactions such as the van der Waals forces) with the surface, followed by robust adhesion. Stages two and three involve colonization, where microorganisms are irreversibly attached to the surface via stronger hydrophilic/hydrophobic interactions, and maturation. Stage four is characterized by a return to transient motility where biofilm cells are detached, due to interactions with either intrinsic or extrinsic factors, and disseminated cells colonize other sites. The attachment stage is the beginning of a biofilm formation, while active detach is

not the end, but the beginning of a new round of biofilm formation, the endless recirculation of biofilm gives the microorganisms the ability to adapt to environmental fluctuations. Indeed, this remodeling is crucial for the biofilm to respond to changes in the environment.

2 Biofilm is a class of complex collective ecosystems more than a collection of individual cells

The classical approach in biofilm study starts from the idea that biofilm represents a sessile developmental stage in the life of unicellular microbial organisms. Based on this view, microorganisms experience a life cycle that involves both sessile and planktonic phases. The cycle begins with microbial attachment to an interface followed by microcolonies formation, biofilm maturation and cells dispersion, which is necessary for microorganism spreading and serve as inoculum for new biofilm initiation. This concept is recently defined the single-cell centric (Penesyan et al. 2021). Penesyan and co-authors (2021) point out that this view derived from the history of research in microbiology, which is centered on planktonic single bacterial cells traditionally considered as the primary and main form of microbial life. However, in recent years, it has become evident that most of bacteria and archaea exist as biofilms in their natural habitats (Flemming and Wuertz 2019). These data introduced a new biofilm-centric view of microorganisms in which single bacterial cells are considered an intermediate dispersal stage of multicellular aggregates. In line with this biofilm-centric view of microbial cells, Penesyan and

Fig. 1 Model of microbial biofilm formation. Biofilm formation consists of five distinct stages: attachment, colonization, proliferation, maturation, and dispersion



co-authors (2021) suggested that the biofilm, which consists of multiple cells cooperating to create a differentiated structure, exhibits characteristics of multicellular organisms. It is known that cells within a multicellular organism have different specialized functions and distinct metabolic rates and that the transport of materials through such multicellular organisms makes concentration gradients of oxygen or pH. It is established those multicellular systems can answer to environmental stimuli using complex regulatory mechanisms that control the behavior of single-cell and coordinate their responses. All these features of a multicellular organism are perfectly superimposable with some properties of microbial biofilm.

Furthermore, several authors (Flemming et al 2016, 2021) describing the properties of biofilm report that it belongs to the class of complex collective ecosystems, such as coral reefs, forests, and beehives. Like such communities, microbial biofilms play as “protective and internally homeostatic fortresses at a scale much larger than the living organisms who built them” and fall under the concept of “extended organisms”(Flemming et al. 2016).

2.1 Physiological heterogeneity

By generating a matrix, bacteria in biofilms create a physically distinct habitat that provides protection and produces nutrients and gases gradients (Flemming et al. 2016). These gradients create microenvironments with specific physical and chemical properties allowing the presence of microorganisms with very different physiological requirements (Flemming and Wingender 2010; Hung et al. 2013). In biofilms present in shallow aquatic environments, for example, microorganisms are distributed according to their metabolic properties. The upper layers are dominated by aerobic organisms that are exposed to light and oxygen, these aerobic cells respiring consume oxygen faster than it diffuses through the biofilm, which results in the formation of anaerobic zones in deep layers of the biofilm principally occupied by anaerobic microorganisms (Kragh et al. 2016). Physiological heterogeneity in biofilms allows the three-dimensional organization of mixed-species biofilms. In many cases, the presence of one group of organisms determines the ability of the other group to survive in the biofilm. For example, phototrophic microorganisms generate and release organic substrates, and neighboring species use these substrates for their metabolic activity (Ward et al. 2008). In a mixed-species biofilm, a division of labor, based on their functional ability, between species belonging to a different taxonomic group is frequently reported (van Gestel et al. 2015; Joshi et al. 2021).

A further advantage of biofilm as a collective organization is the improved ability to acquire nutrients from the environment. It is achieved through resource capture and

retention of cellular products and debris that can be used as a reservoir. This advantage is related to the matrix’s ability to promote the accumulation of nutrients (Flemming and Wingender 2010) and to create an extracellular digestion system made by different hydrolytic enzymes. Indeed, the hydrolytic enzymes present in the biofilm are not only a supply for the microorganisms that produce them but also become a resource for all members of the biofilm community (Worm et al. 2000; Nicolaisen et al. 2012), this phenomenon has been called the ‘social function of extracellular hydrolysis’ (Flemming et al. 2016).

2.2 Social interactions

Biofilms, as complex collective ecosystems, can regulate the behavior of the different cells via extracellular signaling. The organization of bacterial biofilms allows to a variety of organisms to interact, this enables the exchange not only of metabolites/substrate but also of signaling molecules. It is well established that microorganisms are capable of intercellular communication (Heilmann and Götz 2010; Pereira et al. 2013; Zhou et al. 2015; Dong and Zhang 2016), this can occur by the exchange of small organic molecules or proteins, and also the transmission of electrical signals (Prindle et al. 2015). The chemical signaling mechanisms imply the release of signaling molecules by bacteria in response to population size, and the sensing of these molecules by neighboring cells, to induce the coordinated expression of specific genes (Keller and Surette 2006). Therefore, this mechanism infers the accumulation of signaling molecules at high concentrations. Interestingly, in natural environments, such as the open ocean, individual planktonic cells are not supposed to meet such high concentrations of signaling molecules. The biofilm providing a confined system in which signaling molecules can be concentrated facilitates intercellular communication (Seviour et al. 2019; Redfield 2002).

Cell–cell communication and coordinated division of labor (van Gestel et al. 2015) have motivated the introduction of the anthropomorphic term ‘sociomicrobiology’ (Parsek and Greenberg 2005), in this view of biofilm community, both cooperative (Elias and Banin 2012) and competitive (Rendueles and Ghigo 2012) interactions between cells can exist. Aminoacidic auxotrophy is a classic example of cooperation behavior by which cell communities reduce the collective metabolic problem of biosynthesis (Fredrickson 2015), these mutualistic interactions explain also the exchange of amino acids and sugars (Zelezniak et al. 2015). An interesting example of the cooperation between different species in a biofilm is that of cyanobacteria and fungi in biofilms on desert rocks, in this consortium, the cyanobacteria supply nutrients for the fungi, which, in turn, release

essential metals from the rock that advantage the cyanobacteria (Gorbushina and Broughton 2009).

Competitive or neutral species–species interactions have been reported in biofilms (Foster and Bell 2012), the mechanisms that characterize the competition include the production of antibiotics, bacteriocins, extracellular membrane vesicles as weapons to reduce the competitor presence in the biofilm (Rendueles and Ghigo 2012). Although counter-intuitive to the idea of a biofilm as a collective ecosystem or a unified multicellular entity, some authors suggest that competitive behaviors may contribute to the biofilm community fitness and development (Penesyan et al. 2021). The ability of microorganisms to produce antimicrobial compounds can protect the all biofilm community against dangerous external colonizers/predators (Matz et al. 2008). Moreover, exposure to antimicrobial molecules can also induce the development of antimicrobial resistance within biofilm cells (Santos-Lopez et al. 2019) boosting their survival. In this view, the recent discovery of antimicrobial producer and antimicrobial-resistant microorganisms in a 13,000-year-old cave (Paun et al. 2021) underline that these microbial traits have evolved before the production and use of commercial antibiotics.

2.3 Protection and adaptation

It is widely accepted that biofilm formation offers protection of cells from hostile environmental conditions such as ultraviolet radiation, extreme pH, high salinity, extreme temperature, high pressure (Yin et al. 2019), and tolerance to desiccation (Flemming and Wingender 2010). In addition, biofilms have an enhanced resistance or tolerance to antibiotics and other antimicrobial agents compared with planktonic cells. Biofilms can serve as a defence against antimicrobials substantially quenching the activity of antimicrobial substances that diffuse through the biofilm (Nicole et al. 2016) in a form of inhibition identified as diffusion–reaction inhibition (Daddi Oubekka et al. 2012), which can involve chelation by the complex formation and/or enzymatic degradation of antimicrobials. In addition, slow growth within sections of the biofilm and the generation of dormant persister cells further increase the survival of cells to antibiotic actions (Stewart 2015).

Biofilms are not only a shield against external insults but can recognize and respond effectively to environmental stresses. In general, the time scales associated with different abiotic and biotic stresses can vary considerably, the stresses that occurs on intermediate time scales usually cause transient regulatory responses mediated by genetic regulation mechanisms. In *Listeria monocytogenes* biofilms, for example, considerable modulation of protein abundance is observed as a function of temperature (Santos et al 2019). Diego O. Serra and Regine Hengge (Serra and Hengge 2014)

examined how different stresses induce profound differences in biofilm physiology in terms of stratification of the matrix, microscopic architecture biophysical properties, and directly visible morphology of macrocolony biofilms.

Long-term exposure to constant stresses typically results in a process of mutation/selection that results in a progeny with new genetic characteristics. The biofilm features allow a rapid and efficient adaptation to long-term stress. Indeed, the high cell density that characterized the biofilm and accumulation of mobile genetic elements within the matrix offers an ideal set of factors for efficient horizontal gene transfer (Fux et al. 2005). In addition, the matrix provides a stable physical environment for cell-to-cell contact ideal for plasmid conjugation, indeed conjugation has been demonstrated to be more effective in biofilms compared with free-living bacteria (Kumar et al. 2017). In biofilms, the accumulation of genomic changes allow microbial evolutionary processes to occur in a quite short period, and this creates new phenotypes that have advantages within the biofilm environment (Penesyan et al. 2019), as reported in the case of the evolution of mucoid phenotypes of *Pseudomonas aeruginosa* associated with increased persistence in chronic cystic fibrosis infections (Boucher et al. 1997).

Thus, biofilms provide a relatively stable and protected microenvironment for the cells where they can change and adapt to environmental conditions via transient changes in gene expression, as well as through permanent genomic changes that are fixed in populations under natural selection. These features make biofilms unique and central to microbial evolution and adaptation.

3 Biofilm as a successful survival strategy in extreme environments

On Earth, there are environmental niches characterized by conditions described as impossible, in the belief of the common life, for any biological activity. Exploration of these extreme terrestrial environments has turned up a different variety of microorganisms that not only live but also prosper, in these inhospitable niches (Amils et al. 2007; Gasser et al. 2008). Microorganisms that can survive in these extreme environments are called extremophiles (Shu and Huang 2021), they are adapted for millions of years prospering under adverse conditions like extreme temperature, up to the boiling point of water or temperatures below the water freezing point, high radiation, acidic or alkaline pH values, heavy metal pollution, and high salinity. Numerous taxonomic studies have discovered that these organisms are distributed in the three domains of life, Archaea, Bacteria, and Eukarya, and most of the microbial communities found in extreme environments are distributed and assembled in extensive biofilms and microbial mats.

3.1 Extreme temperatures

Extreme temperature produces several challenges, from the structural damage produced by ice crystals at one extreme to the denaturation of biomolecules at the other edge, to face this extreme condition, the microorganisms evolved several peculiar adaptations.

In thermophiles, a modification of cell membranes by increasing the ratio of saturated to unsaturated fatty acids, for bacteria, or by adopting a lipid monolayer in the case of archaea was well documented (Lewin et al. 2013). Their ability to produce heat-shock proteins (Schlesinger 1990) and to maintain DNA stability by a high G + C content are reported (Lewin et al. 2013). At the other extreme, psychrophiles can grow at very low temperatures by modifying the lipid composition of cell membranes to maintain fluidity (D'Amico et al. 2006) and by producing specialized molecules like the cold-shock proteins, antifreeze, ice-binding molecules and osmolytes (Parrilli et al. 2021). The observation that in environments characterized by extreme temperature the microbial cells are frequently present as biofilm indicated that also the ability to form biofilm is a key feature for colonizing these sites. As in the case of biofilm of alkaline spring Bison Pool in Yellowstone National Park (Shu and Huang 2021) or as the Chimney biofilm of Loki's Castle (a deep-sea hydrothermal vents site) (Shu and Huang 2021; Stokke et al. 2015). A study (Coleri Cihan et al. 2017) on the growth of different thermophilic bacteria at different temperatures revealed that the cells can form biofilm and that its amount is temperature dependent. Similar works (Ricciardelli et al. 2019; Liao et al. 2016) on psychrophilic bacteria show temperature-dependent biofilm production. For Yin and co-authors (Yin et al. 2019) biofilm formation allows microorganisms to become more resilient to damage produced by temperature stress, since it acts as a "protective clothing" providing a fitting habitat for their survival in extreme temperature conditions. At high temperatures, the biofilm can withstand to the external high temperature and make the inside adapt for growth and reproduction, on the other hand, biofilm can also stabilize the inner environment when the outside is extremely cold, causing no freeze of the cells and enabling them to survive (Yin et al. 2019).

3.2 High radiation

Different environments on Earth are affected by several types of radiation, with the most extreme radiation arising from human-made radioactive-contaminated sites. Ionizing radiations as UV radiation, X-rays, or gamma rays can impact on microbial cells via direct and indirect (e.g., the formation of reactive oxygen species) actions (Greiner et al. 2012). Radiation-resistant microorganisms have been shown to survive exposure to extreme radiation (kGy), including

exposure to space conditions, for hundreds of days (De Vera et al. 2012). Microbial adaptations to radiation involve mainly genome redundancy (multiple copies of the genome), differences in DNA repair mechanisms (Byrne et al. 2014), use of smaller amino acids (Sghaier et al. 2013), production of pigments (Mojib et al. 2013), and accumulation of Mn(II) (Krisiko and Radman 2013). Several studies (Panitz et al. 2019; Elasri and Miller 1999) indicate that biofilm formation is another strategy for microorganisms to survive at radiation exposure. The presence of biofilms of naturally radioactive hydrothermal spring caves revealed that this way of life is suitable to an environment characterized by high radioactivity (Enyedi et al. 2019). Moreover, it was demonstrated by Frösler and co-authors (Frösler et al. 2017) that *Deinococcus geothermalis* DSM 11,300 biofilm is more UV tolerant than planktonic cells, the authors speculated that it is related to the ability of the biofilm matrix to retain reactive oxygen species produced by the photodissociation of water.

3.3 Extreme pH values and heavy metals

Highly acidic extreme environments are usually linked with volcanic activity and mining processes. The oxidation and dissolution of the sulfidic minerals exposed to water and oxygen are responsible for acid production (García-Moyano et al. 2012). As the same time, low pH increases the metal solubility in water, particularly cationic metals, so extremely acidic environments tend to have high concentrations of heavy metals. Several studies have identified several specific traits of acidophilic bacteria that partially explain their ability to survive at very low pH. They are characterized by a peculiar pH homeostasis strategy based on a restricted proton influx into the cytoplasm by highly impermeable cell membranes. Furthermore, acidophilic microorganisms can maintain intracellular pH using the buffering capacity of the cytoplasm to sequester or release protons. They repair DNA and protein damage caused by low pH by means of chaperones that allow rapid and efficient repair (Baker-Austin and Dopson 2007).

Most of the microbial cells found in acidic extreme environments are distributed and assembled in extensive biofilms (Souza-Egipsy et al. 2021). The chemoautotrophic biofilm within the Richmond Mine at Iron Mountain, United States, is one of the most studied biofilm evolved in highly acidic conditions (Denef et al. 2010). Studies on this biofilm, aimed at investigating the responses of acidophilic biofilm organisms to different pH conditions, revealed pH-dependent niche differentiation and pH-variation in specific functional categories of the dominant species indicating that the main parameter that shapes the community composition is the pH (Belnap et al. 2011). This is clear example of how the *physiological heterogeneity* of biofilm is a key element to survive in a hostile environment. Another feature of biofilm that is

essential in highly acidic extreme environments is the ability of the biofilm matrix to *protect* the cell from high concentrations of heavy metals. Harrison and co-author (Harrison et al. 2007) integrated the mechanisms of metal detoxification in biofilms into a multifunctional model, their results suggest that numerous mechanisms can contribute to tolerance including metabolic *heterogeneity*, metal immobilization, and complexing, reaction with siderophores, genetic mutations, and *phenotypic variations*.

Not only acidophiles but also alkaliphiles generally exist in biofilms (Li et al. 2018). The protection mediated by biofilm matrix has a crucial role also in the defense against alkaline pH, the results of Charles and co-author showed that biofilm matrix by the sequestration of calcium *protects* alkaline pH values up to 13.0.

3.4 High salinity

Hypersaline environments, usually defined as having salt concentrations more than twice that of seawater, such as salt lakes and evaporation ponds used to produce salt from seawater, are found in different sites on the Earth. They are inhabited by a great diversity of microorganisms, called halophiles, adapted to life at high salt concentrations (Oren 2015). Halophiles can maintain osmotic homeostasis by accumulating high levels of inorganic salts in the cytoplasm (Gunde-Cimerman et al. 2018), or they can achieve osmotic balance by biosynthesizing and/or accumulating organic and compatible osmotic solutes (Gunde-Cimerman et al. 2018). As with the previously described extreme environments also in the case of hypersaline environments microorganisms are mainly present in the biofilm community (Häusler et al. 2014) and the ability to produce biofilm is related to salt concentration. For example, the bacterium isolated from a saline wetland in Morocco, *Halomonas stenophila* HK30, is able to form biofilm in a medium with 5% w/v salt (Amjres et al. 2015), and *Kocuria flava* AB402 and *Bacillus vietnamsis* AB403, isolated from mangrove rhizosphere, produce a big quantity of EPS in salt stress conditions (Mallick et al. 2018).

3.5 Low water activity and oligotrophic conditions

There are myriad environments on the surface of our planet that exhibit extremes in one or more physical or chemical conditions, for example, soda lakes are characterized by alkaline pH and high salinity, while in hot spring environments, high temperature, extreme pH, low oxygen availability are present and low temperature and water availability are typical of the cryosphere.

In addition, several extreme environments are characterized by *low water activity* and *oligotrophic conditions*. Both

aspects can be faced better by microorganisms organized in biofilm with respect to cells in planktonic conditions. Indeed, biofilm matrix confers a high tolerance to desiccation, bacteria in the biofilm actively respond to desiccation by the production of EPS molecules (Weaver et al. 2015), which, due to the high amount of hydrated polymers in the EPS matrix, *protects* the biofilm from dryness by acting as a hydrogel that holds water (Flemming and Wingender 2010). Several studies have concluded that biofilm production is enhanced in poor nutrient conditions (Combrouse et al. 2013; Ricciardelli et al. 2019). These observations are explained by the ability of the biofilm to function as a complex sorbent system with different sorption mechanisms. Furthermore, in biofilm are present several binding sites that works as both anionic and cationic exchangers, which implies that a very wide range of compounds can be caught and accumulated for potential use by cells in the biofilm, even when such substances are present at very low concentrations. This ability allows biofilms to grow even in highly oligotrophic environments (Battin et al. 2016).

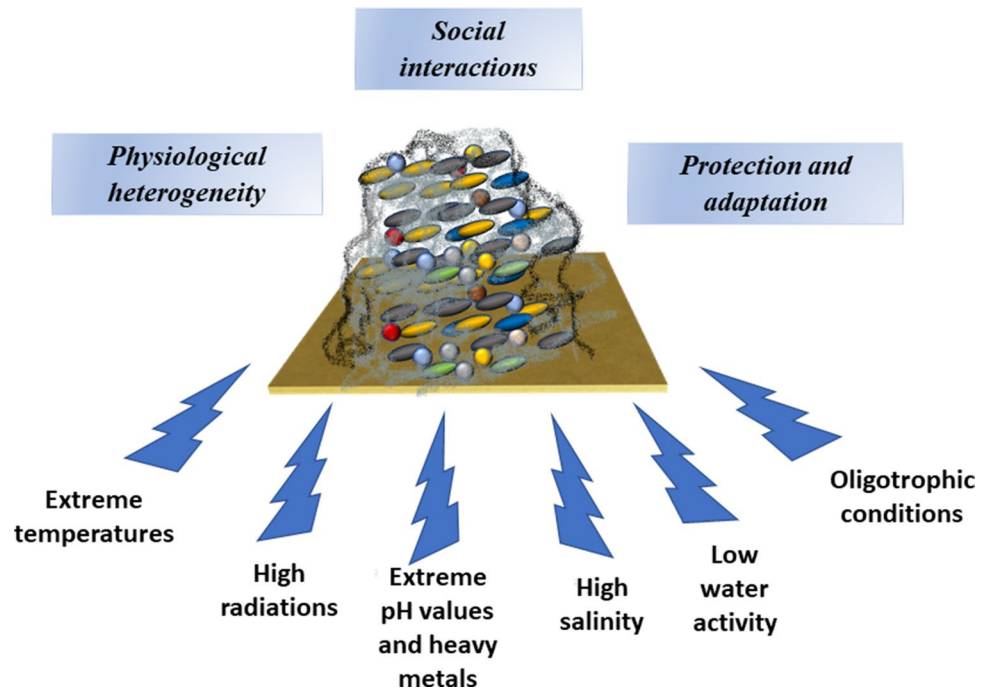
4 Conclusions

All aspects examined (Fig. 2) in this article help to explain why biofilms are a successful survival strategy in extreme conditions and why the ability to establish biofilms is a key trait for microorganisms growing in extreme environments, but also induce the reader to ask: Is Biofilm a successful survival strategy in an extreme environment or the only possible one?

An interesting paper by Blanco et al. (2019) on the EPS composition in twenty phylogenetically different benthic biofilms from different extreme environments established that biofilms isolated from different environments, sharing similar species composition, exhibited very different EPS characteristics and that microorganisms from the same environment achieve similar EPS compositions unrelatedly to their phylogeny. The circumstance that similar biofilm matrix properties are attained by phylogenetically different organisms living in the same environment suggests the action of either convergent evolution over long time scales and/or horizontal gene transfer. This observation brings attention to the role of biofilms in microbial evolution and adaptation to new environments.

As partially summarized in this review, several scientific reports have shown that the biofilm mode of life stimulates the occurrence of genotypic and phenotypic variants (Penesyan et al. 2019; McElroy et al. 2014). Such high diversity has an inherent risk as a fraction of the new individuals could not be able to survive in a given environmental condition. In this situation, the supply of a protective environment, like biofilms, offers a huge advantage by allowing the new

Fig. 2 A schematic representation of the biofilm features and its role in microorganisms ability to survive in extreme environments



generation to be protected from the external selection conditions while continuing to undergo evolutionary processes. So, biofilms provide a protective environment in which the new individuals with reduced fitness to external stress or to the environmental changes, can collect mutations that balance any negative genetic changes. In this way the biofilm helps the microorganisms to adapt and evolve (Penesyan et al. 2021). Furthermore, the ability of biofilms to create gradients of selection conditions, from the maximum environmental impact on the surface toward the minimum impact in the internal part of the biofilm, gives cells a unique opportunity to adapt before being exposed to the external environment by the process of biofilm dispersion. This process is markedly different from what occurs in other systems where the diversity is fixed without the possibility of changes and further selection before regeneration. This role of biofilms as diversity incubators where the dynamic process of generating genotypic and phenotypic variants occurs makes it the most successful strategy to colonize extreme environments and probably the only possible one.

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