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BACTERIAL BIOFILMS AND THEIR BIOLOGICAL SIGNIFICANCE

BIOFILME BACTERIENE ȘI SEMNIFICAȚIA LOR BIOLOGICĂ

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ABSTRACT | REZUMAT

It has long been thought that bacteria, which are unicellular organisms, are found in natural ecosystems only in planktonic form. But the ecologists have observed that the transformation and the circulation of matter in Nature are accomplished under the action of complex and dynamic communities of microorganism, nowadays known as biofilms. The advances in microscopy, in the use of sensors, in high-quality microprobes, but especially in the development of molecular biology techniques and fluorochrome labelling methods, made it possible to study in detail large varieties of natural habitats, knowing that in natural environments bacteria persist attached to the surface of solid supports, or associated with different types of interfaces, on which they multiply and form complex communities of cells embedded in a polymer matrix consisting of exopolysaccharides synthesized by bacterial cells existing in the structure of these communities.

In natural environments, biofilms can be constituted from vegetative cells of a single bacterial species or, as a rule, from the association of several microorganisms (bacteria, microscopic fungi, unicellular algae and protozoa). It has also been demonstrated that bacterial biofilms are biological systems with a high level of organization in which component members form functional, dynamic and specifically coordinated communities, depending on the signals the system receives from its living environment. The complex architecture of biofilm allows direct contact between cells and enables them to produce and receive self-inducing molecules of synchronizing group behaviour so that they act as multicellular organisms.

In bacterial communities organized into biofilms, especially when they are made up of several species, due to their physiological activities, complementary conditions are created for the occurrence of synergistic, neutral or, as the case may be, antagonistic relations.

In the present paper the main data from the scientific literature regarding the factors that influence the formation of bacterial biofilms on solid surfaces, the stages of biofilm formation, the structure of natural biofilms, the mechanisms involved in the regulation of the biofilm formation process, the chemical composition of exopolysaccharides existing in the biofilm structure, ecological advantages offered to biofilm-organized cellular communities, as well as their ecological importance, are presented.

Keywords: bacterial biofilms, development model, biological significance, ecological importance

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Multă vreme s-a crezut că bacteriile, care sunt organisme unicelulare se găsesc în ecosisteme naturale numai sub formă planctonică. Ecologiștii au observat însă, că transformarea și circuitul materiei în natură se realizează sub acțiunea unor comunități complexe și dinamice de microorganisme, care sunt cunoscute în prezent sub denumirea de biofilme. Progresele realizate în microscopie, în utilizarea senzorilor, a microsondelor performante, dar mai ales în dezvoltarea tehnicilor de biologie moleculară și a unor metode de marcare cu fluorocromi, au făcut posibilă studierea detaliată a unor mari varietăți de habitate naturale, stabilindu-se că în mediile naturale bacteriile persistă atașate de suprafața suporturilor solide sau sunt asociate cu diferite tipuri de interfețe, la nivelul cărora se multiplică și formează comunități complexe de celule înglobate într-o matrice polimerică, constituită din exopolizaharidele sintetizate de celulele bacteriene existente în structura acestor comunități.

În mediile naturale, biofilmele pot fi constituite din celule vegetative ale unei singure specii bacteriene sau, de regulă, din asocierea mai multor microorganisme (bacterii, ciuperci microscopice, alge unicelulare și protozoare). S-a demonstrat, de asemenea că biofilmele bacteriene sunt sisteme biologice, cu un înalt nivel de organizare, în care membrii componenți formează comunități structurate funcțional, dinamice și specific coordonate, în funcție de semnalele pe care sistemul le primește din mediul lui de viață. Arhitectura complexă a biofilmului permite contactul direct dintre celule și le oferă posibilitatea de a produce și recepționa moleculele autoinductoare sincronizării comportamentului de grup, astfel încât acestea să acționeze ca organisme multicelulare.

În cadrul comunităților bacteriene organizate în biofilme, mai ales atunci când acestea sunt constituite din mai multe specii, grație activităților lor fiziologice se creeză condiții complementare pentru aparția unor relații sinergice, de neurtalitate sau după caz, a unor relații antagonice.

În prezenta lucrare sunt prezentate principalele date din literatură referitoare la factorii care influențează formarea biofilmelor bacteriene pe suprafețele solide, etapele formării biofilmelor, structura biofilmelor naturale, mecanismele implicate în reglarea procesului de formare a biofilmelor, compoziția chimică a exopolizaharidelor existente în structura biofilmelor, avantajele ecologice oferite comunităților celulare organizate sub formă de biofilme, precum și importanța ecologică a acestora.

Cuvinte cheie: biofilme bacteriene, mod de formare, semnificație biologică, importanță ecologică

Bacteria are unicellular organisms, with a prokaryotic organization and ubiquitous way of spreading, with great plasticity and adaptability to the most varied environmental conditions. They are considered primitive autonomous biological systems with a large surface-to-volume ratio, which greatly influences the ability of cells to come always into direct contact through their cellular envelope with their living environment in order to carry out substances exchanges.

Through their biological activity, bacteria play an essential role in the transformation and the circulation of matter in Nature, which is why they are present in all natural ecosystems.

It has long been considered that vegetative cells of bacteria float freely in liquid media, are independent and indifferent to each other, predominantly in planktonic state (2, 11).

Since 1960, progress in microscopy and molecular techniques has allowed many observations to be made about the existence and lifestyle of bacteria in natural environments.

One of the first important observations regarding the ecology of bacteria in natural environments was that they multiply and persist in the form of aggregates composed of several cells that float in the mass of liquids or attach to the particles and surfaces in their living environment, where they form microcolonies surrounded by large amounts of exopolysaccharides synthesized by the bacterial cells present in the respective microcolonies (2, 3, 5).

Direct investigations by environmental microbiologists in different habitats have shown that most of the bacteria present in any habitat type persist attached to surfaces in the form of communities with a complex organization and three-dimensional architecture that functions as a consortium, within a matrix of exopolysaccharides (1, 5, 8).

It has also been demonstrated that in natural environments the accumulation and multiplication of bacteria present in a habitat are primarily initiated at the interface represented by the current phase of the microorganisms, and the planktonic existence is only a sequence of their life cycle (6).

In Nature, there are many types of interfaces on which biofilms can be formed: liquid-liquid, liquid-gas, liquid-solid, solid-gas, and solid-liquid-gas (25).

Initially, microbiologists have used the terms "dental plaque" or "extensive tartar" to denote communities of microorganisms attached to the surface of teeth or mucous membranes, and marine biologists and naval engineers used the term "biofouling" to define communities of microorganisms on the surface of ships and craft coming into contact with water, or the name "neuston" for the designation of communities of microorganisms that form at the surface of stagnant waters when environmental conditions are favourable to their multiplication (25).

Based on the scientific accumulations made between 1960 - 1970, Costerton (1978) proposed the term "biofilm" to designate the microorganism communities that attach and persist on different types of surfaces, suggesting that this is the ordinary way of life of all microorganisms in the habitats they inhabit (7).

Also, Donlan (2002) provided a widely accepted biofilm description, saying: "Biofilms are sessile communities of microorganisms, represented by cells that have irreversibly adhered to a substrate or interface embedded in a matrix of extracellular polymeric substances they synthesized, and the cells embedded in it express a different phenotype in terms of growth rate and transcription of genetic information" (9).

It has now been shown that microbial biofilms are biological systems with a high level of organization in which the members form functional, dynamic and specifically structured communities according to the signals the system receives from its living environment (5, 21).

In the aquatic environments, in the soil, on the surface of the rocks, on the leaves and roots of the plants, on the skin and the mucous membranes of all the living creatures (the mucosa of the ear, the buccal cavity, the surface of the teeth, the nasal mucosa, the sinuses, the ocular mucosa, the intestinal mucosa, the urethral mucosa, etc.), on the surface of objects made of metal, wood, plastic, of catheters and all types of implants, on the surface of the walls, etc., where more than 99% of known bacterial species are capable to develop as biofilms. (2, 5, 19, 23). In natural environments, biofilms can be constituted from the vegetative cells of a single bacterial species or, more commonly, from the association of one or more bacterial species with microscopic fungi, algae or protozoa (2, 25).

Since 1970, the research has been intensified on the formation of biofilms in natural habitats or on industrial technological surfaces from the medical and veterinary field (23).

FACTORS INFLUENCING BIOFILM FORMATION ON SOLID SURFACES

It is currently demonstrated that the formation of biofilms on solid, abiotic or biotic supports is accomplished through a complex process, involving a collective behaviour based on interdependence relationships from each community microorganism, especially when the biofilm is made up of different species (3, 15).

The collective behaviour of bacteria is a characteristic feature specific to communities that populate a certain ecological niche (e.g. soil, water, air, living organism) that occurs during their long process of adaptation to the environmental conditions the habitat offers (7, 25). Adhesion of bacterial cells to solid supports, both in natural habitats and laboratory conditions, is rigorously influenced by: the considered bacterial species (hydrophobicity of the cell wall, the presence of extraparietal structures such as flagella, pili, fimbriae, the ability to synthesize glycocalyx or capsule), the chemical composition and adhesion surface properties (texture, roughness, hydrophobicity, and the ability to form conditioned film), the volume and characteristics of the fluid in which the biofilm is formed (the volume of liquid, the velocity of the flow, temperature, quantity and type of nutrients, pH value, osmotic pressure, presence or absence of antibacterial substances, etc.) (5, 9, 18).

STAGES OF BACTERIAL BIOFILM FORMATION

The formation of a biofilm on a solid support is a complex and dynamic process which, according to its nature and depending on the support characteristics, is accomplished by one of the following two mechanisms:

- through physico-chemical forces;
- based on molecular recognition phenomena, through direct contact, between the specific surface structures of the bacterium and those of the support on which the biofilm will form.

"In situ" or "in laboratory" investigations carried out to identify the conditions on the bacterial biofilms formation in liquid media, revealed the existence of four (3) or five (2) main stages.

Conditioning of the solid support surface.

Typically, bacterial cells, along with various types of organic or inorganic molecules present in a liquid, are rapidly adsorbed onto the surface of the solid support, on which will be constituted the microorganism communities. By its chemical composition, the conditioning film changes the hydrophobicity and electrostatic charge on the surface of the support and provides the attaching bacterial cells; a rich source of nutrients and trace elements, increasing the adhesion rate.

Adhesion of bacterial cells to the surface of solid supports.

The bacterial cells present in the suspension, depending on their phenotypic characters, can move to the surface of the support by several mechanisms: by diffusion (consequence of the Brownian motion of the cells) under the action of electrostatic and electrodynamic forces, by the action of hydrodynamic forces or by active movement, due to flagella activity, especially when they are chemotactically oriented to the conditioned film where the nutrient concentration is higher.

The adhesion process is faster when bacteria have extraparietal structures (glycocalyx, capsule, flagella, pili, fimbriae), which by their chemical composition opposes the repulsive forces that could prevent the attachment process on the surface of the conditioned support (2). The adhesion of bacterial cells to a surface takes place over a short period of time in two substages: reversible adhesion, when the cells attach briefly to the surface of the support, after which they detach themselves, and then irreversible adhesion, when the bacterial cells remain permanently fixed on the surface of the support and begin to multiply (5).

Bacterial cells have been shown to adhere and fix faster on hydrophobic, non-biased surfaces (plastic, Teflon), as compared to hydrophilic surfaces (glass, stainless steel) (5, 7, 20).

Colony formation and biosynthesis extracellular polymeric substances.

Shortly after the irreversible fixation of planktonic cells on the surface of a conditioned support, they begin to multiply, initially at the liquid-solid-air interface, forming colonies of different sizes, in the form of islands, which will gradually expand over the entire surface of the solid support. During the multiplication process, bacterial cells synthesize exopolymeric substances. Following direct contact between cells and intense metabolism, biochemical signals regulating cell density and initiation, where appropriate, of phenotypic changes occur within the formed cellular community (3).

Biofilm maturation.

As a result of the continuous multiplication of bacterial cells, they come in direct contact with each other through the cell wall; biofilm sizes increase by overlapping the cells in multiple layers, and the biofilm gets a three-dimensional structure and is surrounded by an exopolymeric structure called the mantle. In the last phase of this stage, there is a very large number of cells in the biofilm mass (about 10^{14} UFC/cm^3), and the biofilm can also be observed with the naked eye (5, 7).

Detachment and dispersion of cells from biofilm.

As the bacterial cells inside the biofilm reach the end of the latent phase, and environmental conditions become unfavourable to multiplication (nutrient depletion, oxygen depletion, increased metabolites, shear forces due to hydrostatic conditions, cessation of exopolysaccharide synthesis), living bacterial cells or even biofilm fragments detach and disperse in the medium to fix on new surfaces where they form new biofilms (6).

STRUCTURE OF MATURE BIOFILMS

For a long time, biofilms obtained under laboratory conditions of different types of surfaces were examined with a high resolution electron microscope, but the obtained results did not offer satisfactory results because the preparations to be examined had to be dehydrated, a process that alters the biofilm architecture. At present, for the study of the bacterial biofilm structure, the laser microscope method and confocal scanning are used, for which the samples should not be dehydrated, and the images highlight the three-dimensional structure of the biofilm. This type of microscope can also be used to study the events that occur in each of the development stages of a biofilm when it is formed in special devices called flow cells (4, 16).

The comparative analysis of the three-dimensional structure of "in vitro" biofilms, obtained from a single bacterial species and those formed in natural ecological niches from several microbial species, has shown that most examined biofilms are very similar in structural terms (17).

In both types of biofilm, the bacterial cells were placed in piles, included in an exopolysaccharide matrix, alternating with less dense areas, distributed across the entire biofilm mass constituting a complex channel system through which water circulates. These channels ensure in the biofilm the circulation of nutrients and gases as well as elimination of metabolites released by bacterial cells (1).

At present, in order to study in detail the metabolic activity carried out by the existing bacterial cells in the biofilm, molecular biology techniques based on PCR (Polymerase Chain Reaction) amplification of ribosomal DNA, techniques combining “in situ” fluorochromic hybridization have been used, along with the use of microprobes for the determination of pH profiles, oxygen concentration or the use of microsensors for monitoring concentrations of oxygen, nitrates, nitrites, sulphides, etc (1, 4).

MECHANISMS INVOLVED IN ADJUSTING THE BACTERIAL BIOFILM FORMATION PROCESS

Research on "in vitro" or "in situ" biofilm formation in recent years has shown that all cells present in their mass express the same phenotype, but this is different from the phenotype of bacterial cells of the same species, which are in suspension (3, 17).

Bacterial cells seized inside the matrix of exopolysaccharides come in direct contact with neighbouring cells and release self-inducing signaling molecules that synchronize and intensify the metabolic reactions of all biofilm cells that will react as a multicellular organism. The concentration of self-inducing molecules always correlates with the population density of biofilm cells, a phenomenon known as quorum (2, 10). Self-inducing molecules are captured by the receptors present on the surface of the bacterial cell wall, and are involved with the control of activation of sets of genes existing in the genome of the bacterial cells in the biofilm (10, 24). The assembly of signalling molecules that is synthesized in the cell population, upon reaching the quorum, forms the intercellular communication system. This communication system is theoretically made up of two subsystems called "Quorum sensing" and "Quorum quenching" (2, 10, 19). It has been shown that "Quorum sensing" in Gram-negative bacteria is represented by the main self-inducing molecules that make possible to communication between the cells of a population, that is acyl-homoserine-lactones, while in Gram-positive bacteria, cell density control, after reaching the quorum, is accomplished by modified oligopeptides (2, 15, 24). “Quorum quenching” control system is the set of molecules and mechanisms capable of limiting or abolishing intercellular communication from cells in biofilm (14, 22). Recent research has shown that certain types of enzymes or molecules can stimulate or block intercellular communication in the biofilm and such molecules can be used in microbiological practice either to stimulate the biofilm formation or to prevent their formation in the food industry or medicine (10, 22, 24).

THE CHEMICAL COMPOSITION OF EXISTING EXOPOLYSACCHARIDES IN THE BIOFILMS STRUCTURE

Exopolymers present in the biofilm structure can constitute between 50% and 90% of the organic carbon present in its chemical composition (1, 2).

In the chemical composition of exopolymers existing in the biofilm structure, both organic components and inorganic compounds are found in varying amounts (13, 17).

The main organic components are saccharides, glycoproteins, lipoproteins, enzymes,nucleic acids and humic substances (17). Polysaccharides consist of macromolecules formed by the polymerization of identical units or very similar units. Alternatively, in the chemical composition of the biofilm matrix, non-polymerized saccharides with low molecular weight are present (1, 13, 18). Most researchers claim that polysaccharides and enzymes are the major constituents of the biofilm matrix. Exopolysaccharides in the biofilm structure, with the exception of dextran and levan molecules, as well as all types of enzymes, are synthesized inside bacterial cells in the biofilm and are eliminated outside cells by different mechanisms (16).

ECOLOGICAL ADVANTAGES FOR CELLULAR COMMUNITIES ORGANIZED IN BIOFILM

The ability of bacteria to colonize abiotic or biotic solid supports and to organize themselves in the form of biofilm at the habitats they populate can be considered as one of the indispensable conditions for their persistence and survival against the unfavourable action of environmental factors. Through its physicochemical properties, the biofilm matrix seizes the cellular community, constituting a microniche, where it shows a different behaviour and physiology compared to bacterial cells found in the planktonic state.

Exopolysaccharides in the biofilm structure concentrate on their surface nutrients and growth factors in the environment where bacteria live, simplifies them with the help of exogenous enzymes and transfer them through the channels inside the biofilms to all the constituent cells. By acting as an ion-exchange part, the exopolysaccharides in the matrix composition physically impede or restrict the access of antimicrobial agents (antibiotics, disinfectants) inside of the biofilm.

Exopolysaccharides present in the biofilm structure protect bacterial cells from ultraviolet rays, from pH changes, from osmotic shock, but especially from dryness (2). It has been reported that biofilm exopolysaccharides have the capacity to concentrate cations, toxins and metals (copper, arsenic, cadmium, lead, mercury, zinc, cobalt, cesium and strontium) possibly present in an ecosystem (2).

It should also be emphasized that the complex biofilm architecture allows direct contact between cells and enables them to produce and receive self-inducing molecules to synchronize group behaviour so that they act as multicellular organisms (10, 19).

The microclimate offered to cellular communities at the biofilm level as well as the high cell multiplication rate favour the transfer of genes for the acquisition of resistance to the action of antimicrobial agents to increase pathogenicity (8, 19, 22).

THE ECOLOGICAL IMPORTANCE OF BIOFILMS

Bacterial communities, organized in biofilms, especially when they are made up of several species, play an essential role in the circulation of matter in Nature, on the one hand, as a consequence of the availability of nutritional resources, and on the other hand due to their physiological activities complementary conditions are created for the emergence of synergic, neutral or, where applicable, antagonistic relationships (7, 17).

Besides the positive effect that biofilm bacterial communities have in various fields of activity (natural gas production, waste water treatment, elimination of biodegradable pollutants, soil enrichment in nutrients assimilable by plants, obtaining food, and development of medicines, etc.), bacteria present in the biofilm structure lead to important economic losses in industrial sectors that are experiencing metal biodegradation processes, and in the agro-food sector also to the reduction of nutritional qualities of food or water contamination, and food with pathogenic bacteria capable of causing the consumer infections or food poisoning (12, 20). In the medical and veterinary field, the formation of biofilms creates particular problems in the emergence and treatment of infectious diseases in humans and animals (14, 19, 20, 23).

Although progress has been made so far in the treatment of bacterial infectious diseases, standard antibiotic therapy is capable of producing the healing of the patient only when the involved pathogen is in the planktonic state. In addition to the fact that the bacterial cells embedded in the biofilm exopolysaccharide matrix cannot be influenced by the action of humoral or cellular effectors of natural or acquired immunity, they have many mechanisms that prevent the penetration of the antibiotic into the biofilm or synthesize enzymes capable of neutralizing the biocide's activity before it can cause damage to the cell wall or to other structures inside the cell (1, 3, 11).

The bacterial communities present in the biofilm structure are 10 to 1000 times more resistant to the action of antimicrobial substances, and 65% of infections occurring in humans is produced by these bacteria (2, 3).

The most common infections of humans and animals produced by biofilm-based bacteria are those associated with the existence of an implant (e.g., contact lens keratitis, endocarditis associated with intravascular catheters, bacteraemia associated with urinary catheters, etc (10, 14, 23).

It has also been demonstrated that bacterial biofilms can be organized and cause infections in the mucous membranes or internal organs, infections that usually evolve chronically (e.g., otitis media, periodontitis, prostatitis, cystitis, pneumonia of persons with cystic fibrosis) (23).

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