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REVIEW

Structural and Functional Characteristics of Bacterial Biofilms

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Abstract—Data on the structural and functional traits of bacterial biofilms are presented. The structures involved in different stages of biofilm formation are discussed in detail. Techniques for biofilm modeling and visualization are considered. Results of light and electron microscopic investigations are analyzed. Original data on microscopy of biofilms are presented. The possible formation of dormant and nonculturable forms in the biofilm is discussed.

Key words: biofilms, bacteria, spores, flagella, pili, matrix, microscopy.

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Bacteria exhibit two different behavioral strategies: either free movement, swimming, or a planktonic state, in which individual cells move freely in liquid medium, or an attached state, in which they are tightly pressed to each other and to the surface. Most works in bacterial biochemistry, genetics, and physiology have been based on the assumption that bacterial populations consist of individual cells with identical characteristics or of colonies originating from a single cell. Development of microscopy and molecular genetic techniques promoted the understanding that natural bacterial populations exist mostly in an attached state, in which bacteria may freely exchange signals and exhibit coordinated activity, similarly to the tissues of multicellular organisms. Development of biofilm communities is one of the main strategies for bacteria survival in a certain ecological niche. In the attached state, bacteria integrated into the biofilm are protected from damaging environmental factors and, in the case of infections, from antibacterial preparations applied to the host organism.

A biofilm is presently seen as an uninterrupted multilayer of bacterial cells attached to the surface and to each other and embedded into a biopolymer matrix. Intercellular communications in the biofilm include, among others, the regulatory mechanism termed quorum sensing (QS) [1, 2]. Information exchange involves specific signal molecules, which ensure that the microbial community reacts as a single organism. Since the mechanisms of functioning on microbial communities, including biofilms, presently attract increasing attention, the problem of regulation of bacterial activity via the QS system has been discussed in numerous reviews.

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Special attention is nowadays paid to the role of biofilms in the environment. Numerous problems in medical practice and various areas of economic activity result from the ability of microorganisms to form biofilms on every biotic and abiotic surface. Biofilms are presently known to be among the pathogenesis factors in formation of chronic infectious processes [3]. This is specifically the case for diseases associated with implanted devices (catheters, prosthetics, or artificial heart valves). In natural habitats, biofilms may cause considerable deterioration of the ecological situation. For example, a cyanobacterial community under natural conditions forms a film on a water surface, resulting in undesirable changes in water quality. Due to low sensitivity of biofilm bacteria to biocides and the resulting possibility of water infection with pathogenic microorganisms, biofilms present a serious danger to the drinking water supply. Biofilm formation causes technical problems, decreasing the efficiency of equipment such as oil pipelines, industrial pipelines, etc. Biofilms contaminate the hulls of ships and oil platforms, causing metal corrosion.

However, apart from the negative consequences of microbial colonization, positive aspects of practical application of biofilms, including removal of toxic compounds and manufacture of the products of microbial synthesis, also exist. Another possible application of microbial biofilms is based on their ability to suppress ascidia and algae, which grow on ship hulls [4]. The biofilms formed by bacteria with pronounced antagonistic properties may act as an alternative to toxic chemical protective coatings. The data on *Bacillus subtilis* biofilms suppressing growth of phytopathogens on plant roots are of great interest [5]. Biofilm-forming capacity was shown to be important for attachment of antagonistic bacteria in the rhizo-

sphere. These data certainly do not exhaust the vast and intensely studied issue of biofilm microbial growth. We provide references to reviews dealing with the role of biofilms in various fields of science and practice and demonstrating the recent increase in interest in these microbial communities [6–9].

Biofilm research is developing in several directions. One of them is microscopic investigation in situ, promoting development of an integrated concept of biofilm structure. All biofilm components are closely interrelated and are not easily separated for analysis by other techniques. Light microscopy, especially in combination with cytochemistry and immunocytochemistry, provides the possibilities for nondestructive investigation of biofilms. Epifluorescence microscopy is also used for investigation of biofilms on the surface of various objects. Transmission and scanning electron microscopy (TEM and SEM), confocal laser scanning microscopy (CLSM), and atomic force microscopy (AFM) have been recently applied for biofilm research. Microscopic techniques resulted in successful investigation of the structural and functional characteristics of biofilms, including the cell structures involved in biofilm formation.

STRUCTURES INVOLVED IN FORMATION OF BIOFILMS AT DIFFERENT STAGES OF DEVELOPMENT

Bacterial biofilm is formed as a result of complex coordinated interactions of microorganisms with the surface. The stages of biofilm formation have been examined in a number of publications [9–11]. Generally, the sequence of events is as follows: initial attachment to the surface, formation of a monolayer, movement along the surface with formation of microcolonies, and biofilm maturation and formation of a three-dimensional structure. The surface structures of bacteria (surface projections, flagella, and pili) participate in biofilm formation and provide bacterial motility, which is important for biofilm formation. Mutants with impaired motility are unable to form biofilms [12, 13]. Several types of bacterial motility are known. Swimming and swarming require flagella, while the type IV pili are responsible for twitcing motility. At the initial stage of biofilm formation, directional movement of bacteria by means of flagella results from the presence of attractants. The role of flagella is not limited to motility; they were found to participate in adhesion as well [14].

Adhesion is the key moment in biofilm formation. Data on adhesion or adsorption of bacteria on various surfaces are presented in several reviews [15–17]. Initial contact of bacteria with a solid surface does not imply final attachment. The first, reversible, stage of adhesion is a complex of physicochemical interactions. Adhesins involved in counteracting the forces of repulsion between similarly charged surfaces participate in transition to the irreversible stage of biofilm formation. Bacterial adhesins fall into two major groups. The first includes adhesins related to organelles (fimbriae); the second comprises adhesive factors of nonfimbrial nature. The nonfimbrial group includes exopolysaccharides and other compounds. The cell wall *S* layers consisting of ordered protein or glycoprotein units are also considered as adhesins [18]. Fimbrial adhesins are intensively investigated by microscopic techniques. Electron microscopy of fimbriae is usually carried out by negative staining and shadowing (Fig. 1a). The major classes of fimbriae of gram-negative bacteria include conjugative F fimbriae (F pili), IV type fimbriae (toxin-coregulated fimbriae), fimbriae organized by extracellular precipitation (curli), and fimbriae requiring chaperons for proper assemblage conformation [19]. The fimbriae or pili are most fully investigated in *Escherichia. coli*. *E. coli* cells are known to express pili of several types simultaneously. The pili of type 1, which were found, apart from *E. coli*, in *Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella* spp., *Serratia marcescens, Shigella flexneri*, etc. Type 1 pili facilitate bacterial adhesion to the substrate and ensure intracellular contacts. They are elongated structures of the protein pilin, 1.5 μm long and 5–10 nm in diameter with peritrichous localization. *E. coli* possesses also the surface structures termed curli [19]. The curli-shaped protrusions are thin flexible fibers (4–7 nm) of amyloid nature [20]. The amyloid has a fibrous structure and is stained with Congo red. Bacterial amyloid-like proteins also bind to Congo red, which is used for detection of this type of pili. *Salmonella* express pili similar to those of *E. coli*, including curli or thin aggregative fimbriae (*Tafi*), which are the most significant structures for biofilm formation, providing for stabilization of intercellular interactions [21, 22]. The role of *Tafi* in attachment of salmonella to plants, multicellular behavior, and retention of a natural nidus of salmonellosis was demonstrated. Type IV pili form a special group. Unlike type 1 pili, they are important for biofilm formation at the stage of microcolony formation. Type IV pili belong to types IVa and IVb, differing in the length of their signal peptide [21]. In *E. coli*, bundle-forming type IV pili (BFP) were found [23]. Unlike other types, type IV pili are responsible for flagella-independent motility and provide for twitching motility of attached bacteria. The *P. aeruginosa* mutants deficient in type IV pili formation are adhered to the surface, form a monolayer, but do not produce microcolonies and biofilms. Type IV pili of *P. aeruginosa* are 0.5–7 μm long and 4–6 nm in diameter [24]. The presence of various types of fimbriae is known also for *Burkholderia*, bacteria, which are common in natural environments. The role of *B. cepacia* in pathogenesis of lung diseases in patients with the cystic fibrosis genetic disease is presently establihsed. Biofilm formation after lung infection in such patients is a serious problem in modern infection pathology. Several morphological variants of pili were found in different

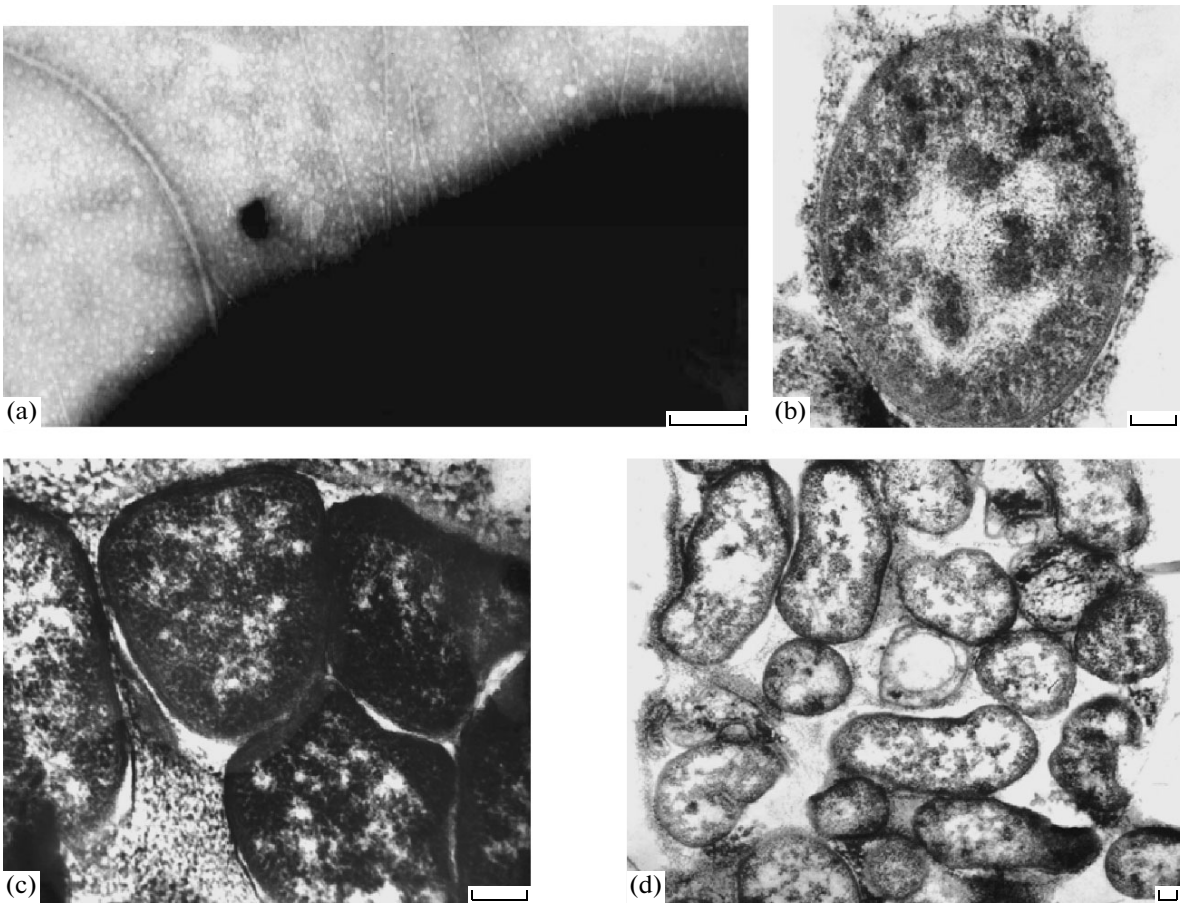

Fig. 1. Electron micrographs of *S. typhimurium*: fragment of a planktonic cell with flagellum and fimbriae (negative staining) (a), planktonic cell (b), biofilm on the broth surface (c), and biofilm on plastic (ultrathin sections) (d). Scale bar is 100 nm.

B.cepacia strains, including *Cbl* pili, which form cablelike structures and promote formation of cell aggregates [25]. The variety of pili types in these bacteria is associated with colonization of different econiches. Pili involved in biofilm formation have been recently discovered in gram-positive bacteria [26]. Contact with a surface affects expression of flagella and pili. In some gram-negative bacteria, surface-induced expression of the *laf* genes was revealed. These genes are responsible for the synthesis of lateral flagella, correlating with decreased motility by means of the polar flagellum [27]. In *E. coli* attached to a plastic surface, curli are produced simultaneously with the loss of ability to form flagella. This change of the type of cell protrusions decreases cell motility and increases the capacity for adhesion [28]. A number of bacterial cell functions are known to be induced in the attached state. Detection of the pili used for bacterial adhesion to various surfaces is important for the selection of antiadhesive means to counteract biofilm formation at an early stage. However, molecular mechanisms of bacterial interaction with surfaces of various natures are poorly known.

Matrix, the Major Biofilm Component

Subsequent development of the biofilm requires formation of a matrix, a product of cellular metabolism. A matrix is the major structural component of the biofilm [29]. Exopolysaccharides form a significant part of the matrix. Similarly to eukaryotes, in prokaryotes polysaccharides form the surface layer of the cell envelope (glycocalix). In bacteria, exopolysaccharides form capsules and mucous layers and may be released into the environment. In the course of biofilm maturation, significant amounts of extracellular polymer substrate (EPS) are produced, which binds the neighboring cells and forms the matrix. Exopolymers and bacterial cells are responsible for 85 and 15% of the biofilm mass, respectively. Thus, bacteria in a biofilm are embedded into a polymer matrix and the matrix's characteristics determine the interactions inside the cell community and those with the environment. While, in different bacterial species, the matrix differs in physical characteristics and chemical composition, it is, however, usually an anionic polymer. The matrix EPS are mainly homo- and heteropolysaccharides. The EPS contains uronic acids (mostly glucuronic) and aminosugars. The EPS composition for some bac-

Properties and functions of the extracellular polymer substrate (EPS)

Function of the EPS component	Nature of the EPS component	Role in the biofilm
Structural	Neutral and acid polysaccharides	Structural component
	Amyloids	Structural component
Sorptional	Charged or hydrophobic polysaccharides	Ion exchange, sorption
Activating	Extracellular enzymes	Polymer degradation
Surface-active	Amphiphiles	Surface-active
	Membrane vesicles	Export from the cells, sorption
Informative	Lectins	Specificity, recognition
	Nucleic acids	Genetic information, structure
Nutritive	Various polymers	Sources of C, N, and P

teria is presently known. *P. aeruginosa* was shown to produce alginate, a copolymer of mannuronic and glucuronic acids [30]. Alginate is also synthesized by *P. fluorescens, P. mendocina*, and *P. putida*. In *E. coli*, the matrix includes colanic acid [31]. The strain *Vibrio cholerae* 01 TS1-4/R synthesizes EPS containing *N*-acetyl-D-glucosamine, D-mannose, 6-deoxy-D-galactose, and D-galactose [32]. *B. cepacia* strains synthesize cepacian, a branched polysaccharide consisting of repeated heptapolysaccharide subunits containing rhamnose, glucose, glucuronic acid, mannose, and three galactoses [33]. Various enterobacteria, including *E. coli* and *Salmonella* spp., synthesize cellulose. In the *Salmonella* biofilm, cellulose and aggregative fimbriae are the major components of the biofilm [34]. Apart from cellulose, colanic acid is present in the salmonella biofilms. The differences were revealed in formation of the salmonella biofilm on plastic and on the surface of eukaryotic cells [35]. With mutation in the *wcaM* gene (responsible for colanic acid biosynthesis), biofilm formation was impaired only on the surface of eukaryotic Hep-2 cells, while mutation in the *yhjN* gene (responsible for cellulose synthesis) prevented biofilm formation both on Hep-2 cells and on plastic. Apart from exopolysaccharides, proteins, nucleic acids, lipopolysaccharides, lectins, and minerals are required for formation of a mature biofilm.

Lectins play an important part in the intercellular interactions related to biofilm formation. The roles of a polysaccharide, protein, and an extracellular polymer, polyglutamic acid, as components of the matrix have been discussed [36]. Biofilm formation in bacilli is presently an object of intense study. In *B. subtilis* biofilm matrix, three proteins were recently found, encoded by a three-gene operon *yqxM-sipW-tasA* [36]. Microscopy revealed that the *eps* and *tasA* mutants form a weak unstructured biofilm, while the double mutants did not form a film at all. Thus, the presence of both polysaccharide and protein in the matrix is required. The functions of the matrix are diverse. Together with the "skeleton" function providing for stability of the biofilm, the protective function is the most important.

The matrix EPS is known to protect cells from antibacterial agents and damaging environmental factors (UV light, radiation, pH changes, osmotic shock, or drying). EPS sorbes metals and minerals, dissolved organic compounds, it concentrates nutrients, enzymes, and growth factors. In econiches where elimination is possible, the matrix EPS fixes the bacterial cells.

The role of the matrix in formation of polymicrobial biofilms was established. The interaction of bacteria with a surface results in surface colonization by a single bacterial species. Formation of a biofilm favors the capture of other bacteria, which are incapable of primary colonization, but possess a glycocalix similar in composition to that of attached bacteria. In mixed bacterial populations, coaggregates often occur resulting from cell agglomeration due to EPS. Lectins play an important part in selective coaggregation of bacteria. Coaggregation is important for microbial communities. It both promotes formation of mixed biofilms containing different synergistic bacteria and "cleans" the medium from pathogenic bacteria in the course of interaction with their bacterial antagonists. The known components of the matrix and their functions are presented in the table.

Methods of Biofilm Visualization

Methods for obtaining biofilms in artificial systems exist presently, facilitating their investigation under controlled conditions. Biofilm formation is usually modeled in 96-well plates. The biofilms are quantified spectrophotometrically by determination of cell-bound dye in the biofilm [37]. This method makes it possible to analyze the genetic mechanisms of biofilm formation in parallel with light and electron microscopy of the biofilm on the well surface. Biofilm formation is modeled also in flow chambers, test tubes, on cover slips, and on other surfaces. Fixed preparations stained with gentian violet or other stains are analyzed.

Phase contrast and interference microscopy are used for investigation of live cells. For stained preparations, the dyes are used that are specific for the matrix as the main biofilm component. Among these is the vital stain Congo red, which binds to cellulose and curli pili when staining salmonella biofilms. Congo red is therefore used to select the strains capable of multicellular behavior according to the characteristic morphotype *rdar* (*red, dry, rough*) [38]. Visualization of both the matrix and the cells is achieved by staining with Congo red and fuchsine [39]. Congo red interacts also with the structured protein *S* layer of the cell wall [40]. Calcofluor, a fluorescent vital stain, is also used for screening as a cellulose indicator. Comparative analysis of calcofluor staining and capacity for biofilm formation in *S. enteritidis* environmental isolates demonstrated that this test was applicable for assessment of the biofilm-forming capacity [41]. Calcofluor is recommended for selection of azospirilla mutants in exopolysaccharide synthesis [42]. Congo red and calcofluor are used for detection of biofilms formed by various bacteria on various types of surfaces. Apart from Congo red and calcofluor, ruthenium red and alcian blue are used, the stains interacting with mucopolysaccharides. Biofilms on opaque objects (plastic, stainless steel, rubber, etc.) are visualized by epifluorescence microscopy. Fluorescence microscopy with the stains revealing proteins, nucleic acids, lipids, or calcium makes it possible to determine the chemical composition of the biofilm. The presence of live bacteria is established with the Live/Dead stain [43, 44]. Knowledge of the matrix composition is important to counteract mature biofilms. Analysis of complex investigations involving microscopy may make it possible to recommend specific enzymes and chemical preparations to degrade the matrix. Investigation of biofilms by transmission electron microscopy (TEM) under standard conditions does not visualize exopolysaccharides in thin sections. In electron microscopy of biofilms, for exopolysaccharide visualization by Luft, ruthenium red is used, which interacts with osmium tetroxide, a component of the fixative [45]. This method reveals the reaction product at the external side of the cytoplasmic membrane, mostly on the cell surface. Ruthenium red was initially used for investigation of the biofilms formed on implants [46]. It is presently applied for exopolysaccharide staining in biofilms of any origin formed either on inert surfaces or in biological material. By staining with ruthenium red and alcian blue, external polysaccharides were revealed in a number of bacteria [47]. EPS is a highly hydrated product that is difficult to visualize by standard electron microscopic techniques involving dehydration and drying. In TEM, the stains not only enhance the EPS contrast, but also stabilize it. Application of the freeze-substitution technique resulted in good preservation of this material in *P. aeruginosa* PAO1 [48].

In some cases, biofilms are visualized on ultrathin sections even without staining. This technique reveals biofilm structures invisible by other methods. For example, ultrathin sections were used to investigate the biofilm formed by bacteria under different conditions [49, 50]. Cryotransmission electron microscopy (cryo-TEM) was suggested for visualization of the native hydrated structures in the biofilm [51]. Scanning electron microscopy (SEM) is widely used in the study of biofilms. However, sample preparation for SEM includes drying and metal coating, which inevitably result in artifacts. The dehydration-sensitive external exopolymer changes significantly, forming tubular structures or finger-shaped extrusions [32, 52]. According to the images, the matrix in salmonella may sometimes be preserved by curli pili [53]. For stabilization of the glycocalix, staining is used for SEM investigation of biofilms [54].

Accumulation of data on the distribution and role of biofilms in natural processes, medicine, and industry required new methods for their investigation. Confocal scanning laser microscopy (CSLM) is presently used for biofilm visualization. It enables direct observation of native biofilms. For observation of biofilm formation under CSLM, flow chambers, systems with continuous flow of the nutrient medium, are used. The chambers are equipped with windows for direct observation of formation and development of an undisrupted biofilm. Research in flow chambers demonstrated that biofilms are polymorphic and adapt structurally to changing nutrient content. CSLM, in combination with other methods, makes it possible to observe bacteria and the matrix simultaneously. For example, CSLM with fluorescent dyes makes it possible to visualize bacteria stained with propidium iodide and matrix polysaccharides bound to Con A lectin conjugated with FITC [55]. Lectins of different carbohydrate specificities conjugated with fluorochromes are used for analysis of biofilm composition. For example, CSLM with labeled lectins was used to study the biofilm formed by *Deinococcus geothermalis* on stainless steel [56]. The biofilm interacted intensely with the WGA lectin, indicating the presence of *N*-acetyl-D-glucosamine and *N*-acetyl neuraminic (sialic) acid. Currently, for cell visualization by epifluorescence or CSLM, bacteria are labeled by insertion into the chromosome (by means of a plasmid vector) of a sequence encoding a fluorescent label (e.g., green fluorescent protein (GFP)). Direct in vivo observation of GFP expression in individual cells and cell populations is possible in situ. GFP and its analogues are highly convenient reporter proteins. GFP-labeled salmonella were used to investigate the distribution of bacteria embedded in a matrix of the *rdar* morphotype colonies and of mutants forming no matrix, while labeled *Enterobacter agglomerans*, for investigation of mixed biofilms [34, 57].

Atomic force microscopy (AFM) is presently used in biofilm research. AFM was used to investigate the

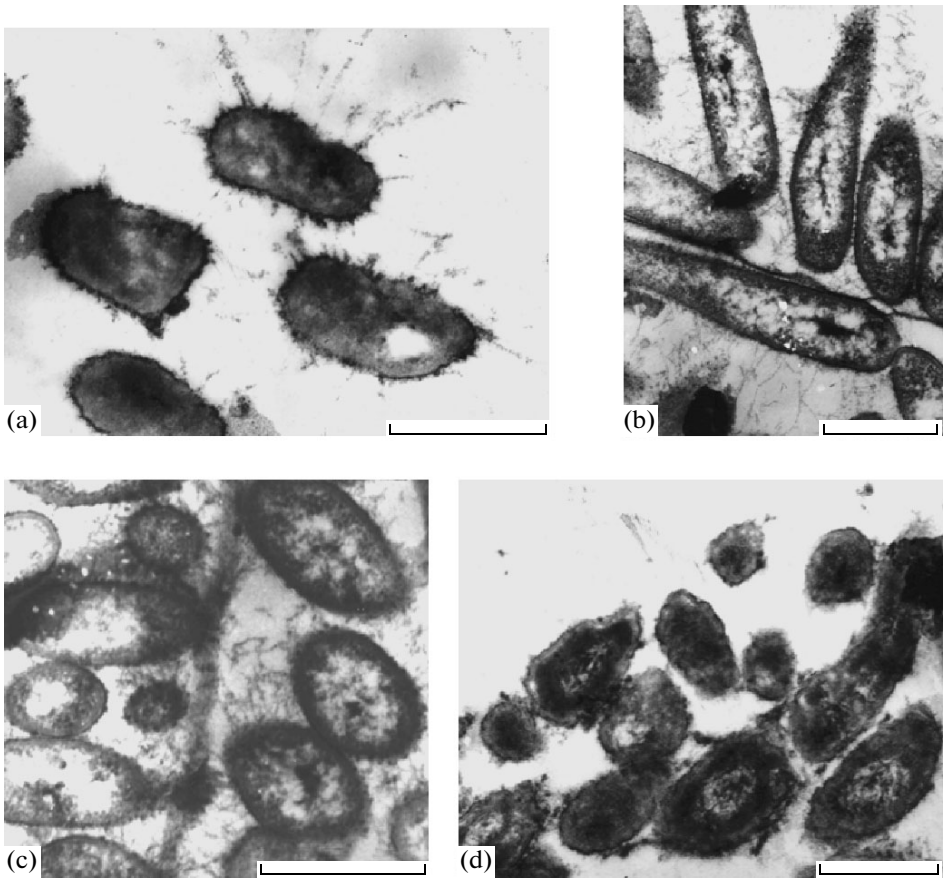

Fig. 2. Electron micrographs of *S. typhimurium*, staining with ruthenium red: planktonic cell (a) and biofilm on plastic (ultrathin sections) (b–d). Scale bar is 1 μm.

colonies and biofilms of salmonella synthesizing cellulose, Bap A surface protein, and curli pili [58].

Morphology of Mature Biofilms

A model of biofilm constructed based on CSLM data is presently accepted, wherein the biofilm consists of mushroom-shaped formations including microcolonies and exopolymeris matrix, where liquid-filled channels are located. This model agrees with the results obtained by electron microscopy. Our electron microscopic research of biofilms revealed some characteristics of their structure. Analysis of ultrathin sections demonstrated that, unlike planktonic cultures, in biofilms, exemplified by those of *Salmonella* and *Burkholderia*, morphologically altered bacteria were common. A planktonic cell of *S. typhimurium* is presented in Fig. 1b, and cells forming films on broth surface and on plastic are shown in Figs. 1c and 1d. Planktonic cells stained with ruthenium red exhibited radially divergent fibrils not connecting the neighboring cells (Fig. 2a), while on samples of the biofilm on plastic, fibrillar material tropic to ruthenium red was observed, which promoted cell contacts (Figs. 2b–2d). In a *B. cepacia* biofilm, apart from typical cells similar to planktonic ones, unusual, smaller cells with electron-dense cytoplasm were present, morphologically comparable to uncultured cells of *S. typhimurium* (Fig. 3c) [59, 60]. For *Salmonella* and *Burkholderia* biofilms, dense packing of the cells is usual, unlike planktonic cultures with individually located cells. Such dense cell packing has been previously reported for salmonella biofilms [41]. This location of the cells in the biofilm favors intercellular communication and transmission of intercellular signals, as well as more successful exchange of genetic information [61]. The presence of numerous extensive membrane structures found previously in *Neisseria gonorrhoeae* biofilms [62] and found by us in *Burkholderia* (Fig. 3d) is another characteristics of biofilms. These structures probably have a barrier function and provide for the mechanical hardness of the biofilm required for its structural integrity. Research on ultrathin sections of biofilms formed by binary or multispecies bacterial communities is promising. Ultrastructural analysis (TEM) makes it possible to reveal the traits of cell interactions in mixed cultures of antagonistic bacterial strains and sensitive cultures [63]. Bacteria in biofilms often possess membrane bubbles or vesicles [64]. In some cases, membrane vesicles act as virulence factors

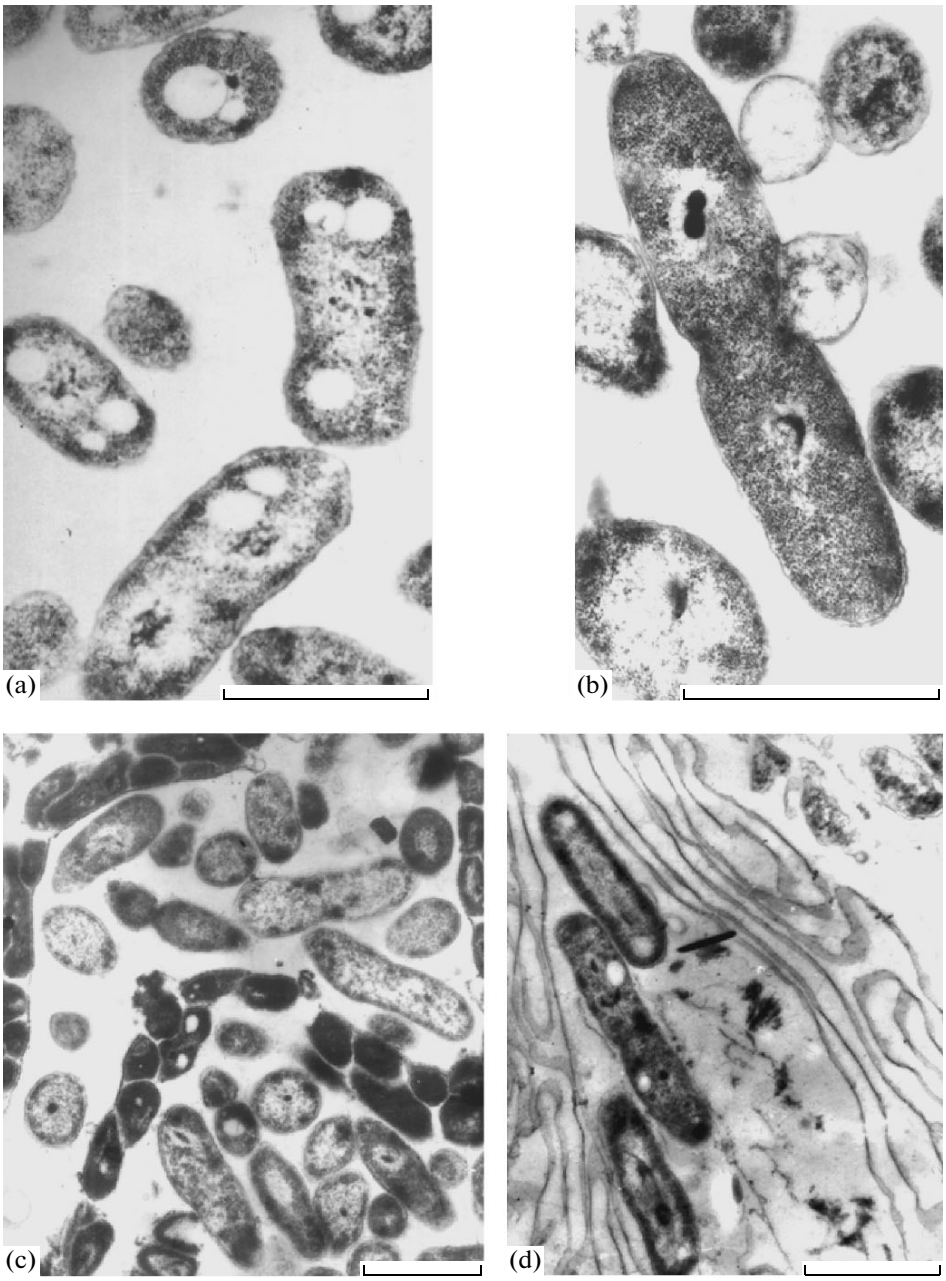

Fig. 3. Electron micrographs of *B. cepacia*: planktonic cells (a) and biofilm on plastic (ultrathin sections) (b–d). Scale bar is 1 μm.

[65]. On ultrathin sections, we revealed membrane vesicles in a *Burkholderia* biofilm on a plastic surface (Fig. 3b). The vesicles of *P. aeruginosa* PAO1, which contain protease, alkaline phosphatase, phospholipase C, and peptidoglycan-lysing enzymes, have been studied in detail. The periplasmic hydrolytic enzymes to be transported to the surface are packed into bubbles. The main function of bubbles in biofilms is probably lysis of some cells, which serve as sources of growth factors for the remaining community members. Survival of a multicellular community in a single ecological niche requires an altruistic strategy, and biofilms indeed exhibit primitive altruistic behavior [66]. Differential expression of the genes of the members of a biofilm community indicates that individual members may perform different functions, enhancing the survivability of the whole community.

A mature biofilm is known to disintegrate and become disperse after some time, with its members becoming planktonic. Observations in flow chambers demonstrate separation of individual cells and cell aggregates after some time. Single cells and small aggregates are most often separated [67]. Since this process results in dissemination of infection or con-

tamination of industrial equipment, it is of significant importance. Bacterial surfactants, alginate lyase, and other polysaccharide lyases are involved in biofilm decomposition. The role of bacteriophage enzymes in decomposition of biofilms is presently actively discussed. According to some authors, since they acquire new features, including antibiotic resistance, new planktonic cells separated from the biofilm present special danger.

Biofilms and Dormant Forms

At the stage of biofilm maturation, the integrated bacteria begin to experience natural "planned" stress impacts. These are mainly the stresses resulting from exhaustion of nutrients and space, i.e., the same factors that occur at the stationary phase of batch cultures [68]. Under biofilm conditions, stress impacts are evidently intensified. This results in emergence of dormant and nonculturable forms in biofilms, together with the metabolically active cells. Sporulation in biofilms, or formation of dormant forms (DFs) in general, is an intriguing issue. The spores of *B. subtilis* and *B. cereus* were shown to form in biofilms at the air–liquid boundary [69]. In this microenvironment, the conditions are favorable for spore formation and the subsequent dispersion of the biofilm favors spore propagation. Apart from those formed in the biofilm, spores and other DFs may arrive from the surrounding environment. The "adhesive" polysaccharide nature of the matrix results in the biofilm acting as a trap for all microorganisms, including their dormant or persistent forms. Due to their resistance to environmental factors and surface characteristics, spores and other DFs may be the first in colonization, especially under extreme conditions. Under favorable conditions, spores germinate and the growing vegetative cells occupy various econiches, including formation of biofilms. Thus, spore-forming bacteria are able to colonize new surfaces. Hydrophobicity of spores and other DFs is known to exceed significantly the hydrophobicity of vegetative cells. The spores may differ in hydrophobicity, depending on the structure of their external layers; this may be determined from their adhesion to hexadecane [71]. Hydrophobicity of microbial cells determines to a significant degree their capacity for adhesion and subsequent colonization. Analysis of adhesive properties of hydrophobic *B. cereus* spores, which have an exosporium, and more hydrophilic *B. subtilis* spores revealed that the number of spores attached to the surface was higher for *B. cereus* than for *B. subtilis*. In vegetative cells, surface appendages carry out the adhesive function. Appendages have been also described for the spores of clostridia and bacilli [71, 72]. The spores of entomopathogenic *B. thuringiensis*, a species widespread in nature and involved in insect control, possess appendages similar to the pili of gram-negative bacteria [73]. Appendage morphology in *B. thuringiensis* spores is variable. Thin tubular extrusions with a channel well discerned under negative staining are the most common structures. The spores are components of entomocidic preparations used for treatment of forests and cultivated fields. They support development of entomopathogenic bacteria in the environment. Capacity of the spores with appendages to adhesion to various objects, including plant leaves, was demonstrated [74]. Adhesive capacity of *B. cereus* spores was also shown. Thus, spores are capable of adhesion, which may be responsible for initiation of surface colonization.

CONCLUSIONS

The information on the structure of biofilms and the patterns involved in their formation was analyzed in this review. These data supplement the information on growth, metabolism, physiology, genetics, and cytology of bacteria obtained by investigation of freely floating (planktonic) cells. Bacterial cells integrated in a biofilm undergo structural changes associated with their attached state and possess specific traits of functional activity caused by their collective existence. As a result, cells emerge in a biofilm bacterial community that are morphologically different from free-living ones. In attached bacteria, the surface structures of planktonic cells are replaced by others that are required for attachment to surfaces. Dense packing of the cells in a biofilm is accompanied by changes in their shape and ultrastructural organization. Interpretation of microscopic data is therefore somewhat difficult. Further complex research of biofilm communities will promote our understanding of the specific traits of their organization. The issue of dormant and nonculturable bacterial forms in biofilms has been insufficiently considered in the literature. Their formation and existence within a microbial community, which is protected from external influences, is important for species preservation along the chain of generations and for survival of microorganisms under changing and extreme environmental conditions. Understanding of the mechanisms of biofilm formation and techniques for their detection are especially important for development of the procedures to counteract biofilm formation in medical, as well as industrial and household, settings.

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