Biofilms – highly organized systems of microorganisms

The Social Life of Bacteria

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Biofilms are complex, dynamic structures in which individual microorganisms function as a multicellular social organism

Contrary to popular opinion, microorganisms rarely occur in the natural environment as free-living individual cells. They usually form agglomerations attached to a base, coated in a thin layer of mucus. Far from being loose collections of cells, such agglomerations are highly organized interconnected systems of individuals acting together, known as a biofilm. Adjacent microbes form a sheath around themselves, known as a matrix, into which they secrete various substances. The main components of the matrix are extracellular polymeric substances (EPS), mainly polysaccharides, and water. Water may account for even much as 97% of biofilm matrices. The ability of polymers to periodically accumulate and expel water gives the matrix the properties of a hydrogel with excellent viscoelasticity. This makes biofilms extremely difficult to remove from their base. Additionally, the hydrogel matrix is effective in protecting biofilm microorganisms against drying out.

The matrix is not just what keeps a biofilm together, but also – perhaps most importantly – a system of communication between the cells inside and a means of providing stable conditions. Through its abundance and physical properties it protects the cells from varying physical conditions in their surroundings, UV radiation, temperature and pH fluctuations, and high concentrations of harmful substances.

On the phase boundary

Biofilms can form on any surface that constitutes a phase boundary, regardless of its type: on solid surfaces in air or immersed in water, on the top surfaces of fluids, or on the boundary between two different fluids. The substances constituting the surface also do not matter, which is why biofilms are found everywhere, both in natural environments and those transformed by humans. They can be observed on rocks, in the sediment of water reservoirs, on stones in streams, on the surfaces of buildings and plants, and in soil. They occur in kitchen sinks, on bathroom tiles, and on the surfaces of food prod-

The closed gold and arsenic mine in Złoty Stok in Lower Silesia is now a tourist attraction. The microorganisms in the mine form an unusual biofilm, shown here covering a rock

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ucts in the fridge. They grow at all depths of the ocean and at all temperatures; they are as common on arctic rocks as they are in hot springs. They also grow on the bodies of animals and humans.

Although they occur in extremely varied environments, they usually take on a similar form: in dynamic (fast-flowing) media they form flat surfaces formed of long filaments (known as mats), while in still media they occur as gelatinous, irregular shapes similar to slimy fungi.

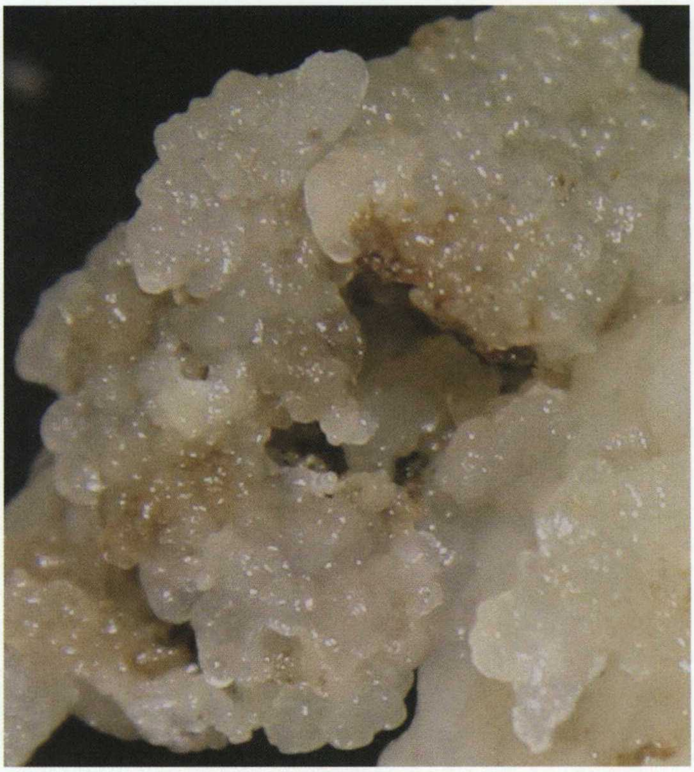
Quantity into quality

Biofilm growth is a complex process which depends both on the properties of the organisms that comprise it and on environmental factors where it develops. However, it is always initiated by individual cells attaching to a base and changing their properties. The immobilized cells lose their flagella, reduce their growth rate, secrete polymeric substances, and change the regulation of certain genes. When a sufficiently large number of cells accumulate, a phenomenon known as quorum sensing occurs. The accumulated bacteria secrete specific signaling substances (such as N-acyl homoserine lactones or short peptides), which migrate throughout the matrix and interact with cells situated further away.

In a biofilm structure one can distinguish empty spaces, canals, scaffolding, and areas with a greater matrix density. Microbe cells can be diffused throughout the matrix or concentrated in microcolonies. Spatial variation is accompanied by physiological variation. Concentrations of various chemical substances in biofilms vary depending on the distance from the surface and the type of micropopulations of organisms in a given location. Cells coexisting in a single biofilm both compete with one another for nutrients and better location, and cooperate with one another in processing environmental components.

Biofilms are complex, dynamic structures that can be regarded as a sophisticated survival mechanism in which individual cells function as though they formed part of a multicellular, social macroorganism. It is a variable and at once enduring system. Under adverse conditions, biofilm organisms, protected by the matrix, remain inactive and as such insensitive to most chemical substances.

Karolina Tomczyk-Żak



Biofilms are highly organized, three-dimensional structures formed by microorganisms immersed in a jointly constructed matrix

Biofilms can consist of one, two, or multiple species. They are most commonly formed by bacteria and archaea, although they can also be formed by fungi and algae (creating water blooms common during summer months).

Biofilms, in particular those formed of a single species, can be grown artificially by providing the microbes with a surface, moisture, and an appropriate medium that is not too rich.

A major challenge

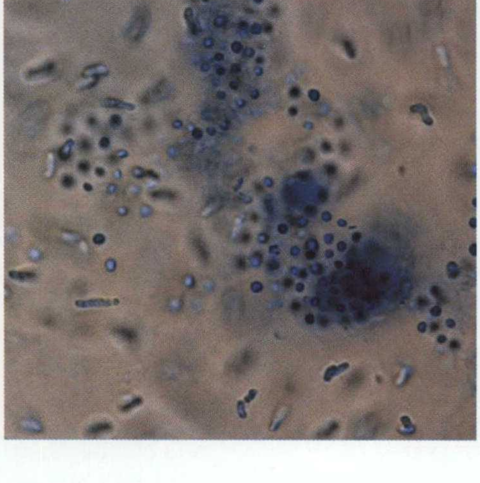
In one specific type of biofilm, called a flocculation, microscopic granules are tightly packed into cylindrical structures, where microbial cells that are close together are coated with a thin matrix film. In contrast to other types of biofilms, flocs are not attached to any surface but remain in large agglomerations by forces of mutual attraction. The physical proximity of cells of different species of bacteria and archaea in granules (electron donors and recipients, respectively) allows for the highest energy-process efficiency known in nature. Granules arise in calm, liquid, most commonly anaerobic environments. They are widely used in biological treatment processes.

Under favorable conditions, biofilm growth can be very abundant, often even dangerous. This occurs when biofilms grow inside pipes causing blockages, or on the outside of metal constructions leading to corrosion. They become a serious problem when they settle on the human body. Found on the teeth as dental plaque, or settling in wounds, catheters and implants, they constitute a major challenge to today's medicine. The physical properties of biofilm matrices and low metabolic activity in cells within biofilms make them resistant to antibiotics, even when individual cells of the pathogen are sensitive to them.

Strength in unity

In the natural environment, monospecies biofilms are found rarely, usually under extreme conditions - for example, Acidithiobacillus forms thick mats on the surface of acidic mining waters (frequently at pH below 2). Pathogenic microbes also frequently grow as monospecies biofilms. Common examples are *Candida albicans, Pseudomonas aeruginosa,* and *Burkholderia cepacia.*

Studying multispecies biofilms poses a major challenge. The interrelations between individual cells, formed over long periods of time, cannot be transferred simply to laboratory conditions. It is assumed that no more than 1% of species from complex environments can be grown artificially. A common reason behind this is the close metabolic relationship between the different species coexisting in the biofilm structure.



Microbe cells, as seen in this optical microscope image, can be diffused throughout the matrix or concentrated in microcolonies

Małgorzata Górecka

Microbes go underground

We have already mentioned above that biofilms can thrive in astonishing places. For instance, the closed gold and arsenic mine Złoty Stok in Poland has constant environmental conditions: the temperature is steady at around 10°C, with almost 100% humidity and darkness. An important feature of this mine is its low content of organic compounds and the presence of numerous toxic compounds (cadmium, lead, radon, hydrogen sulfide, arsine), and in particular a very high concentration of arsenic ions (6.99mg/l). Those conditions make the survival of individual organisms extremely difficult.

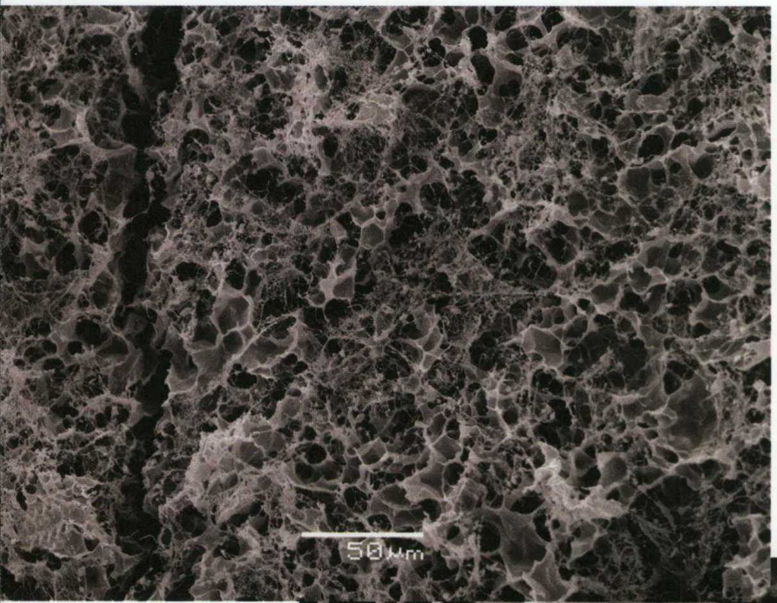
In the farthest section of the corridor "Gertruda," flooded with water and inaccessible to tourists, the mine walls have developed an unusual biofilm. Agglomerations of morphologically distinct bacteria are distributed in the spongy, slimy structure. The biofilm's matrix contains high levels of silica and trace amounts of organic compounds. Such matrix composition suggests the presence of chemolithotrophic microbes, capable of carrying out metabolic reactions in which the energy they require for survival is obtained from inorganic compounds.

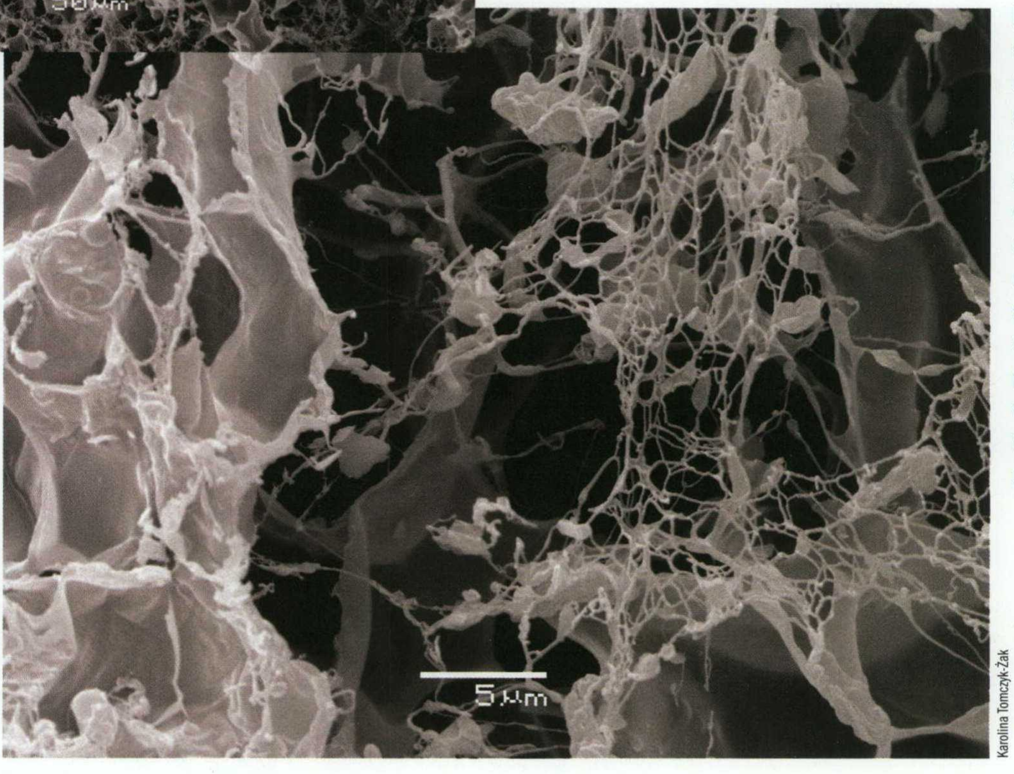
We are conducting in-depth analysis into the diversity of microbes forming this biofilm by using cultures and metagenomic techniques. Metagenomics is the study of metagenomes, or genetic material recovered directly from environmental samples. We have used samples of the biofilm to grow over 70 different bacterial species. As many as 45 are Actinobacteria, known as secondary metabolite producers, numerous in soils and environments low in nutrients.

Concurrently we have conducted sequence analysis of the gene 16S rRNA, a taxonomic marker, obtained from total biofilm DNA through pyrosequencing using technology developed by a subsidiary of Roche. This has allowed us to show that the biofilm contains at least 500 species of bacteria and approx. 20 species of archaea. The dominant group of microorganisms in the biofilm is Rhizobiales, an order of alpha proteobacteria, typical of soil environments. It seems that the biofilm on the mine walls was first formed by bacteria brought in from the surrounding soil by seeping water. Those organisms, capable of chemolithoautotrophic nutrition, were later joined by other species, including some brought in to the mine by people working in it.

We also discovered, again through the use of metagenomics, that the biofilm contains bacteria with functional genes capable of metabolizing arsenic: arsenate reductase and arsenite oxygenase.

Together with the Laboratory of Environmental Pollution at the University of Warsaw, we have shown that bacteria contained within the biofilm secrete high levels of siderophores (iron-chelating compounds), likely to participate in the processes of releasing iron and arsenic ions from non-soluble compounds in the rocks.





In biofilm structures, seen in these scanning microscope images, one can distinguish empty spaces, canals, scaffolding, and areas with a greater matrix density. The main components of the matrix are extracellular polymeric substances (EPS), mainly polysaccharides, and water. Biofilm photos taken in a scanning microscope

Karolina Tomczyk-Żak

The formation of the biofilm opened up the way for the collective growth of many organisms on the rocks of the Złoty Stok mine, and their cooperation is likely to contribute to an increased release of arsenic ions to the environment. ■

Further reading:

Hall-Stoodley L., Costerton J.W., Stoodley P. (2004) Bacterial biofilms: from the natural environment to infectious diseases. *Nature Reviews Microbiology*, 2, 95-108.