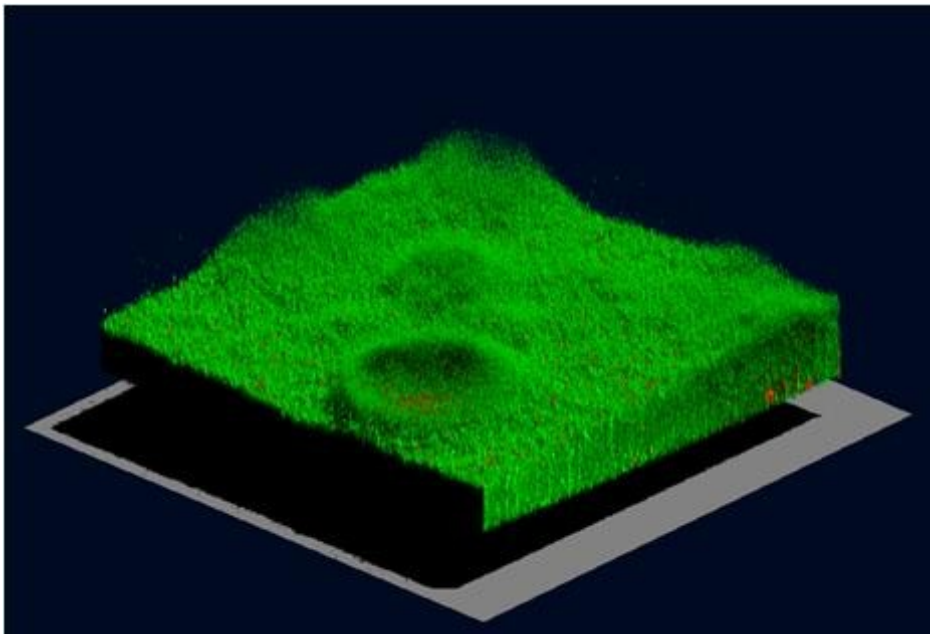
Healthcare industry BW

Jörg Overhage investigates how bacteria react to stress

Bacteria have been around for a very long time and they will probably still be around in the future. They are able to adapt to any factor that is potentially fatal to cells: heat, hunger, radiation, toxic chemicals or immune attacks. However, tolerance to stress is not only characteristic of individual bacteria; bacteria join forces whenever inhospitable conditions arise. A team of researchers led by Dr. Jörg Overhage at the Karlsruhe Institute of Technology (KIT) is investigating how clinically and industrially relevant bacteria react to stress. The researchers are focusing on the complex signalling systems that trigger the cells' ability to counteract stress as well as the molecular signalling cascades that trigger this team spirit in bacteria.

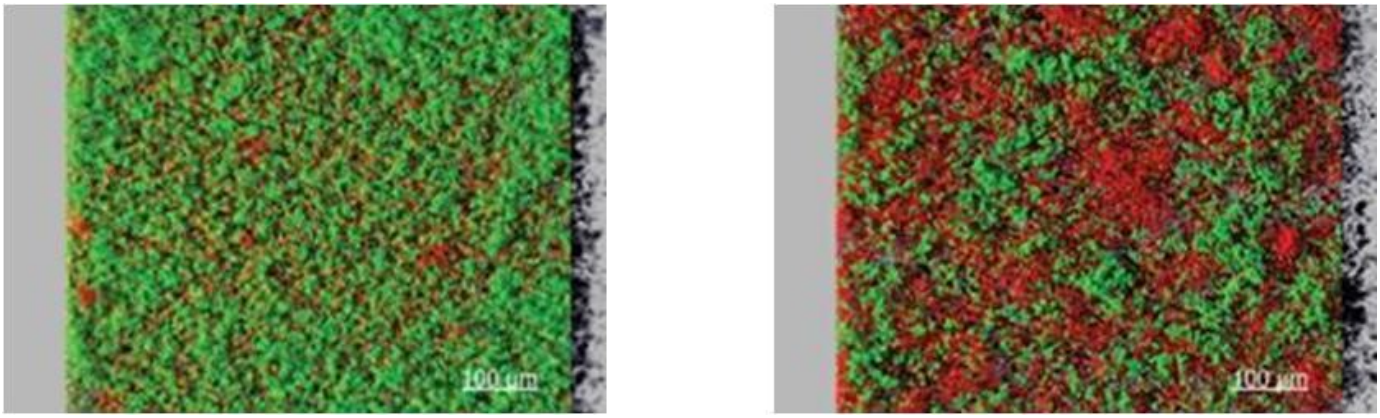


Lateral view of a four-day-old Pseudomonas aeruginosa biofilm analysed with a confocal laser scanning microscope. The biofilm was stained using fluorescent (live/dead) stains. Green: living bacteria; red: dead bacteria.

Many manufacturing processes in the pharmaceutical industry (as well as the biotechnology and food technology industries, etc.) need to be carried out under sterile conditions; drinking water is specifically treated in order to remove microbes; bacteria are unwanted guests on implant surfaces and catheter cannulas, and they are even more unwelcome in the lungs of patients on intensive care wards. However, disinfecting agents and antibiotics are often ineffective when it comes to eradicating bacteria. Microorganisms have been found to survive repeated treatment with antibacterial chemicals, whether they are experiencing stress or not. Bacterial cells are able to sense inhospitable

environmental conditions, which triggers the ignition of signalling cascades that in turn trigger cellular protection mechanisms. The bacterial cells join forces with thousands of cells and create biofilms that protect them against environmental stress. "We would like to understand the molecular signalling processes that regulate the bacteria's ability to protect themselves against stress," said Dr. Jörg Overhage, head of the Bacterial Stress Response and Process Engineering junior research group at the Karlsruhe Institute of Technology (KIT).

Dreaded in hospitals and intensive care wards



Live/dead stain of a Pseudomonas aeruginosa biofilm under the confocal laser scanning microscope. Green fluorescence: alive; red: dead. The biofilm on the right was treated with an antibiotic at a dose 32 times higher than the minimal inhibitory concentration. Despite the 32-fold minimal inhibitory concentration, the biofilm still contains many living cells. Planktonic (i.e. free floating cells) would have been killed at the lowest (1/32) concentration of the antimicrobial used.

Over the last three billion years, bacteria have learned to protect themselves against environmental influences. Bacteria are omnipresent and can survive in Arctic ice or in the heat of volcanic springs. The genome of the bacterium Pseudomonas aeruginosa, which Dr. Overhage's team uses as model organism, encodes around 500 regulators and signal sensors that are involved in the recognition and processing of abiotic stress such as heat, salt, radiation and antibiotics. This means that as much as ten per cent or so of the bacterium's genome consists of so-called stress regulators. And this makes P. aeruginosa one of the most dangerous human pathogens, in particular in people with a weakened immune system. P. aeruginosa becomes resistant to antibiotics with extraordinary speed, which is why it is a particularly dangerous and dreaded pathogen, especially in hospitals. P. aeruginosa is found in most man-made environments. It thrives in drinking treatment systems where it forms biofilms that are really quite resistant to disinfecting agents. Biofilms are communities of microorganisms that are attached to a surface and embedded in an extracellular polymeric matrix (EPS), a layer consisting of proteins, nucleic acids and carbohydrates that surrounds bacterial colonies and protects them against chemicals, protozoan grazing or being killed by the human immune system. In addition, some of the bacteria in a biofilm enter a dormant (persister) state during which they are more or less asleep: "going persister" reduces the bacterial metabolism, which is why they cannot be killed by standard antimicrobials that target bacterial metabolic and cell division processes. When the stress (antibiotics) comes to an end, they wake up again and start to divide.

In one of their projects, Overhage and his team have been able to show how Pseudomonas bacteria react to exposure to a commonly used disinfecting agent, which is used, amongst other things, for the treatment of drinking water. "This agent normally induces the formation of reactive oxygen species in the bacteria," said Overhage going on to add "the agent destroys molecular structures such as DNA, proteins and lipids, thereby interfering with the work of the membrane proteins that

are part of the bacteria's respiratory chain." However, the oxidative stress caused by such agents does not usually kill the bacteria completely. The researchers from Karlsruhe found that sublethal concentrations stimulated biofilm formation. "We also found that an increase in the intracellular concentration of cyclic di-GMP was responsible for this," said Overhage explaining that cyclic di-GMP is a well-known and important second messenger, i.e. a signalling molecule. Increased expression of the molecule is triggered when a bacterial cell detects the disinfecting agent by way of a previously unknown signalling cascade. "The elevated intracellular concentration of cyclic di-GMP triggers the formation of biofilms by Pseudomonas bacteria," said Overhage. Overhage and his team will carry out further investigations to find out how the transfer of signals functions in detail as well as finding out more information about the molecules that the biofilm-forming bacteria use to communicate with each other.

Cells that are invulnerable

In another project, the Karlsruhe researchers have discovered a membrane protein that detects stress signals. This protein, a histidine kinase, is located in the membrane of the bacterial cells and detects molecules of the human immune response (so-called antimicrobial peptides), amongst other things. The protein is also able to detect antibiotics. When the protein has detected an antibiotic, for example, it interacts with an intracellular transcription regulator that up- or down-regulates the transcription of numerous bacterial genes, including the gene that codes for lipopolysaccharide (LPS), which is an integral component of the bacterial cell wall. LPS renders the bacterial cell wall impermeable to antibiotics and other chemicals. This is one of several mechanisms bacteria use to protect themselves against harmful substances. "In terms of therapy, it is important to understand the mechanisms that bacteria use to counteract stress factors as well as understanding the underlying mechanisms in detail," said Overhage. "And perhaps, at some stage we will be able to circumvent or even prevent the mechanisms from occurring."

Overhage and his team use a holistic, systems biology approach whose objective is to gain an in-depth understanding of the network of the regulatory components involved in the recognition and transmission of signals as well as in reactions that are associated with the exposure of bacteria to abiotic stress. The researchers are screening the Pseudomonas aeruginosa genome with the goal of identifying further molecules that are involved in this complex process. One of the researchers' more long-term goals, as already mentioned, is to identify molecular targets in order to be able to interfere with biofilm formation and bacteria's resistance to antibiotics. Biofilms can also be beneficial for humans, for example bacterial communities in the human intestine or in sewage plants can help to degrade organic nutrients and toxic substances.

Further information:

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Stress and molecular defence mechanisms