New ways to interfere with bacterial strategies

Bacteria themselves provide the key to their destruction. New insights into the growth and interaction between different pathogens are used in microbial genetics to develop new methods and active substances for combatting multidrug-resistant bacterial strains.

You have to know your enemy to fight it effectively. For this reason, Prof. Dr. Friedrich Götz and his team at the University of Tübingen are exploring the colonization and growth strategies of human and animal pathogens. The researchers are particularly interested in biofilm-forming bacteria. The formation of bacterial biofilms on implants often leads to long-term infections, which are also known as foreign body associated infections. Biofilm bacteria change their metabolism and often show much greater resistance to antibiotics than their free-living counterparts, which is why infections caused by biofilm bacteria are difficult to treat. Prof. Götz uses molecular biology methods to search for weaknesses in bacterial life and tests new active substances for their efficiency in treating bacterial infections. "We are specifically interested in the interaction of biofilm bacteria and we use a proteomics approach to find out more. What we are interested in are the proteins bacteria use to communicate and interact with each other," Götz explains.

Interfering with bacterial adhesion

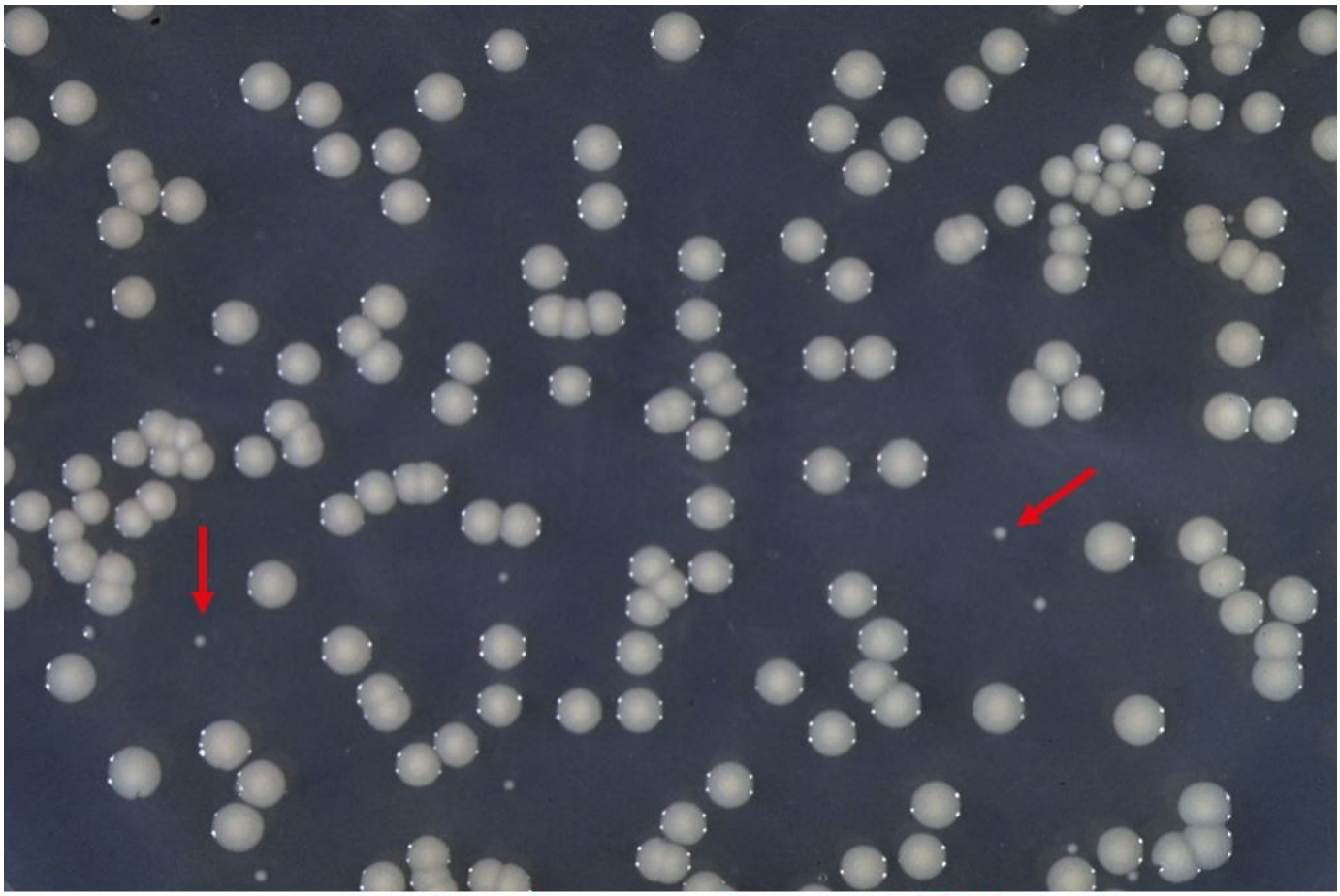
A central question relates to how biofilm formation is initiated and triggered on the molecular level. Götz and his team have shown that biofilm formation proceeds in two stages. It begins with the attachment of free-floating bacteria to the surface of tissue or an implant. The bacteria adhere to the surface through proteins known as adhesins. The latter are special bacterial cell-surface components, proteins or sugar polymers. "Adhesins can adhere to almost all non-organic surfaces and are equally good at adhering to metals as they are to plastics such as silicon and polycarbonates," says Götz, referring to the problem that implants often become colonized with bacteria, which leads to infections. Götz's group has already identified numerous adhesins and is now looking for active agents that can prevent bacterial cells from producing adhesins. Götz's team has established and optimized numerous methods for testing compounds for their ability to inhibit biofilm formation. The researchers have already identified some candidates and are now studying them in greater detail.

The adhesion phase is followed by an aggregation phase during which a larger number of bacteria adhere and divide. The bacteria secrete a type of slime (extracellular polymeric substance) and form a protective, multilayer matrix as the biofilm grows. Götz's team identified and analyzed proteins produced by the bacteria for the purpose of aggregation. The researchers then went on to look for active substances that were able to inhibit the production of aggregation proteins. In a BMBF-funded project, the researchers from Tübingen have worked with EVOTEC AG with the aim of identifying substances that inhibit the formation of the bacterial cell wall. This project was based on the idea

Prof. Dr. Friedrich Götz is the head of the Department of Microbial Genetics and is specifically focused on finding effective ways to treat bacterial infections.

that the growth of bacteria without an intact cell wall was massively disturbed. Penicillin and other antibiotics from the beta-lactam group inhibit bacterial cell wall biosynthesis. However, these antibiotics are the most widely used group of antibiotics and many bacteria have become resistant to the majority of them. Therefore, the goal is to identify substances that belong to a different substance group. Although Götz's team of researchers has succeeded in identifying substances that work by inhibiting the biosynthesis of the bacterial cell wall, the project nevertheless produced disappointing results. Götz explains: "We found that we could not stop the bacteria from aggregating once they had managed to adhere. This means that it is necessary to interfere with the formation of biofilm at an early a stage as possible. We now need to concentrate on finding substances that prevent the adhesion of bacteria to surfaces."

Dual infection sheds light on novel strategies for combatting bacteria



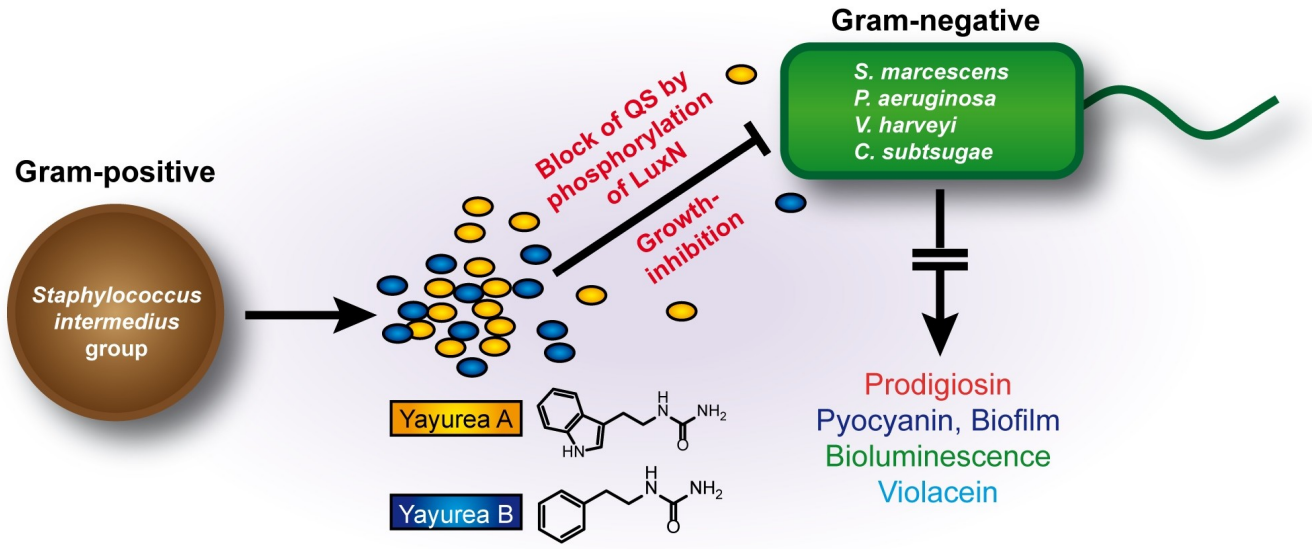
Staphylococcus aureus variants that form very small colonies on culture plates are particularly dangerous bacterial specimens. Although these SCVs (small colony variants) produce relatively low amounts of toxin and are less virulent than staphylococci that form larger colonies, they have the ability to survive successfully in host cells.

Götz eventually went for a completely different strategy based on findings he obtained when investigating the interaction of staphylococci with other pathogenic bacterial species such as Pseudomonas aeruginosa. P. aeruginosa causes pneumonia in humans and leads to chronic infections in people with cystic fibrosis. "Cystic fibrosis is one of only a handful of diseases that are caused by the co-infection of Staphylococcus aureus and Pseudomonas aeruginosa. "This leads to hostilities and physical conflict between the bacteria themselves and between the bacteria and the host," explains Götz. P. aeruginosa secretes toxins that damage the lung epithelial cells of the host and also of the S. aureus cells. Although S. aureus bacteria can react to this situation by altering their metabolism and becoming resistant to such toxins, they nevertheless become less virulent and grow much more slowly. The latter becomes obvious from the rather small colonies on the culture medium. These strains are referred to as SCV (small colony variants).

In cooperation with a group of researchers led by Prof. Dr. Georg Peters (Department of Medical Microbiology at the University of Münster), the team of researchers from Tübingen engineered a stable respiratory-deficient S. aureus mutant. "This mutant produced a much smaller number of toxins, grew very slowly and its acute virulence was thus weakened. However, it is able to persist for much longer in the host cells than the original S. aureus strain and is resistant to gentamicin and related aminoglycoside antibiotics," says Götz. The bacteria's ability to persist for a relatively long

time in the host cells is what makes SCVs so dangerous. Hidden away in the host cells, SCVs evade the host immune response and are not responsive to antibiotics. This observation leads to an important finding with regard to the clinical application of antibiotics. "Care must be taken when using gentamicin for the treatment of acute infections as gentamicin and related antibiotics select for SCVs. The application of gentamicin and related antibiotics increases the risk of an acute infection becoming chronic," says Götz.

The fight for co-existence leads to new anti-infective drugs



Co-existence mechanism of gram-positive staphylococci and gram-negative bacteria. The staphylococci secrete two compounds (Yayurea A and B) that inhibit the growth and chemical communication (quorum sensing, QS) of the Gram-negative bacteria.

The researchers from Tübingen then began looking for natural Staphylococcus species that could co-exist with P. aeruginosa. They identified and studied five species in greater detail. Götz's team of researchers found that these species secreted low-molecular compounds, which inhibit the growth of P. aeruginosa, and a large number of gram-negative bacteria. More specifically, the compounds inhibit the chemical communication, i.e. quorum sensing, between the bacteria. Quorum sensing (QS) is bacteria's ability to coordinate gene expression according to the density of their local population by way of signalling molecules. QS controls the production of proteins that are responsible for bacterial virulence and persistence. Compounds that disturb bacterial quorum sensing systems are referred to as quorum quenching molecules. The researchers have isolated quorum quenching compounds from animal pathogens and will now investigate their suitability for treating infections caused by gram-negative bacteria. If this strategy turns out to be effective, a smart new method will in future be available for the treatment of infections caused by multidrug-resistant bacteria. The group from Tübingen has isolated two substances, Yayurea A and Yayurea B, which suppress quorum sensing signalling and inhibit the growth and biofilm formation of a broad range of gram-negative bacteria such as P. aeruginosa and Vibrio cholerae. Götz and his team have also been able to show that Yayurea A and B are effective against infections caused by colistin-resistant P. aeruginosa bacteria. In cooperation with Prof. Dr. Stephanie Grond from the Institute of Organic Chemistry at the University of Tübingen, Götz and his team succeeded in chemically synthesizing Yayurea A and B as well as 20 additional analogues. Initial investigations have shown that the quorum sensing quenchers have an excellent antimicrobial effect on gram-negative bacteria and they will now be further developed into marketable drugs.

Further information:

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Multiresistant pathogens – a self-inflicted threat?

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