Soil bacteria explored as source of new antibiotics

Effective antibiotics are a precious commodity. They are the most important medical weapon against infectious diseases when the immune system is unable to fight off intruders on its own. Antibiotics prevent the reproduction of or kill bacteria. Unfortunately, the overuse and inappropriate use of antibiotics over the past few years have led to the spread of bacteria that are unresponsive to standard antibiotics. Therefore, researchers around the world have been focusing on the discovery of new substances that are able to combat bacterial infections effectively. Two scientists from the Interfaculty Institute of Microbiology and Infection Medicine (IMIT) at the University of Tübingen, PD Dr. Evi Stegmann and Dr. Yvonne Mast, are exploring the biosynthesis of antibiotic substances with the aim of modifying them to make them suitable for application in the fight against multidrug-resistant bacteria.

PD Dr. Evi Stegmann and Dr. Yvonne Mast, microbiologists in the Department of Microbiology and Biotechnology at the Interfaculty Institute of Microbiology and Infection Medicine (IMIT) at the University of Tübingen, have major research interest in actinomycetes. These harmless bacteria are primarily found in soil and produce an extraordinarily large number of secondary metabolites such as antibiotics. Antibiotics are natural substances that bacteria use to defend themselves against other bacteria, but have long become essential drugs in the treatment of human infections caused by bacteria. Actinomycetes produce a broad range of different substances that protect them against bacterial and fungal pathogens. These substances, which are also used as cytotoxic drugs for the treatment of cancer, are an excellent starting point in the search for new active drug ingredients. Stegmann's and Mast's working groups are part of the cooperative research centre 766 entitled "The bacterial cell wall: structure, function and infection interface" and are also involved in research carried out in cooperation with the German Centre for Infectious Disease Research (DEZIF).

Screening actinomycete libraries for new antibiotics

Over the past 50 years, the IMIT has established a comprehensive collection of actinomycete strains that are now used by researchers to identify new antibiotics. Bacterial strains are cultured in suitable growth media and stimulated to produce antibiotics. The bacterial cultures are subsequently chemically analysed using HPLC or HPLC-MS – high-performance liquid chromatography combined with mass spectrometry – procedures that are used to separate and characterise the molecules. The resulting spectra provide initial information about the chemical structure of the molecules produced by the bacteria. The biological activity of the antibiotic candidate is then assessed with several standard pathogens and investigated in further detail if the tests are positive.

Subsequently, the biologists are attempting to find out how the antibiotic under investigation is synthesised, i.e. they are interested in the process and order of the individual enzymatic steps

The scientist Dr. Evi Stegmann and her group of researchers at the University of Tübingen are focused on the biosynthesis of balhimycin, a vancomycin-like antibiotic.

Dr. Yvonne Mast from the IMIT is focused on the development of actinomycetes pristinamycin, a last resort drug to combat multidrug-resistant bacteria.
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involved in the production of the antibiotic. "We are looking closely at how the antibiotics are synthesised by analysing the individual biosynthesis steps using biochemical, genetic and chemical methods," said Dr. Stegmann. "We need to understand the process of antibiotic biosynthesis in every detail before we are able to trigger or inhibit specific reactions. One of our goals is to make the bacteria produce the largest possible quantities of antibiotics, but we also want to interfere with the biosynthesis process so as to also make the antibiotic effective against antibiotic-resistant bacteria and expand its host range."

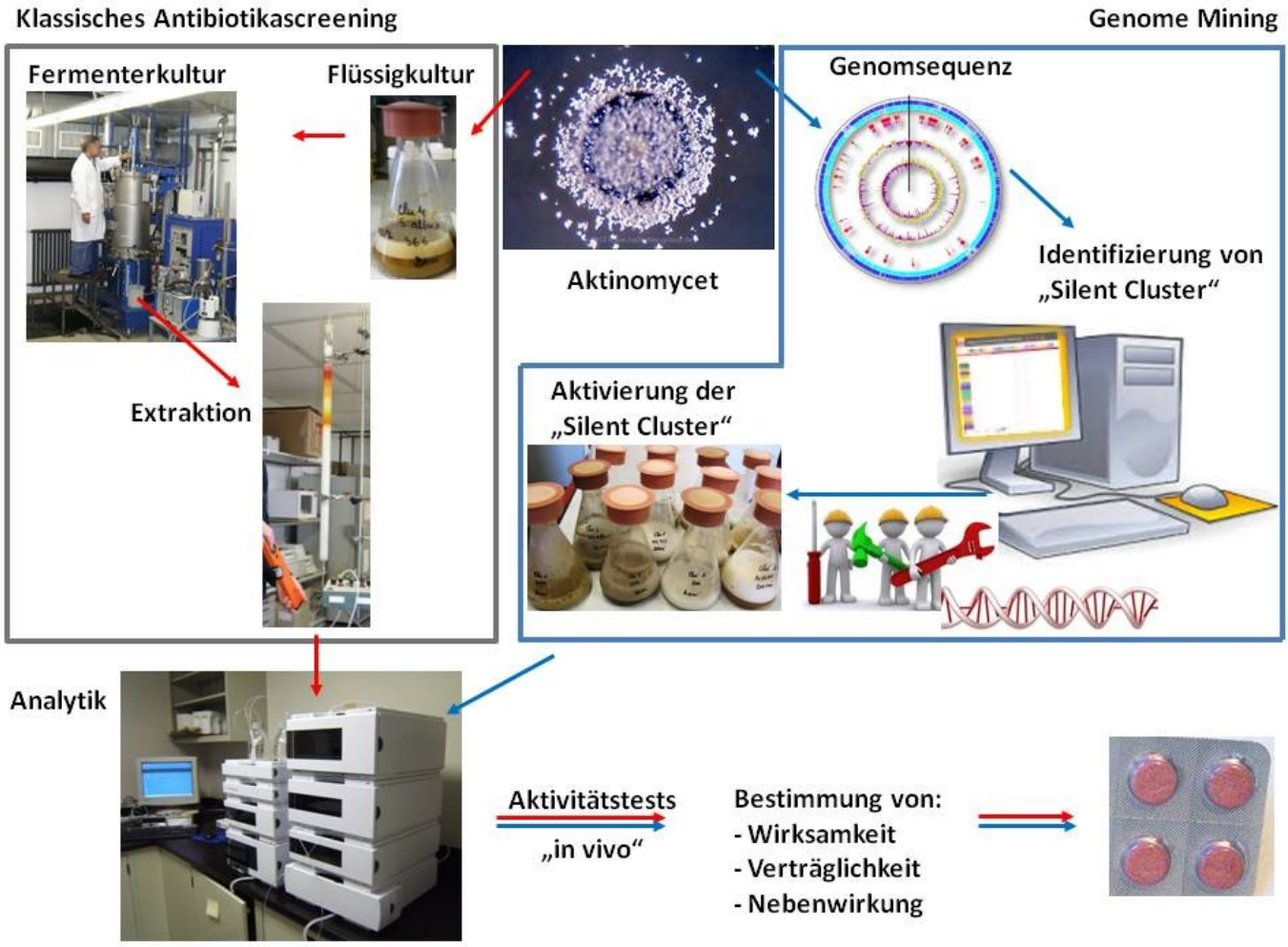
Once the researchers have obtained detailed insights into the antibiotic biosynthesis pathway, they will be able to use state-of-the-art synthetic biology and metabolic engineering methods in order to engineer a microorganism with the sought-after characteristics, including higher productivity, broader host range and improved tolerability.

Antibiotics screening using genome mining

In addition to classical screening methods, the IMIT also uses a state-of-the-art method known as genome mining in order to find novel secondary metabolites that may be useful for drug development. The genome sequences of the majority of the institute's actinomycete strains are known and can be used for finding novel microbial metabolites. "Many strains have a greater antibiotic production potential than previously thought," said Dr. Stegmann. "We have been working with many strains for many years, but they only produce a specific antibiotic under the growth conditions we've used." This is why the researchers from Tübingen also use sequencing data to look for so-called silent clusters, i.e. gene clusters in which the genes are not expressed or expressed at very low levels. The scientists from Tübingen and their colleagues from Groningen have developed

“antiSMASH” (antibiotics & Secondary Metabolite Analysis SHell), a software that allows the rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genomes. The research groups of Dr. Stegmann and Dr. Mast now have the primary goal to activate silent clusters and to isolate and characterise novel secondary metabolites.

Glycopeptides as potential new antibiotics



Schematic showing how the researchers are looking for new actinomycetes antibiotics.
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Amongst other things, Dr. Stegmann’s research group is focused on the synthesis of glycopeptides. They have already succeeded in obtaining detailed insights into the biosynthesis of balhimycin, a glycopeptide antibiotic that is extremely similar to vancomycin, the “last resort” antibiotic used for the treatment of resistant Gram-positive pathogenic bacteria. However, vancomycin not only has the drawback that it can lead to kidney damage, but also a growing number of bacteria is becoming resistant to the antibiotic. Balhimycin is produced by the bacterium Amycolatopsis balhimycina, which is a model organism for investigations into glycopeptide synthesis. The researchers have established a specific genetic system, with which they were able to clarify the biosynthesis of balhimycin in detail. This knowledge then enabled them to specifically interfere with its biosynthesis and use synthetic biology and metabolic engineering methods to produce balhimycin derivatives. The researchers are currently mainly focused on elucidating the bacteria’s resistance mechanism in order to find out how A. balhimycina protects itself against the antibiotic substance it produces. As the bacteria are able to transfer antibiotic resistance genes to pathogenic bacteria by way of horizontal gene transfer, knowledge about the bacteria’s anti-balhimycin mechanisms is of great

importance with regard to future glycopeptide-resistant pathogens.

Pristinamycin – a strong weapon against highly resistant pathogens

Dr. Mast's team is also working on a 'last resort' antibiotic to combat multidrug-resistant pathogenic bacteria. The antibiotic pristinamycin is a mix of two components with synergistic antibiotic action. The antibiotic consists of pristinamycin I (PI) and pristinamycin II (PII) and is marketed as Synercid® by Sanofi. Each compound inhibits the growth of bacteria; however, the combination of both substances in a specific ratio acts synergistically and leads to bactericidal activity of up to 100 times that of the separate components, thus killing the pathogens. The biosynthesis genes are located in the pristinamycin supercluster, which is the largest antibiotic supercluster known to date. "Our aim is to explore the function of the gene regulators and understand how the pristinamycin producer, Streptomyces pristinaespiralis, is able to synthesise PI and PII in a ratio that is required for optimal antibiotic effect," Dr. Mast explains. "Based on this knowledge, we are able to produce a bacterial strain that is highly effective in producing pristinamycin. As this antibiotic is one of only a few last resort antibiotics that are effective against MRSA infections, the efficient production of pristinamycin is an important basis for the fight against highly resistant pathogens."

Further information:

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Multiresistant pathogens – a self-inflicted threat?