Microorganisms are like tiny factories. They can be genetically modified to produce the desired compound in their cells. To make production easier, researchers have developed a process that won't require any living cells. This process can be used, for example, to synthesize peptides from their DNA blueprint in a very short amount of time.

PHOTO: KATRIN BINNER

GERM-FREE

TEXT: TILL HEIN

Comparing molecules to cars might seem like a bit of a stretch. They have certain similarities when it comes to their production process, though: some molecules are actually produced within a cell as if in an assembly-line. Helge Bode and Tobias Erb of the Max Planck Institute for Terrestrial Microbiology in Marburg are looking to modify these molecular production lines so that they can be used to make new antibiotics, which are urgently needed to fight resistant pathogens.

Even a common middle ear infection can now be fatal because many of the bacteria that can cause such infections have become resistant to antibiotics. And that resistance is growing: the World Health Organization (WHO) projects that as early as 2050, as many as 10 million people a year could lose their lives due to infections by multi-resistant bacteria. That's why new antibiotics are desperately needed. An increasing number of pathogens are becoming resistant to proven active ingredients because they are often used carelessly. To make matters worse, while the development of new antibiotics doesn't take any longer or require any more resources than that of other clinical drugs, it's less lucrative for the pharmaceutical industry.

PHOTO: KATRIN BINNER

Candice Jones is head of the MaxGenesys Biofoundry at the Max Planck Institute in Marburg. At the facility, large quantities of DNA and proteins can be designed and tested for various applications.

That's why Bode and Erb are looking for a new way to produce antibiotics and other medical agents, focusing on compounds that microorganisms produce themselves. Bacteria, for example, produce a wide variety of natural products within their cells that perform vital functions in metabolism, communication with other cells, and defense against competitors. Many of these compounds are known as peptides, which consist of amino acid chains just like proteins, except they are much shorter. Their versatility makes them ideal for use in medicine and industry. Peptides form the basis for the antibiotic penicillin, the cancer drug romidepsin, and the immunosuppressant cyclosporin. Roughly 60 percent of all therapeutic drugs used in human medicine today are based on biomolecules produced by bacteria and fungi.

The scientists in Marburg aim to harness the vast reservoir of naturally occurring peptides for new functions but also – drawing inspiration from nature – to develop entirely new peptides. "Unlike molecules produced in a lab without a natural model, peptides existing in the natural world can be assumed to have a specific function or effect. Otherwise, evolution would have weeded them out long ago," explains Helge Bode, Director at the Max Planck Institute for Terrestrial Microbiology. That's why he and Tobias Erb, fellow Director at the Institute in Marburg, see peptides derived from bacteria and fungi as a virtually inexhaustible reservoir of new active ingredients. "Bacteria and other microbes lived alone on Earth for 2 billion years before the first cells with a nucleus ever formed. So, they had plenty of time to produce new molecules for a wide variety of purposes," Erb explains.

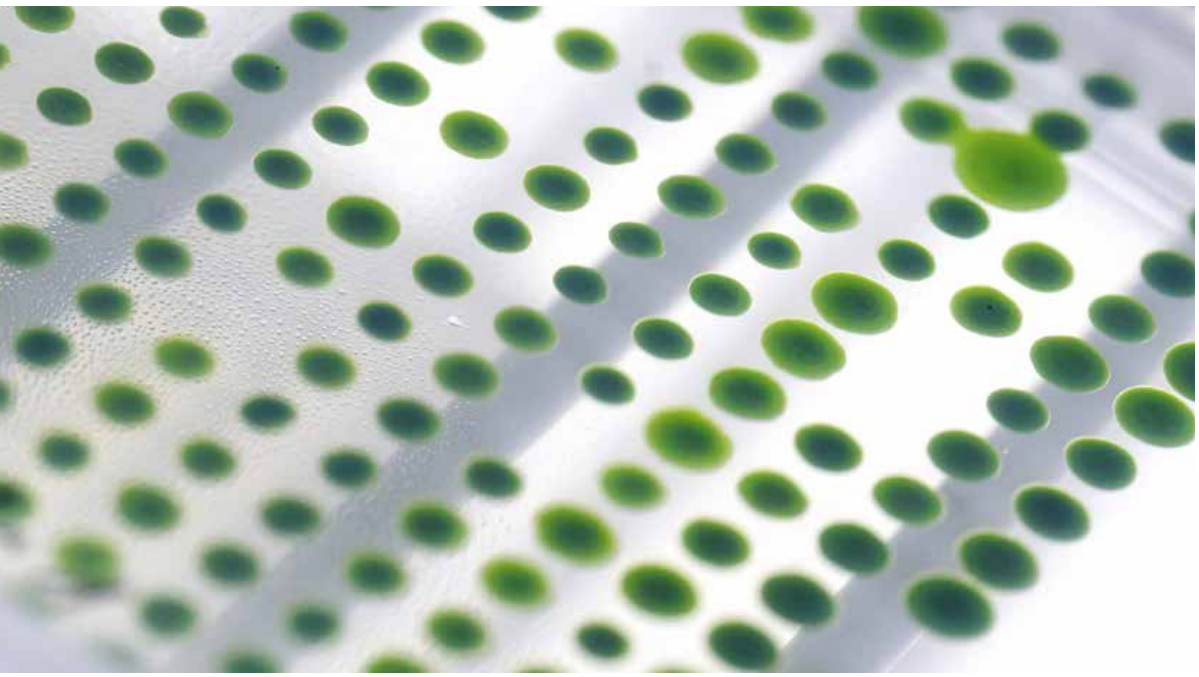
Better than Nature

Nevertheless, evolution has not always found the optimal solution, or else it has had to balance multiple requirements, leading to a compromise in the effectiveness of a given peptide. So, the Directors at MPI Marburg are interested first in optimizing peptides that are already known. For medical use in humans, peptides must not only be highly effective, but also distribute themselves rapidly and evenly throughout the body, remain available there for a long time, and be well tolerated. At the same time, their groups are also designing entirely new molecules. For all the diversity of peptides produced by microorganisms, there are still countless molecules that nature has not yet invented – and perhaps never will. "Evolution is rather conservative," Erb notes, "It mostly relies on tried-and-true methods; only rarely does something completely new emerge."

PHOTO: DAVID AUSSERHOFER

"It's like a car that starts out on the production line as a VW model but then is modified into a Tesla."

HELGE BODE



A microtiter plate containing *Chlamydomonas reinhardtii*. The researchers have developed a test platform that enables the production and analysis of thousands of genetically modified algae lines in parallel.

The researchers led by Bode and Erb are much more ambitious in this regard: they're aiming to synthesize the full range of peptides from all theoretically possible building blocks, and then identify which ones are suitable for use as drugs or raw materials for industry. To generate ideas for new peptides, Bode and Erb analyze natural production processes and functions of the molecules in bacteria and fungi. The proteins of a cell and the majority of its peptides are produced via specialized molecular machines called ribosomes. Peptides produced in this way consist of 20 different amino acids with a specific configuration, known as L-amino acids. These can be combined in a wide variety of ways. For peptides with a length of 40 to 50 amino acids, there are already more possible combinations than there are atoms in the entire solar system. How to go about finding new antibiotic peptides within such a vast pool? To this end, Erb is using artificial intelligence trained on known peptides to find new antimicrobial peptides. "It works in manner similar to training AI to write crime novels by having it read a few dozen Sherlock Holmes stories, the difference being that our AI is trained on thousands of variations," says Erb.

SUMMARY

Bacteria produce countless peptides which they use to communicate with each other, defend themselves against enemies, and assert themselves in their environment.

Many of these molecules, linear or cyclic short amino acid chains, are produced by modular enzymes. Researchers can assemble the individual modules into new enzymes to create peptides that do not occur in nature.

Researchers are working to produce antibiotics that kill bacteria via cell-free methods that do not require any living cells.

Bode, on the other hand, is interested in peptides that are not produced in ribosomes. "They contain other amino acids as well, such as D-amino acids, which are mirror images of L-amino acids, and amino acids that have been further modified," Bode explains. These peptides are synthesized by special enzymes called "non-ribosomal peptide synthetases." These act as catalysts in chemical reactions that join one amino acid after another to form a peptide. "A synthetase works on the same principle as an assembly line in the automotive industry," Bode explains. "The selection, activation, linking, and modification of the components take place at different modules of the enzyme. Each module is comparable to an individual robotic station in a factory, responsible for a specific production step."

Interestingly, the modules can be separated from each other and assembled into a new enzyme, which then produces a different peptide. And two different enzymes can also be coupled together. "So, we aren't just exchanging individual stations, we're connecting two conveyor belts in series. It's like a car that starts out on the production line as a VW model but then is modi-

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fied into a Tesla," Bode elaborates. "We can install parts from BMW, Mercedes, or Volvo too if we want." With cars, that may be hard to imagine, but in enzyme engineering it's no problem. Strictly speaking, however, the researchers are not reassembling the enzyme modules themselves, but rather the genes that carry the instructions for their construction. To do this, they cut the DNA into small pieces using gene-editing tools and reassemble these in new combinations. The number of peptides that can be produced in this fashion is astronomical, as even a single molecule built from four amino acids has over 8 billion variants. This is because the peptides used at the Marburg Institute can consist of more than 300 different amino acid building blocks. These include not only the 20 amino acids found in proteins in all living organisms, but also numerous others found in natural biosynthesis pathways or those produced artificially in the lab. Peptides can also be further modified with sugar or fatty acid molecules, giving them additional properties. Together with ETH Zurich, Bode founded the Basel-based startup Myria Biosciences. Now in year three of operations, the company is engaged in further developing the enzyme engineering methods of non-ribosomal peptide synthetases.

Production Without Cells

To generate the quantity of a peptide needed for further study, researchers typically engineer bacteria that carry the corresponding gene. The cells then produce the desired molecule alongside their own. It gets tricky, however, when the target molecule is an antibiotic, whose very purpose is to kill bacterial cells. For this reason, antibiotics can often only be produced in bacterial cells using special methods, such as highly efficient export out of the cell or synthesizing them as inactive precursors. Erb and his team have thus developed a production process that does not require the use of any living cells. "What we have here is a cell-free transcription-translation system that can produce a peptide from its DNA blueprint within a few hours. This allows us to produce and analyze several hundred peptides per day, faster and more cost-effectively than through any other chemical or biological production method," Erb explains. The researchers can thus rapidly gather new data for analysis using AI. Erb's team runs these cycles of production, analysis, and data interpretation at the fully automated MaxGENESYS lab – built up in Marburg over the past three years with funding from the Max Planck Foundation.

Bode and his group are now developing a similar method for the large group of structurally more complex non-ribosomal peptides, which can be produced in bacteria without cells within a few hours instead of days, and in quantities that allow for precise analysis of the substances. In a collaborative effort with the Compound Management and Screening Center of the Max Planck Society in Dortmund, researchers then test, for example, whether a peptide is lethal to various pathogens, whether it can kill cancer cells, and whether it harms healthy human cells.

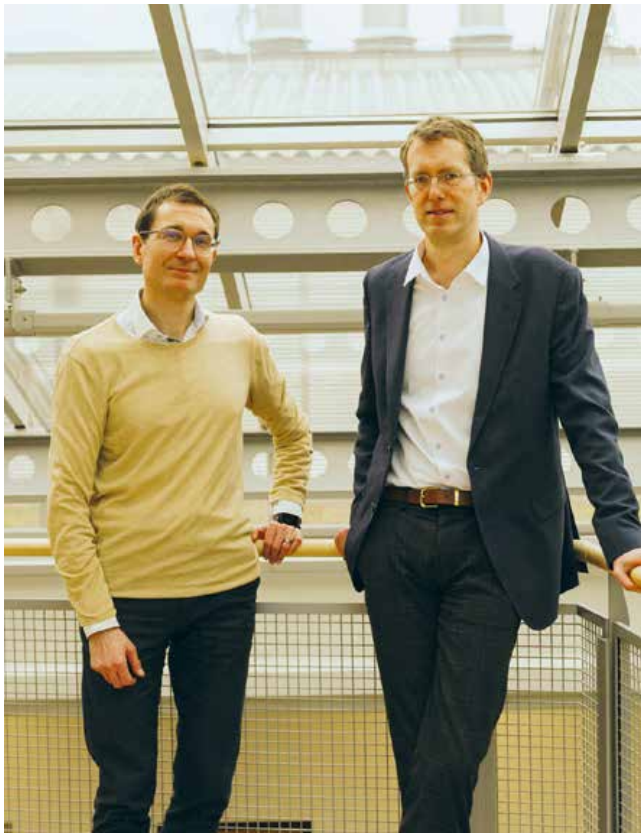

PHOTO: DAVID AUSSERHOFER

Tobias Erb and his colleague Helge Bode, together with their teams, are developing custom-made peptides for medicine and agriculture.

Bode and Erb see their scientific work as part of a long-standing tradition. "Humans have always been inspired by nature in designing technology, yet always made adaptations as needed," says Erb. "Take airplanes, for example. They employ wings to generate lift, just like birds do. But the wings have no feathers, and they don't move. And they have to be that way in order for humans to overcome gravity." The same principle applies to the design of new peptides: inspiration, not imitation.

www.mpg.de/podcasts/medizin-der-zukunft (in German)

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Sabine Schaefer*

Picture: Max Planck Institute for Evolutionary Anthropology

** The entrepreneur and donor – pictured here with Svante Pääbo and Julius Schaefer – provided the initial funding for a research group at the Max Planck Institute for Evolutionary Anthropology led by Nobel Prize winner Svante Pääbo.*

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