Genetic method leverages bacterial transfer mechanism to produce new active ingredients

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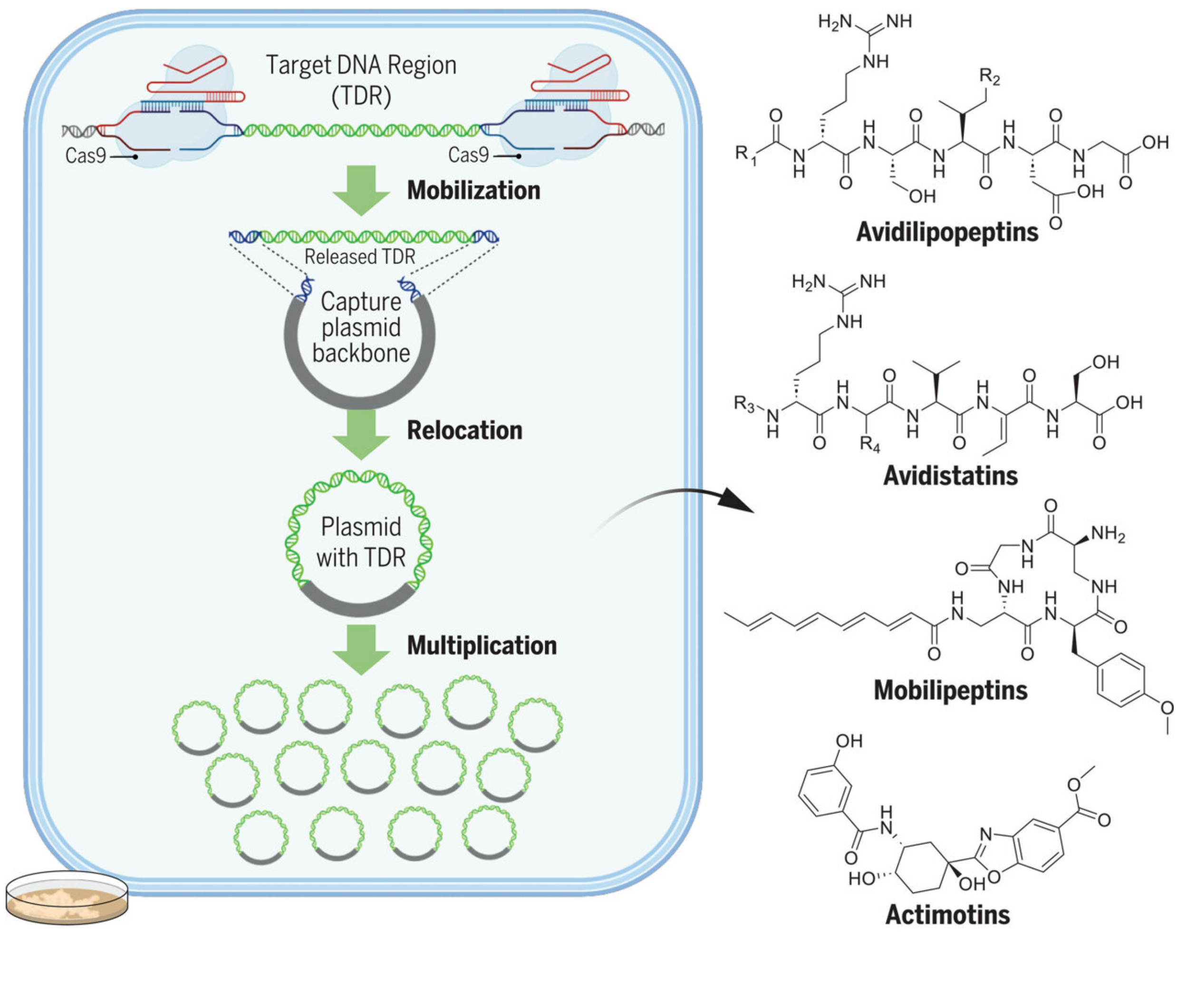

ACTIMOT facilitates the exploration of cryptic biosynthetic potential in bacteria. Credit: *Science* (2024). DOI: 10.1126/science.abq7333

Microorganisms produce a wide variety of natural products that can be used as active ingredients to treat diseases such as infections or cancer. The blueprints for these molecules can be found in the microbes' genes, but often remain inactive under laboratory conditions.

A team of researchers at the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) has now developed a genetic method that leverages a natural bacterial mechanism for the transfer of genetic material and uses it for the production of new active ingredients. The team has published its results in the journal *Science*.

In contrast to humans, bacteria have the remarkable ability to exchange genetic material with one another. A well-known example with far-reaching consequences is the transfer of antibiotic resistance genes between bacterial pathogens. This gene transfer allows them to adapt quickly to different environmental conditions and is a major driver of the spread of antibiotic resistance.

Researchers at the HIPS and the German Center for Infection Research (DZIF) have now harnessed this natural principle to amplify and isolate genetic blueprints for new bioactive natural products from bacteria, known as biosynthetic gene clusters.

Their innovative approach, called "ACTIMOT," makes it possible to either produce the natural products encoded in the gene clusters directly in the native bacterium or to transfer them into more suitable microbial production strains to produce the new molecules there. The HIPS is a site of the Helmholtz Centre for Infection Research (HZI) in collaboration with Saarland University.

ACTIMOT—short for "Advanced Cas9-mediaTed In vivo MObilization and mulTiplication of BGCs"—leverages the CRISPR-Cas9 technology, which has become known as "gene scissors," and accordingly allows precise interventions in the genetic material of bacteria. Since biosynthetic gene clusters are often less active under laboratory conditions, they are extracted from the genome using ACTIMOT and inserted into a mobile genetic unit that is then multiplied by the bacterium itself.

All these steps are performed exploiting the molecular mechanism that also allows bacteria to transfer resistance genes among each other. In many instances, the amplification of the gene clusters on these so-called plasmids is already sufficient to enable the production of the encoded natural products. If this does not succeed, the formed plasmids can be easily transferred into an alternative production strain to produce the encoded natural products. The authors provide successful examples of both approaches in the present study.

"Many biosynthetic gene clusters remain suppressed under laboratory conditions for various reasons, and current efforts to reveal the natural products they encode only address a limited number of them," says Chengzhang Fu, junior research group leader at HIPS and last author of the study.

"Our approach mimics the natural bacterial gene transfer process to directly liberate and amplify entire biosynthetic gene clusters within the native bacterial cell, granting access to previously hidden natural products. Using this technology, we can access the biosynthetic potential of bacteria much faster and easier, as compared to existing methods."

The team has already demonstrated that ACTIMOT can indeed lead to new discoveries: During the study, the researchers discovered 39 new natural products from four previously unknown natural product classes. These discoveries have given the team confidence that ACTIMOT can significantly accelerate the discovery of new drug candidates.

"Microorganisms offer us incredible potential for the production of new chemical matter that we can use, among other things, to develop urgently needed active ingredients," says Rolf Müller, head of department and scientific director of the HIPS and coordinator of the "New Antibiotics" research area at the DZIF, who also took a leading role in the study.

"So far, large parts of this microbial treasure remain hidden from us. ACTIMOT will help us to further exploit the biosynthetic potential of bacteria and thus significantly advance the development of new active agents."

In the current study, ACTIMOT has been used with bacteria of the genus Streptomyces. However, the authors are already planning to expand it to other bacterial species with a high potential for the production of unknown natural products.

Beyond this, ACTIMOT holds potential for application in various other areas, including the large-scale production of high-value natural products , the exploration of unknown gene pathways, and the identification of starting points for natural product optimization.

More information: Feng Xie et al, Autologous DNA mobilization and multiplication expedite natural products discovery from bacteria, *Science* (2024). DOI: 10.1126/science.abq7333

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