Direct cloning method CAPTUREs novel microbial natural products

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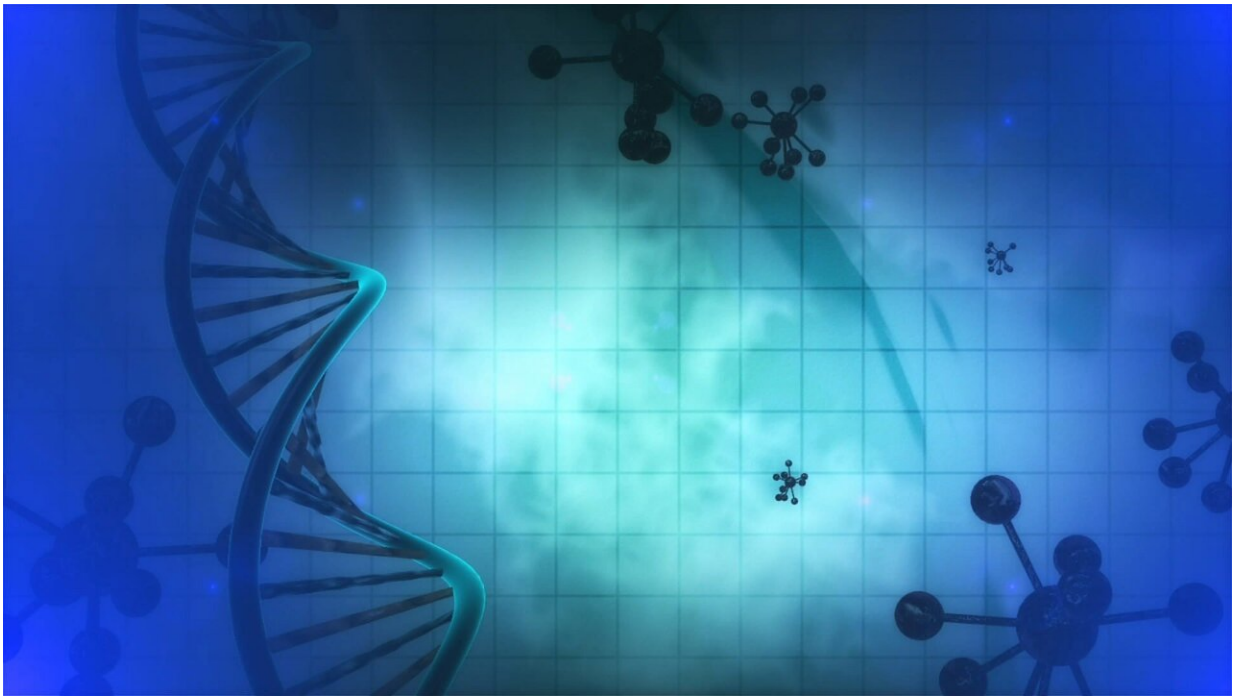

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Microorganisms possess natural product biosynthetic gene clusters (BGCs) that may harbor unique bioactivities for use in drug development and agricultural applications. However, many uncharacterized microbial BGCs remain inaccessible. Researchers at University of Illinois Urbana-Champaign previously demonstrated a technique using transcription factor decoys to activate large, silent BGCs

in bacteria to aid in natural product discovery.

Now, they have developed a direct cloning method that aims to accelerate large-scale discovery of novel natural products. Their findings are reported in the journal *Nature Communications*.

Named Cas12a assisted precise targeted cloning using in vivo Cre-lox recombination (CAPTURE), the method allows for direct cloning of large genomic fragments, including those with high-GC content or sequence repeats. Where existing direct cloning methods fail to effectively clone natural product BGCs of this nature, CAPTURE excels.

"Using CAPTURE, microbial natural product BGCs can be directly cloned and heterologously expressed at an unprecedented rate," said study leader and Steven L. Miller Chair professor of chemical and biomolecular engineering Huimin Zhao, also a member of the Carl R. Woese Institute for Genomic Biology at Illinois. "As a result, CAPTURE allows large-scale cloning of natural product BGCs from various organisms, which can lead to discovery of numerous novel natural products."

Researchers first characterized the efficiency and robustness of CAPTURE by cloning 47 natural product BGCs from both Actinomycetes and Bacilli. After demonstrating nearly 100% efficiency of CAPTURE, 43 uncharacterized natural product BGCs from 14 Streptomyces and three Bacillus species were cloned and heterologously expressed by researchers. The produced compounds were purified and determined as 15 novel natural products, including six unprecedented compounds designated as bipentaromycins. Four of the bipentaromycins exhibited antimicrobial activity against methicillin-resistant Staphylococcus aureus, vancomycin-resistant Enterococcus, and Bacillus anthracis.

"Addressing the current antimicrobial resistance crisis requires discovery of novel molecules capable of treating drug-resistant infections," said Zhao. "Discovery of bipentaromycins not only demonstrates the possibility of discovering novel antimicrobials, but it also provides an example on how this strategy can be applied for discovery of unique bioactive compounds for use in drug development and agricultural applications."

The researchers plan next to characterize these compounds for other bioactivities such as anticancer, antiparasitic and anticancer properties. Preliminary results are already showing anticancer properties for some of the compounds.

"Due to its exceptional robustness and efficiency, CAPTURE will likely become the method of choice for direct cloning of large DNA molecules such as natural product BGCs from genomic or metagenomic DNA for various basic and applied biological applications," said Zhao.

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