Title

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# New keys to unlock the treasure trove of microbial natural products

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This month's Genome Watch highlights how recent advances in computational identification of biosynthetic gene clusters (BGCs), and in their experimental manipulation, are opening new avenues to access novel secondary metabolites.

Secondary metabolism- based compound discovery has produced invaluable medical and agricultural treatments, but the discovery process has been beset with technical complications, from genomic identification to physical isolation.

As genome- scale sequencing has become routine, computational tools have been crucial as a first- pass approach for identifying secondary metabolism pathways. For many years, antiSMASH has been the most comprehensive and accurate tool for predicting secondary metabolite biosynthetic gene clusters (BGCs), and the BiG- FAM database will further advance BGC identification[1]. Constructed by clustering more than a million antiSMASH- identified BGCs from public sequence data into 'gene cluster families' (GCFs), the database allows users to quickly identify groups of closely related BGCs. BiG- FAM is closely tied with the antiSMASH ecosystem, and users can connect antiSMASH webtool result IDs directly with BiG- FAM's data, easily identifying BGC relationships in their own data. Having a system to assist in such comparisons is expected to prove invaluable for advancing secondary metabolism knowledge.

The expression of BGCs is governed by a myriad of tight regulatory systems so that specialized natural products are only produced when required by the host. This presents challenges to researchers attempting to coax host organisms to synthesize these products. To understand the mechanism of regulation, comparative metabologenomic analysis of the polycyclic tetramate macrolactam (PTM) BGC was conducted. The researchers gathered a panel of *Streptomyces griseus* strains, including poor and robust PTM producers[2],

and by comparing PTM BGC and genomic sequences with PTM production, regulatory mechanisms were revealed, including the transcriptional regulator controlling expression, and variations in the promoter that differentiate production. These results highlight the importance of studying closely related organisms to discover regulators of BGC expression and should be broadly applicable to highly sequenced bacterial clades.
Rewiring regulatory systems offers approaches to express BGCs and synthesize their products. Park et al. employed a massively parallel reporter assay to explore a library of >3,000 natural BGC regulatory sequences in *Streptomyces albidoflavus* J1074 (ref.3). Through this approach, the authors found transcription levels spanning a 1,000- fold range and identified key features associated with expression. By expressing global regulatory proteins, the authors could differentially modulate BGC regulatory sequences. Heterologous expression of transcriptional regulators can lead to BGC expression, providing foundational knowledge to overcome control mechanisms, and allowing novel metabolites to be produced on demand.

Cloning and heterologous expression of BGCs is an alternative strategy. However, the large and repetitive sequence of many BGC classes makes their cloning via traditional methods inefficient. ‘CAPTURE’ is a rapid and highly efficient direct cloning method consisting of DNA digestion by Cas12a, DNA assembly and Cre–*lox* in vivo DNA circularization. The technique was successfully used to clone 47 BGCs with varying sizes up to 113 kb (ref.4). Characterizing these BGCs resulted in the discovery of 15 new secondary metabolites, including bipentaromycin, which showed strong antimicrobial activity against multiple bacteria, including methicillin- resistant *Staphylococcus aureus*. Although there are a tremendous number of natural products remaining to be discovered, continuous advancement in genomic technologies, using a multi- faceted combination of computational and laboratory techniques, will be essential for unlocking the metabolic potential of microorganisms.

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