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Genome engineering for microbial natural product discovery

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The discovery and development of microbial natural products (MNPs) have played pivotal roles in the fields of human medicine and its related biotechnology sectors over the past several decades. The post-genomic era has witnessed the development of microbial genome mining approaches to isolate previously unsuspected MNP biosynthetic gene clusters (BGCs) hidden in the genome, followed by various BGC awakening techniques to visualize compound production. Additional microbial genome engineering techniques have allowed higher MNP production titers, which could complement a traditional culture-based MNP chasing approach. Here, we describe recent developments in the MNP research paradigm, including microbial genome mining, NP BGC activation, and NP overproducing cell factory design.

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Introduction

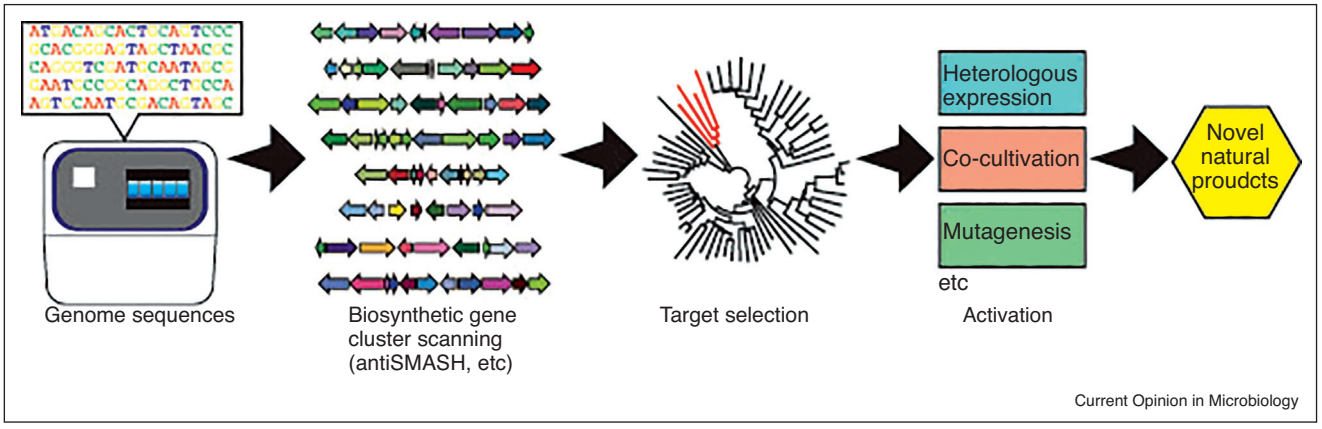
Microbial natural products (MNPs) have been a major storehouse for drug discovery and development for several decades [1,2]. In fact, more than 20 000 active MNPs have been utilized as lead compounds, including antimicrobials, anti-virals, and cytotoxic and immunosuppressive compounds, mainly due to their structural novelty, diversity, and complexity. Since the early 1950s, many MNPs have been discovered and studied, particularly from actinomycetes. *Streptomyces*, the most well characterized genus of actinomycetes, has been considered as one of the most important types of industrial bacteria due to its superior capabilities in producing valuable secondary metabolites. More than 50% of commercially available antibiotics and their lead compounds originate from this genus of actinomycetes. Although the rediscovery issue has led to a slight decline in MNP discovery efforts over the past several decades, especially in the pharmaceutical industry, novel MNP chemical scaffolds continue to generate valuable lead compounds thanks to state-of-the-art genome mining and synthetic biology approaches.

MNP genome mining approach

Because of microbial genome information accumulated in the 1990s to early 2000s and investigations into MNP biosynthetic machinery, there has been an increase in the number of putative MNP BGCs with unknown products [1,3••]. For example, the complete genome sequence of the well-studied *Streptomyces coelicolor* A3(2) contains a higher number of BGCs than that of the known natural products produced by this strain [4•,5]. Such orphan BGCs are expected to be an important source of novel MNP scaffolds. Thus, discovering novel MNPs based on genome information, which is called 'genome mining', has become an emerging trend in the field of natural product science (Figure 1).

Because of advances and low cost in next generation DNA sequencing techniques, numerous genome sequences now become accessible. Furthermore, development of bioinformatic tools has enabled the high-throughput discovery of orphan BGCs for MNPs [3••,6]. There are several programs useful for genome mining of a specific class of MNPs. For instance, NaPDoS was developed by Ziemert *et al.* to analyze ketosynthase (KS) and condensation (C) domains from PKSs and NRPSs, respectively [7]. The polyketide synthases (PKSs)/non-ribosomal peptide synthetases (NRPSs) Analysis Web-site is a tool to analyze the domain organization of PKS and NRPS [8]. NRPSpredictor is one of the most useful tools to predict amino acid selectivity of NRPS [9]. Recently, RiPPMiner was developed by Agrawal *et al.* to analyze the ribosomally synthesized and post-translationally modified peptides (RiPPs) BGCs. antiSMASH was developed by combining several open source bioinformatic tools to comprehensively detect various kinds of BGCs, including polyketides, non-ribosomal peptides, terpenoids, and siderophores BGCs [10]. Therefore, it is now considered as one of the most

Figure 1



Schematic representation of genome mining. The genome sequencing using next generation genome sequencer rapidly provides information of microbial genomes. The putative BGCs can be discovered by bioinfomatic tools, such as antiSMASH. After the selection of a target gene by phylogenetic analysis, the function of each BGC should be analyzed. To awaken a sleeping BGC, several methods may be examined. Comparative metabolomics is useful to find the novel natural products that are produced by the awakened BGC.

important programs for genome scanning of MNP BGCs [11,12,13•]. In addition, other useful tools available for genome mining are also summarized in the following web site (https://omictools.com/secondary-metabolite-biosynthetic-pathways-category). Furthermore, there are several databases focusing on BGCs. Integrated Microbial Genomes-Atlas of Biosynthetic Gene Clusters (IMG-ABC, https://img.jgi.doe.gov/cgi-bin/abc/main.cgi) organized by the Joint Genome Institute and Minimum Information about a Biosynthetic Gene cluster (MIBiG, http://mibig.secondarymetabolites.org/index.html) organized by the Genomic Standards Consortium are important databases that provide useful information for identifying candidate BGCs for genome mining.

Selection of target BGCs is the most important first step in genome mining. Target BGCs can be selected by either core biosynthetic genes, such as PKS and NRPS genes, or tailoring enzyme genes that are responsible for modification of the core scaffolds of natural products [7]. In both cases, comparative or phylogenetic analysis of BGCs is essential for selecting BGCs involved in the production of novel natural products [3••,7,14,15]. Hypothetical antibiotic-resistance genes can also be used as clues for the selection of BGCs [16,17]. This method is useful for identifying secondary metabolites with novel modes of action.

BGC awakening strategies

There are still some difficulties in genome mining since many orphan BGCs are silent or barely expressed under typical laboratory culture conditions [1,18]. Therefore, the development of awakening strategies for these sleeping BGCs is very critical in order to convert the potential derived from genome mining into reality. Various strategies to awaken sleeping BGCs have been reported, including regulatory network optimization, ribosome engineering, chemical elicitors, co-cultivation, and heterologous expression [6,19–27].

It has been well characterized that many MNP BGCs are tightly regulated at the transcriptional level by complicated regulatory networks. To induce silent BGC expression, the promoter swapping method using strong promoters, such as the *ermE** and *kasO* promoters, has been successfully applied. LAL pathway-specific regulators, identified from silent type I polyketide BGCs in *Streptomyces ambofaciens*, were overexpressed under the control of the strong constitutive *ermE** promoter, leading to a production of four novel 51-membered glycosylated macrolides stambomycins A-D [20,28–32]. Ribosomal engineering using *rpoB* and *rpsL* manipulations also resulted in the production of antibiotics in various actinomycetes. In the case of *Saccharopolyspora erythraea*, mutant (H437R) of *rpoB* demonstrated a fourfold increase in erythromycin production as well as a marked elevation of cryptic BGC transcription [33]. Addition of chemical elicitors also induced the production of MNPs. For instance, supplementation of *N*-acetylglucosamine or diphenyl ether derivatives was revealed to induce or enhance production of MNPs in various *Streptomyces* strains. The paper by Rigali and colleagues showed that a high concentration of *N*-acetylglucosamine was transmitted to antibiotic pathway-specific activators through the pleiotropic transcriptional repressor DasR in *S. coelicolor* [34]. Craney and colleagues screened small molecules that activated the biosynthesis of secondary