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Review

Genome mining of rare actinomycetes and cryptic pathway awakening

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ABSTRACT

Actinomycetes have been major storehouse microorganisms for the discovery of natural products in the last several decades. Although many of the discovered natural products have been successfully isolated from the most dominant actinomycetes genus, *Streptomyces*, novel natural products are increasingly coming from the non-*Streptomyces* family, known as rare actinomycetes. Moreover, some of the natural-product biosynthetic gene clusters in actinomycetes are silent or cryptic, meaning that the gene clusters are not expressed under typical laboratory culture conditions. Accordingly, genome mining has become a widely practiced alternative way to identify potentially valuable cryptic natural products from actinomycetes. Unlike the many advances in *Streptomyces* genomes mining, however, relatively few genome mining cases are available in rare actinomycetes. In this mini review, we discuss the biotechnological potential of rare actinomycetes as natural product storehouses, followed by a survey of the natural products that have been identified through the traditional processes of actinomycete screening. We focus in depth on several known examples of novel secondary metabolites as identified through the genome mining of rare actinomycetes. In addition, various approaches that have been applied in awakening techniques for actinomycete cryptic metabolite pathways are summarized.

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1. Introduction

Since the discovery of penicillin in the twentieth century, microbes have been important in the production of more than 23,000 active compounds (i.e., antimicrobials, antivirals, cytotoxic and immunosuppressive compounds) [1]. Although many groups have attempted to identify new microorganisms and new metabolites, less than 1% of all of the existing microorganisms have been identified and characterized [2]. There is still a large pool of uncultured microorganisms and various natural products that are scattered in natural environments. Nevertheless, many of the natural products that were newly discovered in the twentieth century have turned out to be rediscoveries of previously known compounds. More recently, the occurrence of antibiotic resistance and new diseases has increased the importance of the discovery of new bioactive compounds for pharmaceutical drug leads. Research on the remaining 99% of new microbes from unexplored habitats plays an important role as a source of novel antibiotics and other therapeutic agents against drug-resistant bacteria.

2. Natural products in rare actinomycetes

Actinomycetes are a group of high G + C Gram-positive bacteria that are spread widely throughout various natural environments [3,4]. Actinomycetes possess a large number of genes that encode for numerous bioactive metabolites. Since the early 1950s, many natural products have been discovered and studied, particularly from the category of actinomycetes. *Streptomyces*, the best-characterized genus of actinomycetes, is considered one of the most important types of industrial bacteria due to its superior capabilities in producing valuable secondary metabolites, including antibiotics, anti-cancer drugs, immunosuppressants, and enzyme inhibitors [5–7]. More than 50% of the commercially available antibiotics and their lead compounds originate from this genus of actinomycetes [1]. Unfortunately, recent reports have demonstrated that the discovery of novel secondary metabolites from the *Streptomyces* species has dramatically decreased. Currently, more new discoveries are coming from the non-*Streptomyces* actinomycetes, called rare actinomycetes, which consist of more than 200 genera [8]. Rare actinomycetes are typically defined as genera in which the isolation frequency by conventional methods is significantly lower than the isolation frequency of the *Streptomyces* species. Rare actinomycetes include *Actinomadura*, *Actinoplanes*, *Amycolatopsis*, *Actinokineospora*, *Acrocarpospora*, *Actinosynnema*, *Catenuloplanes*, *Cryptosporangium*, *Dactylosporangium*, *Kibdelosporangium*, *Kineosporia*, *Kutzneria*, *Microbiospora*, *Microtetraspora*, *Nocardia*, *Nonomuraea* (*Sebekia*), *Planomonospora*, *Planobispora*, *Pseudonocardia*, *Saccharomonospora*, *Saccharopolyspora*, *Saccharothrix*, *Streptosporangium*, *Spirilliplanes*, *Thermomonospora*, *Thermobifida*, and *Virgosporangium* [9]. The ABL database (Antibiotic Literature Database) notes that approximately 61.6% of antimicrobial products, which are discovered according to their origin, are produced by actinomycetes. Among these 61.6% actinomycete products, non-*Streptomyces* (i.e., rare actinomycetes) metabolites comprise 16% of the total products [9]. Whereas in 1970 rare actinomycete products represented only 5% of the total actinomycete products, 26% of all recent compounds from actinomycete species are derived from rare actinomycetes [10]. Although the majority of known natural products have been discovered in terrestrial environments, emerging rare actinomycetes from other habitats, including marine environments, will also be important storehouses for natural products in the future [8]. Although many researchers have tried to discover novel strains, most screening results have rediscovered streptomycetes as the dominant species. In contrast, because rare actinomycetes are more difficult to isolate and culture, they represent an important aspect of new drug discovery research by acting as a huge resource of structurally diverse natural compounds with unusual biological activities [11]. According to a previous report by Bérdy, rare actinomycetes can produce diverse, unique, impressive, and occasionally complicated compounds with excellent antibacterial potency and low toxicity [10]. Fortunately, the development of a wide variety of methods, such as specialized sampling techniques, various pretreatment procedures and selective media, is currently in progress for the assessment of novel rare actinomycetes. Table 1 shows antibiotic compounds belonging to diverse chemical classes that have been discovered as products of various rare actinomycetes [11–220].

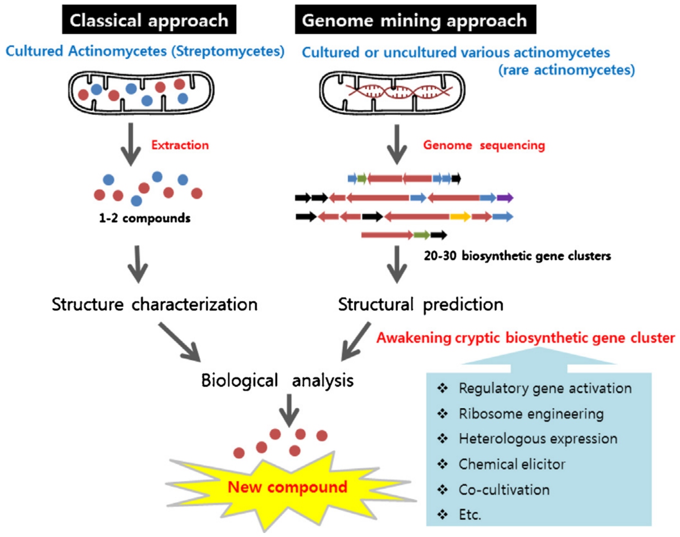

Fig. 1. Overview of the classical approach and the genome-mining approach to natural product screening.

3. Genome mining of rare actinomycetes

Rare actinomycetes have become a recent focus in the search for novel natural products with a broad range of biological activities and pharmacological properties. Moreover, the advancement of genome-mining technologies has recently resulted in the discovery of diverse natural products from rare actinomycetes. Genome mining is a powerful paradigm for the discovery and characterization of natural product biosynthetic genes [221]. As shown in Fig. 1, the advantages of the genome mining approach over the classical approach include that it is possible to obtain uncultivable rare actinomycetes and a massive amount of genomic data (cryptic gene clusters). Since the first reports of the complete sequences of the *S. coelicolor* and *S. avermitilis* genomes, numerous *Streptomyces* genome sequences and analyses have revealed a large number of cryptic gene clusters that encode secondary metabolism [222]. Although spiramycin and congocidine (netropsin) from *Streptomyces ambofaciens* were discovered many years ago by traditional fermentation, the recent genome mining approach revealed many additional gene clusters of potential valuable metabolites, including kanamycin derivatives and stambomycin (Fig. 2, [223]). The genomes of actinomycetes are hiding a huge degree of chemical diversity that is encoded in silent (i.e., cryptic) biosynthetic gene clusters. It is exceedingly difficult to isolate and characterize these cryptic gene clusters that encode natural products within organisms using conventional methods. Thus, numerous researchers have focused on genome mining to address cryptic gene clusters encoding potentially valuable novel natural products. The complete or draft sequencing of the whole genomes of rare actinomycetes is