Discovery of Novel Natural Products by Refactoring Cryptic Pathways

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Introduction. Microorganisms are a major source of new therapeutic agents. Sequenced genomes and metagenomes provide a tremendously rich source for discovery of novel gene clusters involved in natural product biosynthesis (1-2). However, due to the lack of tools to efficiently identify a complete biosynthetic gene cluster, determine the role of each involved gene, and subsequently designate a function to the target gene cluster, only a tiny fraction of those putative natural product biosynthetic gene clusters have been characterized (3-5). To overcome this limitation, we have developed a new genomics-driven, synthetic biology enabled method to discover and produce novel natural products from sequenced genomes and metagenomes.

Methods. By taking advantage of the highly efficient yeast *in vivo* homologous recombination mechanism, this method refactors the target cryptic pathway together with the genetic elements needed for DNA maintenance and replication in *S. cerevisiae*, *E. coli*, and a target heterologous host using a plug-and-play scaffold and a set of heterologous promoters that are functional in the target heterologous host.

Results. As proof of concept, we awakened the silent polyketide spectinabilin pathway from *Streptomyces spectabilis* in *Streptomyces lividans* (Fig.1) and activated a cryptic pathway containing a polyketide synthase-non-ribosomal peptide synthetases from *Streptomyces grieseus* in *Streptomyces lividans*, which led to the discovery of two novel tetramic acid natural products that have never been reported in literature (Fig. 2).

Conclusions. The synthetic biology strategy we present here is simple, generally applicable, and potentially scalable. Our method bypasses the traditional laborious processes to elicit pathway expression and represents a new platform for discovering novel natural products.

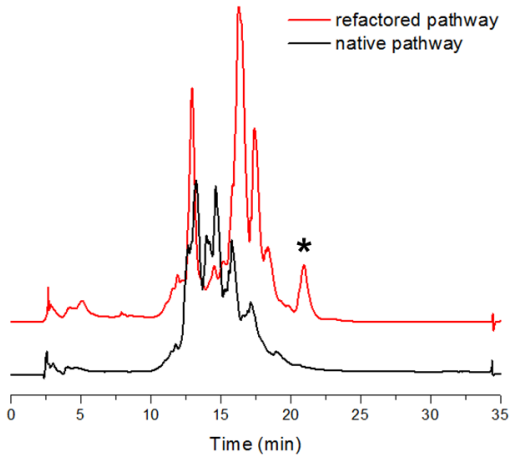

Fig.1 HPLC analysis of the extract from the *S. lividans* strain carrying the refactored pathway involved in the synthesis of spectinabilin.

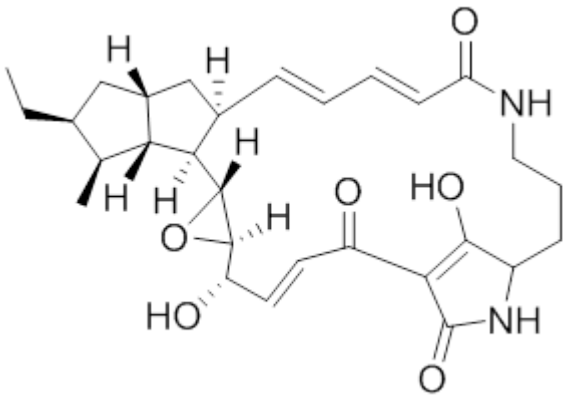

Fig.2 Chemical structure of one of the two novel tetramic acid natural products.

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