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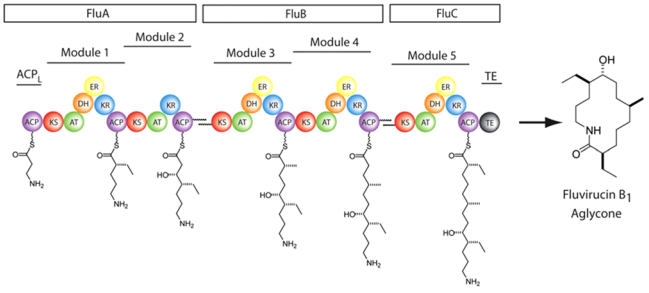
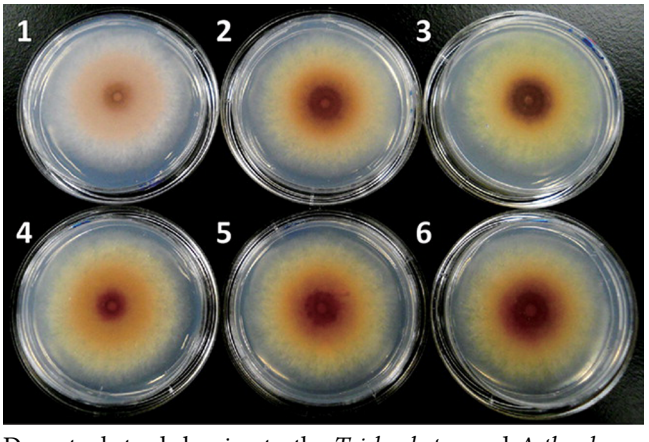
ANALYSIS OF THE FLUVIRUCIN B1 POLYKETIDE SYNTHASE



Polyketides are an enormous class of natural products with important pharmaceutical activities ranging from antibiotic and antiviral to anticancer and immunosuppressive. Nature builds these molecules via biosynthetic machines termed polyketide synthases, which function in a manner similar to an industrial assembly line. Products are built stepwise through addition of two-carbon units and subsequent tailoring of functional groups along the assembly line before being released from the synthase by cyclization or hydrolysis. Here, Lin et al. (DOI: 10.1021/sb4000355) report the discovery and bioinformatics-based characterization of one such polyketide synthase responsible for the production the antifungal/antiviral molecule fluvirucin B1 produced by *A. vulgaris*.

Identification of these types of pathways opens doors to genetic engineering for generating novel natural products with improved pharmaceutical properties. The fluvirucin polyketide synthase contains a unique and promising arrangement of enzymes for engineering purposes and, as a result, will offer increased potential for structure diversification.

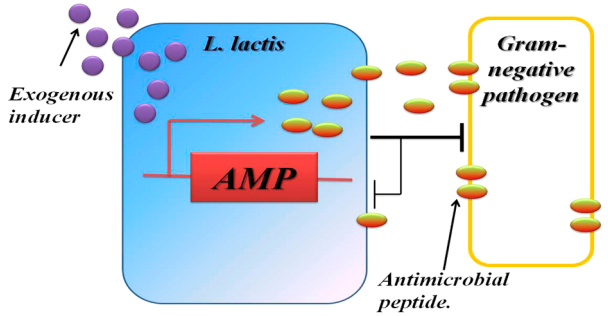
DISCOVERY OF CRYPTIC POLYKETIDE METABOLITES FROM DERMATOPHYTES



Dermatophytes belonging to the *Trichophyton* and *Arthroderma* genera cause skin infections in humans and animals. Secondary metabolites play important roles in the pathogenesis of these microorganisms and genome sequencing of numerous dermatophytes revealed that each genome encodes several secondary metabolite clusters. In this paper, Yin et al. (DOI: 10.1021/sb400048b) successfully transferred some of these dermatophyte gene clusters into a model host, *Aspergillus nidulans*, and demonstrated that these cryptic pathways produce neosartoricins B, C, and D, which are related to a previously described immunosuppressive compound.

In order to clone and stably maintain the large DNA fragments in a plasmid, the authors created an *E. coli*-yeast-*Aspergillus* shuttle vector. This allowed the facile assembly of >20 kb DNA fragments. This system also creates a new method of analyzing biosynthetic pathways from difficult to handle organisms.

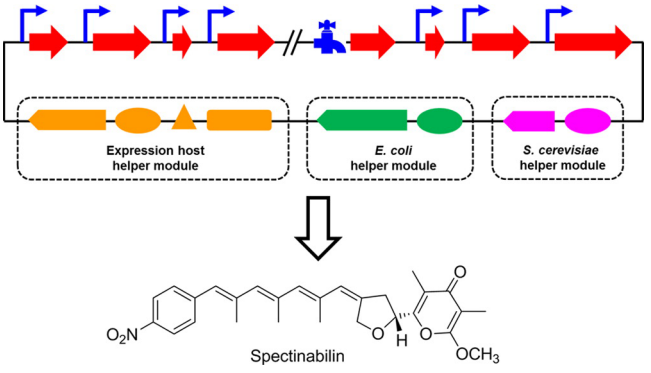
ANTIMICROBIAL PEPTIDES TARGETING GRAM-NEGATIVE PATHOGENS



The emergence of antibiotic resistant bacteria is an issue of growing concern. A significant source of drug-resistance development is the widespread use of antibiotics in animal production. Antibiotics are often administered to cattle, pigs, and poultry, to promote growth and improve feed efficiency, even in the absence of infection. This subtherapeutic administration of antibiotics results in drug-resistant bacteria that infect humans through food. Thus, new technologies are needed to reduce the use of antibiotics for animal growth in agriculture and to treat food-borne gastrointestinal infections in humans caused by antibiotic-resistant bacteria.

Now, Volzing et al. (DOI: 10.1021/sb4000367) report the design of recombinant *Lactococcus lactis* that produce and secrete heterologous antimicrobial peptides with activity against Gram-negative pathogenic *Escherichia coli* and *Salmonella*. This system provides a potential alternative to the use of antibiotics in agriculture.

REFACTORING SILENT BIOSYNTHETIC GENE CLUSTERS



Natural compounds are complex molecules with antibacterial, antiviral, anticancer, and other important biological properties.

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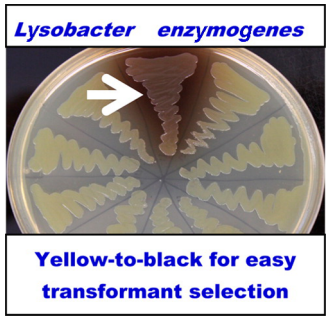
Now, Shao et al. (DOI: 10.1021/sb400058n) describe a new synthetic biology strategy to activate silent biosynthetic pathways from sequenced genomes and metagenomes for discovery of novel natural products.

The authors use a plug-and-play scaffold and a set of heterologous promoters that are functional in a heterologous host under target culturing conditions to refactor the silent biosynthetic pathway of interest. As proof of concept, they demonstrate the activation of the silent spectinabilin pathway from *Streptomyces orinoci* in *Streptomyces lividans*. This strategy offers a new platform for *de novo* cluster assembly and genome mining for discovering new natural products.

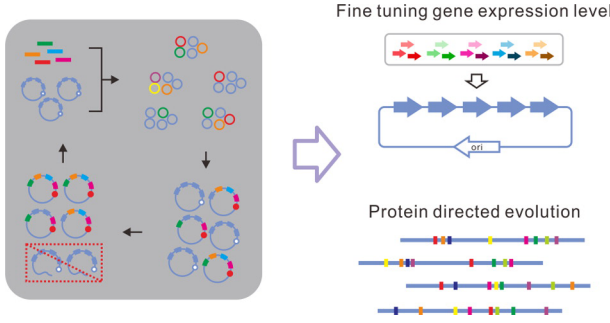
A FACILE METHOD FOR SITE-SPECIFIC GENE INTEGRATION



Over the last several years, there continues to be a pressing need for new antibiotics. Recently, *Lysobacter* has emerged as a new source for bioactive natural products. However, the efforts have been hampered due to the lack of efficient tools for its genetic manipulation. Here, Wang et al. (DOI: 10.1021/sb4000806) describe the development of a facile method for quick-and-easy identification of *Lysobacter* transformants from a large population.

The method described here is based on a distinct yellow-to-black color change as a visual selection marker for site-specific integration of the gene of interest. As proof of principle, the authors constructed a series of expression vectors for a regulator gene and, using this method, integrated the constructs into *Lysobacter enzymogenes*. This resulted in a 2−7 fold yield increase for two new antibiotics. This work represents the first successful metabolic engineering of *Lysobacter* and paves the path toward manipulating antibiotic production in these largely unexplored bacteria.

MULTIPLEX ITERATIVE PLASMID ENGINEERING



Engineering complex biological systems typically requires extensive optimization to achieve the desired functionality. Here, Li et al. (DOI: 10.1021/sb400051t) present Multiplex Iterative Plasmid Engineering (MIPE), a method that enables the simultaneous fine-tuning of several components in a synthetic gene network.

MIPE uses synthetic oligonucleotides for the introduction of modifications in plasmid sequences, which allows it target up to 20 sites simultaneously. This, in turn, enables the generation of very large libraries. The authors used MIPE to optimize the gene expression level in the 5-gene riboflavin biosynthetic pathway and successfully isolated a clone with 2.67-fold improved production in less than a week. This method of optimization of diverse biological systems is easily accessible and cost-effective since it requires only the recombineering strains and synthetic primers.