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Preview

Exploiting *Pseudomonas putida* for Drug Development

In this issue of *Chemistry & Biology*, a strategy that combines large DNA fragment recombineering in *Escherichia coli* and heterologous expression in *Pseudomonas putida* is described. The work focuses on myxochromide S, a natural compound produced by *Stigmatella aurantiaca.*

An important source of drug leads is natural products. As many as 60% of successful drugs are of natural origin, and some of the most potent anticancer, antibacterial, and antifungal drugs are natural products. The search for novel natural compounds was very successful between 1940 and 1980. Automated instrument systems, robots, and high-throughput screening platforms have enabled the pharmaceutical industry to screen thousands of microorganisms and plants for novel compounds. Despite these efforts, the paucity of drug discoveries since 1990 is coupled with the fact that natural products are produced in small quantities in their native hosts, making the drugs too expensive to harvest [1].

Approximately 20 years ago, scientists developed methods to introduce DNA into *Streptomyces* species [2]. A few years later, a new technology called *combinatorial biosynthesis* was born [3]. In principle, this technology makes it possible to alter the structure of a given molecule in the living organism, and even complex structures can be modified that cannot be altered by chemical synthesis. Since 1999, drug discovery strategies for pharmaceutical applications have been experiencing a revolutionary period. The completion of several genome projects has provided thousands of biosynthetic gene clusters of as yet unknown compounds. More recently, genes and enzymes of these clusters have been used for the generation of novel compounds. Metabolic engineering has begun to have an effect on natural product drug discovery in fundamentally new and practically useful ways. Metabolic engineering involves much more than simply introducing genes into a cell—it often involves carefully balancing the genes in the new metabolic pathway so that no gene is drastically overexpressed or underexpressed, both of which prevent synthesis of the desired compound. As such, the control of gene expression and the control of metabolic flux balances is a core issue in the production of drugs using metabolic engineering [4].

In this issue of *Chemistry & Biology*, Rolf Müller and colleagues [5] describe a straightforward strategy that makes possible the expression of a large biosynthetic gene cluster in *Pseudomonas putida*. The group focused its work on myxochromide S, a natural compound produced by the myxobacterium *Stigmatella aurantiaca*. Myxochromides S are cyclic peptides connected to unsaturated polyketide side chains [6]. Genes that are involved in the biosynthesis of these compounds are large in size and together comprise more than 60 kb. Müller's group began their work by isolating a cosmid containing parts of the myxochromide biosynthetic gene cluster. Biosynthetic genes missing additional regulatory elements were then introduced into the cosmid via genetic engineering using the Red/ET recombination system. It is worthwhile to mention that one of the regulatory elements was the toluic acid–inducible P_m promoter, which was inserted in front of the first gene of the cluster. All experiments described so far were performed in *Escherichia coli.* The engineered construct could then be transferred into *Pseudomonas putida*, and after induction the recombinant strain was producing approximately 40 mg/l myxochromide S, five times more than *Stigmatella aurantiaca.* The work performed by Müller's group is opening the door to a new chapter in natural product biology and chemistry. A strategy is provided that promises to express clusters of unknown function as discovered by genome sequencing in many organisms and also to express large DNA fragments isolated from noncultivable bacteria. In addition, this work will make it possible to combine different gene clusters on one DNA fragment, increasing the chance for the production of novel natural products.

There are a few issues not already discussed in the study. The successful expression of biosynthetic gene clusters in *Streptomyces* strains or even in *E. coli* have been described in the past [7–9]. However, a general method for the expression of gene clusters has never been described, and, although data have not been published, scientists all over the world have failed to do so countless times. The challenge now for Müller's group will be to provide further successful examples and describe the limitations of this new technology.

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