Minireview

Principles of microbial alchemy: insights from the *Streptomyces coelicolor* genome sequence

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Abstract

The world's most creative producers of natural pharmaceutical compounds are soil-dwelling bacteria classified as *Streptomyces*. The availability of the recently completed *Streptomyces coelicolor* genome sequence provides a link between the folklore of antibiotics and other bioactive compounds to underlying biochemical, molecular genetic and evolutionary principles.

Streptomyces, which belong to the bacterial order Actinomycetales, are similar in appearance to fungi and undergo a program of colonial morphogenesis that is coordinated with the excretion of bioactive compounds that are often colored (Figure 1) [1]. The biosynthesis of these compounds, many of which are antibiotics, occurs typically in response to a breakdown of balanced metabolism that interrupts or arrests growth [2,3]. On solid media this triggers formation of aerial filaments that septate and mature into a chain of spores.

Streptomyces species are considered exceptionally well endowed for 'chemical warfare', presumably allowing them to eliminate bacterial and fungal competitors in soil ecosystems. This was first realized over fifty years ago, and since then the majority of known antibiotics have been isolated from *Streptomyces*. Although thousands of antibiotics have been described, these are thought to represent only a small fraction of the repertoire of bioactive compounds produced by *Streptomyces* [4,5]. In addition, empirical screening using various assays has revealed that *Streptomyces* culture supernatants contain other pharmaceutically active compounds, such as anti-viral and anti-cancer compounds, modulators of immune responses, and various enzyme inhibitors, as well as herbicides, insecticides, and anti-parasitic compounds [4,6,7]. Furthermore, chemical screening methods have uncovered the remarkable structural diversity of these compounds. Genome sequences of *Streptomyces* species are now providing an overview of the genetic elements responsible for this metabolic diversity, both within one bacterium and the entire genus. Analysis of the completed genome sequence of *Streptomyces coelicolor* [8] and its comparison with other species will give further insights into availability and nature of potentially useful bacterial metabolites.

Dozens of different *Streptomyces* species (Figure 1a) [1] can be selectively isolated from virtually any rich soil sample. Tens of thousands of *Streptomyces* strains are screened annually by pharmaceutical companies as potential sources of novel chemical compounds. High-throughput chromatographic analyses typically reveal that each strain has its own unique profile of secondary metabolites (metabolites that are secondary to the growth and maintenance of the cell), mostly compounds that are not found in synthetic chemical handbooks or combinatorial chemical libraries. The next step of this commercial process, namely increasing yields to make large-scale production of the compound feasible, has relied on screening for better culture conditions and overproducing mutants. Mutations that result in higher yields of certain metabolites can now be combined into one organism by genomic shuffling [9], thus reducing the time needed for developing industrial production strains. Although such empirical approaches have been very fruitful for individual secondary metabolites, the improvements are strain-specific, so the acquisition of useful knowledge is not cumulative.
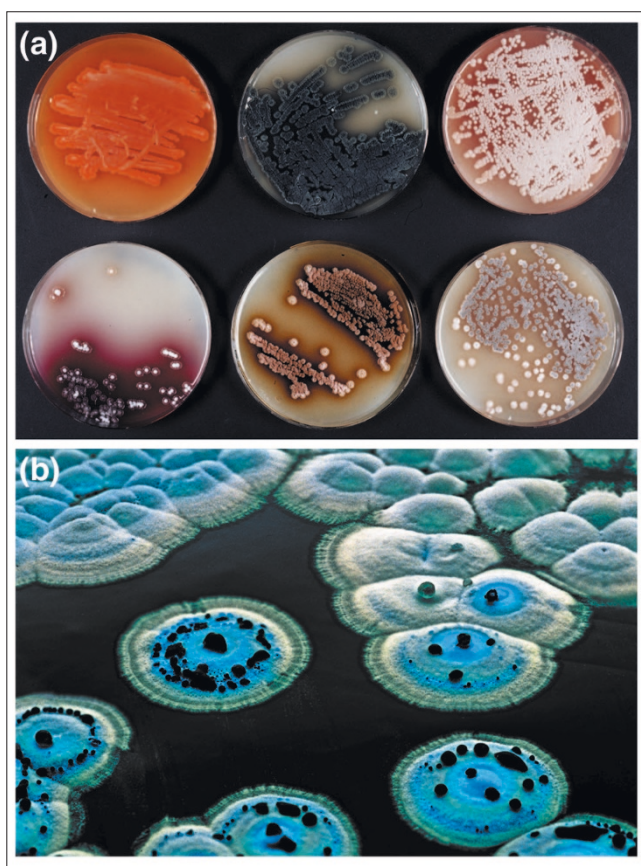
Figure 1
Production of pigmented secondary metabolites by *Streptomyces* colonies.
(a) Typical colonial morphologies of *Streptomyces* isolated from the soil.
Colonies often excrete colored pigments, providing a visual recording of
secondary metabolite biosynthesis. The chemically diverse compounds
represent a vast array of bioactive compounds that often have
pharmaceutical applications. **(b)** A panoramic view of *Streptomyces
coelicolor* colonial morphology. Both peripheral and aerial mycelia develop
from the central mass of the colony. Metabolites, including the blue
antibiotic actinorhodin, are excreted into the medium and into aqueous
droplets on the hydrophobic surface of the colony. Images courtesy of
Tobias Kieser, Celia Bruton and Jennifer Tenor.

Thus, new *Streptomyces* secondary metabolites are still
accessible only through expensive and time-consuming
screening techniques.

The diversity of secondary metabolite structures defies
simple chemical classification, but many of the most useful
and best understood compounds are synthesized by two
families of multifunctional enzymes that can assemble
unusual carbon and peptide chains. Polyketide and peptide
synthases catalyze condensation reactions that can be used
to assemble acetyl CoA or amino-acid derivatives into
polyketide and polypeptide structures, respectively. Polyketide
synthases are structurally and functionally homologous to
fatty acid synthases. 'Classical' fatty acid synthases initiate
the assembly of fatty-acid chains with acetyl CoA, increase

chain length by condensation with malonyl CoA and fully
reduce the carboxyl residues at each step of extension.
These have evolved into two different types of polyketide
synthases: type I polyketide synthases, which catalyze a
reaction in which the growing acyl chain is sequentially
transferred to a series of modular reaction centers of the
enzyme, typically generating macrocyclic lactone rings char-
acteristic of so-called macrolide antibiotics; and type II
polyketide synthases, which typically condense malonyl CoA
iteratively, using the same active condensation site to generate
aromatic compounds. Polypeptide-based antibiotics are
often synthesized by non-ribosomal peptide synthases,
which can condense more than 15 amino acids or unusual
analogs into linear or cyclic structures.

Rapid progress in understanding these complex multi-
functional enzymes was achieved through a combination of
genetic and chemical approaches [10]. It is now possible to
predict and engineer the structure of both polypeptides [11-14]
and polyketides [11-13,15]. The modules of non-ribosomal
peptide synthase and type I polyketide synthase enzymes are
arranged in an order that is colinear with the component
residues in the mature polymer. Furthermore, these enzyme
modules can be genetically engineered to reprogram the
order and modifications of residues within the polymers.
Using this technology, dozens of derivatives of the polyketide
erythromycin have been synthesized by reprogramming the
erythromycin polyketide synthase [16]. Domain swapping
between peptide synthases has demonstrated the feasibility
of engineering peptide antibiotics [14]. Engineering addi-
tional domain modules will increase the repertoire available
for introducing random changes or designing new antibiotics.
It remains to be seen, however, whether these approaches
will rival the diversity and specificity of compounds that have
evolved in biological systems over hundreds of millions of
years. For example, natural products are already specifically
preselected to avoid negative interactions with other protein
domains that might make them toxic to bacteria that produce
them and likewise to humans. So how can genome sequencing
help provide better access to natural products?

A remarkable revelation of the *S. coelicolor* genome sequence
was that the numbers of secondary metabolites that can be
made by a single *Streptomyces* isolate may be even more
extensive than was previously recognized [8]. Homology-
based searches using only polyketide and peptide synthases
revealed many unrecognized metabolic capabilities in
S. coelicolor (Figure 1b) and *Streptomyces avermitilis*, a
species that has been used for many years as a source of the
anti-parasitic compound avermectin for human and veteri-
nary medicine. The *S. avermitilis* genome is predicted to
encode at least 25 different secondary metabolites [17], includ-
ing melanins, carotenoids, siderophores (microbial iron-
binding agents required for iron import), type I and type II
polyketides, and non-ribosomally assembled peptides. Forty
years of genetic studies of *S. coelicolor* identified only a few of

its secondary metabolites, namely prodigiosins (red pigmented antibiotics), two type II polyketides (the antibiotic actinorhodin as well as a gray spore pigment encoded by the *whiE* locus), and the non-ribosomal peptide calcium-dependent antibiotic (CDA). The *S. coelicolor* genome sequence now predicts 18 additional secondary metabolites, including polyketides synthesized by polyketide synthases of type I and type II and by a third type found primarily in plants, peptides assembled by non-ribosomal peptide synthases, siderophores, hopanoids (sterol-like bacterial lipids), butyrolactones (bacterial pheromones), terpene, geosmin (the predominant natural odor of soil), and others [8].

The genes of a secondary metabolic pathway are typically coregulated, clustered within the bacterial chromosome, and functional in heterologous species. Early mapping studies [18] revealed synteny (conserved order and orientation) of genes within homologous gene clusters encoding compounds of antibiotic biosynthesis pathways in different species. This probably reflects common ancestry and common adaptive functions for these gene clusters. Similarly, early linkage analyses of *Streptomyces* chromosomes suggested synteny of auxotrophic markers in different species [19]. Antibiotic biosynthetic clusters were not found in the same places in different *Streptomyces* chromosomes, however, suggesting that these clusters were incorporated later. Sequence analysis of *Streptomyces* chromosomes supports these initial findings and provides further evidence that secondary metabolic islands (SMILES), such as the antibiotic biosynthetic clusters, were acquired by horizontal transmission. Like pathogenicity islands - genomic segments in bacterial pathogens that encode virulence factors and may have evolved through horizontal transfer - genes for illegitimate recombination, such as those encoding integrases and transposases, are occasionally identified within or adjacent to antibiotic gene clusters in *S. avermitilis* [17] and *S. coelicolor* [8]. For example, genes found associated with the secondary metabolite clusters *whiE* and *cda* in *S. coelicolor* are such neighbors. Because the GC content of these clusters is indistinguishable from that of the adjacent chromosome sequences and their regulatory systems are incorporated with those of other *Streptomyces* genes, integration of SMILEs in the genome may have occurred long ago. The retention of apparently intact clusters, which contain no obvious pseudogenes, suggests that their products are at present important but are synthesized only when primary metabolic precursor pools can no longer support efficient growth.

The existence of so many different secondary metabolites probably indicates specific adaptations to different habitats. Mycobacteria, which are also members of the Actinomycetales, are also primarily saprophytic soil bacteria. But the genus also includes *Mycobacterium tuberculosis* and the leprosy-causing *Mycobacterium leprae*, two of the most dangerous pathogens known to man. The *M. tuberculosis* and *S. coelicolor* chromosomes show synteny within a region that is

defined as the central core sequence in *S. coelicolor* (about 5 Megabases) [8]. Interestingly, the *M. tuberculosis* chromosome contains 18 polyketide synthase gene clusters [20]. The fact that six of these clusters were also found in the severely reduced *M. leprae* chromosome suggests that they serve indispensable functions in the bacteria [21]. Although many of the clusters may provide cell-wall components, in *Mycobacterium ulcerans* (the causative agent of a tropical skin disease), one produces mycolactone, a toxin involved in pathogenicity [22]. Mycobactin, a siderophore synthesized by a combination of polyketide synthases and non-ribosomal peptide synthases, plays an essential role in mycobacterial virulence [23]. The fact that *Streptomyces* produce potent immunomodulators, such as tacrolimus [24] (also called FK506 [25]) and rapamycin [26], suggests that secondary metabolites may provide other virulence functions, such as the immunosuppression that accompanies mycobacterial infections. Such compounds may have enormous therapeutic value because lethal pathogenicity effectors could potentially be turned into life-saving pharmaceutical compounds. Similarly, other actinomycetes that have not been as extensively exploited because of isolation and cultivation difficulties may also produce large numbers of unusual SMILE-encoded compounds with undiscovered and unanticipated biological activities [1,4,7] that may reflect each species' interactions with soil metazoans.

Because the *S. coelicolor* genome sequence is now available, large-scale methods of gene expression and site-directed mutagenesis can be exploited to identify even more secondary metabolites and to better understand the regulatory mechanisms that coordinate pathways of primary and secondary metabolism. In an early proteomic analysis using two-dimensional gel electrophoresis of *Streptomyces hygroscopicus* cultures, that had been pulse-labeled at various time points during growth, we used statistical analyses of global gene-expression patterns to identify secondary metabolic enzymes [27]. Analyses of the patterns displayed by 400 spots on the two-dimensional gels showed that the proteins encoded within the SMILE for biosynthesis of the antibiotic and herbicide bialaphos fell into discrete kinetic groups. Similar, but more extensive two-dimensional gel analyses have now been initiated in *S. coelicolor* by us and others, and several hundred protein spots have been identified [28,29]. In addition, Cohen and colleagues [30] were able to correlate transcriptional expression patterns with chromosome position by using powerful gene chip studies of *S. coelicolor* cultures, coupled with a knowledge-based algorithm. This approach allowed them to identify contiguous genes in four SMILEs corresponding to known polyketide synthase or non-ribosomal peptide synthase gene clusters. The same principle might be used to predict SMILEs producing novel compounds that are not encoded by polyketide or peptide synthases. In fact, kinetic analysis also revealed other blocks of coordinately regulated genes. These genes may be involved in the synthesis of previously unrecognized

secondary metabolites in *S. coelicolor*. Although there are many secondary metabolites that are not dependent on polyketide synthases or non-ribosomal peptide synthases, few corresponding enzymes have been identified.

The genome of *S. coelicolor* provides an overview of the genetic framework that defines one streptomycete and its SMILEs. In future, the availability of genome sequences of other *Streptomyces* species will allow us to identify common features that define their shared genetic heritage and links with acquired secondary metabolic genes in different species. Statistical analyses of global gene-expression patterns, in combination with 'metabolomic' studies of the pools of metabolic intermediates and products, will facilitate both understanding and manipulation of the primary and secondary metabolic pathways of these bacteria thus ensuring their continued importance to man.

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