Imaging Mass Spectrometry Reveals Highly Specific Interactions between Actinomycetes To Activate Specialized Metabolic Gene Clusters

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ABSTRACT **The genomes of actinomycetes contain numerous gene clusters potentially able to encode the production of many antibiotics and other specialized metabolites that are not expressed during growth under typical laboratory conditions. Undoubtedly, this reflects the soil habitat of these organisms, which is highly complex physically, chemically, and biotically; the majority of the compounds that make up the specialized metabolome are therefore adaptive only under specific conditions. While there have been numerous previous reports of "waking up" the "sleeping" gene clusters, many involving genetic interventions or nutritional challenges, the role of competing microorganisms has been comparatively little studied. Now, Traxler et al. [M. F. Traxler, J. D. Watrous, T. Alexandrov, P. C. Dorrestein, and R. Kolter, mBio 4(4):e00459-13, 2013, doi:10.1128/mBio.00459-13] have used the recently described technique of microscale imaging mass spectrometry to analyze in detail the stimulation of specialized metabolite production by the model actinomycete *Streptomyces coelicolor* A3(2) by growth in proximity to other actinomycetes. The striking finding from these experiments was that growth of *S. coelicolor* close to each of the five other actinomycetes studied caused it to produce many specialized metabolites that were not made when it was grown in isolation and that the majority of the compounds were interaction specific, i.e., they occurred only in one of the five pairwise combinations, emphasizing the highly specific nature of the interactions. These observations contribute substantially to the increasing awareness of communication between microorganisms in complex natural communities, as well as auguring well for the discovery of useful specialized metabolites based on microbial interactions.**

The Actinomycetes are the major class within the Actinobacteria, the high-G+C branch of the Gram-positive bacteria. These filamentous organisms are responsible for the production of some two-thirds of the antibiotics used in human and veterinary medicine, as well as that of key anticancer, antiparasitic, and immunosuppressant agents (1, 2). Among the Actinomycetes, members of the genus *Streptomyces* are the main players, but other genera such as *Saccharopolyspora* and *Amycolatopsis* also contribute important compounds, and this trend is continuing. While it is now clear that marine actinomycetes are an up-and-coming source of novel "specialized" metabolites (previously referred to as "secondary" metabolites) (3), the great majority of known members of the class inhabit terrestrial soils.

Soils are a particularly competitive range of habitats, and so it is not surprising that streptomycetes have evolved the ability to produce antibiotics as agents in their struggle for survival. A particularly telling argument, first proposed by Chater and Merrick (4), is that the main driving force for the evolution of antibiotic production by filamentous microorganisms is defensive rather than offensive, serving to protect the resources of the colony as it breaks down the biomass represented by the vegetative mycelium to release the building blocks needed for the development of spores, the resting and dispersive phase of colony development.

Given that antibiotics are, by definition, involved in interactions with other soil inhabitants, the question arose as to whether or not their production can sometimes be triggered by the presence of such competitors. There is a large literature spanning several decades to indicate that antibiotic production is seldom constitutive but is highly regulated, typically at several levels, with pathway-specific activators embedded within the clusters of biosynthetic genes responding to pleiotropic regulators that coordinate the production of a specific antibiotic with that of other specialized metabolites, as well as with the switch from vegetative growth to sporulation (5). These studies have emphasized nutritional signals on the reasonable hypothesis that a decreasing level of available nutrients should be the major trigger causing the organism to commit to the irreversible step of ceasing vegetative growth, degrading the vegetative mycelium, and entering the terminal stage of the developmental cycle, with the concomitant production of defensive antibiotics (6).

More recently, several groups have found that the production of specialized metabolites by actinomycetes results directly from an interaction with another microorganism in their vicinity. A particularly striking example of such a discovery is the report by Onaka et al. (7) that certain mycolic acid-containing bacteria, themselves members of the *Corynebacteriaceae* family of Actinobacteria, stimulate antibiotic production when grown in association with an actinomycete, a technique they called "combined culture." They present evidence that such stimulation requires intimate cell-to-cell contact between the partners, but proof of this hypothesis and elucidation of its precise mechanism remain to be established.

Traxler et al. (8) have now taken the study of the biotic stimulation of specialized metabolite production by a streptomycete to a whole new level. Working with *Streptomyces coelicolor* A3(2),

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genetically the model streptomycete (1) and the first to have its genome completely sequenced (9), they investigated variations in its secreted metabolome when grown close to five different actinomycetes (four *Streptomyces* species and one of *Amycolatopsis*) on a medium that does not support the early production of the well-characterized specialized metabolites of *S. coelicolor*. The background to this study was the finding from the complete genome sequence that the organism has the genetic potential to make some two dozen specialized metabolites, of which only a handful had been revealed by decades of study of the organism growing under typical laboratory conditions. This observation, soon followed by the genome sequence of *Streptomyces avermitilis* with a similar conclusion (10), gave rise to the concept of large numbers of specialized gene clusters in the genomes of actinomycetes which are "sleeping" under normal screening conditions and which require specific environmental conditions to "awaken" them.

The power and novelty of the analysis by Traxler et al. stem from the use of the recently developed techniques of nanospray desorption electrospray ionization (NanoDESI) and microbial matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) imaging mass spectrometry (IMS), which enable the detection and characterization of minute quantities of metabolites produced by microbial colonies growing *in situ* on an agar plate.

The most important general conclusion from these detailed experiments was that growth of *S. coelicolor* close to each of the five other actinomycetes caused it to produce many specialized metabolites that were not made when it was grown in isolation. Moreover, and this was the most novel aspect of the results, the majority of the compounds were interaction specific, i.e., they occurred only in one of the five pairwise combinations. This finding is both exciting and challenging. On the one hand, it implies highly specific regulatory controls capable of sensing chemical stimuli coming from competing organisms, which will be fascinating to unravel. On the other, if coculture is to be used as a general means of waking up sleeping gene clusters in order to reveal novel and potentially useful specialized metabolites, use of several stimulating microorganisms, or even complex mixtures of organisms, may be needed in order to access a good proportion of the potential specialized metabolome.

Much of the paper is devoted to a detailed study of siderophores produced by the stimulating organism, the target organism (*S. coelicolor*), or both, including the discovery of a whole new family of acyl-desferrioxamines. Multiple iron-chelating siderophores have been postulated to be involved in competition for available iron, a growth-limiting resource in many habitats (11), so these compounds may perhaps represent a special case. Nevertheless, their dominance in these experiments does not detract from the important overall conclusions of the work.

These studies, and the technical breakthroughs that underpin them, have great interest from an academic viewpoint as microbiologists increasingly ask genomics-based questions about the life of microorganisms, not under the artificial conditions of laboratory pure culture, which have been the mainstay of microbiology for well over a century, but in the rough-and-tumble of complex natural habitats. They are also highly relevant to the renewed search for novel specialized metabolites with medical applications, especially in the increasingly desperate attempt to discover antibiotics to deal with the rise of multiply drug-resistant pathogens. Since the realization of the huge untapped genetic potential of the actinomycetes to make novel chemical structures, diverse approaches have been taken to the challenge of waking up their sleeping specialized metabolic genes, many of them involving genetic interventions (12, 13) and others involving nutritional challenges (6). The work of Traxler et al. suggests that biotic interactions should be high on the list of promising approaches.

In conclusion, it is exciting that, although this narrative is devoted exclusively to the actinomycetes, there is much evidence to suggest that all of the main conclusions will apply to the filamentous fungi, myxobacteria, cyanobacteria, bacilli, and other antibiotic-producing microorganisms. Thus, the possibilities for discovering chemical novelty by exploiting untapped gene expression are indeed enormous.

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