Hot Topics in Pharmacognosy: Some Rambles Through Recent Literature On Bacterial and Fungal Secondary Metabolites

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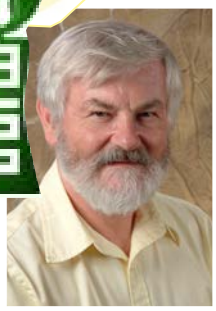

Fungi are extremely prolific producers of secondary metabolites but are often overlooked as sources, perhaps due to the "strange" (at least to people used to growing bacteria) methods of growing them and increasing yields. Although static cultures of actinomycetes are sometimes used in studies on secondary metabolite production, the majority of such microbes are grown in shaking cultures or stir tank reactors. In contrast, fungi, particularly the filamentous fungi, are "happy and productive" when allowed to grow on static surfaces, from agar plates to corn cobs (or individual corn "grains") and at times, on grain-based human cereals such as "Cheerios" or their equivalents from other cereal manufacturers.

The genomic information on fungi and hence being able to identify potential secondary metabolites, is quite sparse when compared to the now thousands of actinomycetes that have had their genomes sequenced and their "innermost" secrets (biosynthetic gene clusters, BGCs) revealed. Most of the BGCs identified have not been characterized as to their exact function but have been catalogued, with recent published examples being those discussed by actinomycete taxonomists such as Dr. David Labeda at the United States Department of Agriculture (USDA) and his colleagues.[1, 2] In the first paper in 2014, they analyzed 830 genome sequences which included 344 specifically obtained for this particular study, deriving a mass spectroscopic technique to identify secondary metabolites from microbial metabolomes (178 strains), and linking back to specific gene clusters. In the second paper, interrogation of over 10,000 actinomycete genomes enabled the identification of natural products containing phosphonic acids, an unusual secondary metabolite series.

Though somewhat similar in concept to the systems derived by Dorrestein and coworkers using different methodologies and bacterial sources[3] and those described by Trautman and Crawford,[4] the scale is materially different and approaches that of interrogations of the human microbiome. Recently, Medema and Fischbach extended the area covering a variety of computational techniques for interrogating genomic data.[5] This paper contains an excellent coverage of the current methodologies plus the databases available (all URLs are given). We can add the paper on IMG-ABC to these examples, which aptly demonstrates the differences between current knowledge of the bacterial genomes versus those of fungi.[6]

However, and this is a very big "however," the eubacterial world (particularly the actinobacteria and some cyanophyta) is well covered, with data from over 10,000 genomic sequences available just for the streptomycetes; but in the case of the eukaryotic fungi, very little comparable information is available, though many of the most important therapeutic agents were isolated from fungi. Examples are the ß lactams (though there have been reports from some Streptomyces over the years) and the basic "statins" and cyclosporins, just to name a few of the blockbuster drug types. Fungi are frequently labeled as only producing "toxins" due to agents such the fumosins, the aflatoxins, or insecticidal agents such as the beauvericins, which are members of the ionophore class known as the enniatin antibiotics.[7]

One of the "perceived problems," until work in the early 2000s by the Keller group at the University of Madison, Madison, Wisconsin, was that the fungi, being eukaryotes, would have all of their biosynthetic clusters spread across their chromosomes, and/or they have a multiplicity of different organelles from the nucleus out-

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wards that could be involved in the production of secondary metabolites. However, the paper by Bok et al in 2006 demonstrated that, contrary to the current dogma, at least in the *Aspergillus* strain used, the biosynthetic clusters were not spread across all the chromosomes but were "nested" on proximal chromosomes.[8] This paper should be read in conjunction with a review the previous year by Keller et al.[9] In 2012, Keller demonstrated that in multiple strains of *A. nidulans* there were at least 33 to a then maximum of >70 identifiable biosynthetic clusters, ignoring terpene synthases, which could be a significant additional number.[10]

In the years since the 2006 paper, workers in groups other than those associated with Keller, have proceeded to investigate the complex control mechanisms involved in fungal secondary metabolite control and expression. Topics include the transport amongst organelles and whether or not these metabolites can be the equivalent of quorum sensing agents for both the fungus and, if an endophyte, the host organism as well. Some papers relevant to such discussions are the following from the Brakhage group,[11, 12] plus the methods described from the Larsen group in University of Denmark, Lyngby, Denmark covering predictions from fungal sequence data,[13] and Inglis et al.[14] In addition to these, two very recent papers have added to the methodologies for computational analyses of fungal genome data to search for secondary metabolites, namely those of Li et al,[15] and van der Lee and Medema.[16]

Techniques for rapid growth and subsequent interrogation of fungal secondary metabolites (for microbes that can be fermented) have recently been described by Barkal et al,[17] and should be read in conjunction with the recent paper from the Keller group.[18] Addressed are uses of fungal secondary metabolites as "fungal protective agents", similar to the comments earlier on quorum sensing agents.

There are two very intriguing papers that have recently been published that are relevant to the comment above; these discuss the use of nitric oxide (NO) as a signaling agent at various times on the growth cycle of *Aspergillus*[19] and the use of the same "messenger" in the establishment of fungal infection in plants.[20] As is well known, NO is a secondary messenger in animals but is also used in plants to close stomata against microbial invasion.[21] This brings up a very interesting question as to "is the fungus circumventing the plant's defenses by producing NO?"

Finally, the number of endophytic fungi now known to be functional in plants that produce "interesting drug compounds" (camptothecin, taxol, podophyllotoxin, etc.), that also produce them when fermented outside of the plant, has been recently covered in an excellent review in *Natural Product Reports* by the Kaltenpoth group at the Max Planck Institute for Chemical Ecology, Jena, Germany.[22] This paper should be read, in particular the data in the supplementary information, in conjunction with the examples given above of the multiplicity of fungal secondary metabolite clusters. Also, the very recent paper from Baccile et al on the production of isoquinoline alkaloids by a plant-like biosynthetic pathway in *A. fumigatus* should be read.[23] It raises all sorts of questions as to the role of a host plant. ■

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