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Exploiting the natural product potential of fungi with integrated -omics and synthetic biology approaches

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Abstract

Fungi are rich, underexploited reservoirs for natural products that may serve as medicines, commodity chemicals, insecticides, pesticides and other valuable chemicals. Moreover, the biochemistry of natural product formation may be repurposed with emerging synthetic biology tools to make valuable non-natural compounds such as biofuels. However, the pathways that lead to these products are poorly understood and frequently inactive under lab conditions making discovery challenging. Recent advances in -omics approaches and synthetic biology tools provide powerful new methods to elucidate and tap this wealth of novel chemistry. In this review, we describe cutting-edge approaches to activate and characterize natural product formation, and discuss the potential of established and emerging fungal systems for natural product discovery.

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Fungi, Natural products, Synthetic biology, Integrated -omics.

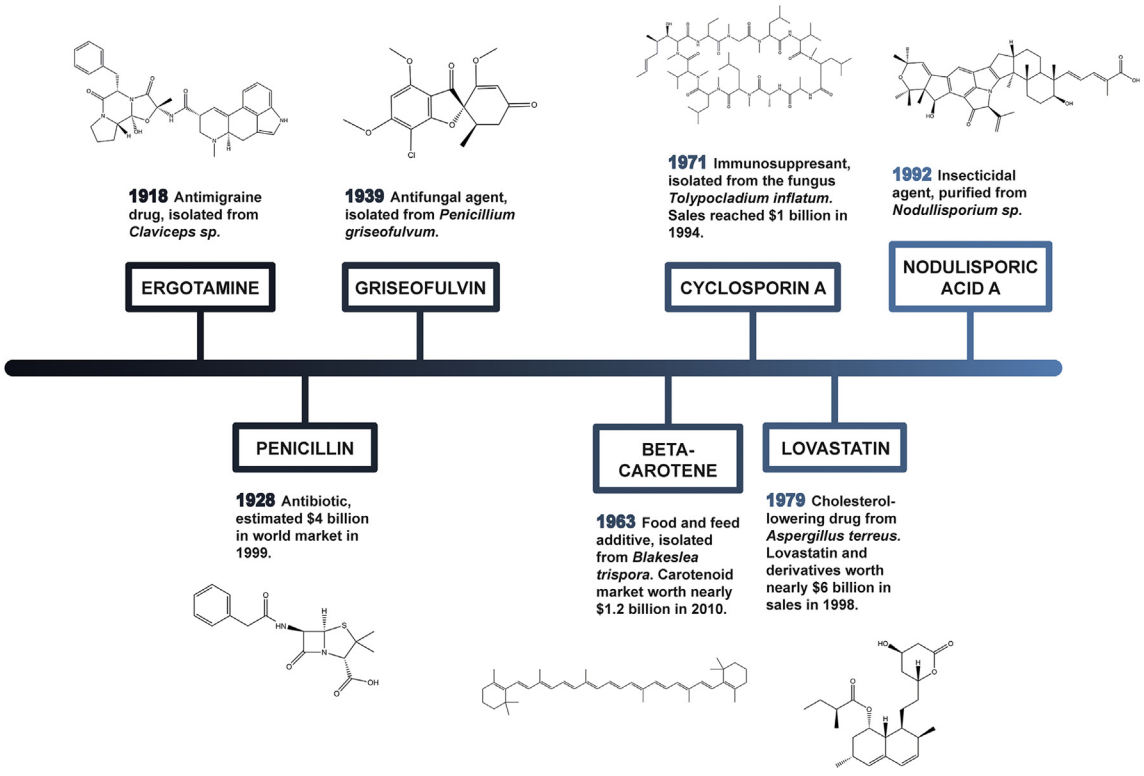
Introduction

Fungal secondary metabolites, or natural products (NPs), form an increasingly important portion of the pharmaceutical, agricultural, and industrial sectors (Figure 1). In the pharmaceutical industry alone, single molecules such as the powerful fungal NP-derived antibiotic amoxicillin are each estimated to top $1 billion USD in annual sales worldwide [1]. Among fungal NPs are cholesterol-lowering statins (e.g. the blockbuster drug lovastatin [2]), common antibiotics (e.g penicillin and cephalosporin [2]), insecticides (e.g. nodulisporic acids [2]), and pigments (e.g. carotenoids [3]). NPs are frequently bioactive and chemically diverse due to their role in conferring a selective advantage to the producing fungus in competitive environments [4]. Bolstered by these compounds, fungi are able to proliferate and thrive in diverse ecological niches including competitive soil environments [5] and the digestive tracts of humans and other mammals [6,7]. However, of the estimated 5 million fungal species, less than 2% have been described and fewer than a 1000 have been sequenced and studied in detail [8–10]. Thus, many unclassified fungi with rich reservoirs for new NPs remain to be discovered within plain sight in locations such as urban parks and farm animals [11,12].

Fungal NPs are generally classified into one of five categories based on their biosynthetic origin: polyketides, non-ribosomal peptides, terpenoids, prenylated tryptophan derivatives, or hybrid compounds such as polyketide/non-ribosomal peptide hybrids that incorporate components from several pathways (Box 1) [13]. From these basic pathways, NPs diversify into a wide range of carbon lengths (typically C6–C30), potentially containing heterocyclic rings, and several stereocenters [14,15]. Of the different classes, polyketides represent the majority of the fungal natural products [16]. Well-known polyketides include statins, the most profitable class of drugs worldwide, and many antibiotics [17]. Common to these NP pathways, however, is a requirement for several enzymes of diverse catalytic function that must be coordinately expressed.

Although fungi do not use operons or polycistronic mRNA, they frequently organize NP biosynthetic genes into co-localized clusters (biosynthetic gene clusters; BGCs) to more easily coordinate pathway expression [16,20]. The first identified fungal BGC was a 3 gene, 56.9 kb penicillin cluster found in the fungi *Aspergillus nidulans* and *Penicillium chrysogenum* [21,22]. However, these BGCs need not be contiguous. Due to the size and number of proteins needed (typically >40 kb), NP formation is tightly regulated and expressed only at times when they are necessary for survival. Thus, many potential NPs evade detection as the majority of putative NP gene clusters are cryptic and remain silent under laboratory conditions.

Figure 1



Value of natural products isolated from fungi. Fungal natural products represent a multi-billion dollar industry with diverse applications. Presented are example molecules along with their isolation year, function, and estimated market value [73–79].

Box 1. Biosynthetic origin of fungal natural products

Fungal polyketides are synthesized from large multidomain polyketide synthases (PKSs) that iteratively construct the molecule with an acyl extender unit, commonly malonyl-CoA, through a decarboxylative Claisen condensation. The incorporated ketide unit may be subsequently functionalized and/or undergo redox reactions to generate chemical diversity before the next extender unit is added. This mechanism is similar to more familiar Type I bacterial PKSs that proceed non-iteratively via modular domains, which are specific for the incorporation and functionalization of a given ketide unit (e.g. DEBS PKS that produces erythromycin) [18]. Fungal PKSs may be further classified as either Type I or Type III. Type I PKSs use an acyl carrier protein to activate and tether the growing polyketide molecule to the PKS while Type III PKSs remain untethered from the molecule [19]. Modular multidomain non-ribosomal peptide synthetases (NRPSs) create non-ribosomal peptides in a similar fashion to Type I PKSs. However, each module of the NRPS adds an amino acid in a non-template driven synthesis reaction before functionalization [16]. Fungal terpenoid synthesis creates isoprenoid starter units via the mevalonate pathway, rather than the bacterial methylerythritol phosphate pathway [16]. These starter units (isopentyl pyrophosphate and dimethylallyl pyrophosphate; IPP and DMAPP) are condensed and cyclized by terpene synthases and cyclases to form diverse terpenoids through allylic carbocation addition [16]. Dimethylallyl tryptophan synthases may also transfer the dimethylallyl moiety from DMAPP to a tryptophan starter unit, which is then modified by methyltransferases and some tailoring enzymes to form prenylated tryptophan derivatives [16].

This review covers recent advances in fungal NP discovery from the prediction of BGCs with bioinformatic tools, to their activation and validation with synthetic biology and -omics approaches. We discuss the success of this developing pipeline for NP discovery and highlight emerging fungal systems with potential for NP discovery along with new synthetic biology tools to repurpose their NP biosynthetic potential.

Prediction and validation of fungal natural products

Despite challenges in the detection of NPs, BGCs that catalyze their formation may be readily identified and mined from genomic sequences. The characteristic catalytic domains of BGCs are strongly conserved and recognized in routine homology searches of the genome with Hidden Markov Models (HMMs) and local alignments [23]. Popular bioinformatics tools SMURF [24] and antiSMASH [25] based on these principles annotate fungal genomes and have been used to identify BGCs including more than 80 non-redundant NP BGCs in *Aspergillus fumigatus* alone [26]. Similarly, analysis of *Penicillium* genomes revealed 89 putative BGCs from which NPs products were subsequently detected [27]. Next generation genomes-to-natural-products (GNP) platforms are building on this foundation to propose the chemical structure of an NP directly from its BGC