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Development of fungal cell factories for the production of secondary metabolites: Linking genomics and metabolism

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ABSTRACT

The genomic era has revolutionized research on secondary metabolites and bioinformatics methods have in recent years revived the antibiotic discovery process after decades with only few new active molecules being identified. New computational tools are driven by genomics and metabolomics analysis, and enables rapid identification of novel secondary metabolites. To translate this increased discovery rate into industrial exploitation, it is necessary to integrate secondary metabolite pathways in the metabolic engineering process. In this review, we will describe the novel advances in discovery of secondary metabolites produced by filamentous fungi, highlight the utilization of genome-scale metabolic models (GEMs) in the design of fungal cell factories for the production of secondary metabolites and review strategies for optimizing secondary metabolite production through the construction of high yielding platform cell factories.

Contents

1. Introduction

Microbial secondary metabolites are widely exploited for their biological activities to ensure the well-being of humans. Secondary metabolites are used as antibiotics, other medicinals, toxins, pesticides, and animal and plant growth factors [1]. Although the antibiotic effects of certain molds have been reported earlier, it was Flemings' persistence in the usability of the antimicrobial activity of penicillin, which initiated what is known as the *golden era* of antibiotic discovery [2]. Despite the fungal origin of penicillin, produced by several members of the *Penicillium* genus [3], most research on secondary metabolites has focused on bacteria, mainly soil isolates of actinomycetes with the majority of compounds originating from the *Streptomyces* genus [4]. Some of the pioneering work that paved the way for antibiotic discovery was conducted by Nobel laureate Selman Waksman, who's systematic screening of *Streptomyces* isolates, led to the identification of several antibiotics, including streptomycin and neomycin which have found extensive applications in the treatment of infectious diseases. However, to

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ensure translation of these findings for commercial production it was necessary with further product optimization and fermentation characterization of microbial physiology, and this resulted in the birth of industrial microbiology as a discipline, with Arnold Demain as one of the founding fathers.

Today we know that although most living organisms can produce secondary metabolites, the ability to produce them is unevenly distributed. Among all known microbial antibiotics and similar bioactive compounds (altogether 22,500), 45% are from actinomycetes, 38% are from fungi and 17% are from unicellular bacteria [4]. Among this wealth of compounds, only about a hundred are in practical use for human therapy, with the majority being derived from actinomycetes [4]. However, it is worth mentioning that in addition to penicillin, several other fungal secondary metabolites have successfully reached the pharmaceutical market, including cholesterol lowering statins [5], the antifungal griseofulvin [6] and the immunosuppressant mycophenolic acid [7].

Biosynthesis of secondary metabolites takes place from a limited number of precursor metabolites from the primary metabolism (Fig. 1). In fungi, these precursors are mainly short chain carboxylic acids (e.g. acetyl-CoA) or amino acids, which are linked together by backbone enzymes such as polyketide synthases (PKSs), non-ribosomal peptide synthetases (NRPSs), dimethylallyl tryptophan synthetases (DMATSs) or terpene cyclases (TCs). The resulting oligomers are then subject to chemical modification by tailoring enzymes which are often controlled under common transcriptional regulation as the backbone enzyme [8]. A hallmark trait of the genes involved in a secondary metabolite pathway is that they, in most record cases, physically cluster in the chromosome in biosynthetic gene clusters (BGCs) [9].

The characteristic clustering of genes as well as the conserved motifs of backbone genes can be exploited for computational detection of BGCs from sequence data. Tools like SMURF [10], antiSMASH [11], PRISM [12] and SMIPS/CASSIS [13] utilize these features to reliably and with a high accuracy detect BGCs of known compound classes in fungi. Other algorithms detects BGCs without relying on specific motifs or the presence of backbone genes, which enables identification of BGCs beyond PKS, NRPS, DMATS and TCs [14–17]. Tools and implementations of BGC mining algorithms have been extensively reviewed [18–23].

A limitation of secondary metabolite production is the low yields that are naturally achieved in most microbes, partly since many secondary metabolites are favored under suboptimal growth conditions [8,24] and because their biosynthesis compete with essential pathways of metabolism, involved in growth related processes (Fig. 1). Applying metabolic engineering to circumvent these limitations can be greatly assisted by utilization of the mathematical representation of metabolism in genome-scale metabolic models (GEMs), which concepts and applications have been reviewed elswhere [25–27]. These models, however, often neglect secondary metabolite biosynthesis, hence their potential in studying secondary metabolism has not been fully tapped. Additionally, with the efficient gene editing tool CRISPR-Cas9 being developed for a number of fungal model organisms [28–30], a great potential exists for implementing the necessary genetic modifications for the development of improved secondary metabolite producers. In this review, we will describe methods for linking BGCs to compounds and show how metabolic modeling can aid in translating the improved secondary metabolite discovery rate into metabolic engineering strategies for the development of fungal platform strains for the production of secondary metabolites.

2. Linking BGCs to compounds

In order to industrially exploit secondary metabolites for production, it is a major advantage to know the genetic basis of the biosynthesis. This allows for employing metabolic engineering strategies for optimizing the production performance of an organism and making the process economically feasible [31]. Among the known secondary metabolites, the vast majority have not had their biosynthetic mechanisms elucidated or linked to a BGC, and are commonly referred to as orphan compounds. Understanding the genetic foundation of secondary metabolite biosynthesis further allows for redesigning the pathways to produce novel compounds [32], as previously shown by widening the product portfolio of β-lactam antibiotics from the penicillin pathway of *Penicillium chrysogenum* [33]. Genome sequencing combined with genome mining, strongly facilitates the process of connecting BGCs to compounds (Fig. 2) and a number of computational tools have been developed to specifically address this challenge either from a targeted or untargeted approach, or by using metabolomics.

2.1. Targeted approaches

A simple approach, for identifying the BGC of a target compound is to compare the number of similar BGCs between two or more

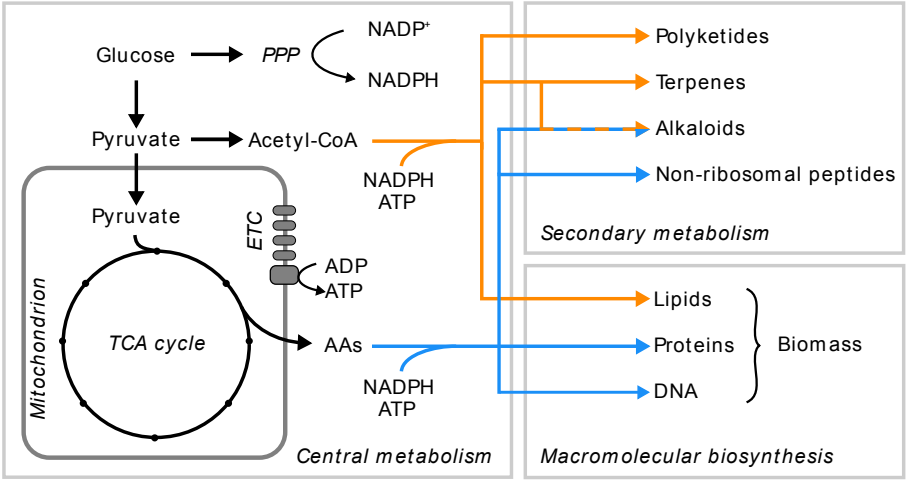

Fig. 1. Biosynthesis of secondary metabolites from precursors of the central carbon metabolism. PPP: Pentose Phosphate Pathway. ETC: Electron Transport Chain. TCA: Tricarboxylic Acid. AAs: Amino Acids.