Journal of Biotechnology 163 (2013) 179–183

Contents lists available at SciVerse ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Engineering fungal secondary metabolism: A roadmap to novel compounds

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ARTICLE INFO

Article history:
Received 23 March 2012
Received in revised form 26 June 2012
Accepted 29 June 2012
Available online 20 July 2012

Keywords:
Fungal secondary metabolism
Natural products
Genetic engineering

ABSTRACT

Natural products play important roles not only in the environment but also as useful compounds in various applications like in medicine or plant protection. An enormous number of such compounds have derived from microorganisms colonizing various habitats. Traditionally, new isolates of bacteria or fungi have been screened for their potential to produce biologically active compounds. In the post genomic era, however, there is a growing number of novel methods based on genetic engineering to obtain new metabolites. In this review, we summarize the recent progress made in the development of novel promising approaches for natural product discovery in fungi using genome mining, activation of silent gene clusters, heterologous expression of biosynthesis genes, exchange of enzyme modules as well as redesign of metabolic flux.

1. Introduction

Natural products are low molecular weight compounds whose biological function has been maintained and optimized during evolution. Some of these compounds are of major importance for various applications in medicine or plant protection. Between the years 1983 and 1994, 520 novel drugs were approved. From these, 40% were based on natural products. In 1999, 9 of the 20 most sold drugs were based on natural products including compounds derived from fungi such as the immunosuppressant cyclosporine, cholesterol-lowering statins or the antibiotic cephalosporin (Barber et al., 2004; Fernandes and Miska, 2002). Usually, the known compounds were identified from different microbial sources grown under laboratory conditions. However, since most of the microorganisms in nature cannot be cultivated in the laboratory yet and furthermore, many microorganisms do not reveal their true biosynthetic potential in the laboratory, novel methods needed to be established to discover novel compounds of microorganisms. Until today, classical methods have been very successful. In addition, however, in the post-genomic era there are several novel tools available to discover or create novel compounds (Brakhage and Schroeckh, 2011). With the help of genetic engineering it is possible to activate silent biosynthesis gene clusters, to generate derivatives of known compounds or create tailor-made/artificial new biosynthesis pathways for previously unknown metabolites. Many of these techniques have been already applied to fungi in order to expand our arsenal of drugs. Nevertheless, further improvements like automatisation are required to make these techniques even more attractive for industry and high-throughput screening programs. These technologies can be expected to help generating numerous new compounds in a time and cost effective manner. There are several excellent reviews covering aspects of this area of research in fungi (Brakhage and Schroeckh, 2011; Li, 2011; Martin et al., 2010; Sanchez et al., 2012; Schmitt et al., 2004; van den Berg et al., 2010; Wallwey and Li, 2011; Yin and Keller, 2011). In the following review, we focus on recent developments in genetic engineering to generate novel natural products in fungi.

2. Strategies to obtain novel compounds

2.1. Activation of silent gene clusters

Thousands of natural products can be expected awaiting discovery because the synthesizing microorganisms have not been cultivated yet, or, if they are cultivated, did not show their true biosynthetic potential in the laboratory because the biosynthesis genes remain silent (Hertweck, 2009b). Until today, several methods based on genetic engineering have been described for the activation of such silent gene clusters in fungi. A straight forward method was described by Bergmann et al. (2007) who identified silent biosynthesis gene clusters of the fungus *Aspergillus nidulans* by genome mining. The authors took advantage of a special

Abbreviations: PKS, polyketide synthase; NRPS, non-ribosomal peptide synthetase.

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http://dx.doi.org/10.1016/j.jbiotec.2012.06.027

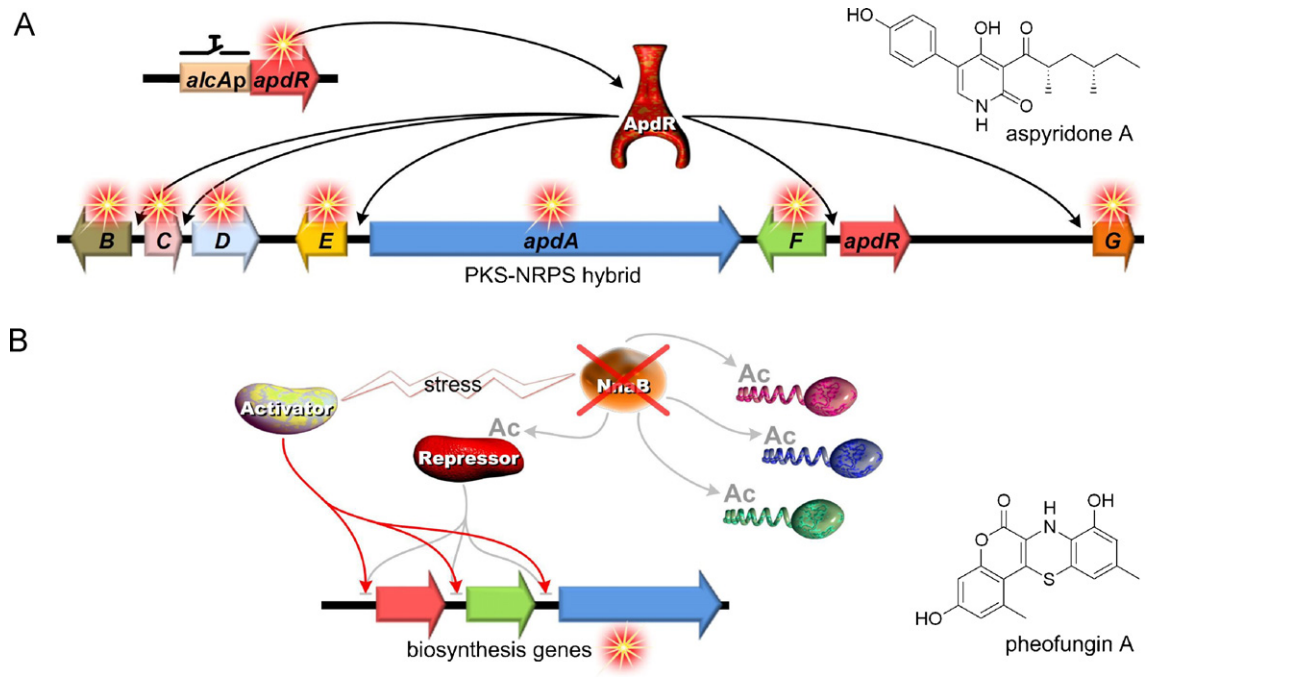

Fig. 1. Examples for induction of biosynthesis pathways by manipulation of a regulatory gene: induction of silent aspyridone biosynthesis cluster in *Aspergillus nidulans* by overexpression of the *apdR* transcription factor gene (Bergmann et al., 2007) (A). Production of pheofungins induced by the deletion of the *nnaB* gene in *Aspergillus nidulans* (Scherlach et al., 2011) (B).

feature of fungal secondary metabolism gene clusters: many of them encode a pathway-specific regulatory gene. By overexpression of the transcription factor gene *apdR* that is part of the selected silent *apd* gene cluster using an inducible promoter, transcription of all the cluster genes was activated and cytotoxic compounds never before described for *A. nidulans* named aspyridones were identified (Fig. 1A). This method was also applied to activate a so far unknown gene cluster of *A. nidulans* designated *inp*. Surprisingly, overexpression of the putative pathway-specific transcription factor gene *scpR* (secondary metabolism *c*ross *p*athway *r*egulator) not only activated the *inp* gene cluster but also the *afo* gene cluster on a different chromosome encoding the asperfuranone biosynthesis genes (Chiang et al., 2009). Further studies showed that the transcription factor ScpR not only induces the genes of the *inp* cluster but also the asperfuranone transcription factor gene *afoA* that subsequently activated the asperfuranone biosynthesis genes (Bergmann et al., 2010). Hence, a putative pathway-specific transcription factor is able to activate another gene cluster enabling a cross talk between different gene clusters. It is conceivable that such a cross talk between gene clusters exists for many fungal gene clusters adding another level of complexity that could form the basis of combinatorial biosynthesis. Similarly, the inducible *alcA* promoter was used to replace the native promoter of the *afo*A transcriptional regulatory gene of the asperfuranone biosynthesis gene cluster. This was carried out in a strain lacking production of the major polyketide sterigmatocystin as a result of a targeted knock-out of the sterigmatocystin pathway gene *stcJ*. This deletion apparently made the PKS precursor molecule malonyl-CoA available for the production of a novel polyketide designated asperfuranone (Chiang et al., 2009). These examples show that overexpression of pathway specific regulators is a valuable tool to activate silent biosynthesis gene clusters leading to novel compounds, although the success and the specificity of such a genetic manipulation cannot be predicted yet.

It is also possible to manipulate wide-domain regulators to influence secondary metabolism. Deletion of a protein N-acetyltransferase in *A. nidulans* led to the formation of pheofungins that represent novel metabolites related to pheomelanins in red hair displaying cytotoxic activity (Scherlach et al., 2011). The biosynthesis of pheofungins requires the orsellinic acid biosynthesis pathway. The mechanism underlying the activation is unknown. It is conceivable that the missing N-acetylation of proteins causes stress in the fungus and thus results in the production of pheofungins (Fig. 1B). Alternatively, a regulator of the pathway could have been influenced by the missing post-translational modification, which leads to the activation of the pheofungin biosynthesis. This example shows that enzymes involved in post-translational protein modifications represent interesting targets for engineering the secondary metabolome. Another prominent example for a wide-domain regulator is LaeA that exhibits a putative methyltransferase involved in the regulation of secondary metabolism in filamentous fungi. Overexpression of the *laeA* gene increased the production of various secondary metabolites in several fungi (Bok and Keller, 2004; Kale et al., 2008; Kosalkova et al., 2009; Sugui et al., 2007). LaeA and the light-regulated developmental factor VeA are both part of the velvet complex (Bayram et al., 2008), suggesting, at least in part, a link between secondary metabolism and development. There are several other wide-domain regulators shown to be involved in the regulation of secondary metabolite gene clusters such as the CCAAT binding complex CBC and the pH regulator PacC which were shown to regulate at least the penicillin biosynthesis in *A. nidulans* (Brakhage et al., 2009; Espeso et al., 1993; Litzka et al., 1998). The production of monodictyphenone and emodin derivatives was induced by targeted deletion of the *cclA* gene, whose product is a component of the COMPASS (complex associated with Set1) complex of *A. nidulans*, which methylates lysine 4 of histone H3 and therefore contributes to epigenetic regulation (Chiang et al., 2010). The Saga/Ada complex, that regulates many cellular processes by coordination of histone acetylation, contains the histone acetyltransferase GcnE and was recently reported to have a major influence on the regulation of the penicillin, sterigmatocystin and orsellinic acid biosynthesis in *A. nidulans* (Nützmann et al., 2011). Another successful method was reported for the brevianamide F synthetase in *Aspergillus fumigatus* (Maiya et al., 2006). Upon