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Mini-review

Fungal secondary metabolite biosynthesis – a chemical defence strategy against antagonistic animals?

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ABSTRACT

Analogous to their hypothesized benefits in plants, many fungal secondary metabolites may serve as a chemical shield that fends off fungal feeders or competing saprophagous animals. We review different approaches providing increasing evidence that some secondary metabolites may mediate resistance to antagonistic animals, reducing their negative effects on fungal fitness. Because secondary metabolism is under tight regulatory control, that allows adjustment of secondary metabolite formation to diverse ecological challenges, we argue that natural selection has favoured at least some fungal secondary metabolites and the underlying regulatory machinery through antagonistic animals. Yet, whether animals indeed operate as selective agents contributing to the evolution of secondary metabolites as part of a fungal defence strategy remains elusive. We suggest combining eco-evolutionary concepts and methods with genomic and transgenic tools to close this knowledge gap. We predict an increase in fungal species discovered to be amenable to this functional ecological genomic approach.

Introduction

Natural selection has produced a high, often bizarre, diversity of ways in which animals and fungi interact with each other, including mutualistic, predatory, pathogenic, and competitive interrelationships (Vega & Blackwell 2005; Rohlfs *et al.* in press). Given the vital role fungi have in the functioning of many terrestrial ecosystems (Hättenschwiler *et al.* 2005) and their influence on humans and human-related activities (Deacon 2006), we need a better understanding of the causes and consequences determining fungal population dynamics in relation to interactions with animals.

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In soil ecosystems, fungi are under heavy attack by fungal grazers (Ruess & Lussenhop 2005) that comprise micro-, meso-, and macrofaunal elements, including protozoa and nematodes, mites and collembola, and earthworms and insects, respectively. Moreover, saprotrophic filamentous fungi exploiting rich food sources such as fruits, seeds and carrion may be engaged in competitive interactions with animals that depend on the same resources (Janzen 1977; Rozen *et al.* 2008; Rohlfs *et al.* in press). Both fungivores and competitors can seriously harm fungi in different phylogenetic affiliations and may thus negatively affect fungal evolutionary fitness (Guevara *et al.* 2000; Rohlfs 2005b; Rohlfs *et al.* 2005; Tordoff *et al.* 2006; McGonigle 2007; Boddy & Jones 2008). Sophisticated feeding structures in many fungivorous arthropods (Ruess & Lussenhop 2005) or collective ''attack'' of fungal colonies by competing insects (Rohlfs 2005a) indicate that antagonistic animals challenge fungi in various ways. Moreover, laboratory and field data suggest that food choice in fungal consumers is not random but appears to be driven by preferences for certain species of fungi over others (e.g., Maraun *et al.* 2003; Jørgensen *et al.* 2005), which may be explained by variation in nutrient concentration and composition of fungal diets. Additionally, fungal secondary metabolites (SMs) have repeatedly been proposed to reduce the nutritional value of fungi and may hence function as deterrents against fungal grazers (Vining 1990; Demain & Fang 2000; Karlovsky 2008). In contrast, recent work indicates that antibiotic microbial compounds might have been misjudged regarding their proposed importance in warding off other microbes (Dietrich *et al.* 2008; Mlot 2009), but may play basic roles in the metabolism of microbial communities.

This review aims to: (1) provide a brief summary of the molecular mechanisms that may equip fungi with a refined set of means to combat antagonistic animals; and (2) to summarize and evaluate approaches, which militate in favour of the so-called ''chemical shield'' hypothesis in animal–fungus interactions. We focus on interactions of mainly filamentous fungi with invertebrate animals, although the conceptual basis can be extended to relationships with vertebrates and other fungal life forms, such as those producing large fruit bodies (Sherratt *et al.* 2005).

Secondary metabolite biosynthesis and regulation: lessons from *Aspergillus*

Fungal SMs are usually separated into four groups: alkaloids, non-ribosomal peptides, polyketides, and terpenes (Hoffmeister & Keller 2007). The genes encoding the underlying pathways of SM production typically are organized into gene clusters (Keller & Hohn 1997). In *Aspergillus nidulans*, about 50 SM clusters have been identified, 36 in *Aspergillus fumigatus* and 56 in *Aspergillus oryzae* (Keller *et al.* 2005). The aflatoxin and sterigmatocystin gene clusters are particularly well characterized. In *A. nidulans*, only sterigmatocystin is produced, whereas in *Aspergillus flavus* and *Aspergillus parasiticus*, sterigmatocystin is the precursor to aflatoxin synthesis (Hamasaki *et al.* 1973; Barnes *et al.* 1994; Hicks *et al.* 2002; Yu *et al.* 2004). A total of 21 genes of the aflatoxin gene cluster have been functionally characterized in some detail, while the functions of six other genes are poorly understood (Yu *et al.* 2004). Almost all of the functionally assigned genes encode different biosynthetic enzymes (Yu & Keller 2005). One gene, *aflR*, encodes a binuclear zinc cluster transcription factor that is specific for fungi (Keller & Hohn 1997; Fernandes *et al.* 1998; Yu & Keller 2005). Both sterigmatocystin and aflatoxin clusters contain an *aflR* gene that is essential for expression of all other cluster genes (Yu *et al.* 1996). Deletion or mutation of *aflR* leads to strong downregulation of sterigmatocystin and aflatoxin gene expression (Fig 1). A second gene, *aflS*, also regulates the expression of aflatoxin, yet the precise role of the AflS protein in aflatoxin formation remains elusive (Georgianna & Payne 2009).

In addition to pathway-specific regulation of SM production, global genetic regulators appear to play an important role. For instance, LaeA controls the expression of genes involved in sterigmatocystin production and also in the production of many other SMs, including lovastatin, penicillin, and pigments in *A. nidulans* or gliotoxin in *A. fumigatus* (Bok & Keller 2004; Perrin *et al.* 2007). Deletion of *laeA* results in loss of *aflR* gene expression and sterigmatocystin and aflatoxin synthesis in *A. nidulans* and *A. flavus*, respectively (Fig 1). Over-expression of *laeA* increases transcription and subsequent product formation (Bok & Keller 2004). Given its homology to arginine and histone methyltransferases, LaeA might function through chromatin remodelling of metabolic gene clusters (Bok *et al.* 2006b). LaeA has been shown to positively regulate 13 of 22 secondary metabolite clusters in *A. fumigatus* (Perrin *et al.* 2007). Interestingly, reproductive traits such as conidiospore formation and patterns of nutrient utilization in Δ*laeA A. nidulans* are rather similar to those of the wild type, indicating a primary role of LaeA in regulation of metabolic gene clusters (Bok *et al.* 2006a). Recently, it has been shown that LaeA forms a heterotrimeric complex with two proteins, VeA and VelB (the VeA-VelB-LaeA complex), which appears to regulate both light-dependent sexual reproduction and secondary metabolism in *A. nidulans* (Bayram *et al.* 2008). In addition to the global effects of LaeA, a histone deacetylase has been shown to suppress gene expression of metabolite clusters during early fungal growth (Shwab *et al.* 2007); yet, as demonstrated by Williams *et al.* (2008), even more epigenetic regulatory elements may be involved in driving the expression of a still-unknown diversity of SMs.

The potential of several regulators to epigenetically control the expression of fungal SMs may provide fungi with an efficient means for displaying adaptive chemical responses to multiple natural enemies and competitors. As in plant–herbivore systems (Wu & Baldwin 2009), however, this proposed fungal response requires the ability to sense animal attacks and to transmit corresponding signals to the relevant regulators. While the signalling network involved in transmitting environmental and developmental signals acting on SM biosynthesis has at least partly been disentangled (Georgianna & Payne 2009) (Fig 1), nothing is known about how animal antagonists induce changes in this regulatory system. Also completely unknown are the molecular mechanisms (e.g., the possible role of fungivory-associated molecular patterns, FAMPs) underlying sensing of, for example, feeding