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MiniReview

Origins and significance of ergot alkaloid diversity in fungi

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Abstract

Ergot alkaloids are a diverse family of indole-derived mycotoxins that collectively have activities against a variety of organisms including bacteria, nematodes, insects, and mammals. Different fungi accumulate different, often characteristic, profiles of ergot alkaloids rather than a single pathway end product. These ergot alkaloid profiles result from inefficiency in the pathway leading to accumulation of certain intermediates or diversion of intermediates into shunts along the pathway. The inefficiency generating these ergot alkaloid profiles may have been selected for as a means of accumulating a diversity of ergot alkaloids, potentially contributing in different ways to benefit the producing fungus.

Keywords: Ergot alkaloids; Clavines; Mycotoxin; *Neotyphodium*; *Claviceps*; *Aspergillus fumigatus*

1. Introduction

The ergot alkaloids are a complex family of mycotoxins derived from prenylated tryptophan in several species of fungi. They are well known from their historical role in human toxicoses. In mammals, ergot alkaloids affect the central and sympathetic nervous systems, as well as immune and reproductive systems, resulting in symptoms such as muscle contractions, changes in blood pressure, lowered immune response, reduced lactation and reproductive capability, disturbances in sleep/wake cycles, hallucinations, and gangrene of the extremities [1–4]. Different ergot alkaloids exert their effects by acting in some cases as partial agonists or, in other cases, antagonists at receptors for 5-HT (5-hydroxytryptamine or serotonin), dopamine, and noradrenaline [1,3,5]. Ergot alkaloids also affect other organisms including bacteria, nematodes, and insects [2,6–10]. Less is understood about the mechanisms behind these activities. Whereas the pharmacological effects of ergot alkaloids have been subjects of considerable research, the ecological significance of the alkaloids, which probably transcends their effects on mammals, is poorly understood.

Ergot alkaloids are produced by several fungi representing two different orders. Certain fungi in the Clavicipitaceae (order Hypocreales) produce ergot alkaloids. These include various ergot fungi in the genus *Claviceps* [1,4,11,12] and several fungi in the genera *Epichloë* and *Neotyphodium*, which live as endophytic symbionts in grasses [2,8,11]. *Aspergillus fumigatus*, a common imperfect fungus and opportunistic human pathogen with close relatives in the order Eurotiales, also produces a set of ergot alkaloids [11,13–15], many of which differ from those of the clavicipitaceous fungi. Several *Penicillium* spp., also likely derived from ascomycete ancestors in the Eurotiales, also have been reported to produce ergot alkaloids [11,14]. Not all fungi in either of these orders produce ergot alkaloids and no members of the

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lineages in between them have been reported to produce these alkaloids.

Ergot alkaloids range in complexity from simpler tricyclic alkaloids such as chanoclavine or 6,7-secolysergine to more complex tetracyclic alkaloids with tripeptide-derived side chains such as the ergopeptines (Fig. 1). They often are classified into three groups – clavines, simple amides of lysergic acid, and ergopeptines. Clavine alkaloids are the simplest ergot alkaloids and lack amide-linked side chains on the ergoline ring system. Certain clavines provide a pathway to lysergic acid, whereas others may be products of shunts off the main ergot alkaloid pathway. Still other clavines may be the ultimate pathway end product in their producing organism. Simple amides of lysergic acid and ergopeptines contain amide linkages from lysergic acid and require the activity of peptide synthetases for their formation [16–18].

Interestingly, ergot alkaloid-producing fungi typically produce a characteristic profile of several ergot alkaloids rather than a single pathway end product (Fig. 1 and Table 1). *A. fumigatus* produces a series of related clavine alkaloids, which accumulate to characteristic concentrations in or on its conidia (asexual spores), in the process of producing the ultimate pathway product fumigaclavine C. Ergot alkaloid producers in the Clavicipitaceae often have profiles that include a combination of clavines, simple amides of lysergic acid, and ergopeptines. In endophyte-infected grasses, e.g., perennial ryegrass infected with the endophyte *Neotyphodium* sp. Lp1, clavines and the ergopeptine ergovaline may be detected at relatively similar concentrations. *Claviceps africana* accumulates clavines and an ergopeptine of the dihydroergot type in its sclerotia (asexual overwintering structures, also called ergots), whereas *C. purpurea* accumulates mainly simple amides of lysergic acid and ergopeptines in its sclerotia.

The ergot alkaloid pathway appears unusually inefficient in that certain intermediates do not flow rapidly through the pathway to an ultimate end product. Instead there are typically points along the pathway at which intermediates may accumulate to concentrations approaching the same order of magnitude as the pathway end product. Also, the pathway in certain producers contains shunts along which intermediates may be diverted to alternate products. The accumulation of intermediates and alternate products (rather than their rapid conversion to the ultimate pathway product) suggests that these alkaloids provide some benefit to the producing fungus that differs from those conferred by the pathway end product.

If an inefficient pathway has been selected for because a diverse profile of alkaloids provides an advantage to the producing organism, then two predictions follow: (a) the pathway should be regulated to produce the observed profile, as opposed to being a collection of enzymes operating at randomly uncoordinated rates; and, (b) alternate end products or accumulating intermediates should have activities that differ from those of the ultimate end products. This minireview will focus on studies that address these particular points, as well as on the means by which diverse profiles of ergot alkaloids may be generated.

2. Origins of diversity in ergot alkaloid profiles

2.1. Diversification of ergot alkaloid profiles within individual producers by inefficiency

The accumulation of intermediates observed in some ergot alkaloid-producing fungi allows those intermediates to serve as de facto products as well as intermediates for the next step in the pathway. Examples of such accumulating intermediates include chanoclavine in *Neotyphodium* sp. Lp1, festuclavine and fumigaclavine A in *A. fumigatus*, and festuclavine and dihydroelymoclavine in *C. africana* (Table 1 and Fig. 1). In theory, the observed accumulation may result from differences in concentrations and/or activities of the relevant enzymes, or partitioning (by secretion or compartmentalization) of intermediates from downstream enzymes.

The hypothesis that inefficiency in the pathway is controlled rather than random is supported by studies on alkaloid accumulation in pathway knockout mutants, mRNA accumulation, and enzyme activity. Knockout of the gene encoding lysergyl peptide synthetase 1 (LPS1), controlling a late step in the pathway (Fig. 1), resulted in changes in the regulation of upstream steps in *Neotyphodium* sp. Lp1. Concentrations of clavine intermediates from the middle portion of the pathway (e.g., chanoclavine) were maintained near wild-type levels, whereas 6,7-secolysergine, believed to be produced from an early shunt in the pathway (Fig. 1), increased in concentration [18].

Coordinated regulation of genes in the ergot alkaloid pathway at the mRNA level has been demonstrated by studies on accumulation of transcripts from known and hypothesized ergot alkaloid biosynthesis genes in *C. purpurea*. Accumulation of mRNAs from genes encoding dimethylallyltryptophan (DMAT) synthase, LPS1, and LPS2, as well as seven closely linked genes in the ergot cluster (*cpox1, cpox2, cpox3, cpP450-1, cpcat2, orfA*, and *orfB*) (Fig. 2) was coordinately reduced in response to increased phosphate concentration, which represses ergot alkaloid production [16,19].

Early biochemical studies with *C. purpurea* indicated that feedback inhibition of enzyme activity also contributes to control of the pathway. Studies with semi-purified chanoclavine cyclase, which catalyzes the cyclization of chanoclavine to agroclavine, showed

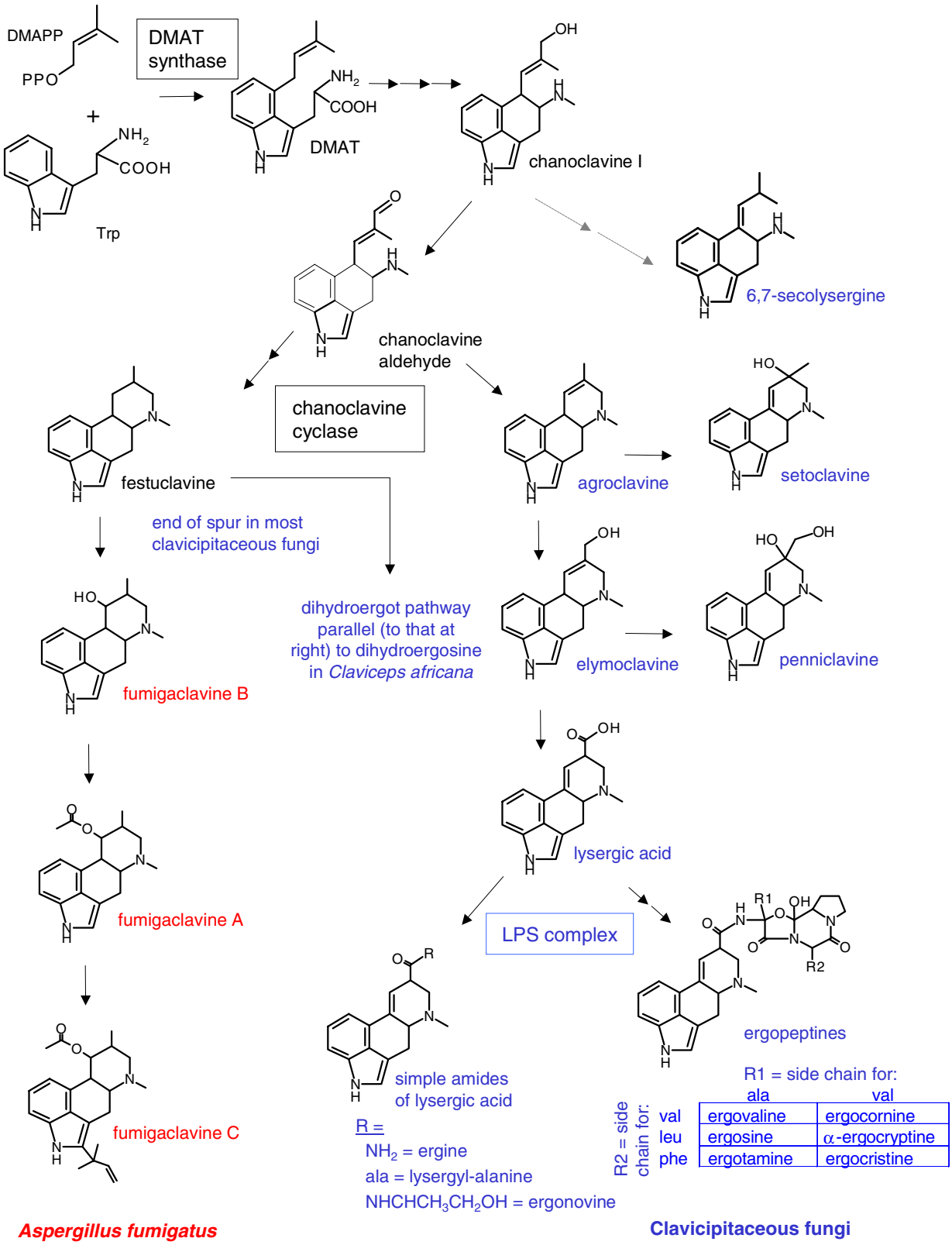

Fig. 1. Critical intermediates and end products of the ergot alkaloid pathways of clavicipitaceous fungi and *A. fumigatus*. Names of alkaloids common to both pathways are in black. Red text indicates alkaloids unique to *A. fumigatus* and blue text those alkaloids or steps found only in clavicipitaceous fungi. Enzymes emphasized in the text are boxed near the steps that they catalyze. Dihydroergot alkaloids (not illustrated) of *C. africana* have a saturated "D" ring (fourth ring to form) like festuclavine and are named with a dihydro-prefix. Structures for side chains on simple amides of lysergic acid and ergopeptines are indicated in tables below the respective general structure. Abbreviations: DMAPP, dimethylallyl pyrophosphate; DMAT, dimethylallyl tryptophan; and, LPS, lysergyl peptide synthetase.

Table 1
Abundant ergot alkaloids and their relative concentrations (mean ± standard error) in four different fungi

	Aspergillus fumigatus (μg/g conidia)[a]
Clavines	
Chanoclavine	3 ± 0.3
Festuclavine	530 ± 92
Fumigaclavine B	36 ± 6
Fumigaclavine A	360 ± 74
Fumigaclavine C	5200 ± 72
	Neotyphodium sp. Lp1-infected perennial ryegrass leaves (ng/g plant tissue)[b]
Clavines	
Chanoclavine	960 ± 150
6,7-Secolysergine	490 ± 46
Setoclavine	84 ± 9
Simple amides of LA[c]	
Ergine	138 ± 21
Lysergyl-alanine	36 ± 4
Ergopeptines	
Ergovaline	810 ± 110
	Claviceps purpurea sclerotia from tall fescue (μg/g sclerotium)[d]
Clavines	
Chanoclavine	4 ± 3
Agroclavine	2 ± 1
Simple amides of LA	
Lysergyl-alanine	3 ± 1
Ergonovine	68 ± 26
Ergopeptines	
Ergovaline	2 ± 0.4
Ergosine	194 ± 50
Ergotamine	66 ± 18
Ergocornine	74 ± 20
α-Ergocryptine	60 ± 11
Ergocristine	54 ± 16
	Claviceps africana sclerotia from sorghum (% of ergot alkaloid) [41][e]
Clavines	
Festuclavine	4
Dihydroelymoclavine	14
Ergopeptines	
Dihydroergosine	80

[a] Alkaloids were extracted and analyzed by HPLC as previously described [13,15].
[b] Alkaloids were extracted from leaf blades [18]. Quantification of chanoclavine [13] and the remaining ergot alkaloids [18] was by HPLC as described previously.
[c] LA = lysergic acid.
[d] Alkaloids were extracted from sclerotia by soaking them in 2-propanol/water/lactic acid (50:50:1) for 2 h. Clavines [13,15], simple amides of lysergic acid [18], and ergopeptines [18,40] were quantified according to previously published HPLC methods.
[e] Data retyped from Blaney et al. [41].

that lysergic acid and elymoclavine, intermediates downstream of chanoclavine cyclase, reduced chanoclavine cyclase activity [12,20]. The results indicate that control of enzyme activity (which presumably occurs also at other steps in the pathway) may modulate the observed spectrum of ergot alkaloids on a level separate from the demonstrated changes in mRNA accumulation. Collectively, these data indicate that the pathway is not just a collection of enzymes operating at different rates but rather it is closely regulated, indicating that accumulation of particular intermediates is controlled and not random.

2.2. Diversification of ergot alkaloid profiles within individual producers by shunt pathways

Several fungi produce alternate products from their ergot alkaloid pathway that are distinct from accumulating intermediates (though sometimes derived from them) via short shunt pathways off of the presumed primary or main pathway. These shunts not only result in additional products in an individual ergot alkaloid-producer's profile, but also may contribute to the control of intermediates through the main pathway. Noteworthy examples among the shunts are those leading to the production of 6,7-secolysergine in some *Neotyphodium* spp. endophytes, festuclavine in several clavicipitaceous fungi, and setoclavine and penniclavine in plant-associated members of the Clavicipitaceae.

The tricyclic clavine 6,7-secolysergine is presumed to be derived from chanoclavine or an intermediate prior to chanoclavine [18]. The significance of this particular shunt product is that its concentration increases in *Neotyphodium* sp. Lp1 when the peptide synthetase gene *lpsA* (encoding LPS1) is knocked out, whereas other clavines in the main pathway are maintained near wild-type concentrations.

In some clavicipitaceous fungi, festuclavine appears to be a minor shunt product of the ergot pathway, whereas most of the preceding intermediate (chanoclavine aldehyde) is cyclized into agroclavine without reduction of the double bond (Fig. 1). Conversely, in *A. fumigatus* and *C. africana* festuclavine is a critical accumulating intermediate in these microorganisms' main pathways to production of fumigaclavine C or dihydroergosine, respectively.

Setoclavine (and its stereoisomer isosetoclavine) and penniclavine/isopenniclavine have been reported from grasses infected with *Neotyphodium* sp. endophytes and from sclerotia of certain *Claviceps* isolates [11,18]. Interestingly, many peroxidases have the ability to oxidize agroclavine and elymoclavine to setoclavine and penniclavine, respectively [21]. Feeding of agroclavine or elymoclavine to endophyte-free grasses resulted in the conversion of the alkaloids to their oxidized counterpart, indicating that an endophyte-infected plant can contribute to the profile of ergot alkaloids observed in that plant [18].

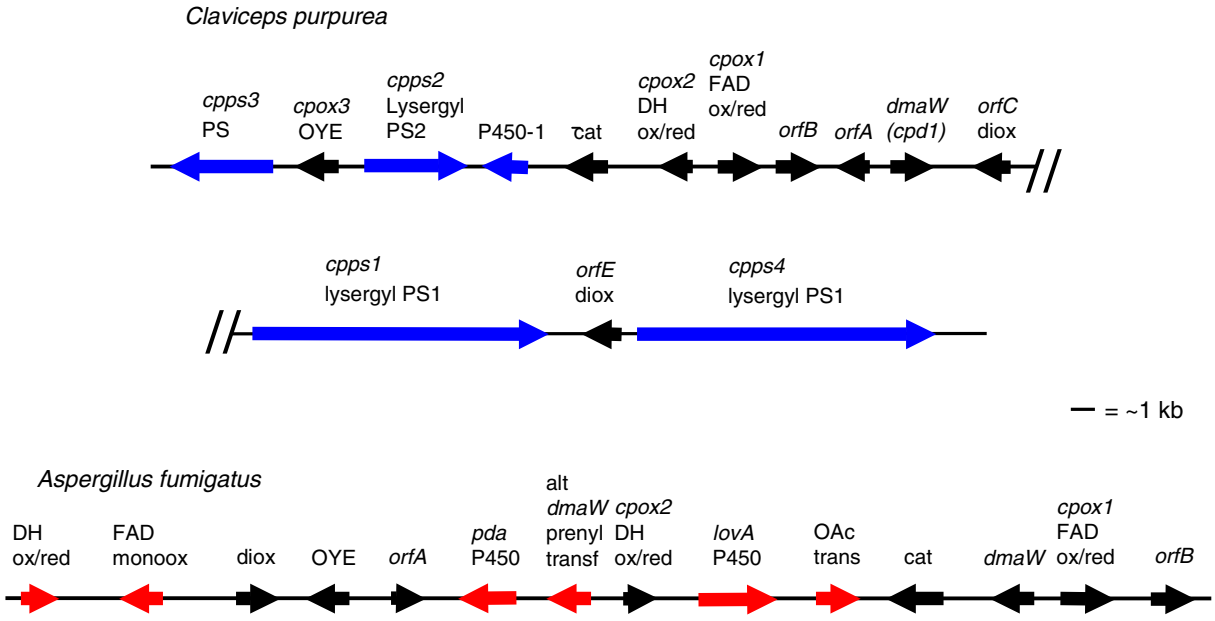

Fig. 2. Parts of the ergot alkaloid gene clusters of *C. purpurea* and *Aspergillus fumigatus* redrawn from previously published work [13,19,30]. Arrows indicate the orientation of transcription. Color scheme is analogous to that in Fig. 1 in that genes indicated by black arrows are common to clusters from both fungi; those marked in blue are found in *C. purpurea* but not *A. fumigatus* and the converse is true for those marked in red. Abbreviations: Cp, *C. purpurea*; *ps* or PS peptide synthetase; ox or ox/red, oxidoreductase; OYE, old yellow enzyme; P450, cytochrome P450 monooxygenase; cat, catalase; DH, dehydrogenase; orf, open reading frame; diox, dioxygenase; monoox, monooxygenase; *pda* P450, similar to P450 involved in pisatin demethylase activity; alt, alternate; trans, transferase; OAc trans, *O*-acetyl transferase; *lovA* P450, similar to P450 involved in lovastatin biosynthesis. The scale applies to both clusters.

2.3. Diversification of ergopeptines within a producer by multiple peptide synthetases and/or by peptide synthetases with low substrate specificity

In *C. purpurea*, the ergopeptines represent a diverse group of ergot alkaloids, all of which could be considered alternate pathway end products. Ergopeptines are assembled by the lysergyl peptide synthetase (LPS) complex made up of LPS2, which binds D-lysergic acid and activates it by adenylation, and LPS1, which recognizes and activates three amino acids and assembles the ergopeptine from the activated components [22,23]. Table 1 lists the concentrations of six different ergopeptines isolated from *C. purpurea* sclerotia from tall fescue. These six ergopeptines result from all possible combinations of two variable amino acids at the first position of the tripeptide moiety and three variable amino acids at the second position (Fig. 1). Two factors are likely to contribute to the diversity of ergopeptines observed in this fungus – multiple peptide synthetases with different amino acid substrate specificities and peptide synthetases with relaxed substrate specificity.

Peptide synthetases have separate adenylation domains (in which amino acid substrate recognition is conferred) for each amino acid that will be incorporated into the peptide product. The amino acid substrate recognition pocket of these adenylation domains has been identified [24], and a code of signature sequences (for determining which amino acid will be recognized by a particular domain) has been proposed based on amino acids occupying critical positions in the enzymes [25,26]. *C. purpurea* has more than one (but fewer than six) genes encoding LPS1 [17,19], and DNA sequence data indicate that some of these genes encode enzymes that have different specificity in their amino acid-recognition domains [19].

An alternate means for generating variability would be to have a peptide synthetase with relaxed specificity in amino acid recognition. A single peptide synthetase with low specificity in amino acid substrate recognition can make more than one peptide product by accepting different amino acids for activation during different cycles of peptide synthesis [27]. The signature sequences of fungal peptide synthetases in general do not match the consensus sequences for specific amino acids very well [27], and this is true of the amino acid-recognizing domains of LPS1 molecules in particular [19,28]. A lack of specificity in amino acid substrate recognition would allow an individual enzyme to produce more than one ergopeptine.

2.4. Diversity in ergot alkaloid profiles among producers by pathway divergence

Gene clusters encoding several different enzymes involved, or hypothesized to be involved, in ergot alkaloid

biosynthesis have been identified in *A. fumigatus* [13,29] and *C. purpurea* [16,19,30] (Fig. 2). Analysis of ergot alkaloid profiles of *A. fumigatus* and *C. purpurea*, and comparison of the gene clusters associated with their known ergot alkaloid biosynthesis genes, indicated that the pathways of the two fungi share early steps before diverging to produce different end products [13] (Figs. 1 and 2). The ergot alkaloid profiles observed in these fungi reflect the presence or absence of certain genes in their ergot alkaloid gene clusters. For example, genes encoding LPS1 and LPS2, required for assembly of ergopeptines and simple amides in clavicipitaceous fungi [16–18], are missing in the *A. fumigatus* gene cluster and genome [13]. Conversely, genes encoding activities presumably required for addition of side chains of fumigaclavines A and C (e.g., *O*-Acetyl transferase and alternate *dmaW*-like prenyl transferase of *A. fumigatus*) are present in the *A. fumigatus* gene cluster but have not been found in clavicipitaceous fungi (Fig. 2).

Other species of ergot fungi differ from *C. purpurea* in ergot alkaloid profiles and pathways. *C. fusiformis* accumulates clavines up through elymoclavine (Fig. 1) but the pathway appears to end at that point [1,11]. Consistent with its lack of ergopeptines and simple amides, *C. fusiformis* does not contain a homologue of the gene encoding LPS1 [17], which is required for synthesis of these compounds [17,18]. *C. africana* accumulates dihydroergot alkaloids derived from a pathway apparently analogous to that of the other clavicipitaceous fungi but via festuclavine (which could be considered dihydroagroclavine) and dihydroelymoclavine and culminating in dihydroergosine [31] (Fig. 1). The genetic basis for this difference has not been studied.

The discovery of similar, rare biosynthetic pathways encoded, at least in part, by clustered genes, in relatively distant phylogenetic lineages presents the possibility that horizontal gene transfer has been involved in the observed distribution of the pathway. However, studies of GC content and codon usage bias in *A. fumigatus* and *C. purpurea* provided no support for a recent horizontal transfer (D.G. Panaccione, unpublished data). An alternate explanation for the current phylogenetically discontinuous distribution for the ergot alkaloids is that the ability to synthesize some type of ergot alkaloid was present in the most recent common ancestor of these Ascomycetes. This fundamental biosynthetic capability may have been developed differently in a few lineages (e.g., in the Clavicipitaceae, *A. fumigatus*, and perhaps other fungi) under differing selective pressures leading to the pathway diversity observed today. In many other diverging lineages the alkaloids may have provided no advantage, or became functionally redundant with other secondary metabolites, allowing the eventual loss of pathway genes. Interestingly, in *A. fumigatus*, the ergot alkaloid gene cluster is within 40 kb of the telomere of the long arm of chromosome 2 [13]. In fungi and other eukaryotic microorganisms, genes conferring niche-specific adaptations have been found near telomeres (e.g., [32–35]) and these subtelomeric regions have been proposed to recombine frequently [33,34].

The differences among ergot alkaloid profiles in different fungi raises the question of whether the profiles differ because the core pathway genes are present in different genomes with different capacities to finish the key intermediates, or whether they differ because of different selective pressures applied by the niches that the different fungi occupy.

3. Evidence for functional differences among structurally diverse ergot alkaloids

Numerous studies have been conducted on the biological activities of individual ergot alkaloids from a pharmacological perspective but relatively few studies have addressed potential ecological benefits of ergot alkaloid production. Studies selected for presentation here compared activities of different ergot alkaloids to a common organism or small set of organisms. Whereas the activity of an alkaloid in a laboratory test does not necessarily reflect the true ecological role of that alkaloid, documented differences in responses to various ergot alkaloids support the hypothesis that accumulation of a diverse set of ergot alkaloids can be advantageous to the producing fungus. Based on limited laboratory research, ergopeptines appear to be particularly active against insects and nematodes, whereas clavine alkaloids are less active against these invertebrate eukaryotes [6,7]. Conversely, clavine alkaloids have been shown to have antibacterial and cytostatic activity that is superior to that of ergopeptines or simple amides of lysergic acid [9,10,36].

Ergopeptines (ergotamine, ergovaline, ergosine, and α-ergocryptine at 5 μg/g) deterred feeding of adult black beetle (*Heteronychus arator*) on an artificial carrot based diet [6]. Ergonovine, a simple amide of lysergic acid, also deterred the insects but only at concentrations of 10 μg/g or higher. Ergine (the simplest amide of lysergic acid) and clavines festuclavine and lysergol (isomer of elymoclavine with double-bond shifted to same position as in lysergic acid; refer to Fig. 1) did not deter feeding, even at a concentration of 20 μg/g.

In similar experiments with a different insect subject, fall armyworm (*Spodoptera frugiperda*) larvae fed on corn leaf disks soaked in 100 mg/L α-ergocryptine had reduced survival (about 50% of that observed for larvae fed control leaf disks) [7]. However, leaf disks soaked in the clavines lysergol, agroclavine, and elymoclavine at the same concentration had no significant effect on insect survival. In contrast to the results with black beetle, both ergopeptines (ergotamine and α-ergocryptine) and

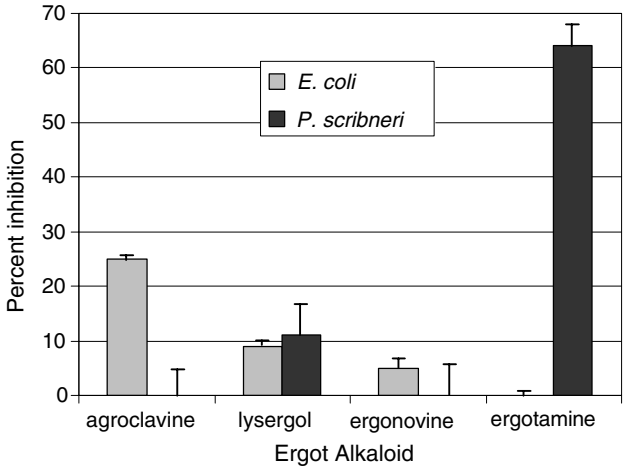

Fig. 3. Inhibition (expressed as percent of no-alkaloid control) of *Escherichia coli* multiplication (lighter bars) or nematode (*Pratylenchus scribneri*) motility (darker bars) by the indicated ergot alkaloids. Multiplication of *E. coli* was measured spectrophotometrically (A_{600}) after incubation with 100 μg/mL of the indicated ergot alkaloids in Luria–Bertani broth (37 °C, 250 rpm) for 5 h. Nematode motility was assessed microscopically after 24 h incubation at 25 °C in 5 μg/mL of the indicated ergot alkaloid. Error bars indicate standard error.

two of three clavines (agroclavine and elymoclavine, but not lysergol) acted as feeding deterrents for this particular insect, reducing larval weight gain and leaf area consumed.

Ergot alkaloids exhibit clear differences in their antimicrobial and cytostatic properties. Clavine alkaloids (particularly agroclavine and festuclavine) were shown to be effective antimicrobial compounds, whereas ergotamine (an ergopeptine) and ergonovine (a simple amide of lysergic acid) were not [9,10]. Similarly, agroclavine and festuclavine (and their synthetic derivatives) had significant cytostatic activity to a mouse lymphoma cell line, whereas four lysergic acid derivatives (including ergine and LSD) did not [36]. Eich and Pertz [9] proposed that agroclavine accumulates to high levels during the early stages of sclerotium development, when it may serve to protect the fungus in a moist, sugar-rich environment, but is found at much lower levels in mature, dry sclerotia, in which microbial activity would be minimal.

Simple in vitro studies (Fig. 3) demonstrate clear differences in the activities among a small set of commercially available ergot alkaloids to the common enteric bacterium *Escherichia coli* and the plant-parasitic nematode *Pratylenchus scribneri*. Agroclavine was the most effective at inhibiting *E. coli* multiplication, whereas ergotamine was highly effective at immobilizing the nematode *P. scribneri*. Again these analyses are not intended as investigations of the natural roles of these alkaloids but clearly demonstrate differences in activities that would provide advantages for accumulation of a diverse set of ergot alkaloids.

The effects of ergot alkaloids on mammals are highly complex and mediated through their interactions with a variety of 5-HT receptor types and subtypes, dopamine D2 receptor subtypes, and certain α-adrenoreceptors [3,5]. Individual ergot alkaloids have unique profiles of affinities for a range of receptors within these three major groups, resulting typically in a complex response. In interactions with 5-HT receptors, a general difference in affinities for ergopeptines and clavines for different subtypes of 5-HT receptors has been noted [3]. Ergopeptines have strong affinity for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, and 5-HT_{2C} receptors, and clavines show moderate affinity for rat 5-HT_{2A} and high affinity for human 5-HT_{1D} and 5-HT_{1F} receptors. In an example involving dopamine D2 receptors, ergopeptines (ergotamine and ergovaline) had approximately 100-fold greater affinity for dopamine D2 receptors that did simple amides of lysergic acid (ergonovine and ergine). A similar difference in response was observed in the ability of these same alkaloids to inhibit vasoactive intestinal peptide-induced cAMP production [37]. Among α-adrenoreceptor subtypes, ergopeptines had higher affinity for α2 as compared to α1 subtypes, whereas simple amides of lysergic acid were active primarily at α1 subtypes [3,38].

Thus, by accumulating a profile containing one or more clavines, lysergic acid amides, and ergopeptines, a fungus would contain alkaloids capable of interacting with a broad range of monoamine receptors, eliciting a variety of biological responses. Moreover, studies described above indicate that a diverse profile of alkaloids provides activities against a range of prokaryotes and invertebrate eukaryotes. Any connection between the greater collective activity and a benefit to the producing fungus, although reasonable to expect, has not been demonstrated. Analyses of gene knockout mutants that contain pathways truncated at different points [e.g., 13,16,17,39] should be helpful in investigating such connections.

4. Summary

Individual ergot alkaloid-producing fungi have profiles of ergot alkaloids that accumulate to characteristic levels based on the structure and regulation of their ergot alkaloid pathways. Certain ergot alkaloid-producing fungi accumulate intermediates to relatively high concentrations. Shunts off the main pathway and multiple peptide synthetases of varying substrate specificity also contribute to the diversity of ergot alkaloids observed in certain fungi. The discovery and sequencing of ergot alkaloid gene clusters has facilitated studies on pathway constitution and regulation.

Different ergot alkaloids clearly have different biological activities and, thus, could play different roles in the biology and ecology of the producing fungus. Ergot

alkaloid pathway inefficiency and other means of alkaloid profile diversification may be beneficial to ergot alkaloid-producing fungi. This hypothesis is being tested by pathway truncation via gene knockout and subsequent analyses of the mutants [13,17,18,39].

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