Overproduction, purification and characterization of FgaPT2, a dimethylallyltryptophan synthase from Aspergillus fumigatus

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A putative dimethylallyltryptophan synthase gene, fgaPT2, was identified in the genome sequence of Aspergillus fumigatus. FgaPT2 was cloned and overexpressed in Saccharomyces cerevisiae. The protein FgaPT2 was purified to near homogeneity and characterized biochemically. This enzyme was found to convert L-tryptophan to 4-dimethylallyltryptophan, a reaction known to be the first step in ergot alkaloid biosynthesis. FgaPT2 is a soluble, dimeric protein with a subunit size of 52 kDa, and contains no putative prenyl diphosphate binding site (N/D)DXD. \( K_m \) values for L-tryptophan and dimethylallyl diphosphate (DMAPP) were determined as 8 and 4 \( \mu \)M, respectively. Metal ions, such as \( \text{Mg}^{2+} \) and \( \text{Ca}^{2+} \), enhance the reaction velocity, but are not essential for the enzymatic reaction. FgaPT2 showed a relatively strict substrate specificity for both tryptophan and DMAPP. FgaPT2 is the first enzyme in the biosynthesis of ergot alkaloids to be purified and characterized in homogenized form after heterologous overproduction.

INTRODUCTION

The biosynthesis of ergot alkaloids has been studied for more than 40 years, especially in the fungus Claviceps purpurea (Floss, 1976; Gröger & Floss, 1998; Williams et al., 2000). The lysergyl moiety of ergot alkaloids, e.g. ergotamine (Fig. 1), is derived from L-tryptophan and dimethylallyl diphosphate (DMAPP) (Gröger & Floss, 1998; Williams et al., 2000). Dimethylallyltryptophan synthase (DMATS), the first pathway-specific enzyme of ergot alkaloid biosynthesis, catalyses the prenylation of L-tryptophan. The biochemical properties of this enzyme were investigated by several groups using partially or homogeneously purified protein from different producer strains of the ergot alkaloids (Cress et al., 1981; Gebler & Poulter, 1992; Lee et al., 1976). Tsai et al. (1995) used partial amino acid sequences of the purified DMATS to create degenerate oligonucleotides, which allowed PCR amplification of a gene called dmaW from a cDNA library of Claviceps fusiformis. This cDNA sequence was cloned in a yeast vector, and expressed in Saccharomyces cerevisiae (Tsai et al., 1995). DMATS activity was tested in crude extracts of yeast transformants. However, the enzymatic activity was low, and only a minute product peak was observed by HPLC. Product identification relied on HPLC retention time and the detection of an ion at \( m/z \) 273 (\( M+1 \)) peak in MS. The MS fragmentation pattern and NMR data of the product were not obtained, and therefore the structure of the enzymic product was not unequivocally confirmed. The groups of Tudzynski and Keller (Correia et al., 2003; Tudzynski et al., 1999) cloned and sequenced the biosynthetic gene cluster of ergot alkaloids in C. purpurea by screening a genomic library with the cDNA sequence reported by Tsai et al. (1995), but they did not attempt a biochemical investigation on DMATS from this cluster. In summary, the experimental evidence on the protein encoded by dmaW is limited, and the purification and characterization of this key enzyme of ergot alkaloid biosynthesis after heterologous gene expression has not yet been reported.

Aspergillus fumigatus is a filamentous and ubiquitous opportunistic fungal pathogen of human and animals. It also produces ergot alkaloids of the clavine type, e.g. elymoclavine, festuclavine and fumigaclavines (Flieger et al., 1997; Spilsbury & Wilkinson, 1961; Yamano et al., 1962) (Fig. 1).

Recently, the whole genome of A. fumigatus (strain AF293) was sequenced by an international consortium with The Wellcome Trust Sanger Institute (UK, http://www.sanger.ac.uk/Projects/A_fumigatus/), The Institute for Genomic Research (USA; http://www.tigr.org/tdb/e2k1/afu1/) and other institutions. From the available, but not yet annotated, genome sequence of A. fumigatus, we could identify a DMATS gene, fgaPT2. Here we report the cloning and heterologous expression of this gene, followed by the purification and biochemical characterization of the DMATS (systematic name dimethylallyl diphosphate: L-tryptophan dimethylallyltransferase; EC 2.5.1.34) that it encodes.
METHODS

Computer-assisted sequence analysis. Preliminary sequence data were obtained from The Wellcome Trust Sanger Institute (http://www.sanger.ac.uk/Projects/Afumigatus/). FGENESH (Softberry; www.softberry.com/berry.phtml) and the DNAsis software package (version 2.1; Hitachi Software Engineering) were used for intron prediction and sequence analysis, respectively. Amino acid sequence homology searches were carried out in the GenBank database by using the BLAST program (release 2.9.9).

Bacterial and fungal strains, plasmids and culture conditions. The Escherichia coli–Sacc. cerevisiae shuttle vector pYES2/NT B and Sacc. cerevisiae INVSc1 were obtained from Invitrogen. pGEMT and pSL1180 were obtained from Promega and Amersham Biosciences, respectively. Sacc. cerevisiae INVSc1 was maintained on Yeast Extract Peptone Glucose Medium (Ausubel et al., 1996). E. coli XL-1 Blue MRF’ (Stratagene) and DH5α (Invitrogen) were used for cloning experiments, and were grown in liquid Luria-Bertani (LB) or on solid LB medium with 1.5% agar at 37°C (Sambrook & Russell, 2001). Carbenicillin (50 μg ml⁻¹) was used for selection of recombinant E. coli strains.

BAC clone AfA 32C2 containing the genomic DNA of A. fumigatus strain AF293 was kindly provided by David Harris of the Wellcome Trust Sanger Institute (Cambridge, UK), and used as genomic DNA template for PCR amplification. A Uni-ZAP XR Premade Library of Sacc. cerevisiae pGEMT and pSL1180 were obtained from Promega and Amersham Biosciences, respectively. Russell, 2001). Carbenicillin (50 μg ml⁻¹) was used for selection of recombinant E. coli strains.

DNA isolation, manipulation and cloning. Standard procedures for DNA isolation and manipulation were performed as described by Sambrook & Russell (2001). Isolation of BAC DNA and plasmids from E. coli was carried out with ion-exchange columns (Nucleobond AX kits; Macherey-Nagel), according to the manufacturer’s protocol.

Overproduction and purification of FgaPT2 protein. For construction of the expression plasmid pIU11, two overlapping fragments of the coding sequences of fgaPT2 were amplified independently of each other. A genuine NcoI restriction enzyme recognition site is located in the overlapping part of both fragments. The fragment of 1164 bp at the 5’ end was amplified from genomic DNA on BAC AF293 by using the primers PT2-561_for (5’-CCAAATGCATCCCATAGGCGAACAAATG-3’) and PT2-1720_rev (5’-AGTACGACTTCAAGAATGTTCGAGGTC-3’). Bold letters represent mutations inserted in comparison to the original sequence to give the underlined BamHI restriction site. The fragment of 624 bp at the 3’ end was amplified from cDNA by using the primers PT2-1375_for (5’-CTAGAAGCATTGAGACACGTGTGACCTC-3’), and PT2-2133_rev (5’-CCGGAATTCATCGGTTCTAGCCCGGGA-3’). Bold letters represent mutations inserted in comparison to the original sequence to give the underlined EcoRI restriction site. Both fragments were ligated into pGEMT to give pIU3 containing the fragment at the 5’ end, and pIU4 containing the fragment at the 3’ end. pIU3 was digested with BamHI and NcoI, and the obtained BamHI–NcoI fragment of 818 bp was ligated into pSL1180, which had been restricted with BamHI and NcoI, to give pIU5. pIU4 was digested with NcoI and EcoRI, and the obtained NcoI–EcoRI fragment of 607 bp was ligated into pIU5, which had also been digested with NcoI and EcoRI, to give pIU7. The resulting plasmid containing the complete coding sequence of fgaPT2 was sequenced to identify potential errors by PCR amplification (MWG-Biotech). The whole coding sequence of fgaPT2 was released from pIU7 by digestion with BamHI and EcoRI, and ligated into the expression vector pYES2/NT B, which had been restricted with the same enzymes, to give the expression construct pIU11.

pIU11 was introduced into Sacc. cerevisiae INVSc1 by electroporation (Ausubel et al., 1996). Recombinants were selected and maintained on minimal medium lacking uracil. For gene expression, the cells were grown in baffled 3000 ml Erlenmeyer flasks containing 300 ml liquid minimal medium (with 2% glucose) at 30°C and 200 r.p.m. for 24 h. Then the cells were transferred into medium containing 1% raffinose and 2% galactose for induction, and cultivated for a further 16 h before harvest. Total protein was obtained after breaking of the cells using glass beads (Ausubel et al., 1996) in 50 mM sodium phosphate buffer (pH 7.4) with 5% (v/v) glycerol and 1 mM PMSF. One-step purification of the recombinant His₆-tag fusion protein by affinity chromatography with Ni-NTA agarose resin (Qiagen) was carried out according to the manufacturer’s instruction.

Fig. 1. Structures of ergot alkaloids. Fumigaclavines were identified in A. fumigatus; lysergic acid and ergotamine in C. purpurea; festuclavine and elymoclavine in both A. fumigatus and Claviceps spp. (Fieger et al., 1997).
Protein analysis. Standard protein techniques were used as described by Bradford (1976) and Laemmli (1970). The molecular mass of native FgaPT2 was determined by gel filtration on a HiLoad 26/60 Superdex 200 column (Amersham Biosciences) using 50 mM Tris/HCl buffer (pH 8.0) containing 150 mM NaCl. The column was calibrated with dextran blue 20000 (200 kDa), aldolase (158 kDa), albumin (66 kDa), ovalbumin (45 kDa) and ribonuclease A (13.7 kDa) (Amersham Biosciences).

Assay for DMATS activity. For quantitative determination of enzyme activity, the reaction mixture (100 μl) contained 50 mM Tris/HCl (pH 7.5), 5 mM CaCl₂, 1 mM l-tryptophan, 1 mM DMAPP and 0–1–1 μg purified FgaPT2. After incubation for 10 min at 30 °C, the reaction was stopped with 10 μl TCA (1:5 M). The protein was removed by centrifugation at 13 000 g for 10 min. Enzymic products were analysed by HPLC at 269 nm using a Multisphere RP 18-5 column (250 × 4 mm, 5 μm; C+S Chromatographic Service) at a flow rate of 1 ml min⁻¹. A linear gradient of 20–70% acetonitrile in 0-1% aqueous trifluoroacetic acid in 20 min was used. Authentic DMAT (Yokoyama) (Hikawa et al., 2000) was used as a standard. All quantitative enzyme activity data in this manuscript are mean values from two independent enzyme assays.

Preparative enzymic synthesis and structural elucidation of dimethylallyltryptophan and derivatives. To a 1-5 ml glass vial, DMAPP (1 mM final concentration), l-tryptophan, 5-methyl-dL-tryptophan or 6-methyl-dL-tryptophan (1 mM), CaCl₂ (5 mM), Tris/HCl (50 mM, pH 7-5) and FgaPT2 (75 μg) were added to a final volume of 1 ml. The mixtures were incubated at 30 °C for 16 h. A 100 μl volume of TCA (1:5 M) was added, and the protein was removed by centrifugation. 4-Dimethylallyltryptophan (DMAT) and derivatives were purified by HPLC using the method described above. The products obtained (0:05–0:2 mg) were analysed by ¹H-NMR spectroscopy, H-H-COSY-NMR and positive-ion fast atom bombardment (FAB)-MS.

Nucleotide sequence accession number. The nucleotide sequences of the genomic DNA reported in this study are available at The Wellcome Trust Sanger Institute (http://www.sanger.ac.uk/Projects/A_fumigatus/). The cDNA sequence of fgaPT2 is available at GenBank under accession number AY775787.

RESULTS

Analysis of the A. fumigatus genome for presence of prenyltransferase genes

In order to find putative prenyltransferase genes, the whole genome sequence of A. fumigatus was analysed for presence of sequence homologues of cdp1 from C. purpurea (Tudzynski et al., 1999), which encodes the enzyme DMATS. A homologue of cdp1, termed fgaPT2, encoding a protein with 54 % identity at the amino acid level to the DMATS from C. purpurea, could thus be identified in the sequence of contig 34 (http://www.sanger.ac.uk/Projects/A_fumigatus/).

The genomic sequence of this putative prenyltransferase gene consists of three exons of 1151, 122 and 104 bp, interrupted by two introns of 61 and 74 bp. The existence of these introns was confirmed by sequencing of a PCR fragment amplified with cDNA as the template (see below). TATA and CCAAT boxes were found 68 and 132 bp upstream of the putative start codon of fgaPT2, respectively. The start codon and the introns were predicted by alignment with DMATS sequences from other fungi (Tsai et al., 1995; Tudzynski et al., 1999; Wang et al., 2004) in combination with the prediction obtained from FGENESH.

The predicted gene product of fgaPT2 comprises 459 amino acids, and has a calculated molecular mass of 52.5 kDa. FgaPT2 shows high similarity to putative DMATS from various fungi. For example, FgaPT2 has an identity of 60 % to DMATS from Neotyphodium coenophialum (Wang et al., 2004), 54 % to DMATS from C. purpurea (Tudzynski et al., 1999), 56 % to DMATS from Balansia obtecta (Wang et al., 2004), and 52 % to DMATS from C. fusiformis (Tae et al., 1995). The prenyl diphosphate binding site (ND)DXXD (Liang et al., 2002) was not found in the sequence of FgaPT2.

Cloning of fgaPT2

To eliminate the two introns in the sequence of fgaPT2, a fragment of 624 bp at the 3′ end was amplified using PCR from cDNA of A. fumigatus B5233, which is available in the form of phagemids isolated from a cDNA library, whereas a 5′ end fragment of 1164 bp was amplified from genomic DNA of A. fumigatus AF293 in the form of BAC DNA. The two PCR fragments overlap by 346 bp, and were cloned via a genuine NcoI recognition site in the overlapping region into a cloning vector. Sequencing of the resulting plasmid revealed one nucleotide difference from the published genome sequence of strain AF293, i.e. G instead of T at position 1327 of the coding sequence, which leads to an exchange of serine by alanine in position 443 in FgaPT2. PCR fragments in four clones from two independent amplifications show the same sequence at this position, suggesting that the difference may not be due to an error of PCR amplification. Rather, the sequence of the cDNA library obtained from A. fumigatus strain B5233 may differ from the published genomic sequence of A. fumigatus AF293.

Overproduction and purification of FgaPT2

The coding sequence of fgaPT2 was cloned into the vector pYES/NT B. Soluble proteins obtained from transformants of S. cerevisiae harbouring the expression construct were used for purification with Ni-NTA agarose, and FgaPT2 was purified to apparent homogeneity, as judged by SDS-PAGE (Fig. 2a). The observed molecular mass was 57 kDa, and corresponds very well to the calculated mass of 56 kDa for His₅-FgaPT2. For the His-tagged protein, a protein yield of 0.2 mg pure FgaPT2 per litre of culture was obtained, which was low, but sufficient for the biochemical characterization.

Identification of the enzymic product

HPLC analysis of the incubation mixture of l-tryptophan and DMAPP with the purified FgaPT2 (Fig. 2d) showed a
product peak with a retention time of 18 min, which was absent in mixture containing enzyme that had been heat-denatured prior to the assay at 100°C for 30 min (Fig. 2c). This peak has the same retention time as that of authentic DMAT (data not shown). Product formation showed a linear dependence on the amount of protein (up to 15 µg per 100 µl assay) and on the reaction time (up to 30 min). The reaction was strictly dependent on the presence of both tryptophan and DMAPP.

The enzymic product was subsequently isolated on a preparative scale (see Methods) and subjected to NMR and MS analysis.

Comparison of the 1H-NMR spectrum of the isolated product with that of L-tryptophan (Table 1) revealed that in the spectrum of the isolated compound the doublet at 7.71 p.p.m. for H-4 of L-tryptophan had disappeared. Instead, additional signals for a dimethylallyl moiety were observed at 5.37 (br t, 7.2 Hz, H-2'), 3.79 (d, 7.2 Hz, H-1'), 1.79 (s, 3H-5') and 1.76 (s, 3H-4') p.p.m., respectively. The expected correlations of these protons were proven by a H-H-COSY spectrum. Positive FAB-MS showed an ion at m/z 273 ([M+H]+) confirming the presence of a prenylated tryptophan. The NMR and MS data (m/z (intensity): 93 (58·0), 198 (41·6), 217 (20), 256 (72), 273 (100) [M+H]+, 295 (8) [M+Na]+) proved that the enzymic product is the expected DMAT (Fig. 2b). These data correspond to those of DMAT reported by Gebler & Poulter (1992), except for a 0·25 p.p.m. difference for H-\alpha of DMAT. The chemical shift of this proton is dependent on the pH value of the NMR samples. The spectrum of the isolated compound was taken at pH 2, whereas the literature data were obtained at pH 4·3. For direct comparison, we recorded the 1H-NMR spectrum of L-tryptophan at two different pH values, i.e. pH 2 vs pH 4·3. The signal of H-\alpha of tryptophan was observed at 4·14 p.p.m. at pH 2, whereas an upfield shift to 4·04 p.p.m. was found when the sample was measured at pH 4·3. This corresponds to the downfield shift observed for H-\alpha of DMAT at pH 2 compared to the literature data (Gebler & Poulter, 1992).

Fig. 2. (a) Purification of FgaPT2 after overproduction as a His6-tag fusion protein. The 12% polyacrylamide gel was stained with Coomassie brilliant blue R-250. Lanes: 1, molecular mass standards; 2, soluble protein before induction; 3, soluble protein after galactose induction; 4, eluate from Ni-NTA agarose. (b) Reaction catalysed by FgaPT2. (c, d) HPLC chromatograms of enzyme assay with native FgaPT2 (d) and with heat-inactivated FgaPT2 (c). The reaction mixtures were incubated at 30°C for 30 min. The structure of DMAT was elucidated by NMR and MS analyses (see Results). AU, absorbance units.
Biochemical properties of DMATS FgaPT2

The presence of Mg\(^{2+}\) or Ca\(^{2+}\) enhanced the prenyltransferase activity up to twofold, with 20 and 5 mM being the most effective concentrations, respectively. However, in the absence of divalent cations, the enzyme was still highly active. Even in the presence of 5 mM EDTA, no significant decrease of activity was observed. This finding is in contrast to the absolute requirement for divalent cations reported for most \textit{trans}-prenyltransferases (Liang et al., 2002), and is similar to that observed for the aromatic prenyltransferases CloQ involved in the biosynthesis of chlorobiocin from \textit{Streptomyces roseochromogenes} (Pojer et al., 2003), LtxC in the biosynthesis of lyngbyatoxins from \textit{Lyngbya majuscula} (Edwards & Gerwick, 2004), and DMATS in the biosynthesis of ergot alkaloids from \textit{C. purpurea} (Geble & Poulter, 1992).

The FgaPT2 reaction apparently followed Michaelis–Menten kinetics, and the \(K_m\) values were determined as 8 \(\mu\)M for L-tryptophan, and 4 \(\mu\)M for DMAPP. These data are similar to those reported by Cress et al. (1981) for purified DMATS from \textit{Claviceps} sp. SD58, i.e. 8-8 and 7-2 \(\mu\)M for L-tryptophan and DMAPP, respectively, but are at variance with those reported by Lee et al. (1976), namely \(K_m\) values of 67 and 200 \(\mu\)M for L-tryptophan and DMAPP, respectively. The maximum reaction velocity observed with FgaPT2 was 0-198 \(\mu\)mol min\(^{-1}\) mg\(^{-1}\), corresponding to a turnover number of 0-37 s\(^{-1}\).

FgaPT2 was found to be specific for its substrates L-tryptophan and DMAPP. Low product formation was observed when D-tryptophan (1-8\% of that with L-tryptophan), 5-methyl-DL-tryptophan (11-6\%) or 6-methyl-DL-tryptophan (6-8\%) was used instead of L-tryptophan. The enzymic product of 6-methyltryptophan was identified unequivocally as 4-dimethylallyl-6-methyltryptophan (6-methyl-DMAT) by \(^1\)H-NMR (Table 1) and MS analysis \((m/z)\) (intensity): 212 (100), 231 (34), 270 (66), 287 (76) \([\text{M}+\text{H}]^+\). 5-Methyltryptophan was converted by FgaPT2 to dimethylallyl-5-methyltryptophan, as confirmed by MS analysis \((m/z)\) (intensity): 212 (85), 231 (31), 270 (65), 287 (100) \([\text{M}+\text{H}]^+, 309 (32) \text{[M+Na]}^+\). There was no product formation \((<0-2\%)\) observed with 4-hydroxybenzoic acid, the substrate of the prenyltransferase of ubiquinone biosynthesis (Melzer & Heide 1994), or with 4-hydroxyphenylpyruvic acid or umbelliferone, the substrates of previously identified prenyltransferases of secondary metabolism (Hamerski et al., 1990; Pojer et al., 2003). These results are comparable with those obtained with DMATS from \textit{C. purpurea} (Lee et al., 1976). When DMAPP was replaced with geranyl diphosphate, very low product formation (0-7\% of that with DMAPP) was observed. A low product formation was also observed with isopentenyl diphosphate (3-4\% of that with DMAPP).

Using gel chromatography, the native molecular mass of FgaPT2 was determined as 86–91 kDa, suggesting that the protein is active as a dimer.

**DISCUSSION**

We have cloned and expressed the \(fgaPT2\) gene of \textit{A. fumigatus}, encoding a DMATS, and characterized this first pathway-specific enzyme in the biosynthesis of ergot alkaloids. FgaPT2 catalyses the conversion of tryptophan to DMAT in the presence of DMAPP. The enzymic product was identified unequivocally as DMAT by NMR and MS analyses.

FgaPT2 is the first enzyme involved in ergot alkaloid biosynthesis to be heterologously overproduced and purified
the prenyl diphosphate binding motif (N/D)DXXD of complete absence of divalent cations, and does not contain A. fumigatus Gerwick, 2004), FgaPT2 from they do not require Mg$^{2+}$ catalyse the C-prenylation of an aromatic nucleus, and since of CloQ, LtxC and DMATS are unknown. All three enzymes synthase (Liang et al, 2003) and LtxC from L. majuscula (Edwards & Gerwick, 2004), FgaPT2 from A. fumigatus is active in the complete absence of divergent cations, and does not contain the prenyl diphosphate binding motif (N/D)DXXD of trans-prenyltransferases such as farnesyl diphosphate (FPP) synthase (Liang et al, 2002). The substrate binding sites of CloQ, LtxC and DMATS are unknown. All three enzymes catalyse the C-prenylation of an aromatic nucleus, and since they do not require Mg$^{2+}$ or other divalent metal ions for activity, they apparently differ from FPP synthase in substrate binding and catalytic mechanism (Liang et al, 2002). FgaPT2 shows significant sequence homology only to DMATS from fungi (Tsai et al, 1995; Tudzynski et al, 1999; Wang et al, 2004), but not to prenyltransferases from bacteria, e.g. CloQ and LtxC. Therefore the ‘aromatic’ prenyltransferases from fungi and bacteria may represent different evolutionary groups of prenyltransferases. Information on additional fungal prenyltransferases could support this hypothesis.

Further analysis of the genome sequence of A. fumigatus in the vicinity of fgaPT2 revealed the presence of an additional homologue of cpd1, termed fgaPT1, which showed an identity of 32 % to cpd1 at the amino acid level. fgaPT1 and fgaPT2 are separated by only 10 kb (Fig. 3a). Interestingly, we could identify four additional ORFs, termed fgaOX1, fgaOX2, fgaOX3 and fgaCAT, in the vicinity of fgaPT1 and fgaPT2 (Fig. 3), which showed high sequence similarity to genes from the biosynthetic gene cluster of ergot alkaloids from C. purpurea (Fig. 3), i.e. to cpox1, cpox2, cpox3 and cpox4 (Correia et al, 2003). Only part of the ergot alkaloid cluster of C. purpurea is published, and therefore additional genes surrounding fgaPT2 in A. fumigatus may still be identified. Together with the DMATS, the four genes mentioned above may be involved in the formation of the clavine skeleton, the common structural feature of ergot alkaloids from both organisms (Fig. 1). Fumigaclavine C has an additional prenyl moiety at C-2 of the indole nucleus, which could account for the presence of the second putative prenyltransferase gene fgaPT1. No homologue for the non-ribosomal peptide synthetase gene ccpp1 (Fig. 3) of the ergot alkaloid cluster in C. purpurea (Tudzynski et al, 1999) was found in the vicinity of fgaPT2, consistent with the absence of a peptide moiety in fumigaclavines (Fig. 1). Therefore, the genes surrounding fgaPT2 in the A. fumigatus genome may possibly represent a biosynthetic gene cluster for fumigaclavines and their structural analogues elymoclavine and festuclavine (Fig. 1) (Flieger et al, 1997; Spilsbury & Wilkinson, 1961; Yamano et al, 1962). Future functional investigations of the genes in this putative biosynthetic gene cluster may enhance the understanding of the biosynthesis of the pharmacologically important ergot alkaloids.

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