Lv *et al. Microbial Cell Factories* (2025) 24:226
https://doi.org/10.1186/s12934-025-02853-3

Microbial Cell Factories

RESEARCH Open Access

Characterization of the unconventional meroterpenoid gene cluster from *Stachybotrys* sp. CPCC 401591 for phenylspirodrimanes biosynthesis

Hui Lv[1†], Bailin Song[1†], Lingjin Bu[1], Wenni He[1], Lu Wang[1], Liyan Yu[1*] and Tao Zhang[1*]

Abstract

Background Phenylspirodrimanes (PSDs) are a unique class of meroterpenoids that have been extensively studied due to their diverse biological activities. These metabolites are supposedly biosynthesized through the farnesylation of orsellinate. However, the molecular basis has not yet been elucidated.

Results Herein, using a combination of genome mining, bioinformatic alignment, and gene deletion approach, we characterized the dedicated gene cluster *psd* responsible for biosynthesizing PSDs in the fungus *Stachybotrys* sp. CPCC 401591. RNA sequencing-based transcriptomic analyses broadened our understanding of the genetic basis, regulation, and mechanisms of PSDs biosynthesis. Furthermore, based on the chemical structures of PSD derivatives characterized and deduced gene functions of the cluster *psd*, a hypothetical metabolic pathway for biosynthesizing chartarlactam K (**1**) was proposed. These results revealed the underlying mechanism of phenylspirodrimanes generation, and these findings might facilitate downstream metabolic engineering studies of PSDs or the production of new chemical entities.

Conclusions Genome discovery and gene deletion experiments unveiled a previously uncharacterized biosynthetic gene cluster *psd* involved in the biosynthesizing PSD derivatives in *Stachybotrys* sp. CPCC 401591. In addition, based on the gene cluster data and transcriptomic analyses, a hypothetical biosynthetic pathway of **1** was put forward.

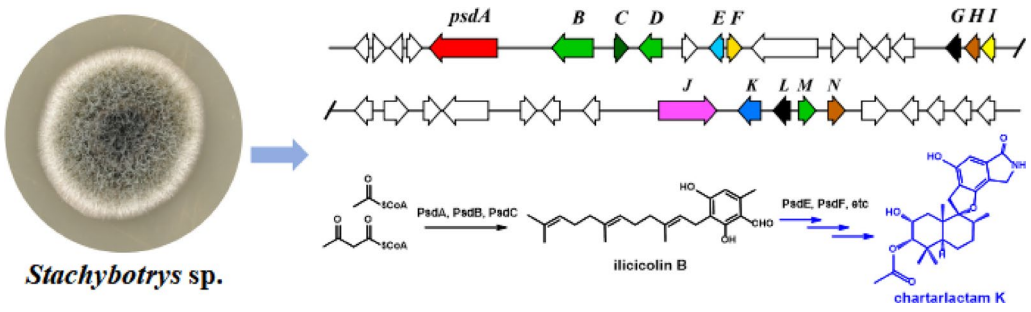
Keywords Phenylspirodrimanes, Genome mining, Gene deletion, Transcriptome, Biosynthesis, *Stachybotrys*

[†]Hui Lv and Bailin Song have contributed equally to this work.

*Correspondence:
Liyan Yu
yly@cpcc.ac.cn
Tao Zhang
evergo218@163.com

Full list of author information is available at the end of the article

Graphical Abstract



Introduction

Phenylspirodrimanes (PSDs), belonging to meroterpenoids, represent a structurally unique family of fungal secondary metabolites [1]. Naturally occurring PSDs with structural diversity have attracted increasing interest from research communities due to their diverse bioactivities, *e.g.*, anti-tumor, antibacterial, anti-diabetic, antiviral, anti-hyperlipidemic, anti-inflammatory, and anti-osteoporosis properties [1–6]. Monomeric and dimeric PSDs comprise the largest group with over 120 compounds bearing a drimane-type sesquiterpene skeleton, spiro-fused to a phenyl moiety *via* a furan ring [4, 7]. The structural differences among PSD dimers are attributed to various dimerization patterns of two monomers to form different linkage units. The linkage units include alkyl diamine chain (e.g., stachybocin E and spirodihydrobenzofuranlactam VI) [6, 8], nitrogen-containing heterocycle (e.g., stachyin B and chartarlactam L) [3, 9], 6/7 oxygen heterocycle (e.g., bistachybotrysin K [10], [5,6]-spiroketal aromatic skeleton (e.g., bistachybotrysins L) [7], C – C coupled bridge with a cyclopentanone core (e.g., stachartarin A and bistachybotrysins F) [11, 12], and C – C coupled connection through two furan rings (e.g., stachybochartins A) [13]. Many PSDs, such as bistachybotrysins M, S, and U, selectively inhibited the proliferation of human tumor cell lines HCT116, BGC 823, Daoy, NCI-H460, and HepG2 with IC_{50} values in the range of 1.8–3.5 μmol/L, making them potential drug leads [7].

Phenylspirodrimanes were mainly isolated from the fungi of the *Stachybotrys* genus, and closely related strains exemplified as *Memnoniella echinata* and *Hansfordia sinuosae* [4, 14, 15]. *Stachybotrys* is a genus including approximately 60 species and is widely distributed, which has been isolated from plant substrates, soil, rhizomes, sponges, and corals [3, 13, 16–18]. Species from this genus are prolific producers of a variety of structurally diversified metabolites, including phenylspirodrimanes, atranones, trichothecenes, dolabellane diterpenes, isoindolinone, cyclosporins, cochlioquinones, xanthones, and alachalasins [2, 19–24]. Recently, postgenomic analyses have accelerated the biosynthetic studies on prenylated aryl-aldehyde of SMs, including stachybisbin B and ascofuranone. Among these metabolic pathways, interestingly, the formation of intermediate ilicicolin B (LL-Z1272β) is associated with a three-gene ensemble that encodes proteins including non-reducing polyketide synthase (NR-PKS), UbiA-family prenyltransferase, and nonribosomal peptide synthetase (NRPS)-like reductase [25, 26]. Biosynthetically, PSDs are believed to be generated from orsellinic acid and farnesyl diphosphate *via* an on-pathway intermediate ilicicolin B (Fig. 1) [27, 28]. However, few studies of molecular basis for the biosynthesis of phenylspirodrimanes has been characterized.

Previously, we isolated the compound **1** from the wetland fungus *Stachybotrys* sp. CPCC 401591, along with seven phenylspirodrimane derivatives (Fig. S1, supporting file) [23]. Herein, we investigated an unconventional biosynthetic cluster in the strain CPCC 401591. Intriguingly, using a combination of bioinformatic analyses, gene deletion, and RNA sequencing-based transcriptomic profiling, a plausible metabolic pathway of compound **1** was also proposed. Deciphering the biosynthesis and identification of the biocatalysts for the formation of PSDs could broaden our knowledge of fungal meroterpenoid biosynthesis. Our study could facilitate future applications for generation of bioactive molecules through metabolic engineering.

Materials and methods

Strains and culture conditions

The fungus *Stachybotrys* sp. CPCC 401591 and the deletant used in this study were deposited in the China Pharmaceutical Culture Collection Center (CPCC) [24]. The fungi were routinely cultivated on potato dextrose agar (BD, USA) and preserved in 15% glycerol at – 80 °C. For genomic DNA (gDNA) extraction, the fungus was cultured in liquid potato dextrose broth (PDB) for one week. For RNA sequencing, the wild-type fungus was cultivated in PDB broth and Trichothecene Biosynthesis Induction (TBI) broth for seven days, respectively [29].

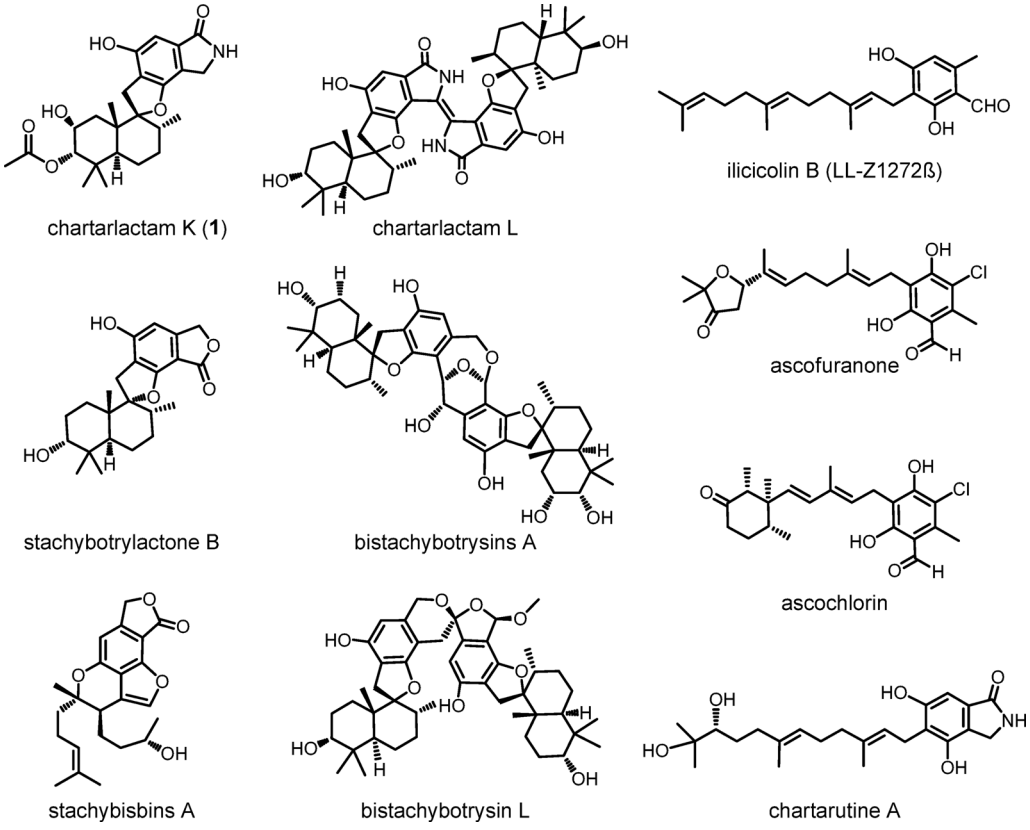


Fig. 1 Chartarlactam K (**1**) and selected fungal meroterpenoids putatively derived from the ilicicolin B

Small-scale fermentation of the parental strain CPCC 401591 and the deletant *ΔpsdA* was performed using rice medium (100 g rice/100 mL of deionized water in a 500 mL Erlenmeyer flask) for thirty days [30, 31]. The cultivation temperature of the strains is 30 °C. DNA cloning was conducted using *Escherichia coli* DH5α as the host. The derived engineered *E. coli* cells were incubated in Luria-Bertani (LB) medium supplemented with kanamycin (50 *μ*g/mL) at 37 °C.

Sequencing and bioinformatic tools

The gDNA from the strain CPCC 401591 was extracted using E.Z.N.A.® Fungal DNA Mini Kit (Omega, USA) as per the manufacturer's instructions [30]. Genome sequencing was performed at Shanghai Majorbio Biopharm Technology Co., Ltd. (Shanghai, China) using the Illumina HiSeq 2500 system. Sequence assembly was performed with SOAPdenovo 1.05 [32]. The systematic bioinformatic analysis of biosynthetic gene clusters (BGCs) was conducted using the antiSMASH platform [33]. Local BLAST searching was performed in which StbA and StbB encoded in LL-Z1272β biosynthetic pathway served as queries. Gene annotations of the BGCs coding sequences were performed on FGENESH platform (www.softberry.com) and then verified manually for BLAST alignments in the NCBI database.

RNA-sequencing and real-time PCR analysis

Total RNA extraction was performed using a PureLink™ RNA Mini Kit (Invitrogen, USA). gDNA was further removed using RNase-free DNase I (Takara, Japan), and the EasyPure® RNA purification kit (TransGen, China) was employed for RNA preparation. RNA concentration was determined using a Nanodrop 2000/2000c spectrometer (Thermo Fisher Scientific, USA). The cDNA synthesis was recovered from RNA (1,250 ng) using HiScript III RT SuperMix (Vazyme, China) as described by the manufacturer. Quantitative PCR was conducted using SYBR qPCR assay (Vazyme, China) according to the standard manual. The primers used in this study are listed in Table S2. The relative quantification of mRNAs was then normalized against the levels of the β-tubulin gene according to the $2^{-\Delta\Delta CT}$ method [31, 34, 35]. Data was calculated with the average value from triplicate experiments. RNA-sequencing was accomplished at Shanghai Majorbio Biopharm Biotechnology Co., Ltd. (Shanghai, China), and an Illumina NovaSeq 6000 sequencer was employed.

General techniques for DNA manipulation

To construct the disruption cassette of the gene *psdA*, the homologous arms of the gene *psdA* were amplified from gDNA of the parental strain using primer sets (*psdA*-Up-F/R and *psdA*-Dn-F/R, respectively) listed in

Table S1 (Supporting Information). PCR amplifications were performed using Phanta Flash Super-Fidelity DNA Polymerase (Vazyme, China). The plasmid pAg1-ble was digested with the *Kpn*I/*Pac*I enzyme sets. Then, the downstream amplicon *psd*-Dn was ligated into the linearized pAg1-ble vector, in which the Clonexpress Ultra One Step Cloning Kit was employed (Vazyme, China). In addition, the upstream amplicon *psd*-Up was ligated into *Sbf*I-digested linear fragment pAg1-ble-Dn to generate the deletion plasmid pAg1-ble-*ΔpsdA*, in which the in vitro recombination cloning strategy was employed (Vazyme, China).

Fungal transformation and generation of the deletant ΔpsdA

Fresh spores of *Stachybotrys* sp. were harvested from PDA plates, which were cultured for 7–8 days at 30 °C. The deletant of the strain CPCC 401591 was created using the standard protoplast-polyethylene glycol (PEG) strategy previously reported [36–38]. The transformants were inoculated into selective PDA medium (200 μg/mL zeocin) with stationary incubation for 7 days. Diagnostic PCRs were further utilized to confirm the genotype of transformants [37, 39]. The specific primers for the construction of deletion cassettes and deletants verification are listed in Table S1.

Fermentation, extraction, LC-MS, and GNPS analysis

The mutant and the wild-type strain CPCC 401591 were initially cultivated on PDA plates for 7 days. Subsequently, 1×10^8 fungal spores were inoculated in 100 mL PDB broth and incubated on a rotary shaker at 200 rpm to afford a seed culture. Then, 10 mL seed culture was inoculated in rice fermentation medium and cultured at 30 °C for thirty days, respectively. The fermented material was extracted three times with ethyl acetate, and the organic layer was concentrated to dryness under vacuum. The extract was redissolved in 1 mL acetonitrile, and the sample (5 μL) was analyzed by high-performance liquid chromatography analysis (Agilent InfinityII 1260, Agilent Eclipse SB-C18 column, 5 μm, 4.6 × 250 mm).

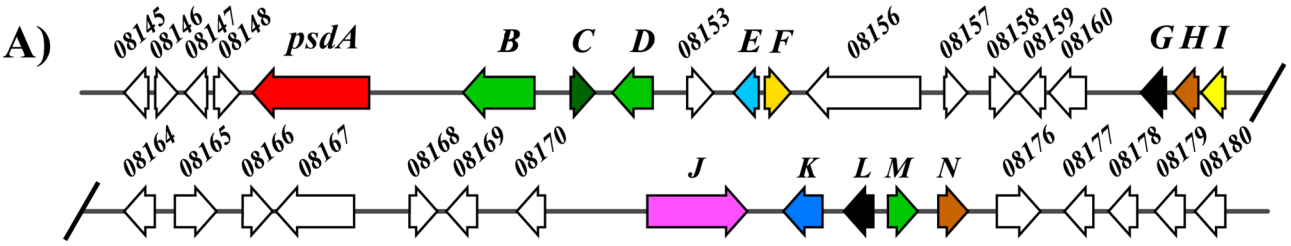
LC-MS/MS measurements were performed using Thermo Fisher Scientific LTQ Orbitrap^{XL} mass spectrometer equipped with a heated electrospray ion source (HESI). Chromatographic separation was acquired using a reversed-phase Agilent Eclipse SB-C18 column (5 μm particle size, 4.6 × 250 mm). The elution was conducted with a linear gradient of 10 – 100% acetonitrile-water (v/v) at a flow rate of 1.0 mL/min^{-1} for 25 min, followed by a 5 min wash with 100% acetonitrile for 5 min. MS/MS spectra were achieved using electrospray ionization in positive ion mode. The optimized detection parameters were set as follows: ion spray voltage, 4.5 kV; ion source temperature: 550 °C; capillary temperature, 220 °C; scan range of *m*/*z*: 150 – 2000 Da, profile mode; a high collision energy of 45 eV was employed for fragmentation. HPLC solvent gradients and MS scan functions were controlled using the Xcalibur data system (Thermo Fisher Scientific).

Results

Mining and bioinformatic analysis of the gene cluster psd in Stachybotrys sp.

To characterize the genetic locus responsible for phenylspirodrimanes biosynthesis, we initially performed the whole genome sequencing of the strain *Stachybotrys* sp. The Illumina HiSeq 2500 sequencing of the fungus generated 276 scaffolds, which concluded 38.44 million non-redundant bases. In the course of antiSMASH-based genome mining endeavors in the fungus, one unique meroterpenoid biosynthetic cluster was acquired, which encodes a conserved (>74.69% identity) non-reducing polyketide synthase (NR-PKS, PsdA), adenylate-forming reductase (PsdB), and UbiA-family prenyltransferase (PsdC) ensemble. It has been reported that a 5-methyl orsellinic acid encoding NR-PKS, a prenyltransferase, and an NRPS-like reductase could function collaboratively to produce ilicicolin B, an on-pathway intermediate during the process of ascochlorin and stachybisbin B biosyntheses [1, 15]. This cluster (Accession no. PX219560) is designated as *psd* (Fig. 2 and Table S2, Supplementary Information).

Furthermore, investigation of the bilateral regions of the skeleton forming genes *psdA*, *psd*B, and *psdC* supported the discovery of other genes encoding post-modification enzymes. The enzymes included one 3-dehydroshikimate dehydratase, one short-chain dehydrogenase/reductase, one oxidoreductase, two methyltransferases, one O-acetyltransferase, one MFS-type transporter, and one pathway-specific transcriptional regulatory factor that correspond to the structural features of phenylspirodrimanes. Nevertheless, the gene cluster *psd* was inserted by many genes that encode proteins involved in primary metabolism or obviously unrelated to the biosynthesis of the PSDs, exemplified as gene 08153 (coding glycosyl hydrolase), gene 08166 (coding alpha-L-arabinofuranosidase), gene 08170 (coding neutral protease). Therefore, these redundant genes were easily eliminated. In the light of the PsdA, PsdB, and PsdC encoded by the cluster *psd* exhibited highly similar identities with cluster *stb* and *asc*, involved in biosynthesizing L-Z1272β and ascochlorin, respectively [1, 15]. We hypothesize that the BGC *psd* might be responsible for phenylspirodrimanes biosynthesis.



B)

Gene ID	Size	Gene description
psdA	2233 aa	Non-reducing polyketide synthase StbA
psdB	1071 aa	Adenylate-forming reductase StbB
psdC	330 aa	Prenyltransferase StbC
psdD	272 aa	Short-chain dehydrogenase/reductase
psdE	320 aa	3-dehydroshikimate dehydratase
psdF	297 aa	Oxidoreductase
psdG	486 aa	Probable mitochondrial chaperone BCS1-B
psdH	259 aa	Methyltransferase
psdI	195 aa	GATA zinc finger domain-containing protein
psdJ	762 aa	O-acetyltransferase
psdK	524 aa	MFS-type transporter
psdL	491 aa	Orsellinic acid biosynthesis cluster protein D
psdM	328 aa	Short chain dehydrogenase/reductase
psdN	361 aa	Methyltransferase

Fig. 2 **A** Genetic organization of the phenylspirodrimanes (PSDs) biosynthetic locus in the *Stachybotrys* sp. CPCC 401591 genome. **B** Putative function of ORFs within the BGC *psd* involved in PSDs biosynthesis

Characterization of the cluster psd responsible for PSDs biosyntheses

To verify the participation of PsdA in the generation of phenylspirodrimanes, deletion of the NR-PKS coding gene *psdA* was performed. We established a protoplast transformation-based gene disruption experiment to support the proposal that *psdA* is functioning in PSDs biosynthesis [36]. The construction of the knockout plasmid was conducted using the scaffold of pAg1-ble by replacing the bilateral fragments of the BleR selective cassette with homologous arms of the target gene *psdA*. Next, after protoplast transformation experiment, of thirty-eight putative zeocin$^+$ *Stachybotrys* transformants analyzed, the mutant *ΔpsdA*-16 was verified by diagnostic PCRs characterization with four primer sets. With one Zeo^R specific primer set, the mutant gave two amplicons of 1,300 bp and 1,392 bp in size, respectively, while the WT had no amplicons (Figure S2, Supporting Information). Further confirming using one primer set resided in the deletion region of the gene *psdA*, two amplicons with 4,553 bp and 4,533 bp in size were recovered from WT, while no amplicons were obtained from the mutant (Figure S2, Supporting Information).

The thus-acquired crude extracts from *ΔpsdA* and the parental strain were analyzed using Liquid Chromatography-Mass Spectrometry (LC-MS) analysis (Fig. 3). Knock-out of the *psdA* gene completely abolished the generation of the compounds stachybotrylactam (t_R=15.15 min) and chartarlactam F (t_R=16.45 min), thereby setting up the linkage between the *psd* biosynthetic cluster and the yielding of the PSDs. At the same HPLC conditions, the peaks of chartarlactam F and stachybotrylactam detected in the parental strain (WT) that two target compounds were further verified along with the evidence of *m/z* = 386.3 $[M+H]^+$ and *m/z* = 771.2 $[2\,M+H]^+$ using LC-MS/MS analysis. However, as shown in Fig. 3, the strain *ΔpsdA* lacked these characteristic molecular ion peaks, indicating the absence of the target two compounds in metabolic profiling of the mutant (Figure S3, Supporting Information). Collectively, the experimental evidence further corroborated with our prediction regarding the function of the gene cluster *psd*,

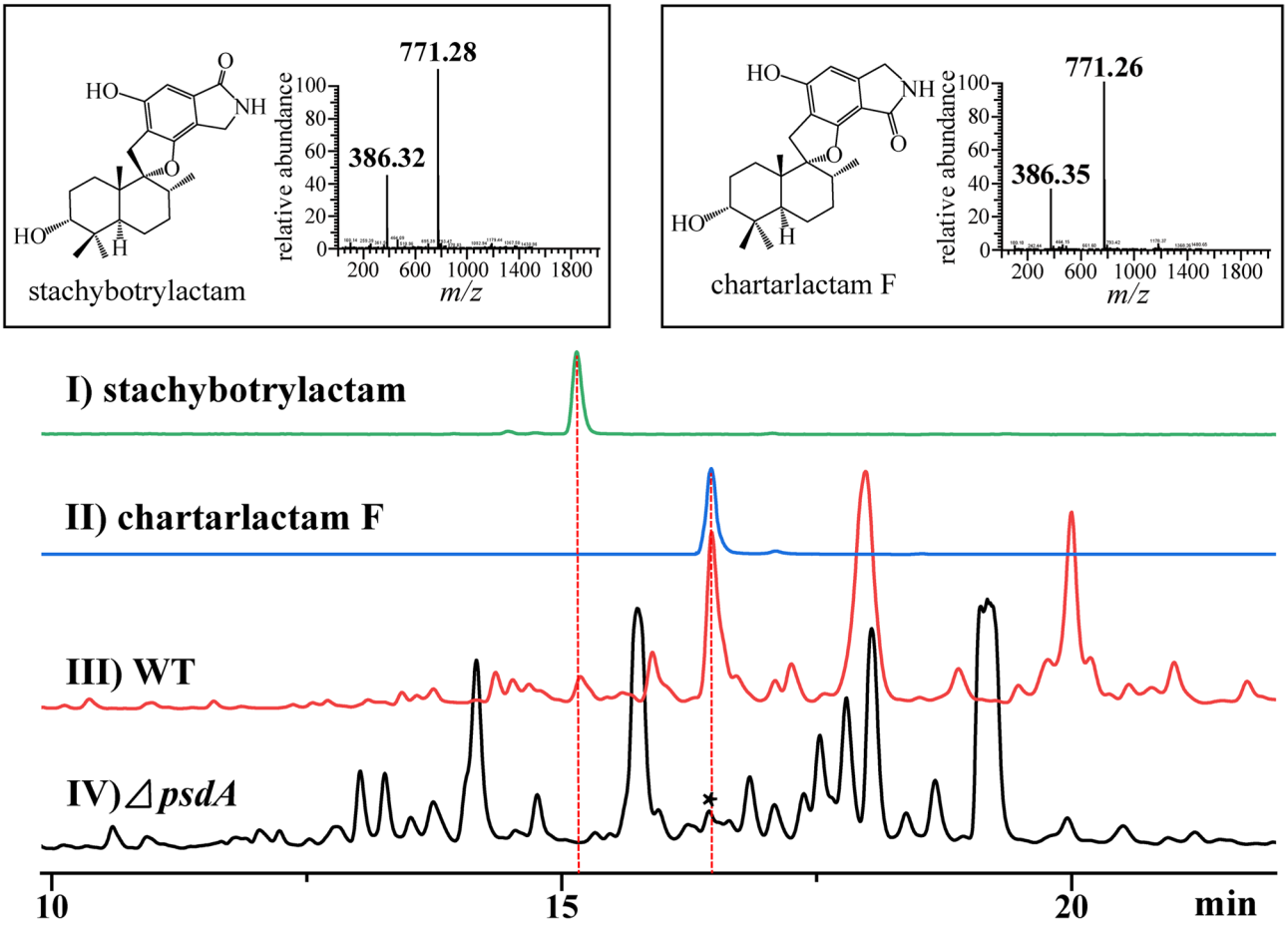


Fig. 3 Gene deletion of *psdA* and characterization of *ΔpsdA* mutants. HPLC profiles of organic extracts obtained from the WT and *ΔpsdA* mutants of *Stachybotrys* sp. The positions of stachybotrylactam and chartarlactam F are indicated in the chromatograms, a peak labeled by * appears at the same retention time as chartarlactam F in deletion strains. This compound exhibited different UV absorbance and EIC values (Figure S3, Supporting Information). The elution was monitored at 230 nm and shown on the same Y-scale. HPLC-MS analysis of *ΔpsdA* metabolites in positive ion mode. Stachybotrylactam, EIC at *m/z* 386.32, $[M+H]^+$; *m/z* 771.28, $[2\,M+H]^+$. Chartarlactam F, EIC at *m/z* 386.35, $[M+H]^+$; *m/z* 771.26, $[2\,M+H]^+$

confirming that the gene *psd*A is a key gene for PSDs biosynthesis in *Stachybotrys* sp.

Preliminary investigation of the genetic basis for PSDs biosynthesis

To reveal the expression level of the gene cluster *psd* during PSDs biosynthesis on TBI and PDB broth, transcriptome sequencing was performed to characterize the differentially expressed genes (DEGs) of intergroup comparison (TBI/PDB) (Fig. 4A). There were 3,874 DEGs, in which the up-regulated and down-regulated genes were 2,053 and 1,821, respectively (Fig. 4B). The RNA-seq data was submitted to NCBI database (PRJNA1309316). Gene Ontology (GO) enrichment analysis manifested that the up-regulated genes were approximately engaged in biological processes including lipid metabolism, organophosphate catabolism, and secondary metabolites biosynthesis (Fig. 4C). The KEGG enrichment data demonstrated that the up-regulated genes were significantly enriched in primary metabolic pathways participating in the processes of fatty acid, amino acid, starch, sucrose, glycolysis and gluconeogenesis (Figure S4, Supporting Information). These pathways were related to primary metabolism, including carbon metabolic reactions for strain growth and secondary metabolism. In addition, significant enrichment was also observed in the biosynthetic pathways of terpenoids and mycotoxins.

Next, when we further examined the cluster *psd*, one phenomenon observed is that there are many dispensable genes within the cluster. Therefore, transcriptomic analyses of the encoded genes might be helpful to distinguish genes directly involved in PSDs biosynthesis. The expression level of *psdA* (gene 08149) and *psdN* (gene 08175, coding methyltransferase) in TBI sample were significantly up-regulated than PDB condition (Fig. 4B, Figure S5, and Table S2, Supporting Information). Further deciphering the genes 08145–08148 and 08176–08180, gene 08148 encoded a hypothetical protein and was

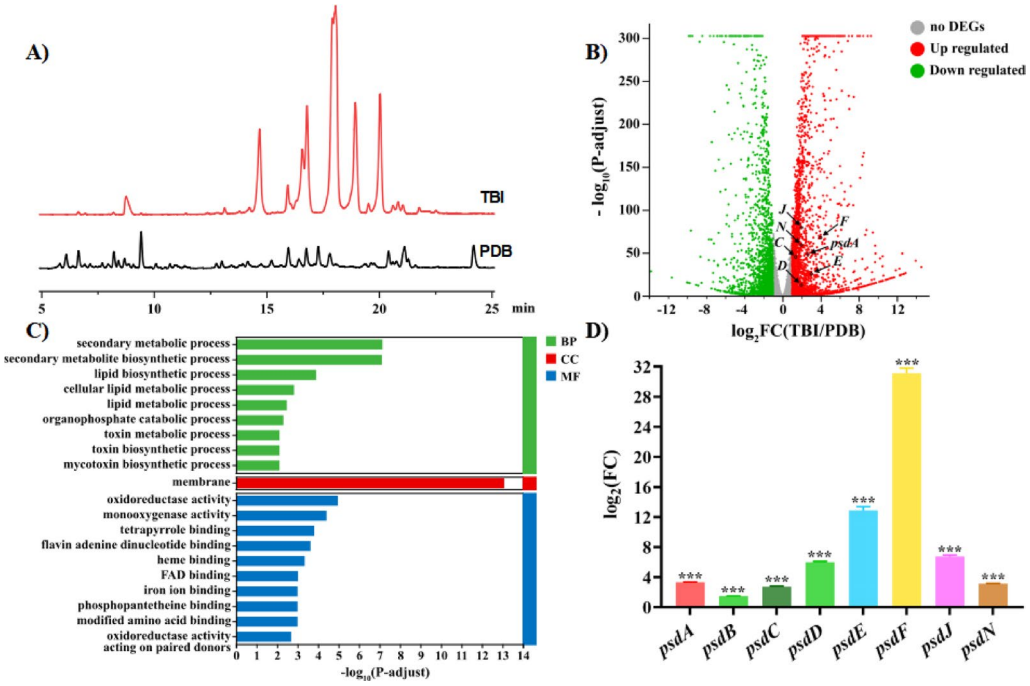


Fig. 4 Mining transcriptome data for phenylspirodrimanes production. **A** HPLC profiles of organic extracts obtained from the strain *Stachybotrys* sp. CPCC 401591 in two fermentation broth. The extracts were analyzed by measuring UV absorbance spectra at 230 nm on an Agilent 1260 Infinity system. **B** Analysis of DEGs. FoldChange (FC) shows the ratio of transcriptional levels (TBI/PDB); $\log_2$(FC) represents the value of differential folds; and -$\log_{10}$(P-adjust) shows the levels of significance. **C** GO enrichment of up-regulated genes in TBI fermentation broth. **D** Differentiated expression levels of the PSDs biosynthetic gene cluster. The error bars are shown as ± standard deviation ($n = 3$). ***$P < 0.001$

significantly down-regulated, while genes 08145–08147, gene 08176, and gene 08180 exhibited no detectable expression level in both conditions. Moreover, the genes (08177–08179) were annotated as phosphatase, mannitol dehydrogenase and accessory effector CFEM5, respectively. These three genes are obviously not associated with secondary metabolism and could be excluded from further analysis. Therefore, we speculated that *psdA* and *psdN* should be located on the left and right boundaries of the cluster *psd* (Fig. 2A).

Similarly, statistical analyses revealed that seven genes (*psdA*, *psdC-F*, *psdJ* and *psdN*) were significantly up-regulated (FC > 2, $p < 0.05$) in TBI condition, demonstrating that corresponding coded proteins should participate in PSDs generation. Also, seven genes (*psdB*, and *psdG-I*, and *psdK-M*) from both culture conditions exhibited high expression level in TPM (transcripts per million) values and moderately up-regulated (1 < FC < 2) in TBI sample, indicating these genes might contribute in PSDs biosynthesis or metabolites transport. Based on the transcriptome data, it was supposed that *psdI* might serve as regulatory factor, which could trigger the catalysis of proteins encoded by the cluster *psd*. In contrast, eleven genes displayed no detectable expression level in both conditions, which are unlikely involved in biosynthesizing PSDs (Figure S5, Table S2, Supporting Information). Real-time reverse-transcription PCR further demonstrated that transcriptional level of the cluster *psd* in TBI condition exhibited much higher than in PDB broth, in which eight genes including *psdA-F*, *psdJ*, and *psdN* were selected for investigation (Fig. 4D). The transcription levels of *psdA*, *psdE* and *psdF* increased remarkably in 3.3, 12.9, and 31.1-fold compared with that of PDB condition, respectively (Fig. 4D). The transcriptional analyses of Psd family genes indicated that TBI broth activated the biosynthesis of PSDs significantly.

Investigation on the biosynthesis of metabolite chartarlactam K

Phenylspirodrimanes are unique meroterpenoids featuring a drimane-type sesquiterpene is spiro-fused to a phenyl moiety *via* a furan ring [40]. The genetic basis for the biosynthesis of ascochlorin was well determined previously, and the early-stage biosynthesis of PSDs should be highly identical to that of ascochlorin [18]. In the light of the molecular structures of stachybisbin B and proposed gene functions of the gene cluster *psd*, a metabolic pathway of metabolite **1** was envisioned [9] (Fig. 5). Domains of the megasynthase PsdA are programmed and generate polyketide chains and an offloading thioesterase domain is used for thioester hydrolyzation and chain release. The following steps involve the prenyltransferase PsdC to accomplish the sesquiterpene chain transferring and an NRPS-like reductase PsdB responsible for aldehyde

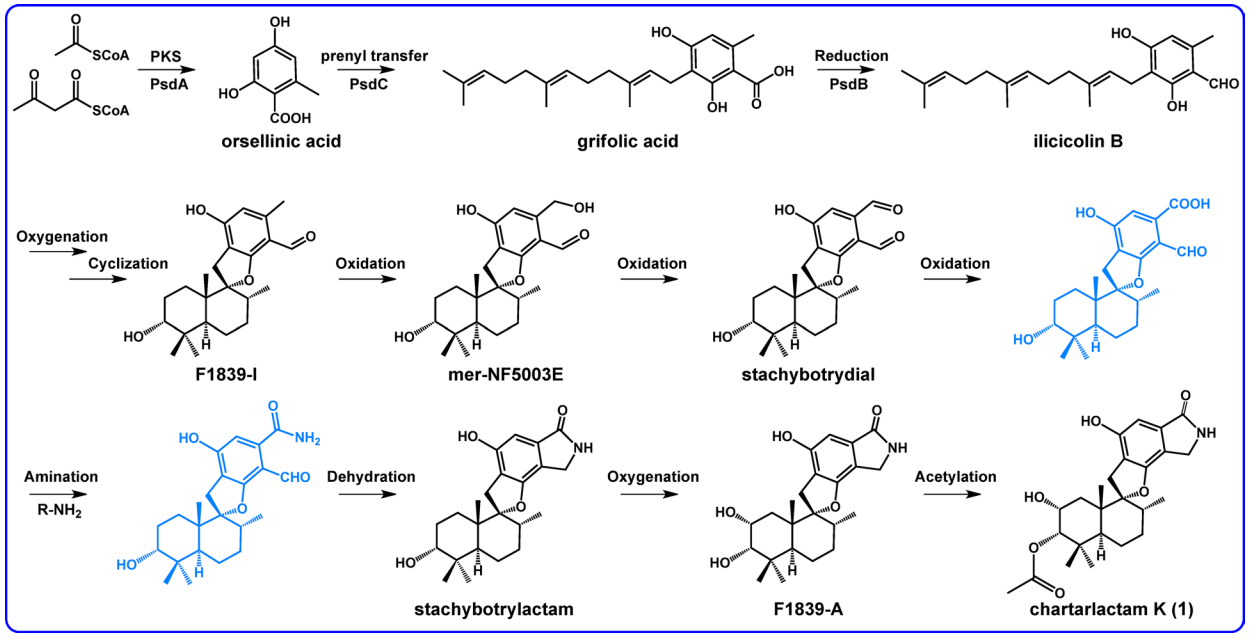


Fig. 5 Proposed scheme of chartarlactam K (**1**)biosynthesis

formation to generate precursor ilicicolin B [9, 18]. In ascochlorin pathway, the intermediate ilicicolin B occurs halogenation catalyzed by AscD and AscE-catalyzed epoxidation for the generation of ilicicolin A, followed by terpene cyclase AscF-mediated cyclization and multiple oxidations [18]. In contrast, the intermediate ilicicolin B might be epoxidized and cyclized to afford the spirocyclic intermediate F1839-I, which was sequentially catalyzed by an uncharacterized flavin monooxygenase and a terpene cyclase in a different manner [22, 33, 41] (Fig. 5).

Subsequently, an additional oxygenation installs the hydroxyl group in the compound F1839-I. Then, the generated intermediate mer-NF5003E is then subjected to oxidative modification, leading to the formation of the precursor stachybotrydial (bearing an O-phthalaldehyde group) [22]. The intermediate stachybotrylactam might be transformed from the precursor stachybotrydial by three successive steps including C22-carboxylation, amination, and dehydration [41]. Subsequently, an additional oxygenation reaction installs the C-2 hydroxyl group in the intermediate F1839-A [33]. The final reaction might be catalyzed by O-acetyltransferase PsdJ and then furnishes the acetyl group to yield **1** (Fig 5). In addition, based on the PSDs isolated from the strain CPCC 401591, we believed that the intermediate stachybotrydial containing O-phthalaldehyde moiety exhibits high reactivity and could be condensed with varied amino acids to synthesize structurally diverse N-containing derivatives (Figure S1, Supporting Information) [41]. Considering the diverse class of PSDs, we preferred that multi-step redox cascade occurs during the biosynthetic process [33, 41]. This proposal hints to the possibility that redox enzymes might exhibit relatively broad substrate scope during PSDs biosynthesis in vivo.

Discussion

To the best of our knowledge, this is the first report to characterize the gene cluster *psd* functioning in biosynthesis of phenylspirodrimanes from *Stachybotrys* species [2]. This genus has been considered to be a treasure for PSDs discovery, a multitude of bioactive derivatives bearing complexified structural modifications have been isolated and gained increasing interest recently [2, 22, 32]. *Stachybotrys* is a distinctive fungal genus due to its metabolic versatility and capability to produce diversified secondary metabolites [2, 26, 27]. In this report, genome guided bioinformatic mining of the fungus CPCC 401591 revealed the presence of an uncharacterized gene cluster *psd*. It carries the conserved locus encoding PsdA, PsdB, and PsdC ensemble, which exhibited higher identities with their counterparts responsible for producing ascochlorin and stachybisbin B, respectively [1, 17]. This finding indicated that PsdA/PsdB/PsdC ensemble is believed to be involved in the conversion of shared intermediate ilicicolin B, which is considered to be an on-pathway precursor in the biosynthesis of many fungal meroterpenoids [1, 12, 17, 20]. A more detailed cluster blast bioinformatic analysis was conducted on genome-sequenced filamentous fungi from NCBI database. Interestingly, all these ilicicolin B-forming genes are associated with the three-gene ensemble. This ensemble consists of genes that encode an orsellinic acid synthase, a UbiA-like prenyltransferase, and an NRPS-like reductase [1, 17]. To further investigate the phylogeny, three conserved proteins

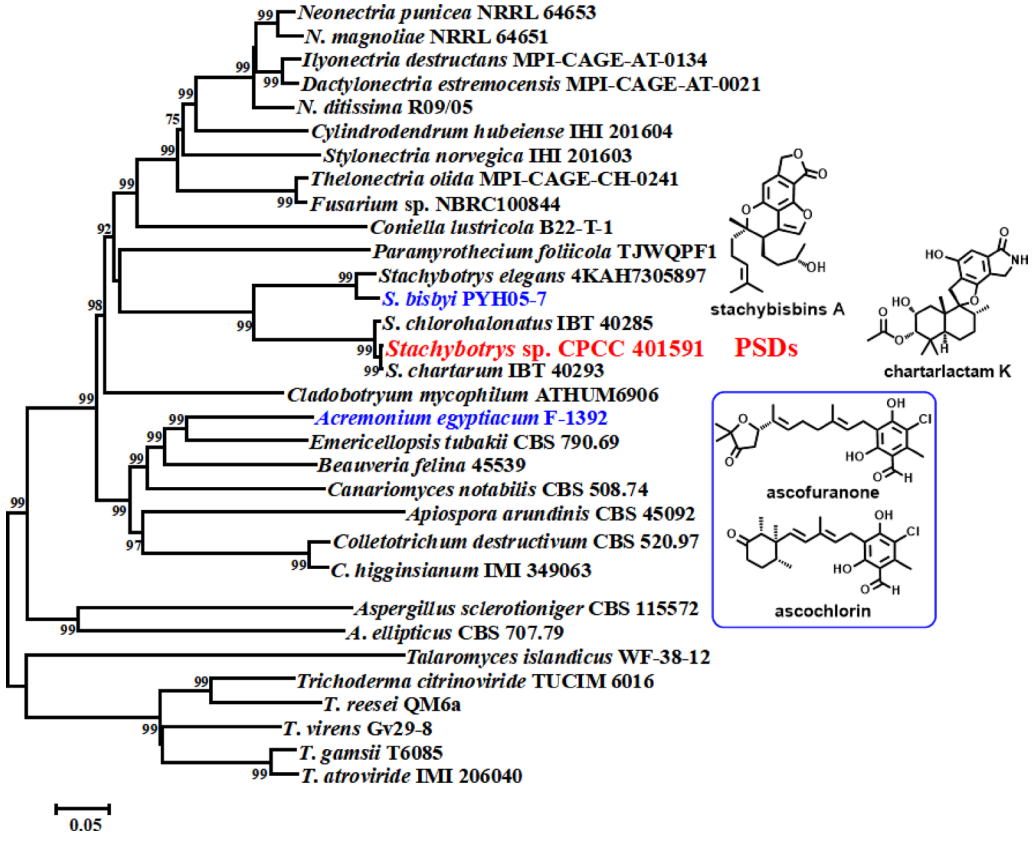


Fig. 6 Phylogenetic tree of fungal three-gene ensemble coding proteins retrieved from characterized or uncharacterized ilicicolin B-forming pathways. The ilicicolin B-forming ensemble from *Stachybotrys* sp. CPCC 401591, *S. bisbyi* PYH 05–7, and *Acremonium egyptiacum* F-1392 was highlighted in red or blue, respectively. The scale represents changes per site; numbers at branches are bootstrap values. For accession numbers and further details, the reader is referred to the Supporting Information Table S3

were selected for Multi-Locus Sequence Typing (MLST) phylogenetic tree construction. As shown in Fig. 6, markedly, the species *Stachybotrys* clustered as a well-supported branch, which constitute an important genus for producing PSDs [6, 12, 20–23, 33, 35, 42]. In addition, the biosynthetic pathways with ilicicolin B serving as pathway intermediate are widely distributed in fungal strains, exemplified as stachybisbins from closely related *S. bisbyi* PYH05-7, ascochlorin and ascofuranone from *Acremonium egyptiacum* F-1392 [1, 17]. This further implies mining other fungal strains clustered in different branch might supply the opportunities to discover novel ilicicolin B-derived meroterpenoids.

Nature's power as a chemist is well represented by the structures of PSD derivatives, which are featured by a distinct spirocyclic drimane fused to a phenyl substituent, and particularly a characteristic spirofuran ring framework [12, 20, 22, 42]. Nevertheless, few reports have described the genetic bases of this class of hybrid metabolites [12]. In our study, the gene deletion-based strategy was performed to demonstrate that the gene cluster *psd* is responsible for the PSDs biosyntheses. Another definitive evidence is that the gene cluster *stb* responsible for ilicicolin B biosynthesis from the fungus *S. bisbyi* PYH05-7 was heterologously expressed in *Aspergillus oryzae* NSAR1 host [2, 26, 27]. A more detailed investigation of the gene cluster *psd* revealed that encoding genes are scattered and distributed separately. Further genome mining of the sequenced *S. chartarum* IBT 40,293 and *S. chlorohalonata* IBT 40,285 also revealed the presence of clusters similar to the cluster *psd* (Supplementary Information, Table S4) [17, 43]. Some strains of *S. chartarum* reportedly produces PSDs, such as chartarutines [44], stachybotrins [45], distachydrimanes [28], bistachybotrysins [7, 23, 45], and therefore, this cluster is also expected to be responsible for the production of phenylspirodrimanes in *S. chartarum* [12]. Considering the presence of several unrelated genes in the cluster *psd* and previous OSMAC experimental data, therefore, we tried to distinguish the redundant genes using transcriptomic analyses. Notably, among the co-localized twenty-seven open reading frames in the gene cluster *psd*, thirteen down-regulated, not expressed, or silent genes were efficiently differentiated. Although the experimental evidence was preliminary, mapping the differential expression profiles could shed light on

identification of candidate genes responsible for PSDs biosynthesis. Regarding the metabolic pathway in generating F1839-I from intermediate ilicicolin B, two key reaction steps might be included, *e.g.*, epoxidation catalyzed by flavin monooxygenase, and cyclization catalyzed by membrane-bound terpene cyclase [1, 12, 17, 28, 40]. Nevertheless, these encoded genes could not be obtained in this cluster [17]. With transcriptome sequencing data, two separate gene clusters are obtained that most likely participated in the catalytic process, and this need to be further characterized through gene deletions. Ultimately, the underlying mechanism of PSDs biosynthesis would be uncovered in the near future.

Using a genome mining strategy, we clarified the gene cluster *psd* functioning in phenylspirodrimanes biosyntheses in *Stachybotrys* sp. The biosyntheses of PSDs and ascochlorin share the common pathway up to the generation of the pathway intermediate ilicicolin B. Also, a bioinformatic analysis of the BGC *psd* was performed. Notably, RNA-sequencing based transcriptomic profiling facilitated to decipher intriguing characteristics of PSDs biosyntheses and eliminate the unrelated genes. This study thus contributes to broadening the knowledge on biosynthesizing this group of meroterpenoids. We have established the method for the genetic manipulation of the fungus *Stachybotrys*, and further manipulation of encoding genes of the *psd* locus will be conducted in our laboratory.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12934-025-02853-3.

Supplementary Material 1.

Author contributions

T.Z. conceived the study, obtained initial funding. T.Z., and L.Y. supervised the work to completion. T.Z., H.L., and B.S. performed experiments and analyzed the data. L.B., W.H., and L.W. advised on the design and partial interpretation of data. T.Z., and H.L. wrote the draft manuscript. T.Z., and L.Y. edited the draft manuscript. All authors reviewed the final manuscript.

Funding

This work was financially supported by the CAMS Innovation Fund for Medical Sciences (2021-I2M-1-055), the National Natural Science Foundation of China (No. 31872617), National Microbial Resource Center (No. NMRC-2025-3), and the central level, scientific research institutes for basic R & D fund business (3332018097).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

Author details

[1]Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China

Received: 4 August 2025 / Accepted: 30 September 2025
Published online: 29 October 2025

References

1. Bhowmik P, Baezid HM, Arabi II. Assessment of antidiabetic activity of three phenylspirodrimanes from fungus *Stachybotrys chartarum* MUT 3308 by ADME, QSAR, molecular Docking and molecular dynamics simulation studies against protein tyrosine phosphatase 1B (PTP1B). J Biomol Struct Dyn. 2024;42:10210–24.
2. Ibrahim SRM, Choudhry H, Asseri AH, Elfaky MA, Mohamed SGA, Mohamed GA. *Stachybotrys chartarum*-a hidden treasure: secondary metabolites, bioactivities, and biotechnological relevance. J Fungi. 2022;8.
3. Li Y, Wu C, Liu D, Proksch P, Guo P, Lin W. Chartarlactams A-P, phenylspirodrimanes from the sponge-associated fungus *Stachybotrys chartarum* with antihyperlipidemic activities. J Nat Prod. 2014;77:138–47.
4. Wu Y, Lu L, Zhang P, Wang L. Chartarlactams U-X: novel phenylspirodrimanes from a marine derived fungus *Stachybotrys* sp. SZU-W23 with anti-inflammatory activity mediated by the NF-kappaB/ROS signaling pathways. Mar Drugs. 2025;23.
5. Liu D, Li Y, Guo X, Ji W, Lin W. Chartarlactams Q-T, dimeric phenylspirodrimanes with antibacterial and antiviral activities. Chem Biodivers. 2020;17:e2000170.
6. Ma X, Li L, Zhu T, Ba M, Li G, Gu Q, Guo Y, Li D. Phenylspirodrimanes with anti-HIV activity from the sponge-derived fungus *Stachybotrys chartarum* MXH-X73. J Nat Prod. 2013;76:2298–306.
7. Liu J, Jia X, Zhao J, Feng J, Chen M, Chen R, Xie K, Chen D, Li Y, Zhang D, Peng Y, Si S, Dai J. Bistachybotrysins L-V, bioactive phenylspirodrimane dimers from the fungus *Stachybotrys chartarum*. Org Chem Front. 2020;8:531–42.
8. Roggo BE, Hug P, Moss S, Stampfli A, Kriemler HP, Peter HH. Novel Spirodihydrobenzofuranlactams as antagonists of endothelin and as inhibitors of HIV-1 protease produced by *Stachybotrys* sp. II. Structure determination. J Antibiot (Tokyo). 1996;49:374–9.
9. Wu B, Oesker V, Wiese J, Malien S, Schmaljohann R, Imhoff JF. Spirocyclic Drimanes from the marine fungus *Stachybotrys* sp. strain MF347. Mar Drugs. 2014;12:1924–38.
10. Jia X, Zhao J, Feng J, Chen R, Xie K, Chen D, Li Y, Liu J, Dai J, Bistachybotrysin K. One new phenylspirodrimane dimer from *Stachybotrys chartarum* with potent cytotoxic activity. J Asian Nat Prod Res. 2020;22:496–502.
11. Ding Z, Ding J, Zhao J, Chunyu W, Li M, Gu S, Wang F, Wen M. A new phenylspirodrimane dimer from the fungus *Stachybotrys chartarum*. Fitoterapia. 2018;125:94–7.
12. Feng J, Zhang M, Jia X, Zhao J, Chen R, Xie K, Chen D, Li Y, Liu J, Dai J. Bistachybotrysins F-J, five new phenylspirodrimane dimers with a central Cyclopentanone linkage from *Stachybotrys chartarum*. Fitoterapia. 2019;136:104158.
13. Zhang H, Yang M, Zhuo F, Gao N, Cheng X, Wang X, Pei Y, Kong L. Seven new cytotoxic phenylspirodrimane derivatives from the endophytic fungus *Stachybotrys chartarum*. RSC Adv. 2019;9:3520–31.
14. Vertesy L, Kogler H, Markus A, Schiell M, Vogel M, Wink J. Memnopeptide A, a novel terpene peptide from *Memnoniella* with an activating effect on SERCA2. J Antibiot (Tokyo). 2001;54:771–82.
15. Wu Z, Li Y, Ma M, Chen J. Salicylic acid derivatives and phenylspirodrimanes from the sponge-associated fungus *Hansfordia sinuosae*. J Asian Nat Prod Res. 2018;20:985–91.
16. Jagels A, Stephan F, Ernst S, Lindemann V, Cramer B, Hubner F, Humpf HU. Artificial vs natural *Stachybotrys* infestation-comparison of Mycotoxin production on various Building materials. Indoor Air. 2020;30:1268–82.
17. Kawahara T, Itoh M, Izumikawa M, Kagaya N, Sakata N, Tsuchida T, Shin-Ya K. New phenylspirodrimane metabolites MBJ-0030, MBJ-0031, and MBJ-0032 isolated from the soil fungal strain *Stachybotrys* sp. f23793. Biosci Biotechnol Biochem. 2020;84:1570–5.
18. Yang B, Long J, Pang X, Lin X, Liao S, Wang J, Zhou X, Li Y, Liu Y. Structurally diverse polyketides and phenylspirodrimanes from the soft coral-associated fungus *Stachybotrys chartarum* SCSIO41201. J Antibiot (Tokyo). 2021;74:190–8.
19. Hinkley SF, Mazzola EP, Fettinger JC, Lam YF, Jarvis BB. Atranones A-G, from the toxigenic mold *Stachybotrys chartarum*. Phytochemistry. 2000;55:663–73.
20. Sasamura S, Kobayashi M, Muramatsu H, Yoshimura S, Kinoshita T, Ohki H, Okada K, Deai Y, Yamagishi Y, Hashimoto M. Bioconversion of FR901459, a novel derivative of cyclosporin A, by *Lentzea* sp. 7887. J Antibiot (Tokyo). 2015;68:511–20.

21. Jagels A, Hovelmann Y, Zielinski A, Esselen M, Kohler J, Hubner F, Humpf HU. Stachybotrychromenes A-C: novel cytotoxic meroterpenoids from *Stachybotrys* Sp. Mycotoxin Res. 2018;34:179–85.
22. Fujioka T, Yao K, Hamano K, Hosoya T, Kagasaki T, Furukawa Y, Haruyama H, Sato S, Koga T, Tsujita Y. Epi-cochlioquinone A, a novel acyl-CoA: cholesterol acyltransferase inhibitor produced by *Stachybotrys bisbyi*. J Antibiot (Tokyo). 1996;49:409–13.
23. Rong X, He W, Guo Z, Li X, Wang L, Gao K, Yu L, Zhang T. Isolation and antitumor activity of phenylspirodrimane derivatives from the fungus *Stachybotrys* Sp CPCC 401591. Nat Prod Res Dev. 2023;35:1348–56.
24. Rong X, Guo Z, He W, Cai G, Gong K, Wang L, Yu L, Zhang T, Gao K. Secondary metabolites exhibiting antitumor bioactivities from the fungus *Stachybotrys* Sp CPCC 401591. Mycosystema. 2023;42:1611–21.
25. Araki Y, Awakawa T, Matsuzaki M, Cho R, Matsuda Y, Hoshino S, Shinohara Y, Yamamoto M, Kido Y, Inaoka DK, et al. Complete biosynthetic pathways of Ascofuranone and Ascochlorin in *Acremonium egyptiacum*. Proc Natl Acad Sci U S A. 2019;116:8269–74.
26. Li C, Matsuda Y, Gao H, Hu D, Yao XS, Abe I. Biosynthesis of LL-Z1272beta: discovery of a new member of NRPS-like enzymes for aryl-aldehyde formation. ChemBioChem. 2016;17:904–7.
27. Zhao J, Feng J, Tan Z, Liu J, Zhang M, Chen R, Xie K, Chen D, Li Y, Chen X, Dai J. Bistachybotrysins A-C, three phenylspirodrimane dimers with cytotoxicity from *Stachybotrys chartarum*. Bioorg Med Chem Lett. 2018;28:355–9.
28. Lin S, Huang J, Zeng H, Tong Q, Zhang X, Yang B, Ye Y, Lin S, Huang J, Zeng H, et al. Distachydrimanes A–F, phenylspirodrimane dimers and hybrids with cytotoxic activity from the coral-derived fungus *Stachybotrys chartarum*. Chin Chem Lett. 2022;33:4587–94.
29. Liew MXX, Nakajima Y, Maeda K, Kitamura N, Kimura M. Regulatory mechanism of trichothecene biosynthesis in *Fusarium graminearum*. Front Microbiol. 2023;14:1148771.
30. Rong X, Zhang L, He W, Guo Z, Lv H, Bai J, Yu L, Zhang T. Exploration of diverse secondary metabolites from *Penicillium Brasilianum* by co-culturing with *Armillaria mellea*. Appl Microbiol Biotechnol. 2024;108:462.
31. He W, Rong X, Lv H, Zhang L, Bai J, Wang L, Yu L, Zhang T. Genetically-modified activation strategy facilitates the discovery of sesquiterpene-derived metabolites from *Penicillium Brasilianum*. Synth Syst Biotechnol. 2025;10:391–400.
32. Li R, Zhu H, Ruan J, Qian W, Fang X, Shi Z, Li Y, Li S, Shan G, Kristiansen K, et al. De Novo assembly of human genomes with massively parallel short read sequencing. Genome Res. 2010;20:265–72.
33. Blin K, Shaw S, Vader L, Szenei J, Reitz ZL, Augustijn HE, Cediel-Becerra JDD, de Crecy-Lagard V, Koetsier RA, Williams SE, et al. AntiSMASH 8.0: extended gene cluster detection capabilities and analyses of chemistry, enzymology, and regulation. Nucleic Acids Res. 2025;53:W32–8.
34. Zhang T, Cai G, Rong X, Wang Y, Gong K, Liu W, Wang L, Pang X, Yu L. A combination of genome mining with an OSMAC approach facilitates the discovery of and contributions to the biosynthesis of melleolides from the basidiomycete *Armillaria tabescens*. J Agric Food Chem. 2022;70:12430–41.
35. Zhang T, Pang X, Zhao J, Guo Z, He W, Cai G, Su J, Cen S, Yu L. Discovery and activation of the cryptic cluster from *Aspergillus* sp. CPCC 400735 for Asperphenalenone biosynthesis. ACS Chem Biol. 2022;17:1524–33.
36. Zhang T, Wan J, Zhan Z, Bai J, Liu B, Hu Y. Activation of an unconventional meroterpenoid gene cluster in *Neosartorya glabra* leads to the production of new berkeleyacetals. Acta Pharm Sin B. 2018;8:478–87.
37. Zhang T, Gu G, Liu G, Su J, Zhan Z, Zhao J, Qian J, Cai G, Cen S, Zhang D, Yu L. Late-stage cascade of oxidation reactions during the biosynthesis of oxalicine B in *Penicillium oxalicum*. Acta Pharm Sin B. 2023;13:256–70.
38. Gao SS, Zhang T, Garcia-Borras M, Hung YS, Billingsley JM, Houk KN, Hu Y, Tang Y. Biosynthesis of heptacyclic duclauxins requires extensive redox modifications of the Phenalenone aromatic polyketide. J Am Chem Soc. 2018;140:6991–7.
39. Zhang T, Cai G, Rong X, Xu J, Jiang B, Wang H, Li X, Wang L, Zhang R, He W, Yu L. Mining and characterization of the PKS-NRPS hybrid for epicoccamide A: a mannosylated tetramate derivative from *Epicoccum* sp. CPCC 400996. Microb Cell Fact. 2022;21:249.
40. Dayras M, Sfecci E, Bovio E, Rastoin O, Dufies M, Fontaine-Vive F, Taffin-de-Givenchy E, Lacour T, Pages G, Varese GC, Mehiri M. New phenylspirodrimanes from the sponge-associated fungus *Stachybotrys chartarum* MUT 3308. Mar Drugs 2023;21.
41. Tribelhorn K, Twaruzek M, Kosicki R, Straubinger RK, Ebel F, Ulrich S. A chemically defined medium that supports Mycotoxin production by *Stachybotrys chartarum* enabled analysis of the impact of nitrogen and carbon sources on the biosynthesis of macrocyclic trichothecenes and Stachybotrylactam. Appl Environ Microbiol. 2023;89:e0016323.
42. Zhao J, Feng J, Tan Z, Liu J, Chen R, Xie K, Zhang D, Li Y, Yu L, Chen X, Dai J. Stachybotrysins A-G, phenylspirodrimane derivatives from the fungus *Stachybotrys chartarum*. J Nat Prod. 2017;80:1819–26.
43. Semeiks J, Borek D, Otwinowski Z, Grishin NV. Comparative genome sequencing reveals chemotype-specific gene clusters in the toxigenic black mold *Stachybotrys*. BMC Genomics. 2014;15:590.
44. Li Y, Liu D, Cen S, Proksch P, Lin WH. Isoindolinone-type alkaloids from the sponge derived fungus *Stachybotrys chartarum*. Tetrahedron. 2014;70:7010–5.
45. Wang Y, Chen K, Xing Q, Zhang T, Xu Y. Stachybotrins G and H, two new phenylspirodrimane derivatives from the fungus *Stachybotrys chartarum*. Planta Med. 2025.

Publisher's Note