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# Genetic basis of *Pseudonectria foliicola* infection in humans and research on DKP-type natural products

Bingqian Zhuo[1†], Na Bao[1,2†], Xin Hong[1,3†], Youqi Ji[1], Wei Xu[1] and Yumei Ge[1,2,3,4,5*]

## Abstract

**Background** Traditionally, *Pseudonectria foliicola* has been considered to have high host specificity, mainly infecting boxwood and causing typical leaf spot disease, with little threat to human health. However, in 2024, the first case of human keratitis caused by *P. foliicola* infection was reported in Zhejiang Provincial People's Hospital, China. As a clinically rare pathogenic fungus, the pathogenic mechanism of *P. foliicola* remains unclear.

**Results** This study analyzed the genetic basis of its human infection using genomics. We found at least 25 secondary metabolite biosynthetic gene clusters, including 417 virulence - related genes (nearly 50% related to human infections), and there is homology of virulence factors with known human pathogenic fungi. Fourteen compounds were isolated and identified, 7 of which contain diketopiperazine structures, predicted to have significant respiratory toxicity, neurotoxicity, etc.

**Conclusion** This study further fills the research gap in the cross - kingdom infection mechanism of this fungus. It provides new insights into the common rules of filamentous fungi breaking host barriers and is of great scientific significance and clinical reference value for preventing and controlling the new public health risks of plant - derived fungi transmitting to humans.

**Keywords** *Pseudonectria foliicola*, Molecular basis, Pathogenic mechanisms, Secondary metabolites, DKP

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## Background

Infections caused by filamentous fungi have become a significant threat to global public health, annually resulting in approximately 6.5 million cases of invasive infections and over 2.5 million direct deaths [1–3]. Currently, the first WHO Fungal Priority Pathogen List released in 2022 has established a priority system for the prevention and control of clinical fungal infections [4]. However, with the increasing number of immunocompromised individuals, the epidemiological characteristics of fungal infections worldwide are undergoing major changes. Besides common pathogenic species, some rare fungal species are becoming increasingly active. In China, fungal infections present a "triple feature": stable superficial

infections, rising invasive infections, and active local/rare pathogenic species. Especially since 2000, rare pathogenic species have been increasingly causing diseases. For example, in 2012, meningitis caused by *Filobasidium uniguttulatum* resistant to azoles was reported, and in 2013, onychomycosis in an immunocompetent individual caused by *Rhodotorula minuta* was first reported [5].

The ability of a pathogen to infect a host and cause disease mostly depends on virulence factors. These virulence factors are key for the pathogen to adhere to, invade, and damage host tissues, while evading the host immune system [6, 7]. Secondary metabolites are non-essential natural products produced by microorganisms under specific conditions. Their ecological significance lies in enhancing the host's environmental adaptability and survival competitiveness. Although secondary metabolites do not participate in the basic life activities of microorganisms, these compounds significantly improve the survival competitiveness of fungi in complex ecosystems (such as inhibiting bacterial colonization and regulating quorum sensing) through biological activities like antibacterial effects [8, 9].

Diketopiperazine (DKP)-type natural products are an important class of secondary metabolites. DKP compounds can form a six-membered cyclic dipeptide basic skeleton through the condensation of two α-amino acid peptide bonds via the NRPS pathway or the cyclodipeptide synthases (CDPSs) pathway. This unique structural formation method endows DKP compounds with high rigidity and stability [10, 11]. On this basis, various types of DKP compounds are derived, such as indole diketopiperazines, epipolythiodioxopiperazines (ETPs), and proline-type diketopiperazines [12].

DKP compounds have rich biological activities, generally including antibacterial activity, cytotoxicity to various cells (such as liver cells, spleen cells, and immune cells), inhibitory effects on specific enzymes (such as angiotensin-converting enzyme), as well as antiviral and antioxidant biological activities [13]. DKP compounds play important roles in the infection and pathogenic processes of fungi, especially ETPs containing disulfide or polysulfide bonds. Current understanding of the mechanisms of ETPs in pathogenesis is as follows: ETPs in the oxidized state can be taken up by cells, and then reduced by glutathione in the cells to generate reactive oxygen species (ROS), which are then captured and accumulated by the cells, ultimately leading to DNA damage. This accumulation is believed to enhance their toxicity; in addition, ETPs can also bind to accessible cysteine residues in enzyme molecules, consume zinc ions related to protein functions, and induce protein denaturation. Therefore, ETPs do not exert their toxicity by targeting a single protein or a specific signaling pathway, but through their disulfide bond moieties, which interact non-specifically with various functionally related biomolecules in the cells, thereby exerting toxic effects [14].

*Pseudonectria foliicola* is a fungal species mainly associated with plant diseases. Traditionally, *P. foliicola* is considered to have strong host specificity, mainly infecting boxwood [15]. However, in recent years, the first case of human keratitis caused by *P. foliicola* infection was reported in Zhejiang Province, China [16]. This suggests that the host specificity of *P. foliicola* is not absolute, and it may break through species barriers to infect humans under specific conditions such as corneal trauma. Regarding the basic biological characteristics and pathogenic mechanisms of *P. foliicola*, apart from Yazmín Rivera's analysis of the genome of *P. foliicola* in 2018, current research remains limited [17–19].

This study aims to explore the molecular basis of *P. foliicola* infecting humans through genomics and metabolomics, approaches, and focuses on identifying some secondary metabolite virulence factors. Our research results will not only deepen our understanding of the pathogenic mechanism of *P. foliicola*, but also provide valuable insights into the comprehensive understanding of the mechanism of cross-kingdom infections by filamentous fungi, which may have important implications for the development of new diagnostic, preventive, and therapeutic strategies for fungal infections.

## Methods

### Fungal materials

The fungus *P. foliicola* used in this study was isolated from a patient with keratitis at Zhejiang Provincial People's Hospital [14].

### Screening of fungal growth conditions

*P. foliicola* was inoculated into six different media using the three - point inoculation method, including *Aspergillus* Minimal Medium (AMM), Chromogenic *Candida* Agar (CANA), Yeast Extract Peptone Dextrose Medium (YPD), Oatmeal Agar (OA), Potato Dextrose Agar (PDA), and Yeast Extract Glucose Chloramphenicol Agar (YGC). The inoculated media were incubated at a constant temperature of 28 ℃ and 37 ℃. After 96 h, the growth morphology was observed. Meanwhile, the fungus - containing media under different culture conditions were extracted with an extraction solution of ethyl acetate: methanol: acetic acid = 90:9:1. The extracted samples were analyzed by LC-MS. The optimal growth conditions for *P. foliicola* were confirmed.

### Fine - scale genome mapping analysis of the fungus

According to the results determined in the previous step, *P. foliicola* was cultured in YPD liquid medium under the conditions of 28 °C, 200 rpm for 3 days. After the cultivation, the mycelia were collected by filtration, freeze

- dried, and then sent to Beijing Novogene Technology Co., Ltd. for fine - scale genome mapping sequencing of the fungus. Based on phylogenetic relatedness, this study selected *Dactylonectria macrodidyma* JAC15-245 (https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_020744425.1/) and *Fusarium graminearum* PH-1 (https://www.ncbi.nlm.nih.gov/datasets/Genome/GCF_000240135.3/) as reference genomes for homologous prediction of *Pseudonectria follicola*, with all genome data sourced from the National Center for Biotechnology Information (NCBI). This study implemented an integrated bioinformatics workflow for homology prediction and comparative genomics analysis of *Pseudonectria foliicola*: the MAKER2 v.2.31.6 pipeline was first employed, incorporating protein homology evidence from *Fusarium graminearum* PH-1 and supported by ab initio prediction using SNAP and AUGUSTUS v.3.2.1, to uniformly annotate the genomes of *P. foliicola*, *D. macrodidyma*, and *F. graminearum*; subsequent genome-wide orthology clustering was performed via the OrthoVenn platform (based on a modified OrthoMCL algorithm) to identify core and species-specific gene sets; buildingon this foundation, a comprehensive functional comparison was conducted using tools including antiSMASH and -base v.4.0 to profile secondary metabolite gene clusters and pathogenicity-related genes, thereby elucidating the genomic basis of *P. foliicola* as a narrow-host-range opportunistic pathogen.

#### Analysis of some virulence - related metabolites of the fungus

After enriching spores of the strain on YPD solid medium, 1 mL of spore suspension (about $1.5 \times 10^8$) was inoculated into 500 mL of YPD liquid medium and cultured on a shaker at 28 °C and 200 rpm for 7 days. The 30 - L fermentation broth was extracted with ethyl acetate (1:1, v/v) to obtain 5.5 g of crude extract. Then, the crude extract was purified by reversed - phase column chromatography (C18 packing, column size: 50 mm × 250 mm, packing particle size 40 μm) and HPLC (LC – 20AR) to obtain 14 pure compounds. During the purification process, a Phenomenex Luna 5 μm C18(2) 100 A column was used with a column temperature set at 40℃. The detection wavelengths were 254 nm and 220 nm, with an injection volume of 100 μL per sample and a collection time of 20 min. The specific separation conditions for each crude fraction were as follows: The 20% and 30% fractions were eluted at a flow rate of 2mL $min^{-1}$ with a mobile phase consisting of 30% acetonitrile and 70% ultrapure water under isocratic conditions. For the 40% fraction, separation was carried out at 2mL $min^{-1}$ using 40% acetonitrile in ultrapure water (isocratic elution). The 50% fraction was separated at 3mL $min^{-1}$ with 40% acetonitrile in ultrapure water (isocratic elution). The 60% fraction was eluted at 3 mL $min^{-1}$ with a mobile phase of 50% acetonitrile and 50% ultrapure water under isocratic conditions.

The structures were analyzed by LC - MS (Zeno TOFTM7600) and NMR (Bruker Avance III 600 MHz). The toxicity was predicted using the ProTox – 3 (https://tox.charite.de/protox3/) platform, and the compounds were compared with the Reaxys/SciFinder databases.

## Results

#### Screening of culture medium for *P. foliicola*

The growth phenotypes of the fungus under different temperature conditions, together with the Total Wavelength Chromatogram (TWC) results obtained by LC-MS, are shown in Fig. 1. *P. foliicola* exhibited a pink and fluffy appearance across all six culture medias. However, significant differences were observed in the formation of its developmental structures under different conditions. At 28℃, the strain showed significantly enhanced developmental structure formation. The characteristic pink and fluffy morphology resulted from dense and well-differentiated aerial mycelia, and the colonies displayed intact structures with regular margins. In contrast, at 37℃, developmental structure formation was markedly impaired. The fluffy appearance was substantially reduced, only sparse aerial mycelia were observed, mycelial differentiation was insufficient, and colony architecture appeared loose and incomplete. These observations indicate that *P. foliicola* is better adapted to near-room-temperature growth conditions.

Medium screening further demonstrated that developmental structure formation was most pronounced in YPD medium. Under this condition, aerial mycelia were thick and evenly distributed, forming a uniform pink and fluffy morphology accompanied by well-organized and layered mycelial differentiation. This growth performance was superior to that observed in CANA and OA media, in which aerial mycelia developed to a moderate extent and mycelial differentiation was relatively well maintained. In contrast, developmental structure formation was clearly restricted in AMM, PDA, and YGC media. These media were characterized by sparse aerial mycelia, a weak fluffy appearance, and poor mycelial differentiation.

Consistently, analysis of the TWC profiles revealed that the number of detected secondary metabolite peaks was highest in YPD medium, followed by CANA and OA media. Taking both developmental structure formation as an indicator of growth performance and the requirements for metabolite detection into account, YPD medium was selected for subsequent experiments in this study.

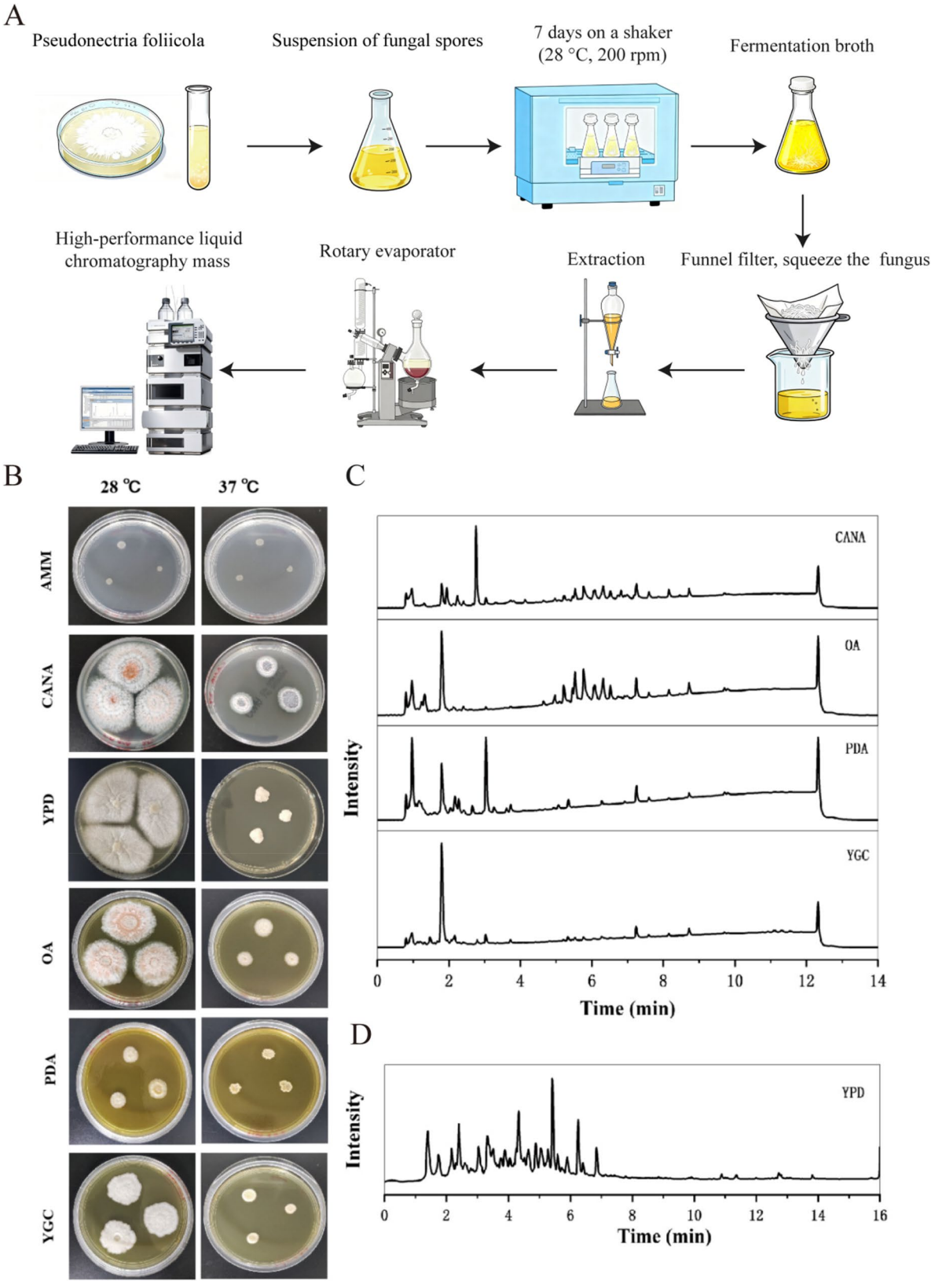


**Fig. 1** Growth characteristics and metabolite profiles of *Pseudonectria foliicola* under different culture conditions. **A** Flow chart of *P. foliicola* culture and its metabolites detection; **B** Colony morphology of *P. foliicola* on six media (AMM, CANA, YPD, OA, PDA, YGC) incubated at 28 ℃ and 37 ℃ for 96 h. Enhanced mycelial growth was observed at 28 ℃; **C** TWC (LC-MS) profiles of SMs under different culture media; **D** TWC profiles of SMs under YPD culture medium

## Analysis of the molecular basis of fungal pathogenicity

### *Analysis of fungal effectors*

**Prediction of secondary metabolite (SMs) gene clusters** Genomic mining analysis using antiSMASH software (version 4.0.2) revealed that the genome of *P. foliicola* contains at least 25 SMs biosynthetic gene clusters, with their specific distribution shown in Fig. 2. These clusters include 7 NRPS/NRPS-like gene clusters, 5 PKS gene clusters, 1 RiPPs gene cluster, 1 indole alkaloid synthesis cluster, and 4 terpene synthesis gene clusters.

**Cytochrome P450(CYP450)** CYP450 is a superfamily of heme-thiolate proteins that participate in the metabolism of endogenous substances and exogenous substances including drugs and environmental compounds. The P450 database classifies genes into 16 categories based on their positions in the InterPro database, and the statistical results are shown in Fig. 3.

The number of matched genes varies significantly among different P450 categories. As shown in Table 1, E-class P450, group I has the largest number of matched genes, reaching 33, which dominates the P450 gene family. The number of matched genes for "Undetermined" and E-class P450, group IV is 20 and 18 respectively, both at relatively high levels. The former indicates that a large number of genes remain unclassified, while the latter plays an important role in the system. Both Cytochrome P450 and P450, indomethylase-like have 11 matched genes, and P450, CYP52 has 5, with relatively limited influence. In contrast, E-class P450, CYP2D has the smallest number of matched genes, only 2. This difference in quantity reflects the unbalanced composition of the P450 gene family.

**Transcription factor analysis** Transcription factors (TFs) are proteins that can bind to DNA in a sequence-specific manner and regulate transcription. They control chromatin and transcription by recognizing specific DNA sequences, thereby forming a complex system that guides genome expression. The amino acid sequences of the target species were aligned with the fungal transcription factor database (http://ftfd.snu.ac.kr/), and annotation results were obtained.

Analysis of the annotation results from the alignment of the target species' amino acid sequences with the fungal transcription factor database showed that, in terms of identity, the mean value was approximately 614.9, and the median was about 530.0. This indicates that the identity of most alignment results is at a certain level, and the data distribution may be somewhat skewed. The distribution of alignment lengths (Align_length) was as follows: the total number was 1103.0, with a mean of 208.3, a standard deviation of 167.68, a minimum value of 0.0, a 25th percentile of 108.0, a 50th percentile of 171.0, a 75th percentile of 265.0, and a maximum value of 2265.0. This suggests that the alignment lengths vary over a large range, with significant differences in length among

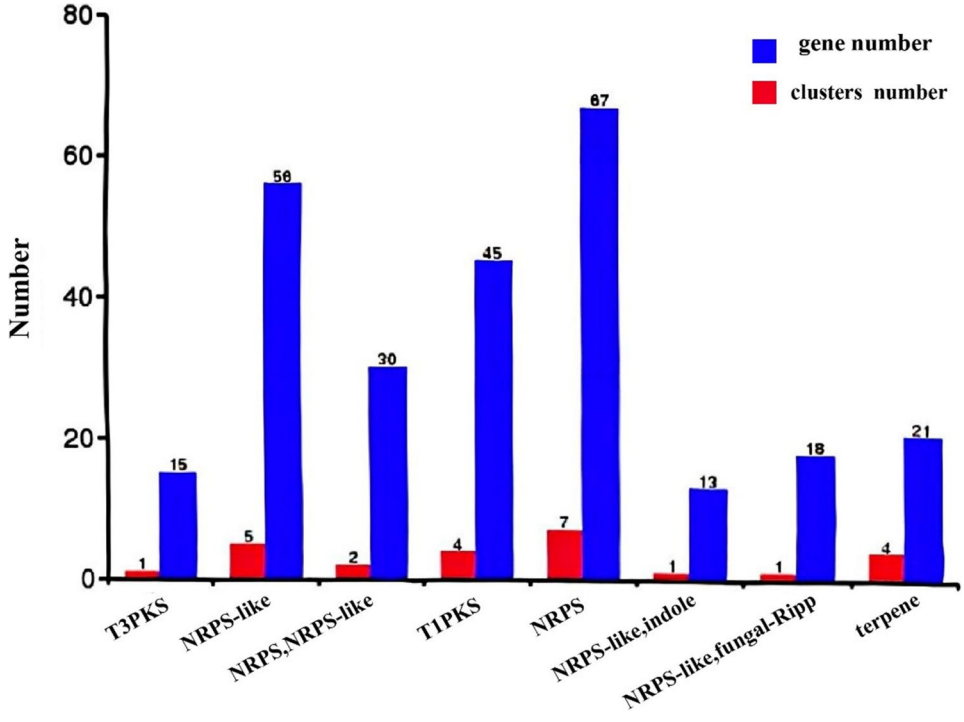


**Fig. 2** Distribution of secondary metabolite biosynthetic gene clusters in the *Pseudonectria foliicola* genome predicted by antiSMASH

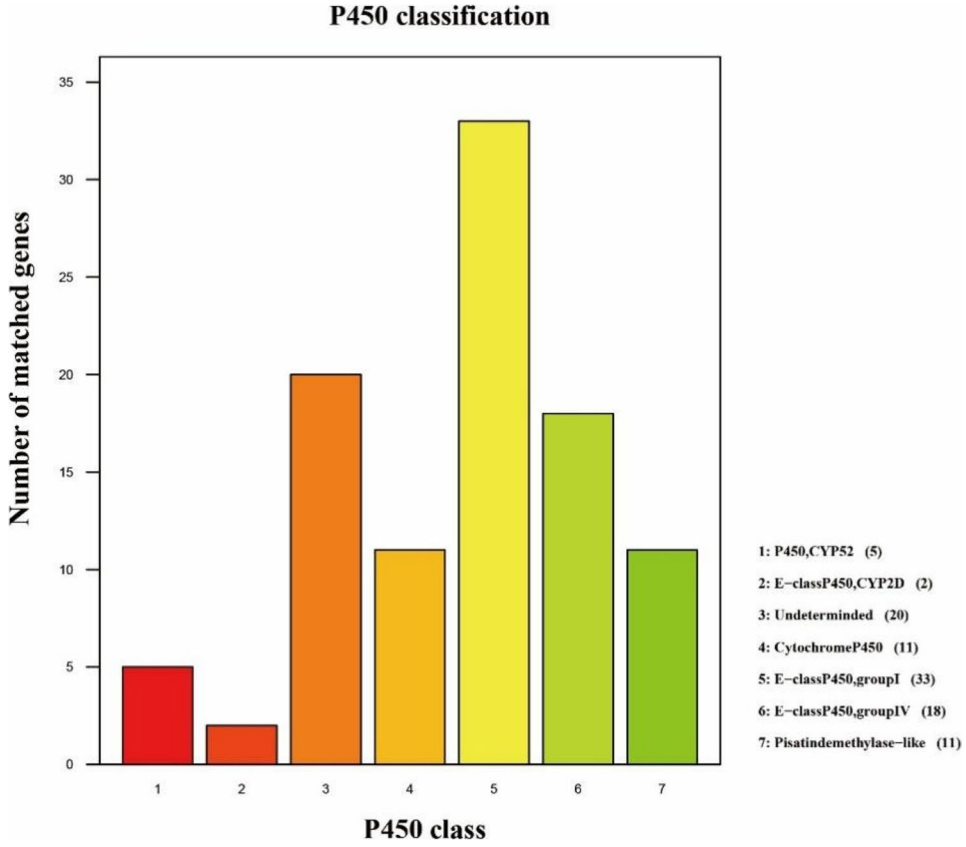


**Fig. 3** Classification and abundance of cytochrome P450 (CYP450) genes in *Pseudonectria foliicola*

**Table 1** The number of matched genes across different P450 classes

| P450_class | class_name | gene_number |
|---|---|---|
| 1 | P450,CYP52 | 5 |
| 2 | E-classP450,CYP2D | 2 |
| 3 | Undeterminded | 20 |
| 4 | CytochromeP450 | 11 |
| 5 | E-classP450,groupI | 33 |
| 6 | E-classP450,groupIV | 18 |
| 7 | Pisatindemethylase-like | 11 |

different alignment tasks. The frequency of occurrence of different subject identifiers (Subject_id) varied; for example, the identifier 42.0 appeared 12 times, showing a relatively high frequency. This reflects that some alignment subjects appear more frequently in the overall alignment, implying that these subjects may match the target species' amino acid sequences more commonly.

Based on the alignment and annotation results of the target species' amino acid sequences against the fungal transcription factor database, a total of 1,103 genes were involved, and the distribution of transcription factor (TF) families exhibited diverse characteristics. Significant differences were observed in the number of genes matched among different TF categories. As shown in Table 2, the ZnCys category contained the highest number of matched genes, reaching 230, and dominated among the TF gene families. The bHLH and Myb categories matched 63 and 130 genes, respectively, also representing relatively high numbers—the former is a core regulatory category within TF families, while the latter plays a key role in regulatory systems. The C2H2 category matched 115 genes, also holding an important position within this family. In contrast, categories such as GRF, GCN5L_Fv, HTH, and YL1_Tvg each matched only one gene, while categories including APSES, BED, and DDT_Fv also contained relatively few genes (4, 2, and 1, respectively), indicating their limited influence within the TF families. This marked disparity in gene numbers reflects the imbalanced composition of the TF gene families. At the family level, some transcription factor families corresponded to a higher number of genes. For instance, the ZnCys_Fv0617 (FVEG_11830.3) family contained eight genes, accounting for 0.73% of the total gene count. In contrast, other families corresponded to relatively fewer genes. Overall, distinct differences were observed in gene numbers among different transcription factor families, which may reflect variations in their functional roles and importance within the target species.

**Table 2** Gene counts by transcription factor family

| TF_class | class_name | gene_number |
|---|---|---|
| 1 | APSES | 4 |
| 2 | AraC | 45 |
| 3 | ARID_Fv | 2 |
| 4 | BED | 2 |
| 5 | bHLH_ | 63 |
| 6 | BTF | 8 |
| 7 | bZIP | 47 |
| 8 | C2H2 | 115 |
| 9 | CCAAT | 7 |
| 10 | CCHC | 25 |
| 11 | CENTB | 1 |
| 12 | DDT_Fv | 1 |
| 13 | DHHC | 5 |
| 14 | DtxR | 2 |
| 15 | FH | 4 |
| 16 | GATA | 10 |
| 17 | GRF | 1 |
| 18 | GH_Fv | 1 |
| 19 | GCN5L1_Fv | 1 |
| 20 | HMG | 85 |
| 21 | HOMEO | 85 |
| 22 | HTH | 1 |
| 23 | HSF | 2 |
| 24 | JUMONJI | 8 |
| 25 | LacI_Fs | 1 |
| 26 | Lambda | 6 |
| 27 | LSD1_Fg | 1 |
| 28 | LuxR | 5 |
| 29 | MADS | 6 |
| 30 | MIZ | 4 |
| 31 | Myb | 130 |
| 32 | NDT80 | 2 |
| 33 | NEG_Fv | 1 |
| 34 | NF-X1 | 2 |
| 35 | Not1_Fs | 1 |
| 36 | OBFOLD | 107 |
| 37 | P53 | 4 |
| 38 | PSQ | 2 |
| 39 | Rad18 | 4 |
| 40 | RFX_Tvg | 1 |
| 41 | SART1_Fs | 1 |
| 42 | SGT1_Fs | 1 |
| 43 | ssDNA | 2 |
| 44 | TEA_Fs | 1 |
| 45 | TFIIS | 6 |
| 46 | TUBBY | 2 |
| 47 | WING | 56 |
| 48 | WRKY_Bt | 1 |
| 49 | YL1_Tvg | 1 |
| 50 | ZnCys | 230 |

### *Functional annotation of fungal genes*

**Gene function annotation** The genome of *P. foliicola* was systematically functionally annotated using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG).

GO functional annotation (Fig. 4) showed that the genes of *P. foliicola* are widely distributed across three major categories: molecular function, cellular component, and biological process. At the molecular function level, genes are mainly enriched in catalytic activity, binding function, and transporter activity, suggesting their core roles in material metabolism and signal transduction. In terms of cellular components, most genes are associated with cells, cell parts, and organelles, reflecting the integrity of the fungal cell structure and the complexity of its functions. At the biological process level, genes are significantly involved in metabolic processes, cellular processes, and biological regulation, indicating their activity in growth, reproduction, and environmental adaptation. These GO classification results reveal the diversity of gene functions in *P. foliicola* and provide a functional basis for understanding its physiological metabolism and pathogenesis-related molecular mechanisms.

KEGG metabolic pathway annotation (Fig. 5) further illustrates the distribution of genes across biological pathways. Among the major categories, genes related to Metabolism are the most abundant, covering pathways such as amino acid metabolism, carbohydrate metabolism, and biosynthesis of secondary metabolites. In the Genetic Information Processing pathway, genes associated with transcription, translation, and protein folding/sorting/degradation account for a significant proportion, reflecting the fungus's ability to regulate gene expression and maintain protein homeostasis. Additionally, annotations of genes involved in infectious diseases and immune-related pathways within the Human Diseases category provide pathway-level clues to the potential mechanisms underlying the cross-kingdom infection of humans by this fungus. Overall, the KEGG annotation results reveal the core biological processes engaged in by *P. foliicola*, laying a foundation for dissecting its metabolic network and pathogenic mechanisms.

### *Analysis of fungal pathogenicity*

The amino acid sequences of the target species were aligned with the PHI database using Diamond software, and the results are shown in Fig. 6A. The amino acid sequences of *P. foliicola* were aligned with the DFVF database, with the results presented in Fig. 6B. The statistical data on the number of genes related to pathogen

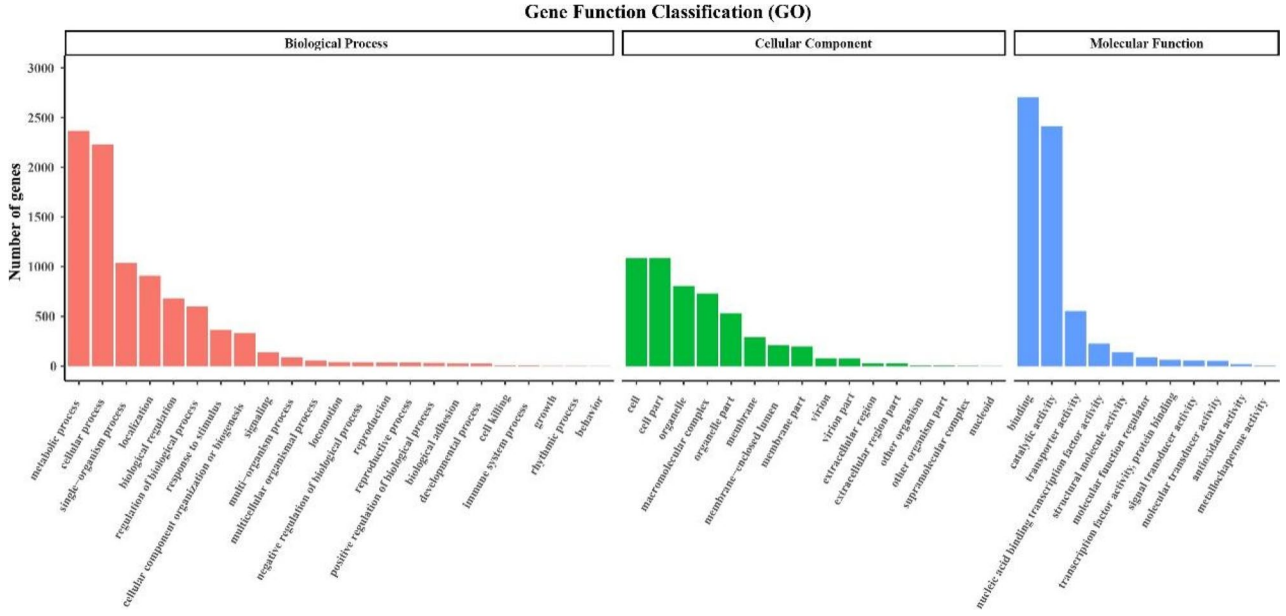


**Fig. 4** Gene Ontology (GO) functional annotation of *Pseudonectria foliicola* genes. Genes are distributed across three main categories: molecular function (enriched in catalytic activity and binding), cellular component (mainly associated with cells and organelles), and biological process (dominant in metabolic and cellular processes)

PHI phenotypic mutation types and the alignment results of *P. foliicola* amino acid sequences with the DFVF database are shown in Fig. 6B.

Systematic analysis based on PHI and DFVF databases revealed the potential pathogenic mechanisms of *P. foliicola*. The analysis indicated that there are 417 virulence-related genes in the genome of *P. foliicola*, among which nearly 50.0% are associated with human infections. Notably, 12.47% of these virulence factors have unknown functions, suggesting the possible existence of novel pathogenic mechanisms. Furthermore, it was found that *P. foliicola* shares significant homology in certain virulence factors with known human pathogenic fungi (e.g., Candida albicans), as shown in Table 1. These findings provide a potential molecular basis for *P. foliicola* to break through species barriers and infect humans (Table 3).

### Analysis of partial secondary metabolite virulence factors

Fourteen compounds were identified by nuclear magnetic resonance spectroscopy and liquid chromatograph mass spectrometer, as shown in Fig. 7. Compound 1 was (3 S,7R,8 S)-7-hydroxy-3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione [20]; Compound 2 was Cyclo-(L-trans-(4-hydroxyprolinyl)-L-phenylalanine) [21]; Compound 3 was cis-Cyclo(L-Ile-L-Pro) [22]; Compound 4 was 2-Aminobenzoic acid [23]; Compound 5 was cis-Cyclo(L-Leu-L-Pro) [24]; Compound 6 was Cyclo[1-[2-(cyclopentanecarbonyl)-3-phenylpropanoyl] pyrrolidine-2-carboxylicacid-(1-carbamoylpropyl)amide] [25]; Compound 7 was Brevianamide F [26]; Compound 8 was N-phenethyl-acetamide [27]; Compound 9 was N-(2-(1 H-indol-3-yl)ethyl) acetamide [28]; Compound 10 was 2-(1 H-Indol-3-yl)ethanol [29]; Compound 11 was cis-Cyclo(L-Val-L-Phe) [22]; Compound 12 was Daidzein [30]; Compound 13 was 3-phenylpropanoic acid [31]; and Compound 14 was Genistein [32]. The retention times of these compounds are 8.7 min, 6.4 min, 7.5 min, 12 min, 7.9 min, 8.7 min, 9.5 min, 13 min, 14.2 min, 15.6 min, 7 min, 11.2 min, 16.9 min and 13.2 min, with corresponding isolated weights of 2.5 mg, 2.4 mg, 3.4 mg, 3.7 mg, 2.4 mg, 3.0 mg, 2.6 mg, 2.5 mg, 3.4 mg, 1.1 mg, 3.0 mg, 6.8 mg, 1.3 mg, 3.8 mg, respectively. Among them, Compounds 1, 2, 3, 5, 6, 7, and 11 contain diketopiperazine (DKP) structures. Additionally, compound 7 has been reported that it can be obtainable from deep-sea-derived *Fusarium* and exhibits certain cytotoxicity [33].

The toxicity profiles of compounds 1, 2, 3, 5, 6, 7, and 11, which possess DKP structures (typically formed via NRPS cyclization), were systematically analyzed using the ProTox-3 online prediction platform, with results shown in Fig. 8. Compounds 1, 2, 3, 5, 6, and 7 exhibited significant respiratory toxicity (P (respi) > 0.7); compounds 2, 5, 6, 7, and 11 displayed marked neurotoxicity (P (neuro) > 0.7); compounds 1, 2, 6, and 7 showed clinical toxicity (P (clinical) > 0.7); and compounds 3, 5, 7, and 11 were predicted to have blood-brain barrier penetration capability (P (bbb) > 0.7).

## Discussion

To further confirm the conserved role of DKP-type compounds in fungal pathogenesis, additional examples from other pathogenic fungi have been supplemented. For plant

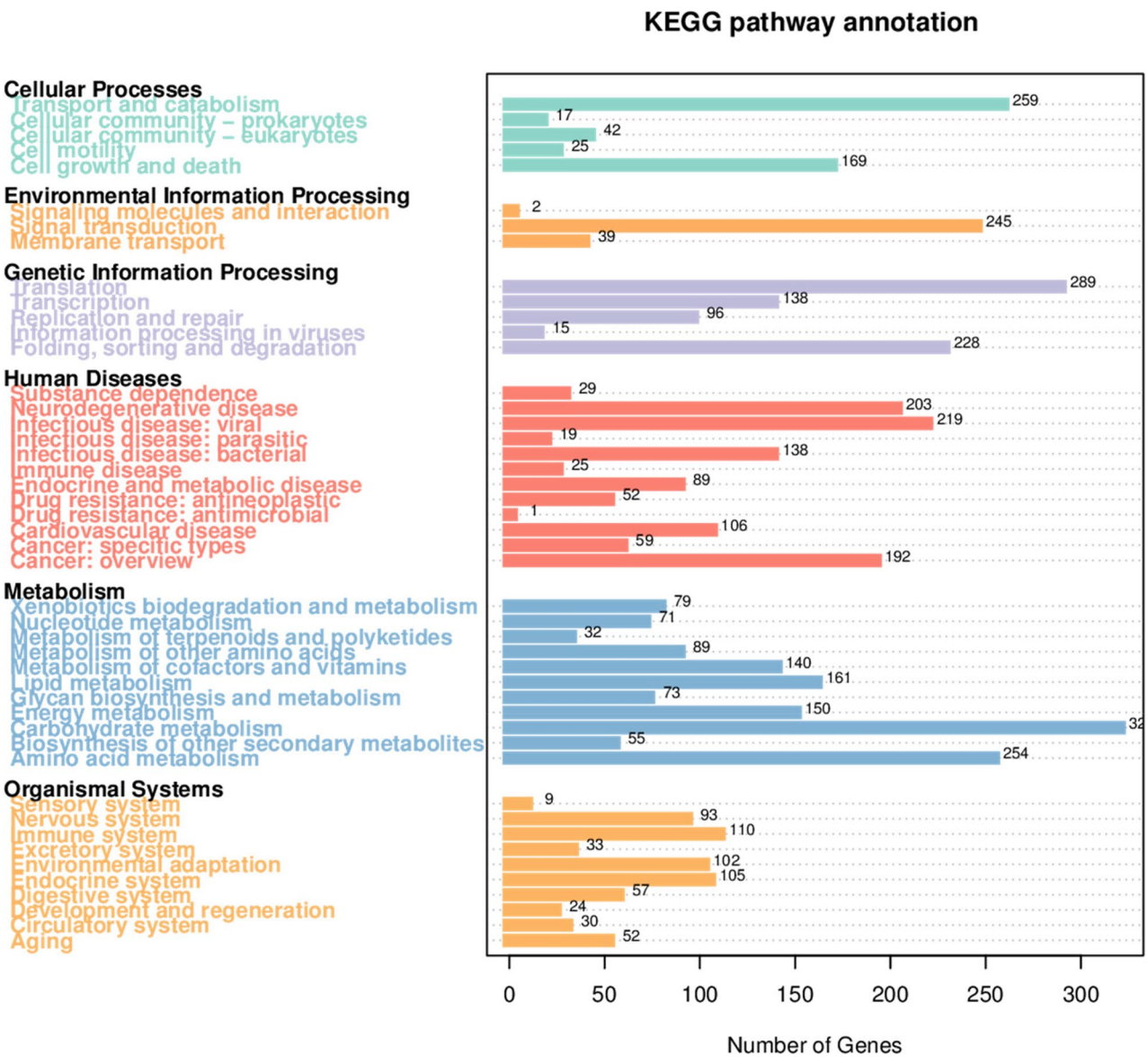


**Fig. 5** KEGG metabolic pathway classification chart for gene function annotation of samples. Most genes are involved in metabolic pathways, including amino acid and carbohydrate metabolism, and biosynthesis of secondary metabolites

pathogenic fungi, *Bacillus amyloliquefaciens*, for secreting a cyclic dipeptide, cyclo(l-leucyl-l-prolyl) (CLP), with efficacies to attenuate the biofilm formation and virulence of the most important oral pathogen, *Streptococcus mutans* [34]. For animal pathogenic fungi, *Candida albicans*, a common human opportunistic pathogen, produces 3-benzyl-6-isobutylidene-2,5-piperazinedione and cyclo(L-Pro-L-Leu), which regulates the morphological transition from yeast to hyphal form—this transition is a key virulence trait enabling *C. albicans* to break through host epithelial barriers and cause deep infections [35]. Beyond their roles in regulating fungal infection and virulence as discussed earlier, DKP-type compounds also possess diverse biological activities that have garnered significant research attention, with potential implications for both therapeutic development and pathogenic mechanism exploration. Based on previous studies on DKP-related compounds, DKP-type compounds generally exhibit not only antibacterial activity but also widespread cytotoxicity against various cells (e.g., lung cancer cells and hepatocytes), making them regarded as a source of antitumor drugs. Additionally, most DKP compounds interact with various ions or neurotransmitters, rendering them potential neuroprotective agents. Some compounds can cross the blood-brain barrier through passive diffusion, thus serving as ideal candidates for treating brain diseases [12, 14, 36]. Among previously identified DKP compounds, gliotoxin produced by *Aspergillus fumigatus* is recognized

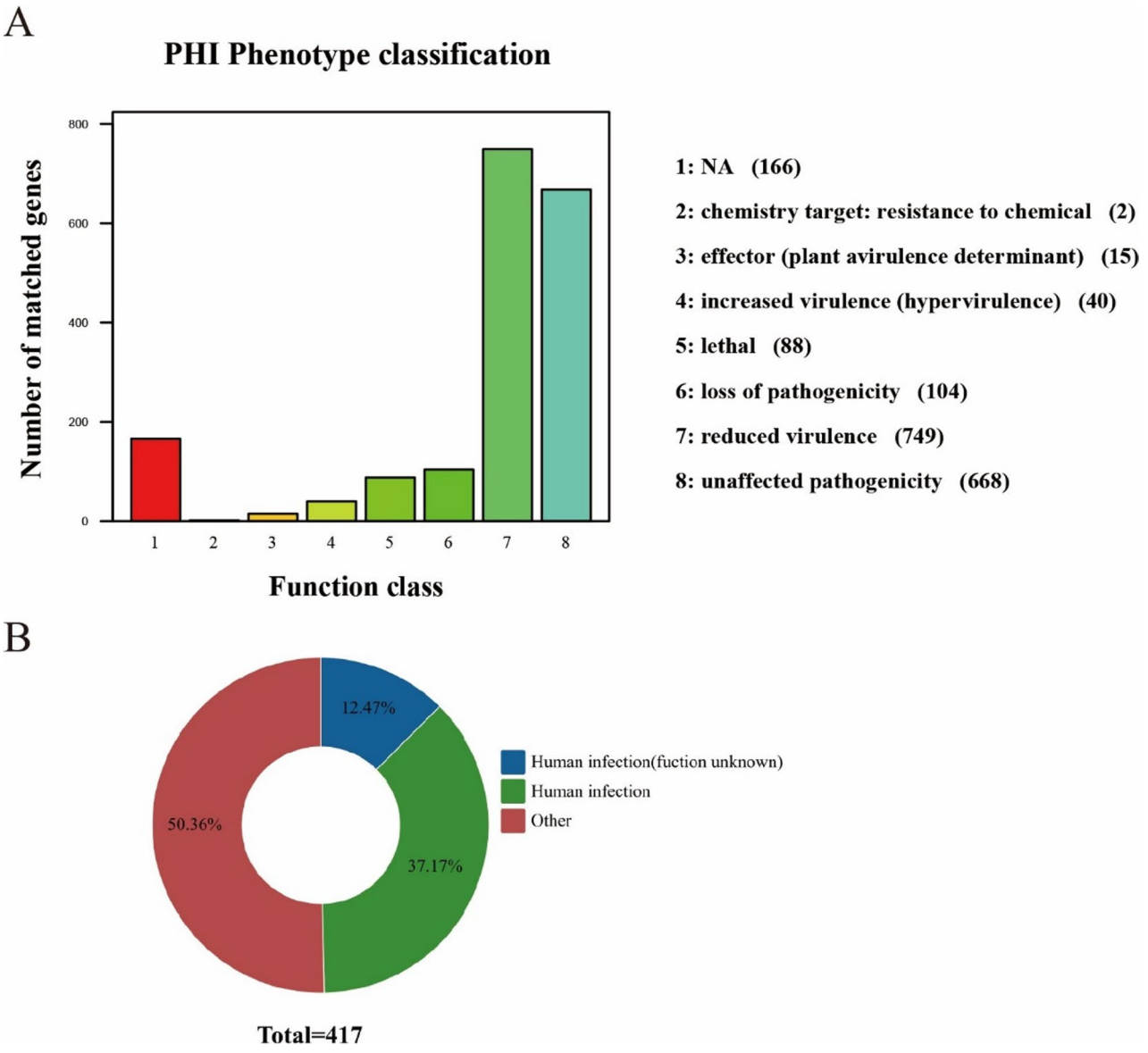


**Fig. 6** Prediction of virulence-associated genes in *Pseudonectria foliicola*. **A** Distribution map of pathogen PHI phenotypic mutation types in sample gene Function Annotation (**B**) Fungal virulence factor prediction

**Table 3** Partially similar virulence factors

| Similar strains | EMBL_ID | Similar virulence factors |
|---|---|---|
| *Candida albicans* | EAK95794.1 | SIMILARITY: Belongs to the small GTPase superfamily. Rab family. |
| *Candida albicans* | EAK97431.1 | SIMILARITY: Belongs to the actin family. |
| *Penicillium marneffei* | AAN04057.1 | SIMILARITY: Belongs to the peroxidase family. Peroxidase/catalase subfamily. |
| *Fusarium sp* | ACZ56011.1 | SIMILARITY: Belongs to the histone H3 family. |

as a typical DKP-type secondary metabolite that promotes fungal infections. It exerts cascading immunosuppressive effects, including inhibiting macrophage phagocytosis, blocking the formation of neutrophil extracellular traps, inducing apoptosis, and suppressing the NF-κB signaling pathway, thereby contributing to *A. fumigatus* infection and pathogenesis [37–39].

DKP-type secondary metabolites identified in this study are proposed as potential virulence-associated compounds based on their known biological activities and their detection in the clinical isolate, rather

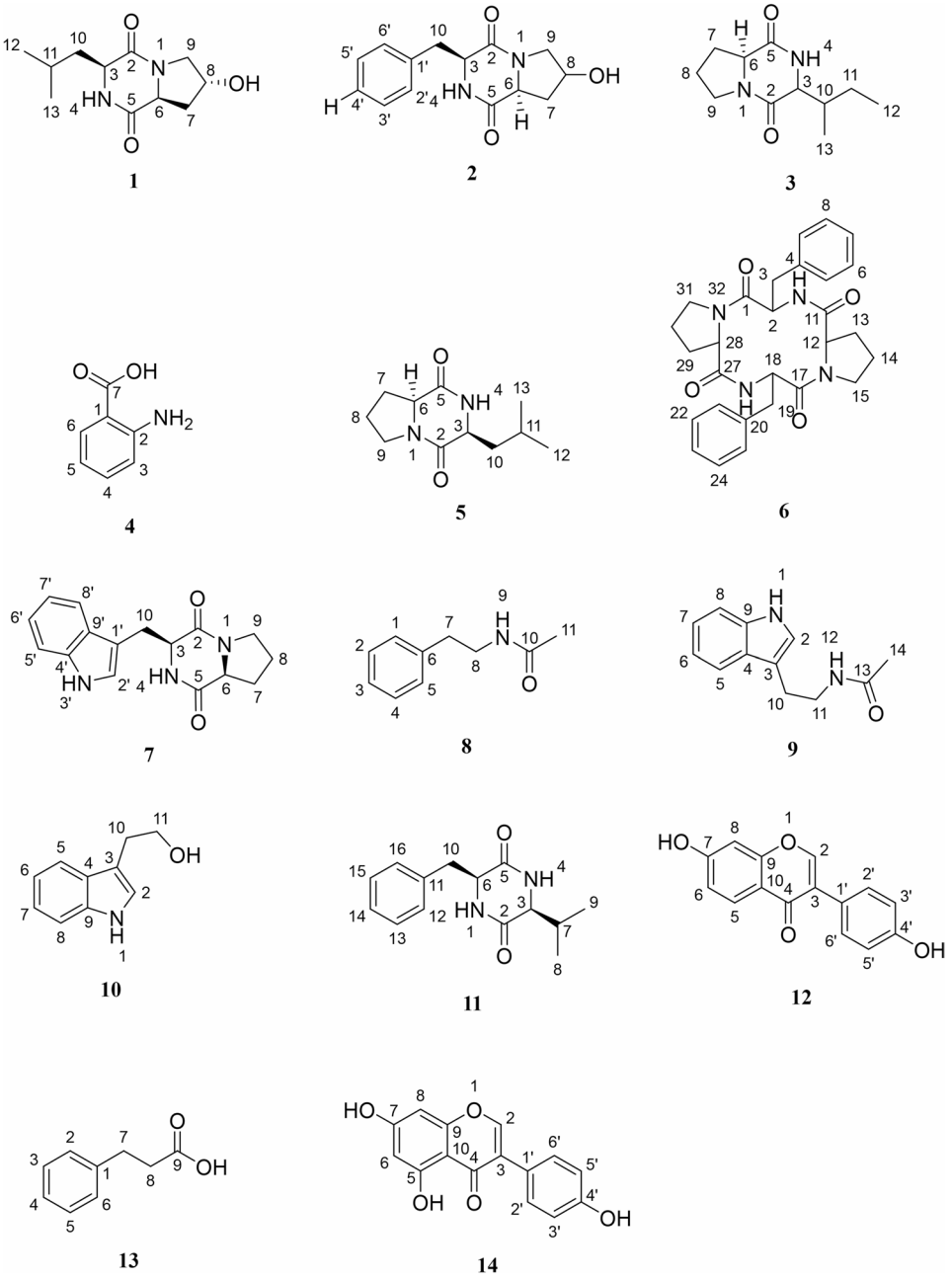

**Fig. 7** Chemical structures of 14 secondary metabolites isolated from *Pseudonectria foliicola*. Compounds 1, 2, 3, 5, 6, 7, and 11 contain a diketopiperazine (DKP) scaffold

than being claimed as clinical isolate-specific markers. Through systematic literature retrieval, we confirmed that all 14 metabolites reported in our study, including 11 typical DKP-type compounds (Compounds 1–3, 5–7, 11) and 3 structurally related derivatives (Compounds 4, 8–10, 12–14) are widely distributed in fungi, bacteria, and even plants, rather than being specific to the clinical isolate of *P. foliicola* [40–43]. Although these compounds are not unique to *P. foliicola*, existing literature indicates that secondary metabolite profiles (including DKPs) exhibit significant strain-specific variation within the same fungal species, which is closely related to ecological niches and host adaptation. Among them, Compound 7 (Brevianamide F) has been extensively investigated. In addition to its antibacterial and antitumor activities, its antithrombotic effects were confirmed using zebrafish models, RNA-seq, and qRT-PCR, with inferences that it regulates the MAPK signaling pathway and coagulation cascade [44]. Furthermore, Compounds 1 and 5 were verified in cellular experiments to exhibit low-level

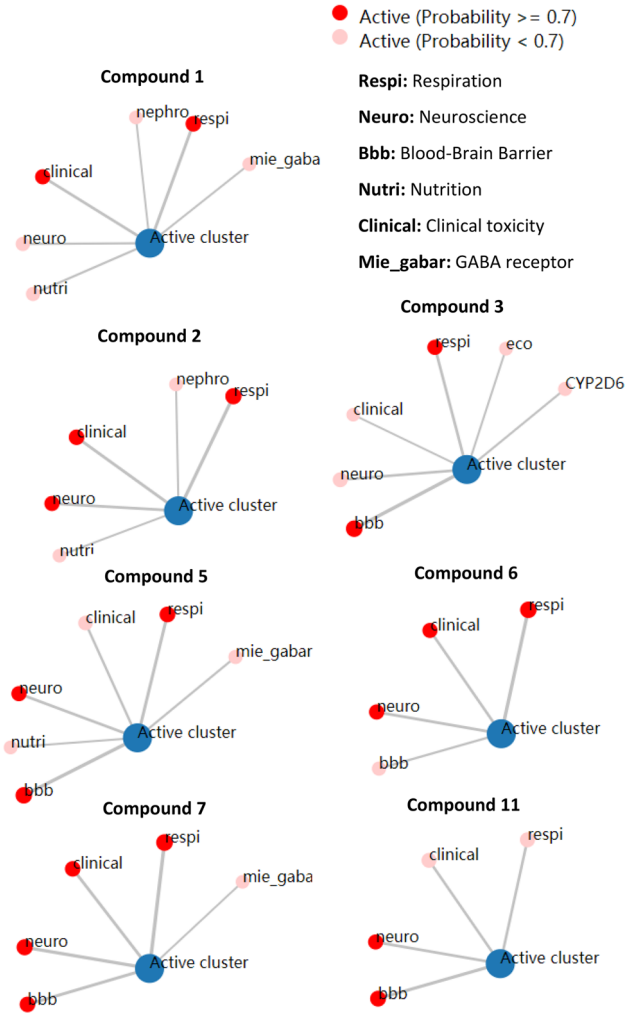


**Fig. 8** ProTox-3 predicted potential toxicity network diagram of selected compounds. Compounds 1, 2, 3, 5, 6, and 7 show high respiratory toxicity ($P > 0.7$); compounds 2, 5, 6, 7, and 11 exhibit notable neurotoxicity; several compounds are predicted to penetrate the blood-brain barrier (BBB)

cytotoxicity [45]. These findings validate the predicted activities of the compounds. Their biological activities, which often induce headaches, are consistent with clinical manifestations such as headache in keratitis patients, indicating that the isolated DKP compounds, as virulence factors, contribute to the pathogenicity of *P. foliicola* to a certain extent.

Importantly, the biological activities of these isolated DKP compounds (e.g., neuroactive effects) are closely associated with clinical manifestations of *P. foliicola*-induced infections. Specifically, their ability to induce headaches is consistent with the clinical symptom of headache observed in keratitis patients infected with *P. foliicola*. This correlation strongly indicates that the isolated DKP compounds act as virulence factors, contributing to the pathogenicity of *P. foliicola* to a certain extent. Comparative analyses involving plant-derived *P. foliicola* and their produced DKP compounds will be essential in future studies to determine strain specificity and to further elucidate the contribution of DKP-type metabolites to pathogenicity.

## Conclusion

This study provides the first systematic elucidation of the molecular mechanisms underlying the cross-kingdom human infection by the plant-derived fungus *P. foliicola*. Genomic analysis revealed that the strain harbors 417 virulence-associated genes, with 50% directly linked to human infection pathways. Notably, these genes are enriched in secondary metabolite gene clusters, including non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS), providing a genetic basis for its adaptation to mammalian hosts. Metabolomic investigation led to the isolation and identification of 14 compounds. Among these, indole diketopiperazine derivatives featuring a diketopiperazine (DKP) structure demonstrated potent neurotoxic and respiratory toxic effects, along with the ability to penetrate the blood-brain barrier. This toxicity profile aligns with neurological symptoms, such as headaches, observed in clinical cases of keratitis, suggesting that these compounds may facilitate pathogenesis by disrupting host cellular respiration, invading neural tissue, and breaching immune barriers. Furthermore, this study reports the first identification of flavonoid biosynthesis-related genes and their products in *P. foliicola*. This finding suggests the potential acquisition of plant-derived metabolic pathways via horizontal gene transfer, offering a novel perspective on cross-kingdom interactions among fungi, plants, and humans.

### Abbreviations

| | |
|---|---|
| TFs | Transcription Factors |
| GO | Gene Ontology |
| KEGG | Kyoto Encyclopedia of Genes and Genomes |
| PHI | Pathogen-Host Interaction |
| DFVF | Database of Fungal Virulence Factors |
| DKP | Diketopiperazine |
| NRPS | Non-Ribosomal Peptide Synthetases |
| PKS | Polyketide Synthases |
| CYP450 | Cytochrome P450 |
| ROS | Reactive Oxygen Species |
| ETPs | Epipolythiodioxopiperazines |
| CDPSs | Cyclodipeptide Synthases |
| RiPPs | Ribosomally Synthesized and Post-translationally Modified Peptides |
| LC-MS | Liquid Chromatography-Mass Spectrometry |
| NMR | Nuclear Magnetic Resonance |
| HPLC | High-Performance Liquid Chromatography |
| AMM | Aspergillus Minimal Medium |
| CANA | Chromogenic Candida Agar |
| YPD | Yeast Extract Peptone Dextrose Medium |
| OA | Oatmeal Agar |
| PDA | Potato Dextrose Agar |
| YGC | Yeast Extract Glucose Chloramphenicol Agar |
| TWC | Total Wavelength Chromatogram |
| WHO | World Health Organization |
| NCBI | National Center for Biotechnology Information |
| GABA | Gamma-Aminobutyric Acid |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-026-12615-z.

Supplementary Material 1.

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**Authors' contributions**
Z. B. and N.B. performed the experiments and drafted the main manuscript text; X.H. and Y.J. processed the data; W.X. prepared figures and visualizations; G.Y. revised the manuscript and provided financial support for this research. All authors reviewed and approved the final manuscript.

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**Data availability**
Sequence data that support the findings of this study have been deposited in NCBI with the primary accession code OR336108.1.

## Declarations

**Ethics approval and consent to participate**
This study was supported by the Ethics Committee of Zhejiang People's Hospital (Ethics Committee Approval of Biomedical Research Involving Humans, Approval No.: 2022JS008) and was carried out in accordance with the ethical standards of the Declaration of Helsinki.

**Competing interests**
The authors declare no competing interests.

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**References**

1. Xiao M, Zhang L, Wang Y, Feng J, Kong W, Lin L, Xu Y. China's innovative National plan to combat fungal diseases and antifungal resistance. Innovation. 2024;6(1):100741.
2. Sedik S, Wolfgruber S, Hoenigl M, Kriegl L. Diagnosing fungal infections in clinical practice: a narrative review. Expert Rev Anti-Infective Therapy. 2024;22(11):935–49.
3. Denning DW. Global incidence and mortality of severe fungal disease. Lancet Infect Dis. 2024;24(7):428–38.
4. World Health Organization. WHO fungal priority pathogens list to guide research, development and public health action. Geneva: World Health Organization; 2022.
5. Chen M, Xu Y, Hong N, Yang Y, Lei W, Du L, Zhao J, Lei X, Xiong L, Cai L, et al. Epidemiology of fungal infections in China. Front Med. 2018;12(1):58–75.
6. Pakbin B, Brück WM, Rossen JWA. Virulence factors of enteric pathogenic *Escherichia coli*: a review. Int J Mol Sci. 2021;22(18):9922.
7. Mohammadzadeh R, Menbari S, Pishdadian A, Farsiani H. *Helicobacter pylori* virulence factors: subversion of host immune system and development of various clinical outcomes. Expert Rev Mol Med. 2023;25:23.
8. Knowles SL, Raja HA, Roberts CD, Oberlies NH. Fungal-fungal co-culture: a primer for generating chemical diversity. Nat Prod Rep. 2022;39(8):1557–73.
9. Rangel LI, Hamilton O, de Jonge R, Bolton MD. Fungal social influencers: secondary metabolites as a platform for shaping the plant-associated community. Plant Journal: Cell Mol Biology. 2021;108(3):632–45.
10. Walker KL, Loach RP, Movassaghi M. Total synthesis of complex 2,5-diketopiperazine alkaloids. Alkaloids Chem Biology. 2023;90:159–206.
11. Borgman P, Lopez RD, Lane AL. The expanding spectrum of Diketopiperazine natural product biosynthetic pathways containing cyclodipeptide synthases. Org Biomol Chem. 2019;17(11):3066.
12. Li K, Zhang JJ, Chen L. Research progress on Diketopiperazine derivatives. Zhong Nan Yao Xue. 2023;21(11):2977–83.
13. Song Z, Hou Y, Yang Q, Li X, Wu S. Structures and biological activities of diketopiperazines from marine organisms: a review. Mar Drugs. 2021;19(8):403.
14. Huber EM. Epipolythiodioxopiperazine-Based natural products: Building Blocks, biosynthesis and biological activities. ChemBioChem. 2022;23(23):e202200341.
15. Bika R, Copes W, Baysal-Gurel F. Comparative performance of sanitizers in managing plant-to-plant transfer and postharvest infection of *Calonectria pseudonaviculata* and *Pseudonectria foliicola* on Boxwood. Plant Dis. 2021;105(10):2809–21.
16. Zhu Y, Nan P, Zhu Z, Ji Y, Zhuo B, Xu W, Ge Y. Pseudonectria keratitis-emerging pathogenic fungi in the eye. Ann Clin Microbiol Antimicrob. 2024;23(1):64.
17. Shin S, Kim JE, Son H. Identification and characterization of fungal pathogens associated with Boxwood diseases in the Republic of Korea. Plant Pathol J. 2022;38(4):304–12.
18. Rivera R, Salgado-Salazar Y, Veltri C, Malapi-Wight D, Crouch M. Genome analysis of the ubiquitous Boxwood pathogen *Pseudonectria foliicola*. PeerJ. 2018;6:e5401.
19. Salgado-Salazar C, Shiskoff N, LeBlanc N, Ismaiel AA, Collins M, Cubeta MA, Crouch JA. Coccinonectria pachysandricola, causal agent of a new foliar blight disease of *Sarcococca Hookeriana*. Plant Dis. 2019;103(6):1337–46.
20. Vagala H, MariaappaNn P. Enantiomerically pure piperazines via NaBH4/I2 reduction of Cyclic amides. Tetrahedron: Asymmetry. 2017;28(1):175–80.
21. Qian YM, Chen QM, Chen WH, Yin QY, Yang JN, Hui Y. Secondary metabolites of endophytic fungi *Aspergillus fumigatus* from scutellaria formosana. Chem Nat Compd. 2024;60(1):192–4.
22. Stark T, Hofmann T. Structures, sensory activity, and dose/response functions of 2,5-diketopiperazines in roasted cocoa Nibs (Theobroma cacao). J Agric Food Chem. 2005;53(18):7222–31.
23. Sultane PR, Mete TB, Bhat RG. A convenient protocol for the deprotection of N-benzyloxycarbonyl (Cbz) and benzyl ester groups. Tetrahedron Lett. 2015;56(16):2067–70.
24. Monbaliu JC, Hansen FK, Beagle LK, Panzner MJ, Steel PJ, Todadze E, Stevens CV, Katritzky AR. A new benzotriazole-mediated stereoflexible gateway to hetero-2,5-diketopiperazines. Chemistry-A Eur J. 2012;18(9):2632–8.
25. Smaoui S, Mellouli L, Lebrihi A, Coppel Y, Fguira LF, Mathieu F. Purification and structure Elucidation of three naturally bioactive molecules from the new terrestrial *Streptomyces* Sp TN17 strain. Nat Prod Res. 2011;25(8):806–14.
26. Carolina Torres-García M, Díaz D, Blasi I, Farràs I, Fernández X, Ariza. Jaume Farràs, Paul Lloyd-Williams, Miriam Royo, Ernesto Nicolás. Side chain anchoring of Tryptophan to solid supports using a dihydropyranyl handle: synthesis of brevianamide F. Int J Pept Res Ther. 2012;18(1):7–19.
27. Li C, Wang MN, Lu XH, Ling Z, Jiang J, Zhang L. Reusable bronsted acidic ionic liquid efficiently catalyzed N-Formylation and N-Acylation of amines. Acs Sustainable Chem Eng. 2020;8(11):4353–61.
28. Guolin W, Tingting L, Wenhui M, Xiping Y, Yu G, Haijun C. One-pot synthesis of tricyclo-1,4-benzoxazines via visible-light photoredox catalysis in continuous flow. Tetrahedron Lett. 2017;58(14):1395–8.
29. Gore S, Baskaran S, König B. Fischer Indole synthesis in low melting mixtures. Org Lett. 2012;14(17):4568–71.
30. Huang R, Ding ZG, Long YF, Zhao JY, Li MG, Cui XL, Wen ML. A new isoflavone derivative from Streptomyces sp YIM GS3536. Chem Nat Compd. 2013;48(6):966–9.
31. Yakukhnov SA, Ananikov VP. Catalytic transfer hydrodebenzylation with low palladium loading. Adv Synthesis Catal. 2019;361(20):4781–9.
32. Selepe MA, Drewes SE, van Heerden. FR total synthesis of the pyranoisoflavone Kraussianone 1 and related isoflavones. J Nat Prod. 2010;73(10):1680–5.
33. Shi JY, Wang CF, Xie MM, Hao YJ, Wang N, Ma HB, Yang XW. Brefeldin A from the deep-sea-derived fungus *Fusarium* sp. Targets on RIPK3 to inhibit TNFα-induced necroptosis. Chem Biodivers. 2022;19(10):e202200696.
34. Gowrishankar S, Poornima B, Pandian SK. Inhibitory efficacy of cyclo(L-leucyl-L-prolyl) from mangrove rhizosphere bacterium-bacillus amyloliquefaciens

(MMS-50) toward cariogenic properties of Streptococcus mutans. Res Microbiol. 2014;165(4):278–89.
35. Jothi R, Hari Prasath N, Gowrishankar S, Pandian SK. Bacterial quorum-sensing molecules as promising natural inhibitors of *Candida albicans* virulence dimorphism: an in silico and in vitro study. Front Cell Infect Microbiol. 2021;11:781790.
36. Chooi YH, Krill C, Barrow RA, Chen S, Trengove R, Oliver RP, Solomon PS. An in planta-expressed polyketide synthase produces (*R*)-mellein in the wheat pathogen Parastagonospora nodorum. Appl Environ Microbiol. 2015;81(1):177–86.
37. Scharf DH, Brakhage AA, Mukherjee PK. Gliotoxin–bane or boon? Environ Microbiol. 2016;18(4):1096–109.
38. Kwon-Chung KJ, Sugui JA. What do we know about the role of gliotoxin in the pathobiology of Aspergillus fumigatus? Med Mycol. 2009;47(Suppl 1):S97–103.
39. Dolan SK, O'Keeffe G, Jones GW, Doyle S. Resistance is not futile: gliotoxin biosynthesis, functionality and utility. Trends Microbiol. 2015;23(7):419–28.
40. Scott PM, Kennedy BP, Harwig J, Chen YK. Formation of diketopiperazines by penicillium italicum isolated from oranges. Appl Microbiol. 1974;28(5):892–4.
41. Wang ZF, Sun ZC, Xiao L, Zhou YM, Du FY. Herbicidal polyketides and Diketopiperazine derivatives from *Penicillium viridicatum*. J Agric Food Chem. 2019;67(51):14102–9.
42. Zhou G, Cai J, Wang B, Diao W, Zhong Y, Pan S, Xiong W, Huang G, Zheng C. Secondary metabolites from the Mangrove Ecosystem-Derived fungi *Penicillium* spp.: chemical diversity and biological activity. Mar Drugs. 2024;23(1):7.
43. Bok JW, Keller NP. LaeA. A regulator of secondary metabolism in Aspergillus spp. Eukaryot Cell. 2004;3(2):527–35. https://doi.org/10.1128/EC.3.2.527-535.2004.
44. Zhang H, Sun C, Xia Q, Li P, Liu K, Zhang Y. Brevianamide F exerts antithrombotic effects by modulating the MAPK signaling pathway and coagulation cascade. Mar Drugs. 2024;22(10):439.
45. Chen C, Ye Y, Wang R, Zhang Y, Wu C, Debnath SC, Ma Z, Wang J, Wu M. Streptomyces Nigra sp. nov. Is a novel actinobacterium isolated from Mangrove soil and exerts a potent antitumor activity in vitro. Front Microbiol. 2018;9:1587.

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