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A high-quality genome assembly of angel-wing mushroom *Pleurocybella porrigens* that causes acute encephalopathy

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Abstract

In 2004, 59 cases of food poisoning linked to the consumption of the mushroom *Pleurocybella porrigens* were reported in Japan, resulting in 17 fatalities due to acute encephalopathy. In 2023, we found three components of this mushroom—a lectin (PPL), pleurocybelline (PC), and pleurocybellaziridine (PA)—which are collectively implicated in its toxicity in mice. Although we reported the genomic data for *P. porrigens* in 2013, detailed annotation was not provided at that time. In addition, the biosynthetic pathway of PA, the compound responsible for acute encephalopathy, has not previously been reported in any Basidiomycetes species, including *P. porrigens*. In this study, we obtained the genome sequence of *P. porrigens* using the PacBio Revio System. *De novo* assembly with hifiasm resulted in a higher N50 value compared to previous assemblies, and gene annotation was conducted through BLAST search, GO analysis, KEGG pathway analysis, and carbohydrate-active enzyme identification. In addition, differential gene expression analysis revealed a significant up-regulation of 1427 genes in the fruiting bodies and 1436 genes in the mycelia. Although the gene encoding TqaL, a PA biosynthetic enzyme reported in the genus *Penicillium*, was not found in *P. porrigens*, a detailed examination of the BLASTx search identified genes with Fe/αKG oxygenase activity similar to TqaL. Three-dimensional structural modeling using AlphaFold3 revealed that the active site cavities of TqaL and the identified protein were in close proximity. Furthermore, the validity of the differential gene expression analysis between the fruiting bodies and the mycelia was confirmed by quantitative reverse-transcription PCR.

Keywords Aziridine, Fungi, Long-read sequence, Pleurocybelline, Poisonous mushrooms, *Tqa* genes

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Introduction

There are many poisonous mushrooms, such as *Amanita phalloides* (Garcia et al. 2015) and *Conocybe filaris* (Horowitz and Moss 2025), which contain toxins that can be fatal even in trace amounts. *Pleurocybella porrigens* (Japanese name, Sugihiratake; English name, Angel-wing mushroom) is a mushroom belonging to the family *Tricholomataceae* and is widely distributed in the Northern Hemisphere temperate regions. This mushroom typically grows in overlapping clusters on decaying stumps and fallen coniferous trees such as cedar and pine (Ainsworth 2008; Matsumoto et al. 2005). One characteristic feature of the fruiting bodies is that they grow in clusters of overlapping ear- or fan-shaped white umbrellas ranging between 2 and 6 cm in diameter (Fig. 1).

In 2004, 59 cases of food poisoning linked to the consumption of the mushroom *P. porrigens* were reported in Japan, resulting in 17 fatalities due to acute encephalopathy. Many of the 17 fatalities originally had symptoms of chronic renal failure and were undergoing hemodialysis before their deaths. Food poisoning caused by *P. porrigens* primarily affects the central nervous system, with initial symptoms including leg weakness and lightheadedness. After several days, involuntary muscle movements appear, followed by rapid paralysis, generalized convulsions, and loss of consciousness. Death typically occurs due to progressive brain edema (Kuwabara et al. 2005). In response, the Ministry of Health, Labour and Welfare of Japan established a study group to investigate the cause of *P. porrigens*-related food poisoning; however, it was disbanded in 2006 after concluding that the cause remained unidentified. Various compounds, including vitamin D analogs (Sasaki et al. 2006), fatty acids (Hasegawa et al. 2007), saccharides (Takata et al. 2009), and hydrogen cyanide (Gonmori and Yokoyama 2009) have been proposed as potential toxic components of *P. porrigens*.

We have previously published research on food poisoning caused by *P. porrigens* (Kawaguchi et al. 2010; Suzuki et al. 2009, 2014, 2021). In 2023, we demonstrated that a mixture of pleurocybelline (PC) and *P. porrigens* lectin (PPL) exhibited exo-protease activity, degrading substrates at both *N*- and *C*-termini without amino acid specificity. Furthermore, when a mixture of PC, PPL, and

Fig. 1 Species image of *P. porrigens*

pleurocybellaziridine (PA), characterized by its aziridine ring structure, was administered to mice, a significant increase in the number of apoptotic cells was observed. Based on these findings, we concluded that the PPL-PC complex disrupts the blood-brain barrier (BBB), and that PA subsequently induces acute encephalopathy (Additional file 1: Fig. S1) (Kawagishi 2023; Suzuki et al. 2023). Although Wakimoto et al. carried out a total synthesis study of PA and demonstrated its existence in nature, its biosynthetic pathway remains unclear (Wakimoto et al. 2011).

In 2021, Bunno et al. reported the biosynthetic and metabolic pathways of PA in the genus *Penicillium*, a group of ascomycete fungi (Additional file 1: Fig. S2) (Bunno et al. 2021). They proposed that the non-heme iron and αKG-dependent oxygenase TqaL oxidizes L-valine to PA, the HAD-like enzyme TqaF opens the aziridine ring of PA through hydroxylation, and the non-heme iron oxygenase TqaM catalyzes oxidative decarboxylation to produce 2-aminoisobutyric acid (AIB). According to the National Center for Biotechnology Information (NCBI) database, the *Tqa* genes are predominantly found in ascomycetes; however, no biosynthetic and metabolic pathways of PA have been reported in Basidiomycetes to date.

In 2013, we reported the genomic data of *P. porrigens* obtained using the Illumina Genome Analyzer (GAIIx) (Suzuki et al. 2013). However, due to the limitations of the technology available at the time, the sequence quality was relatively low by today's standards, and a detailed annotation was not provided. The genomic data from our 2013 study is archived in the integrated genome database A-WING (Yamamoto et al. 2014). In addition, we reported the transcriptomic data of the fruiting bodies and the mycelia of *P. porrigens* using the Illumina NovaSeq 6000 in 2024 (Watanabe et al. 2024).

This study aims to generate a high-quality genome assembly of *P. porrigens* using the long-read PacBio Revio System (PacBio, Menlo Park, CA), and to elucidate the biological functions of *P. porrigens* using BLAST search (Altschul et al. 1990), Gene Ontology (GO) analysis (Ashburner et al. 2000), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis (Mao et al. 2005; Moriya et al. 2007), and carbohydrate-active enzymes (CAZymes) identification. Furthermore, based on the results of these various annotations, we further aimed to identify the biosynthetic pathway of PA, the proposed causative agent of acute encephalopathy, in *P. porrigens*.

Materials and methods

Fungal strain

P. porrigens strain ST (NBRC 00117360) used in this study was originally collected by the authors in Yamanashi Prefecture, Japan. The strain has been deposited in the NITE Biological Resource Center (NBRC, Chiba, Japan) and is publicly available under the accession number NBRC117360. For laboratory experiments, the strain was also preserved at the Center for Bioscience Research and Education, Utsunomiya University. The fruiting bodies of *P. porrigens* were cultivated in potato dextrose broth with 0.5% yeast extract at 25 °C.

Genome sequencing

The genomic DNA of *P. porrigens* was extracted using the cetyltrimethylammonium bromide (CTAB) method (Tanaka et al. 2017). The SMRTbell prep kit 3.0 was used to construct a genomic DNA library, and then long-read sequencing was performed using the PacBio Revio System, a long-read sequencing platform. The raw sequencing data were deposited at the DDBJ as the DDBJ Sequence Read Archive (DRA) under accession no. DRA020122. The RNA extraction method used for transcriptome analysis of *P. porrigens* is described in the author's previous report (Watanabe et al. 2024).

High quality genome assembly and gene prediction

The raw sequence reads were assembled with hifiasm ver. 0.19.8-r603 (Cheng et al. 2021). Genome completeness was assessed using BUSCO version 5.3.2 (Manni et al. 2021), with agaricomycetes_odb10, fungi_odb10, and eukaryota_odb10 lineage dataset, and Merqury version 1.3 (Rhie et al. 2020), a k-mer based assembly evaluation tool. BLAST search of the assembled sequences revealed numerous mitochondrial contigs, which were manually removed — specifically those showing ≥ 98% identity to known mitochondrial sequences.

For gene prediction, both ab initio prediction and RNA-seq–assisted annotation were performed. Ab initio gene prediction was conducted using Augustus ver. 3.5.0 (Stanke et al. 2006) with the model *Coprinus cinereus*. For transcript-supported annotation, RNA-seq reads of *P. porrigens* obtained previously by NovaSeq 6000 (DNA Data Bank of Japan: DRA017296; Watanabe et al. 2024) were mapped to the mitochondrial-filtered genome assembly using HISAT2 program version 2.2.1 (Kim et al. 2015). Subsequent annotation and differential gene expression analysis were performed on the genome assembly data with the mapped RNA sequences. Finally, to ensure that the annotated genes accurately represent biologically active transcripts, the RNA-seq–based gene models were prioritized and used for annotation and differential gene expression analyses.

Bioinformatics analysis

The predicted protein-coding genes were searched using BLASTn and BLASTp. The BLASTn program against the non-redundant nucleotide database 'nt' and the BLASTp program against the protein database 'Swiss-prot' were

conducted with a threshold E-value of 1E−50. GO analysis, KEGG pathway analysis by KofamKOALA (Aramaki et al. 2020), and Pfam (protein families) search using hmmscan version 3.4 (Finn et al. 2011; Mistry et al. 2021) were also performed for functional annotations. dbCAN version 3.3.2 (Cantarel et al. 2009; Zhang et al. 2018) was used for CAZymes identification (HMMER E-value < 1E−15, HMMER Coverage > 0.35). Transcript expression levels were quantified using StringTie version 2.2.3 (Pertea et al. 2015), followed by gene-level read counting with featureCounts version 2.0.3 (Liao et al. 2014). Differential gene expression analysis between the fruiting bodies and the mycelia of *P. porrigens* was performed with EdgeR (Robinson et al. 2010). Differentially expressed genes were defined as those with |log2 fold change (LogFC)| >1 and FDR < 0.05. For GO enrichment analysis, the mean LogFC of genes associated with each GO term was compared to the genome-wide mean LogFC and standardized to compute a *Z*-score, and statistical significance was evaluated using FDR-adjusted *p*-values (Benjamini–Hochberg method). Protein structure predictions were made by AlphaFold3 (Lawande et al. 2024), and structural comparisons were conducted using the PyMOL Molecular Graphics System, Version 3.1, Schrödinger, LLC.

Quantitative reverse-transcription PCR

Quantitative reverse-transcription PCR (qRT-PCR) was performed to quantify the expression of eight genes related to the biosynthetic and metabolic pathways of PA. Total RNA was extracted from 100 mg each of the fruiting bodies and the mycelia of *P. porrigens*. cDNA was synthesized from 10 ng of total RNA using the PrimeScript RT Reagent Kit (Clontech TaKaRa, Shiga, Japan) following the manufacturer's instructions. qRT-PCR analysis was performed with a LightCycler 480 Instrument II (Roche, Basel, Switzerland) with FastStart Essential DNA Green Master (Roche), according to the manufacturer's protocol. The expression level of the actin gene was used as an internal standard (Yu et al. 2020c), and relative gene expression levels were calculated using the $\Delta\Delta$Ct method.

Table 1 Assembly and annotation summary compared to the previous study in 2013

Characteristic	Illumina genome analyzer (2013)	PacBio revio (this study)
Number of genome contigs	31,164	63
N50 length (bp)	1598	4,824,809
Shortest contig length (bp)	173	17,242
Longest contig length (bp)	22,324	8,438,766
Average contig length (bp)	1032	857,459
Total number of nucleotides in genomic contig (bp)	32,149,440	54,019,894
GC content (%)	39.5	42.2
Number of transcripts	3006	28,655
Number of genes annotated by nt database	130	3734
Number of genes annotated by Swiss-prot database	856	11,572
Number of genes annotated by Pfam	2523	25,589
Number of genes annotated by GO	1738	20,757
Number of genes annotated by KEGG	717	13,998
Number of genes annotated by CAZy	73	1352

Results

Sequence and assembly

The genome of *P. porrigens* was successfully sequenced using the PacBio Revio System. *De novo* assembly by hifiasm resulted in 1112 contigs with 104,987,603 bp. The completeness of the *P. porrigens* genome assembly and gene prediction was also evaluated using the BUSCO software with agaricomycetes_odb10 and eukaryota_odb10. The completeness of the *P. porrigens* genome ranged from 97.4 to 99.1% across the agaricomycetes, fungi, and eukaryota datasets. (Additional file 1: Fig. S3). Merqury was also used to assess the *k*-mer completeness of the genome assembly (Additional file 1: Fig. S4). The above genome size was larger than the typical range of genomes in Basidiomycetes (Chen et al. 2016; Floudas et al. 2012; Li et al. 2018; Liu et al. 2012; Mohanta and Bae 2015; Olson et al. 2012). In addition, a BLAST search of the assembly data suggested that these raw assembly data contain many mitochondrial sequences. Therefore, only the genome sequence of *P. porrigens* was extracted by removing mitochondrial sequences from the assembly data. Furthermore, we mapped the RNA sequences of *P. porrigens* (DRA017296) that we reported in 2024 to the genome sequences by the HISAT2 program. As a result, a refined genome assembly consisting of 63 contigs, with an N50 length of 4,824,809 bp, was obtained in this study (Table 1). The contigs ranged from 17,242 bp to 8,438,766 bp with an average length of 857,459 bp. The *P. porrigens* genome consisted of 54.0 Mbp of nucleotides with a GC content of 42.2%, representing an increase compared to the 32.1 Mbp we previously obtained using the Illumina Genome Analyzer (Suzuki et al. 2013). This value is consistent with the expected genome size for Basidiomycetes species obtained with the PacBio Revio System (Cantarel et al. 2009). The completeness of the *P. porrigens* genome ranged from 98.6 to 99.2% across the agaricomycetes, fungi, and eukaryota datasets and was further supported by Merqury (Additional file 1: Figs. S5, S6). The slight increase in BUSCO completeness after removing mitochondrial sequences is likely due to the possibility that, prior to removal, several mitochondrial contigs showed spurious similarity to conserved orthologs and were misclassified by BUSCO as fragmented

or duplicated genes. Furthermore, the BUSCO completeness of the genome data obtained in 2013 using the Illumina Genome Analyzer ranged from 25.1 to 31.4%, highlighting the substantial improvement in genome quality achieved in the present *P. porrigens* assembly compared with the 2013 data (Additional file 1: Fig. S7). These results by BUSCO, Merqury, and other assembly parameters indicated that high-quality genome data of *P. porrigens* was obtained in this study. Detailed annotation was performed on the obtained sequences after mitochondrial removal and RNA sequence mapping.

Functional annotation of *P. porrigens* contigs

After removing the mitochondrial sequences from the *P. porrigens* genome assembly data and mapping the RNA sequences using HISAT2 program, a total of 28,655 transcripts were identified from the open reading frames. In addition to RNA-seq–based annotation, ab initio gene prediction was also performed using Augustus ver. 3.5.0, which identified 7,062 putative genes. The transcriptome-based approach used in this study was supported by high-quality RNA-Seq data derived from both the fruiting bodies and the mycelia (Watanabe et al. 2024), representing the major developmental stages of *P. porrigens*. Because this approach yielded a larger and biologically supported gene set (28,655 genes), we adopted the RNA-seq–based gene models to ensure that the annotated genes accurately reflect biologically active expression.

The predicted protein-coding genes were further annotated using BLAST search, Pfam search, GO analysis, KEGG pathway analysis, and CAZyme identification. The BLAST search revealed that 3,734 genes were significantly similar to those in the nt database, and 11,572 genes were similar to those in the Swiss-Prot database (Table 1).

Next, we performed a Pfam search to identify protein domain families in the genome sequences. The Pfam search showed that 25,589 genes contained structural domains, with 52 Pfam categories containing more than 300 genes (Additional file 1: Fig. S8). The *P. porrigens* genome was found to be enriched in three conserved domains: Protein kinase domain (PF00069; 999 genes), F-box-like (PF12937; 901 genes), and Protein tyrosine and serine/threonine kinase (PF07714; 803 genes).

We then assigned the predicted protein-coding genes to GO terms to gain functional insights. A total of 20,757 genes were assigned GO terms. Considering multiple GO annotations for each gene, 37,051 genes (34.3%) were assigned to biological process (BP), 15,477 genes (14.3%) to cellular component, and 55,413 genes (51.3%) to molecular function (MF) (Additional file 1: Fig. S9). In the biological process category, the most frequent assignments were "oxidation-reduction process" (GO:0055114) and "metabolic process" (GO:0008152). In the cellular component category, "membrane" (GO:0016020) and "nucleus" (GO:0005634) were most assigned. In the molecular function category, "protein binding" (GO:0005515) and "ATP binding" (GO:0005524) were the most frequently assigned.

KEGG pathway analysis was performed on 63 contigs of the *P. porrigens* genome. This analysis revealed that 718 genes were annotated as part of the "metabolic pathways" (map01100) and 295 genes as part of the "biosynthesis of secondary metabolites" (map01110) (Additional file 1: Fig. S10).

CAZymes annotation in the *P. porrigens* genome

CAZymes constitute one of the most critical gene families in fungal genomes. They are involved in lignocellulose degradation as well as various other biological processes, including development and stress responses (Garron and Henrissat 2019; Pallister et al. 2020). CAZyme annotation using Hmmer identified 1352 genes encoding CAZymes in the *P. porrigens* genome. This included 685 Glycoside Hydrolases (GHs), 260 GlycosylTransferases (GTs), 32 Polysaccharide Lyases (PLs), 44 Carbohydrate Esterases (CEs), 287 Auxiliary Activities (AAs), and 44 Carbohydrate-Binding Modules (CBMs) (Fig. 2).

Differential gene expression analysis

Differential gene expression analysis was performed using EdgeR, based on RNA-seq reads mapped to the *P. porrigens* genome assembly. As a result, 1427 genes were up-regulated in the fruiting bodies, and 1,436 genes were up-regulated in the mycelia ($LogFC>|1|$, $FDR<0.05$). Detailed results of the differential gene expression analysis are provided in Material S1 (Additional file 2), and the MA plot of the analysis is shown in Fig. S11 (Additional file 1). GO enrichment analysis was then conducted using the *Z*-score method based on the differential gene expression results. This approach evaluates coordinated expression changes at the functional category level rather than at the level of individual genes. GO terms with significant expression differences ($FDR<0.05$) between the fruiting bodies and the mycelia are listed in Tables S1 and S2 (Additional file 1). A total of seven GO terms, including "protein binding" (GO:0005515) and "ATP binding" (GO:0005524), were significantly up-regulated in the fruiting bodies. Conversely, four GO terms, including "obsolete oxidation-reduction process" (GO:0055114) and "oxidoreductase activity" (GO:0016491), were significantly up-regulated in the mycelia.

Exploration of biosynthetic and metabolic pathways of pleurocybellaziridine (PA)

PA is the causative compound that causes acute encephalopathy by *P. porrigens*. In 2021, Bunno et al. reported on the biosynthetic and metabolic pathways of PA in the

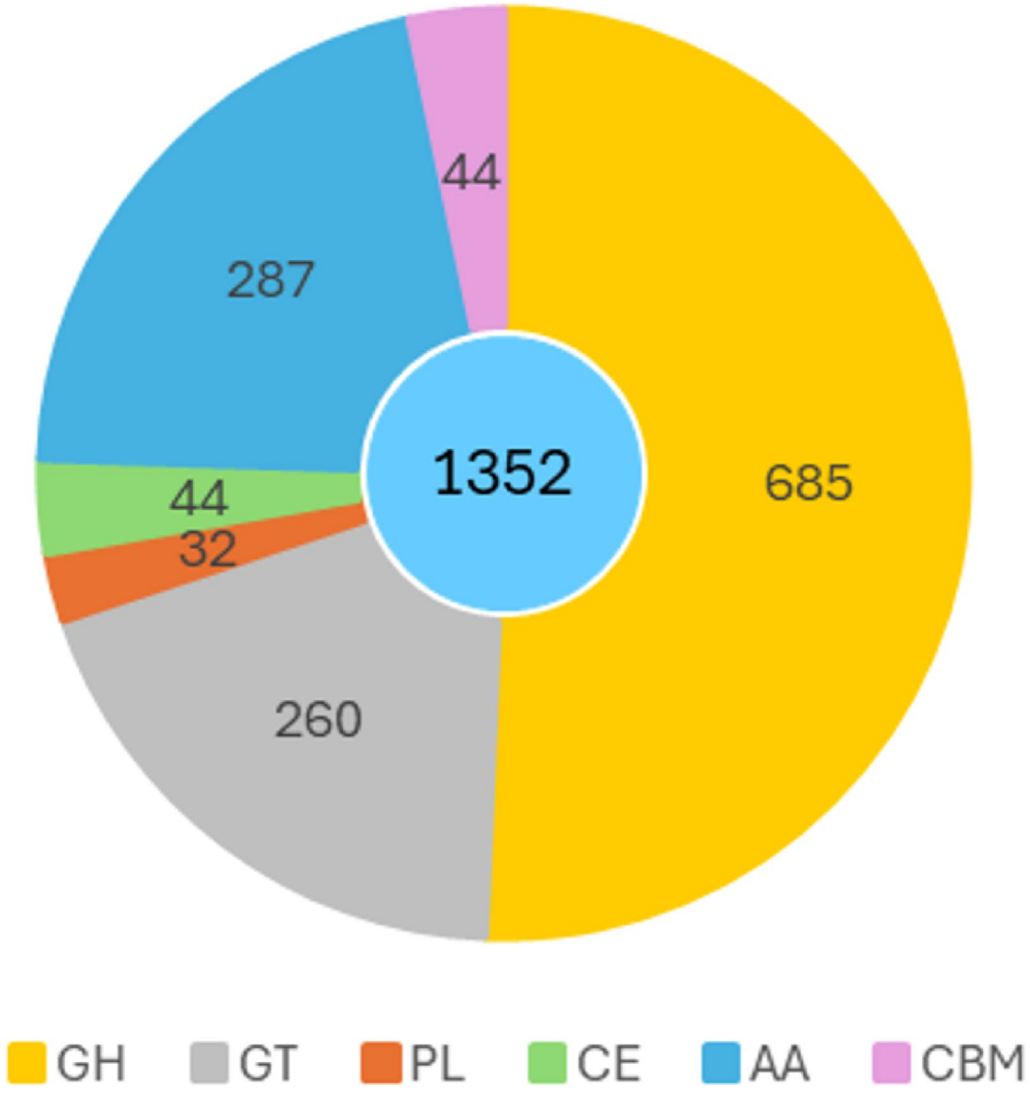

Fig. 2 The distribution of CAZymes categories in *P. porrigens*. Glycoside hydrolases: GH, Glycosyl transferases: GT, Polysaccharide lyases: PL, Carbohydrate esterases: CE, Auxiliary activities: AA, Carbohydrate-binding modules: CBM

genus *Penicillium*. They proposed that the non-heme iron and αKG-dependent oxygenase TqaL oxidizes L-valine to PA, the HAD-like enzyme TqaF opens the aziridine ring through hydroxylation, and the non-heme iron oxygenase TqaM catalyzes oxidative decarboxylation to produce 2-aminoisobutyric acid (AIB) (Bunno et al. 2021). However, the biosynthetic and metabolic pathways of PA in Basidiomycetes species, including *P. porrigens*, remain unclear.

To assess whether *P. porrigens* biosynthesizes L-valine, a proposed precursor of PA, KEGG pathway analysis was conducted based on genome data supported by RNA-seq mapping. This analysis revealed that genes encoding all five biosynthetic steps of L-valine from pyruvate are expressed in both the fruiting bodies and mycelia of *P. porrigens*, suggesting that L-valine is biosynthesized in both the fruiting bodies and mycelia of this fungus (Fig. 3). Differential gene expression analysis showed that the biosynthesis of (*R*)-2,3-dihydroxy-3-methylbutanoate from pyruvate was up-regulated in the mycelia (MSTRG.6805, MSTRG.7867). Subsequently, the biosynthesis of 2-oxoisovalerate from (*R*)-2,3-dihydroxy-3-methylbutanoate was up-regulated in both the fruiting bodies (MSTRG.5730) and the mycelia (MSTRG.2944). Finally, the biosynthesis of L-valine from 2-oxoisovalerate was up-regulated in the fruiting bodies (MSTRG.4918).

We next examined whether *P. porrigens* possesses *Tqa* genes previously identified in the genus *Penicillium* and are involved in the biosynthetic and metabolic pathways of PA, using two types of information: the nucleotide sequences and protein domains of *TqaL*, *TqaF*, and *TqaM*. We first downloaded the protein sequences of the *Tqa* gene cluster (*TqaL*: 15 sequences, *TqaF*: 3 sequences, *TqaM*: 4 sequences) from the NCBI website and created

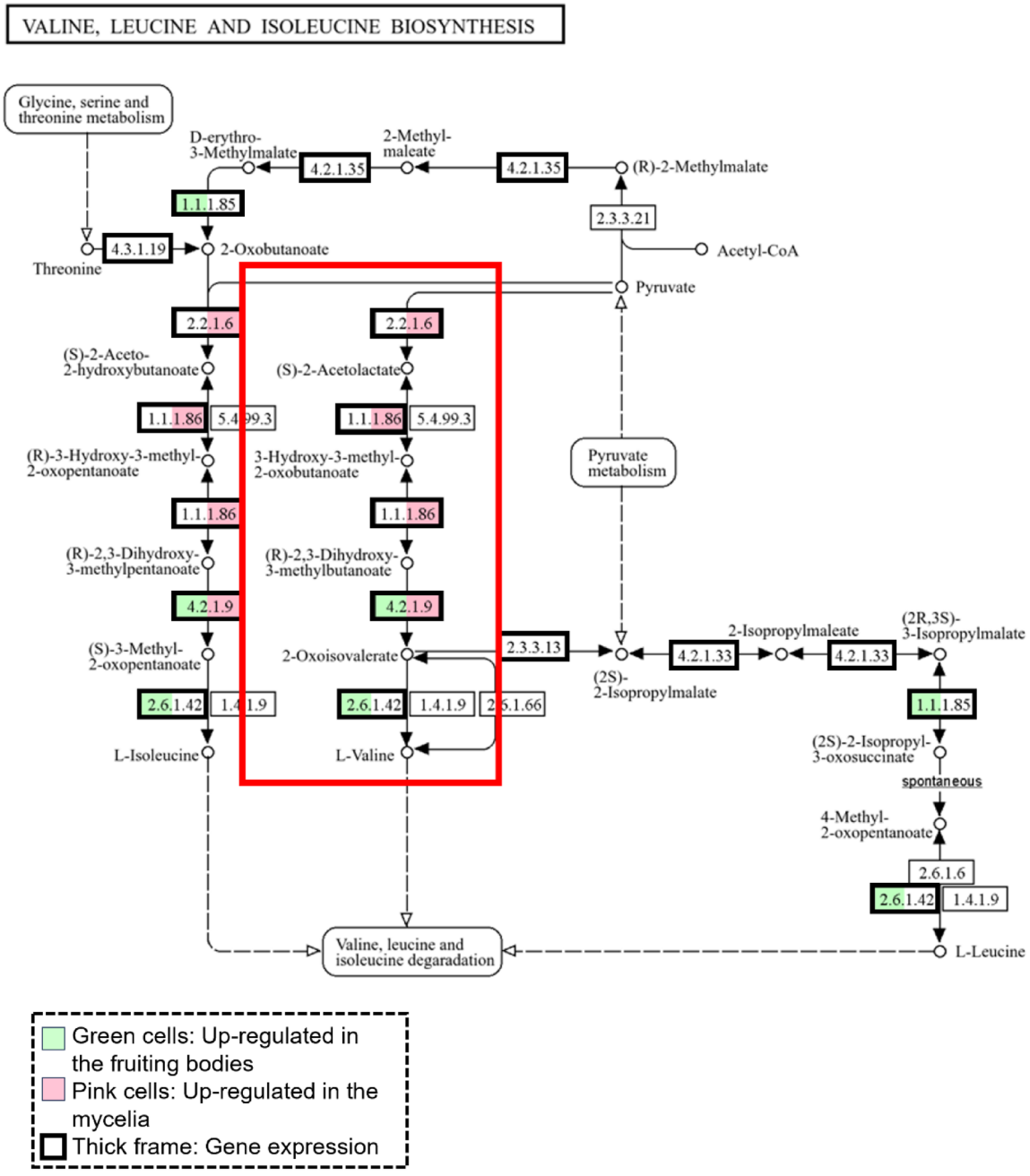

Fig. 3 KEGG pathway analysis for valine, leucine and isoleucine biosynthesis. Green cells: gene expression increased in the fruiting bodies. Pink cells: gene expression increased in the mycelia

a protein sequence database. Then, we performed a BLAST search (with a threshold E-value of 1E−4) using the created database against the genome sequences of *P. porrigens* mapped with RNA sequences (Additional file 1: Table S3). The retrieved sequences were further annotated by BLAST search (with a threshold E-value of 1E−4) against the Swiss-Prot database. However, based on the percent identity, alignment lengths, and annotation results, we concluded that *P. porrigens* does not possess homologs of the *Tqa* genes.

Next, we examined whether *P. porrigens* contains enzymes belonging to the same protein families as the *Tqa* genes. The Pfam domain IDs of *TqaL*, *TqaF*, and *TqaM* were identified in the InterPro protein families database (Finn et al. 2016) as PF10014 (2OG-Fe dioxygenase), PF13419 (Haloacid dehalogenase-like hydrolase), and PF00596 (Class II Aldolase and Adducin

N-terminal domain), respectively. We then checked whether the genomic sequence of *P. porrigens* contained the Pfam protein domains corresponding to the *Tqa* genes. We found that PF10014 was absent, PF13419 was present in 15 sequences, and PF00596 was present in 8 sequences (Additional file 1: Table S4). A total of 23 sequences identified above were annotated through a BLAST search (with a threshold E-value of 1E−4) against the Swiss-Prot database. Although sequence similarity and alignment lengths were not high enough to confirm direct homology, MSTRG.5551.1 and MSTRG.7191.1 were putatively annotated as *TqaF*-like and *TqaM*-like genes, respectively, based on shared Pfam domain composition (Haloacid dehalogenase-like hydrolase and Class II Aldolase) and partial sequence similarity.

Additional exploration of the biosynthetic pathway of pleurocybellaziridine (PA)

To date, our preliminary study indicated that the fruiting bodies of *P. porrigens* contain significantly more PA than the mycelia. Therefore, we attempted to identify candidate PA biosynthetic enzymes based on the results of GO enrichment analysis and BLASTx search.

First, we searched for genes potentially related to PA biosynthesis based on GO enrichment analysis. GO terms associated with "oxygenase," "oxidase," and "dehydrogenase" with Z-score < 0 are summarized in Table S5 (Additional file 1). No GO terms were significantly up-regulated in the fruiting bodies compared to the mycelia. Genes associated with PA biosynthesis were not identified from the results of the GO enrichment analysis.

To identify enzymes in *P. porrigens* with functional similarity to *TqaL*, we searched the BLASTx results from the differential gene expression analysis between the fruiting bodies and mycelia for the term "alpha-ketoglutarate-dependent" which yielded five contigs. Among these, only MSTRG.1696 was annotated as a "dioxygenase." Differential gene expression analysis indicated that MSTRG.1696 was significantly up-regulated in the fruiting bodies (logFC = − 1.34, FDR = 1.79E− 02). The amino acid sequences of TqaL and MSTRG.1696.1.p2 were aligned using BioEdit version 7.2.5 (Hall 1999) to assess structural similarity, revealing several conserved regions between the two proteins (Additional file 1: Fig. S12).

Three-dimensional structural modeling of PA biosynthetic enzyme

Based on the above results, MSTRG.1696.1.p2 was selected as a candidate PA biosynthetic enzyme, and its three-dimensional (3D) structure was predicted using AlphaFold3. The predicted structure of MSTRG.1696.1.p2 was then compared to that of TqaL protein using PyMOL software. The structural model also included non-heme iron and Tris(hydroxymethyl) aminomethane in the structure prediction. The overall structure of MSTRG.1696.1.p2 is shown in Fig. 4A. The iron is coordinated by conserved metal-binding residues His184, Asp186, and His253 within the active site cavity. The aligned active site structures of MSTRG.1696.1.p2 and TqaL are shown in Fig. 4B. The residues His184, Asp186, and His253 in MSTRG.1696.1.p2 are spatially close to the corresponding His180, Asp182, and His329 in TqaL, suggesting that their active site architectures are nearly identical.

Experimental analysis of the fruiting bodies and the mycelia

To examine the relative expression levels of genes in the fruiting bodies and the mycelia, qRT-PCR was performed on eight genes related to the biosynthetic and metabolic pathways of PA (Fig. 5). Five genes (MSTRG.6805, MSTRG.7867, MSTRG.5730, MSTRG.2944, MSTRG.4918) involved in the biosynthetic pathway of L-valine from pyruvate were selected, and three genes (MSTRG.1696, MSTRG.5551, MSTRG.7191) were chosen as candidates for enzymes potentially involved in the PA biosynthesis and metabolism. qRT-PCR primers were designed with length of 20–25 bp and melting temperatures (Tm) of 63–68 °C (Additional file 1: Table S6). The actin gene was used as an internal control.

qRT-PCR analysis revealed that five of the eight genes showed higher expression in the fruiting bodies, whereas three genes were more highly expressed in the mycelia. The predicted expression patterns from EdgeR and the qRT-PCR results were consistent in seven out of the eight genes. MSTRG.6805, which encodes acetolactate synthase, was up-regulated in the mycelia according to EdgeR-based differential expression analysis, while qRT-PCR revealed higher expression in the fruiting bodies. This discrepancy may be attributed to the fact that acetolactate synthase is susceptible to environmental influences such as feedback inhibition, as well as to the use of separate RNA extractions for each analysis and methodological differences between EdgeR and qRT-PCR. Nevertheless, the consistency of the remaining results supports the high accuracy of the differential expression analysis conducted using EdgeR.

Discussion

In this study, we conducted a genome analysis of *P. porrigens* using the long-read PacBio Revio System to obtain high-quality genomic data. The total number of nucleotides in the genomic contigs of *P. porrigens* was approximately 54 Mbp, which is comparable to that of *Russula griseocarnosa*, another member of Basidiomycota with a reported chromosome-level assembly (Liu et al. 2022; Yu et al. 2020a, b). The N50 value and genome completeness indicated that the assembly quality was high with the

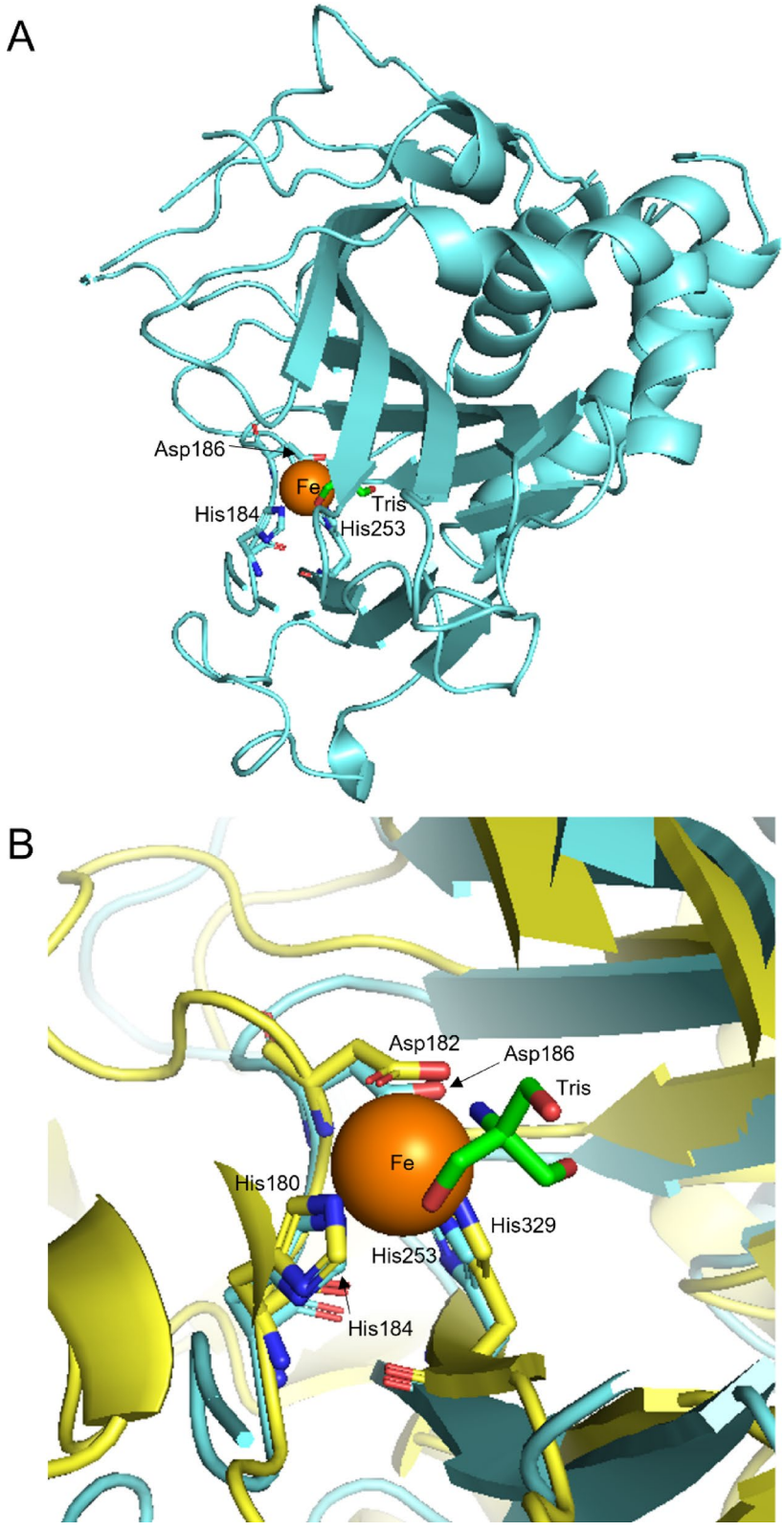

Fig. 4 Overall structure of MSTRG.1696.1.p2 (**A**) and the overlaid active site structure of MSTRG.1696.1.p2 (blue) and TqaL (yellow) (**B**)

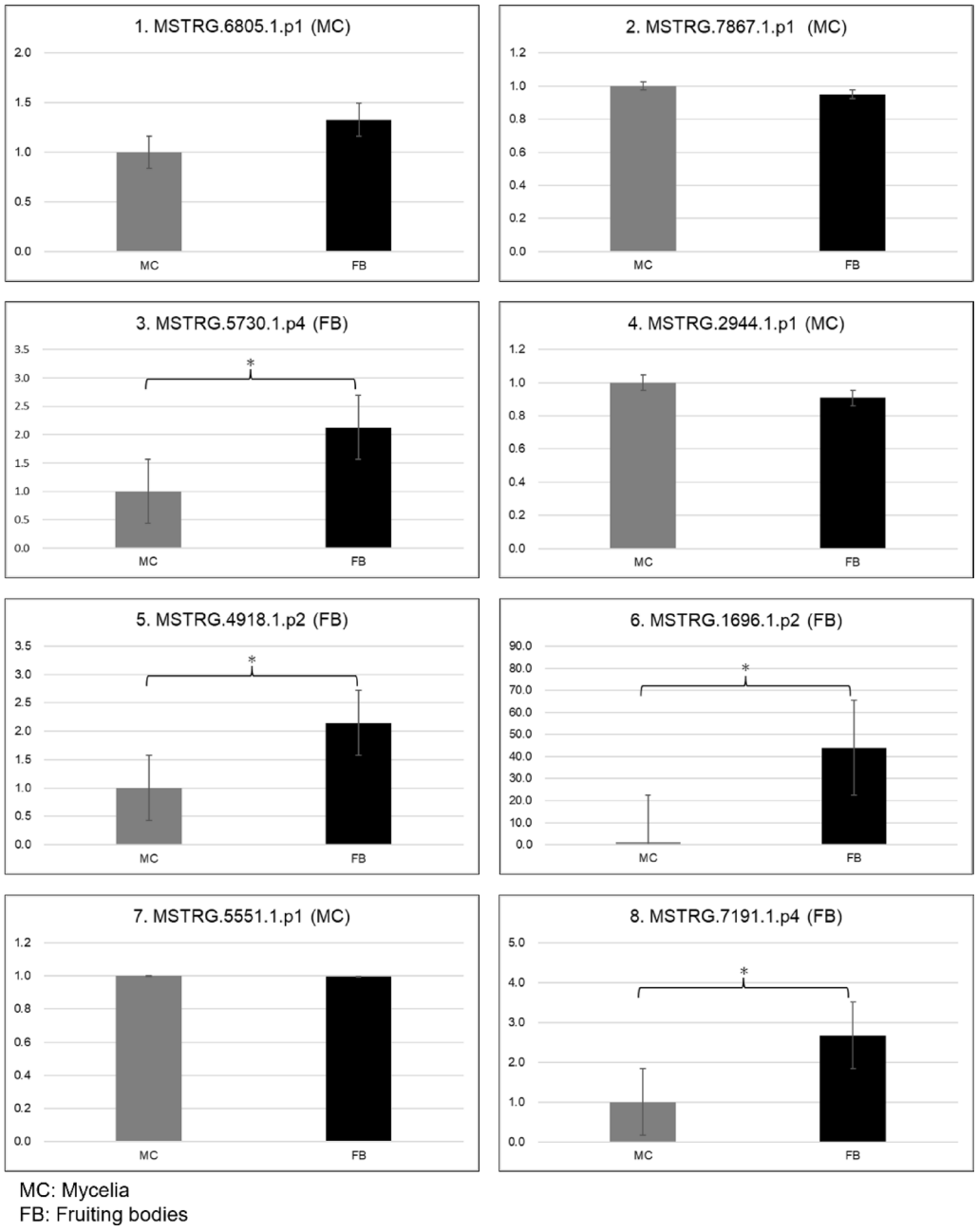

Fig. 5 Differential gene expression analysis based on quantitative reverse-transcription PCR

N50 being approximately 3000 times greater than that obtained in a previous study using the Illumina Genome Analyzer (Suzuki et al. 2013).

We elucidated the basic genetic information of *P. porrigens* through BLAST search, Pfam search, GO analysis, and KEGG pathway analysis. Moreover, the CAZyme annotation revealed that 1352 genes in the *P. porrigens* genome encode CAZymes. Notably, lignin-degrading enzymes (Pollegioni et al. 2015) such as laccase and manganese peroxidase, which belong to the AA1 and AA2 families, were identified, suggesting that these enzymes play a role in lignocellulose degradation during the growth of *P. porrigens* on old stumps and fallen trees of conifers such as cedar and pine (Ainsworth 2008; Matsumoto et al. 2005). When compared to other Basidiomycete species, such as *Pleurotus giganteus*—a closely related species for which comparable long-read sequencing data are available and whose CAZymes were analyzed under the same conditions (Yu et al. 2022)—*P. porrigens* was found to have a higher proportion of glycosyl transferases and carbohydrate esterases.

Regarding the PA biosynthetic pathway, representative toxin-producing Basidiomycetes, such as *Amanita phalloides*, produce well-characterized toxic compounds (α- and β-amanitin), and their biosynthetic pathways have been elucidated as ribosomally synthesized and post-translationally modified peptide (RiPP) systems (Garcia et al. 2015). In contrast, no enzymes responsible for PA biosynthesis have been reported in Basidiomycetes, including *P. porrigens*. In this study, KEGG pathway analysis of *P. porrigens* genome indicated that L-valine, a predicted precursor of PA (Singh and Huang 2025; Watanabe et al. 2024; Zhou et al. 2024), is likely biosynthesized in both the fruiting bodies and the mycelia of *P. porrigens*. Pfam search suggested that *P. porrigens* lacks the gene encoding TqaL (Gao et al. 2011), an αKG-dependent oxygenase, which has been reported to be involved in the PA biosynthetic pathway in the genus *Penicillium* (Bunno et al. 2021), a group of ascomycete fungi. Based on BLASTx results and differential gene expression analysis between the fruiting bodies and the mycelia of *P. porrigens*, a gene with αKG-dependent oxygenase activity similar to *TqaL* was identified as MSTRG.1696.1. Additionally, since *TqaF* and *TqaM*, which are reported to be involved in PA metabolism in the genus *Penicillium* (Bunno et al. 2021), were not found in *P. porrigens*, genes with potential functional similarity to *TqaF* and *TqaM* were searched based on their Pfam domain IDs. As a result, MSTRG.5551.1 was identified as a gene that may potentially have a function similar to *TqaF*, and MSTRG.7191.1 as a gene that may potentially have a function similar to *TqaM*. The 3D structural modeling with AlphaFold3 and PyMOL revealed that the active site cavities of TqaL and MSTRG.1696.1 are in close proximity. Notably, the residues His184, Asp186, and His253 in MSTRG.1696.1 are spatially close to the corresponding His180, Asp182, and His329 in TqaL. These findings suggest that their active site architectures are nearly identical, further supporting the hypothesis that MSTRG.1696.1 may share a similar catalytic function with TqaL and play a role in the PA biosynthetic pathway in *P. porrigens*.

Furthermore, the differential gene expression analysis between the fruiting bodies and the mycelia of *P. porrigens*, conducted using EdgeR software, was validated by qRT-PCR, which confirmed consistent expression differences for seven genes out of the eight genes analyzed. These findings support the reliability of the differential gene expression analysis conducted using EdgeR in the present study. Our preliminary study demonstrated that the fruiting bodies of *P. porrigens* contain substantially higher amounts of PA than the mycelia. Consistently, both EdgeR-based gene expression analysis and qRT-PCR in this study revealed a significant up-regulation of MSTRG.1696.1 in the fruiting bodies, thereby strengthening our hypothesis that this gene is involved in PA biosynthesis. In addition, GO enrichment analysis revealed that transcription-related GO terms were significantly enriched in the fruiting body, including "DNA binding" (GO:0003677; Z-score = – 1.3, FDR = 0.001) and "regulation of DNA-templated transcription" (GO:0006355; Z-score = – 0.65, FDR = 0.042). These results indicate that transcription factor activity is elevated in the fruiting body, and suggest that among the up-regulated genes, some may be involved not only in fruiting body development but also in PA biosynthesis.

The primary aim of this study was to establish a high-quality genomic and transcriptomic resource that enables future investigation of the biosynthetic mechanisms underlying PA production. Although MSTRG.1696 was identified as a candidate Fe/αKG-dependent oxygenase and its expression was higher in fruiting bodies, the involvement of this gene in PA biosynthesis remains a working hypothesis. Since *TqaF* and *TqaM* have been identified in Ascomycetes, our analysis in this study was limited to domain-based similarity searches to identify potential homologs in *P. porrigens*. Therefore, the corresponding PA metabolic pathway may differ substantially or even be absent in this Basidiomycete species. Nevertheless, this approach demonstrates the feasibility of identifying potential biosynthetic genes in this species and provides a foundation for future functional validation. As next steps, we will conduct heterologous expression and gene disruption analyses of these candidate genes to experimentally verify their roles in PA biosynthesis. These functional studies are expected to further clarify whether PA is synthesized in *P. porrigens* and, if so, to elucidate the underlying enzymatic pathway. This work therefore establishes an essential genomic framework for future mechanistic studies and contributes valuable resources for understanding secondary metabolism in Basidiomycetes.

Abbreviations

AAs	Auxiliary activities
AIB	2-Aminoisobutyric acid
BBB	Blood-brain barrier
GAIIx	Illumina genome analyzer
CAZymes	Carbohydrate-active enzymes
CBMs	Carbohydrate-binding modules
CEs	Carbohydrate esterases
CTAB	Cetyltrimethylammonium bromide
DRA	DDBJ sequence read archive
GHs	Glycoside hydrolases
GO	Gene ontology
GTs	Glycosyltransferases
KEGG	Kyoto encyclopedia of genes and genomes
NCBI	National Center for Biotechnology Information
PA	Pleurocybellaziridine
PC	Pleurocybelline
Pfam	Protein families
PLs	Polysaccharide lyases
PPL	*P. porrigens* lectin
P. porrigens	*Pleurocybella porrigens*
qRT-PCR	Quantitative reverse-transcription PCR

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13568-025-01995-2.

Supplementary Material 1.

Supplementary Material 2.

Author contributions

T. Suzuki conceived the project and designed most of the experimental outlines. A.O. and T Suzuki collected ***P. porrigens***. T. Sato and A.O. extracted DNA for sequencing. N.W. and M.K. analyzed the sequencing data. K.M., K.S., S.T., and J.Z. conducted qRT-PCR. J.Z. registered the genome sequence to DDBJ. N.W. and T. Suzuki wrote the manuscript. All authors read and approved of the final manuscript.

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Availability of data and materials

The sequence data have been deposited in DNA Data Bank of Japan (DDBJ) under accession number DRA020122. The draft genome sequences of ***P. porrigens*** obtained in this study were deposited in the DDBJ Mass Submission System (MSS) under accession numbers BAAHNN010000001-BAAHNN010001112.

Declarations

Ethics approval and consent to participate

This article contains no studies with human participants or animals performed by authors.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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