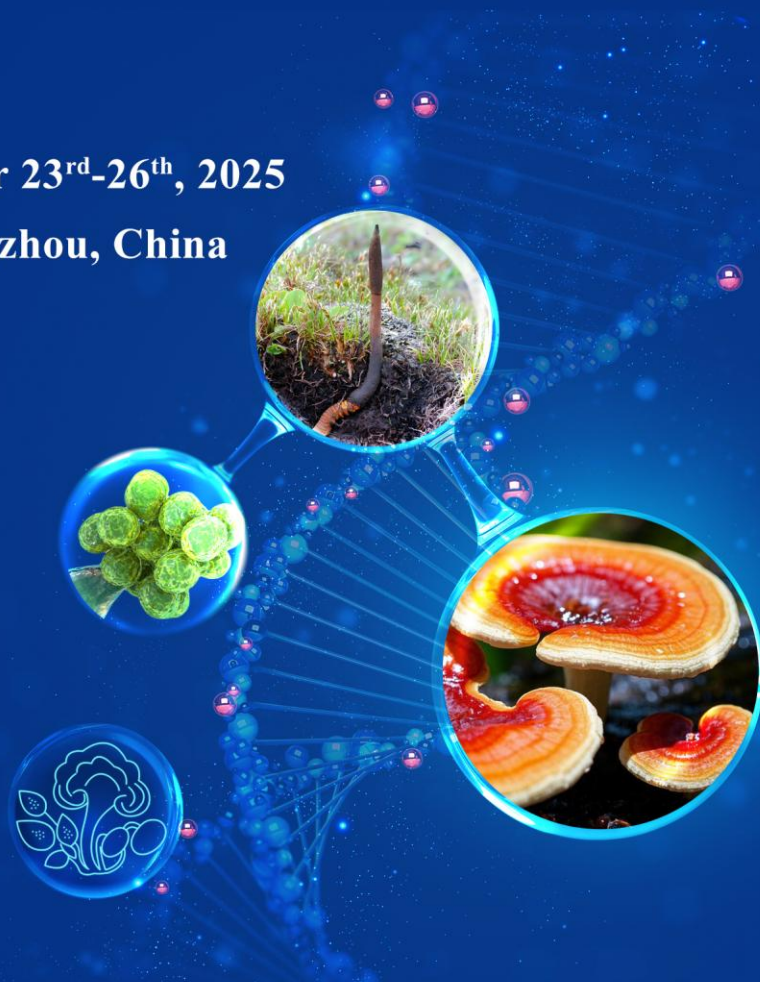




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Keynote speaker



Abstract

New bioactive compounds from invertebrate-associated ascomycetes and basidiomycete**Marc Stadler**

President, International Mycological Association, Germany

In the past years, we have studied certain taxonomic and ecological groups of fungi very intensively for production of bioactive secondary metabolites. An international team, including researchers from over 20 countries and 4 continents, performed the research. The collaborations included intensive field work to collect specimens from which mycelial cultures were obtained on-site in tropical countries like Kenya and Thailand. The taxonomic characterization often revealed undescribed species or even genera. The cultures were grown in solid or liquid scale, subjected to standardized extraction procedures and the extracts were checked by using various bioassays and by HPLC-DAD/MS based metabolomics approaches. Interesting compounds were isolated to purity after scale-up of fermentation and characterized by spectral methods such as NMR spectroscopy and high resolution mass spectrometry.

We have focused on the hypocrealean invertebrate-associated fungi from Thailand, on polypores and other Agaricomycetes from Kenya or from rare species dwelling in ecologically interesting habitats in Europe. Another project concerned the characterization of nematode antagonists that parasitized plant pathogenic worms. Many of the strains produced new secondary metabolite scaffolds, such as new meroterpenoids (which were frequently encountered in basidiomycetes). We also found many unprecedented PKS/NRPS hybrids and complicated polyketides that had strong or selective biological effects in vitro. Examples are biofilm inhibitors that even destroyed preformed biofilm of bacterial and fungal human pathogens.

In total we have published over 50 papers on this matter in the past 5 years. The most interesting findings will be summarized.

Abstract

Gene-for-gene interactions between fungi and insects

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It has long been established that the gene-for-gene (GfG) strategy is employed by microbes in interactions with diverse plant by following the zig-zag model of defense and counter defense. Unlike plants, the cells of different animals including insects lack the apoplast structure and the intracellular nucleotide-binding domain and leucine-rich repeat-containing receptor (NLR)-like proteins. The effector-mediated GfG strategy is thus questioned in microbe-metazoan interactions. However, similar to plant pathogenic fungi, entomopathogenic fungi encodes an array of small-secreted cysteine-rich like effector proteins. By using the model insect *Drosophila melanogaster* as a genetically trackable host, different effector-like genes were deleted and used for virulence assays. Before fungal penetration, we found that a spore surface protein can be detected by a *Drosophila* chemosensing protein to trigger extensive grooming to wipe off fungal spores. Those virulence-required effector genes were further used for screening of putative target in *D. melanogaster*. We found that, to counteract the antifungal Toll pathway, the Tge1 effector is secreted by *Metarhizium robertsii* to block the β -glucan coreceptors GGBP3 and GGBP-like 3, the latter mediates a positive feedback loop activation of antifungal responses. The *Metarhizium* Fkpl effector targets both Psh and CtsK1 of *Drosophila*. Both are the proteases involved in sensing danger molecules of pathogens such as the serine proteases for cuticle degradation. The core component Spätzle (Spz) functions as the Toll receptor ligand to activate the downstream pathway after matured as C106 by its processing enzyme SPE. By using C106 as bait, our library screening identified two effectors of *M. robertsii*: an M28-family aminopeptidase ETS1 and hypothetical protein ETS6. The former degrades both Spz and C106 while ETS6 block the formation C106 dimer or hijack this dimer from Toll receptor, which represents a dual disruption strategy to suppress fly antifungal immunity. Interesting, diverse C106-like ligands from other invertebrates including ticks can be similarly targeted by ETS1 and ETS6. It was also interesting to find that an insect-like trehalase can be secreted by *Cordyceps militaris* to scavenge silkworm blood sugar trehalose, which induces a starvation-like response in caterpillars that facilitates insect feeding and weight gain to benefit fungal fruiting. Overall, in addition to establishing the GfG machinery in fungus-insect interactions, our works provide novel insights into fungal infection of insect hosts.

Keywords: Entomopathogenic fungi; effector; Gene-for-gene interaction; immune suppression

Abstract

Research Advances in Wheat Stripe Rust and Its Control in China

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Wheat stripe rust, a major fungal disease impacting global wheat production, has been systematically studied and controlled in China with significant breakthroughs. Research has defined China's unique epidemic system, pinpointing the Northwest regions as a key over-summering area for the pathogen and a hotspot for rapid virulence variation. Guided by this, a targeted control strategy has been proposed, emphasizing “prioritizing control in the over-summering zone, sustained management in the winter multiplication zone, and comprehensive prevention in the spring epidemic zone”.

Regarding pathogenic mechanisms reproduction-have been clarified. Among these, sexual reproduction facilitated by alternate host *Berberis*, serves as the main driver of pathogen variability, underpinning a "reduce-delay-block" green prevention/control system. Furthermore, the genome of the stripe rust pathogen has been sequenced, revealing key mechanisms related to infection, sugar uptake, and effector function. RNAi-based resistant materials have also been successfully developed.

In addition, studies have elucidated the histocytological and molecular mechanisms underlying wheat-stripe rust interaction, including the hypersensitive response (HR) regulatory network and CBL-CIPK signaling pathway. Key regulatory elements, such as the transcription factor TaWRKY19 and susceptibility gene TaPsIPK1 have been identified. By editing susceptibility genes, novel broad-spectrum resistant materials effective against prevalent stripe rust races and leaf rust have been created, establishing a new breeding strategy based on susceptibility gene modification. Moreover, the world's first landscape of wheat disease resistance gene has been constructed, enabling the cloning of multiple yellow rust resistance genes. And a series of stripe rust resistant varieties have been developed. These integrated advances in basic research and applied technology provide a comprehensive and sustainable solution for wheat stripe rust control, ensuring wheat production security.

Abstract

Mitochondrial DNA Polymorphisms and Inheritance in Fungi

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Aside from the nuclear genome, almost all eukaryotes have a mitochondrial genome. These two genomes differ greatly in their genome size, gene content, and mode of inheritance. In most eukaryotes, during sexual reproduction, while the nuclear genome is biparentally inherited, the mitochondrial genome is predominantly inherited from a single parent. The genetic and evolutionary mechanisms that govern the divergent modes of inheritance remain largely unknown. In this presentation, I will share our research results on fungal mitochondrial genetic studies, including the often-large genome size variation within and among species, diverse modes of inheritance during sexual crosses, potential genetic and evolutionary mechanisms for uniparental inheritance, adaptive significance of biparental inheritance, and evidence for recombination in nature.

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Next-Gen Mycologists: Pioneering Innovations and Novel Perspectives in Mycology Symposium



Abstract

Phylogeny and Taxonomy of the genera *Cantharellus* and *Craterellus* (Hydnaceae, Basidiomycota) in China

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Members of *Cantharellus* and *Craterellus* are usually called chanterelles. They are widely distributed and have significant ecological and economic values. Although there have been several studies especially in recent years focusing on the chanterelle mushrooms in China, the research on their species diversity and phylogenetic relationships is still far from sufficient. In this study, taxonomic and phylogenetic analyses on the species diversity, distribution patterns, and interspecific relationships of *Cantharellus* and *Craterellus* in China were conducted through morphological examination and molecular phylogenetic analyses of multiple loci, including the internal transcribed spacer regions (ITS), the large subunit of nuclear ribosomal RNA gene (nLSU), the second largest subunit of RNA polymerase II (rpb2), the translation elongation factor 1 gene (tef1), and the small subunit of mitochondrial ribosomal RNA gene (mtSSU). In total, 68 chanterelle species are recognized in China, including 42 species of *Cantharellus* and 26 species of *Craterellus*. Among them, there are 20 new species of *Cantharellus* and *Craterellus* were firstly reported in China. Besides, 6 new combination names were proposed. Additionally, one ambiguous taxon was tentatively named as *Craterellus* sp., and five synonyms were redefined. Among the accepted species, 36 known species were described in detail based on observations, and the other 9 species were reviewed. Keys to the accepted species of *Cantharellus* and *Craterellus* in China were provided.

Keywords: chanterelle, Cantharellales, edible fungi, novel taxa, phylogenetics

Abstract

Differential genome structure and allelic expression between two nuclei of *Lentinula edodes* in life cycle

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Edible fungi possess biological characteristics that are completely different from those of plants and animals, with the dikaryotic state being one of the most important distinctions. In current genetic research of edible fungi, the dikaryotic state is often considered as a diploid single-nucleus organism, especially in transcriptomic studies. We assembled the two haploid genomes from a commercial strain of *L. edodes* using Illumina, HiFi, and Hi-C technologies. The total genome lengths were 50.93 Mb and 49.80 Mb for the two monokaryons SP3 and SP30, respectively, with each assembled into 10 chromosomes with 99.63% and 98.91% anchoring rates, respectively. Based on the gene structure analysis of two genomes, identifies 11,271 alleles, accounting for 88% of the entire genome. It also analyzes the expression differences of alleles throughout the life cycle. At the nuclear level, no preference for allelic expression was observed in all stages, but there were preference differences among chromosomes. Meanwhile, the alleles significantly associated with the respective stages exhibited expression preference. During the dikaryon growth and brown film formation, there were no significant preference. However, the two nuclei exhibit different roles. Cis-element analysis indicated that environmental factors like light, oxygen, temperature, as well as circadian clock and phytohormones, regulated the development of *L. edodes* mycelium and fruiting bodies. A total of 5,457 karyotypes were identified as exhibiting a preference ($\log_2$ ratio >1 or $\log_2$ ratio <-1) across all stages of dikaryon 3×30 . Among the identified preferred karyotypes, 178 pairs exhibited a preference for SP3, 200 pairs for SP30, and 2028 pairs demonstrated various preferences at all stages. Both cis- and trans-regulation might be important reasons for the expression differences of allele between the two nuclei. This study helps deepen the understanding of the biological properties of dikaryotic edible fungi and the corresponding mechanisms of internal and external environments during the development of *L. edodes*.

Keywords: *Lentinula edodes*, dikaryon, genome, differential expression alleles, life cycle

Abstract

Microbial Growth Dynamics in Banana Soil: Impact of Fungicides on Fungi-Fungi Interaction

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Banana wilt, primarily caused by the soil-borne fungus *Fusarium oxysporum*, remains a major threat to banana production worldwide. In the Philippines, approximately 18,000 hectares of banana plantations in Mindanao, the region with the largest banana plantations in the country, are affected. Soil also accumulates chemical residues such as fungicides that affect the diverse microorganisms influencing plant health. These chemicals can alter microbial interactions in the soil. To evaluate the effects of environmentally relevant concentrations of the fungicide propiconazole on fungal interactions, we established two experimental setups: (i) direct contact, allowing close proximity, and (ii) non-contact, simulating distance-mediated interactions. We collected soil samples from a banana plantation in Mindanao and isolated soil microorganisms, including *Fusarium* spp. and *Trichoderma* spp. We hypothesized that *Trichoderma* sp. would inhibit *Fusarium* sp. growth both by direct competition and indirectly through volatile-mediated effects, and that these responses would be modulated by fungicide presence. Our results showed that both propiconazole (p value = 5.514×10^{-9}) and interaction setup (solo, contact, non-contact; p value = 0.03102) significantly influenced colony extension rates, while no significant interaction between these factors was observed. *Trichoderma* sp. exhibited a slight growth increase in the nocontact setup without fungicide. In contrast, *Fusarium* sp. showed stimulation in the no-contact setup, with or without propiconazole, and the fungicide effect was significant only under this setup (p value = 0.0194). Comparisons revealed significant differences between contact and non-contact setups, and between solo and non-contact conditions, irrespective of fungicide treatment. Our findings will then be correlated with the soil physicochemical parameters (pH, water-holding capacity, hydrophobicity, aggregation, and texture). These findings highlight how fungicides and spatial arrangements shape fungal interaction dynamics, with implications for managing soilborne pathogens in banana cultivation.

Keywords: agrochemicals, fungal interaction, propiconazole, soil dynamics, soil fungi

Abstract

Systematic knockout efforts highlight conserved transcription factors regulating the initiation of mushroom formation in *Coprinopsis cinerea*

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Mushroom development starts with the formation of hyphal knots (HKs), which represent the transition from vegetative to reproductive growth, and is one of the most complex morphogenetic processes in fungi. To investigate the gene regulatory networks (GRN) underlying HK formation, we systematically knocked out 37 conserved transcription factors (TFs) in the *Coprinopsis cinerea* AmutBmut #326 strain. Most of these TFs are up-regulated during mushroom initiation. The mutants' phenotypes range from the lack of HK formation to arrest at diverse developmental stages, or the production of dense HKs and primordia. Among them, six TFs' mutants showed healthy vegetative mycelia but no HK formation. To further test their function, we examined genetic interactions between six shortlisted TFs. Constitutive expression of one TF (by the *gpd* promoter), referred to as *fluffy1*, in the wild-type strain could produce HK rings precociously, without starvation induction. Overexpression of two other TFs, *Nhk1* and *Nhk2* (no hyphal knot 1 and 2), in other mutants partially or completely restored HK formation, suggesting that these TFs play a key role in the pathway. We also identified two apparent repressors of HK formation. Knockout mutants of one of these, called *Dpr1* (dense primordia 1), could produce dense HKs and primordia without light induction, even under the suboptimal temperature conditions (37 °C), at which the WT does not. Therefore, *Dpr1* may act as a key upstream repressor to regulate HK formation in suitable environments. Combined with RNA-seq data, we inferred a gene regulatory network underlying mushroom initiation in Agaricomycetes. Our work provided new insights into mushroom development at the molecular level and also provides potential benefits for genetic modification and strain breeding.

Keywords:

Basidiomycota, CRISPR/Cas9, Evolution, Morphogenesis

Abstract

Insight into the Etiology of Cherry Diseases in China

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Sweet cherry (*Prunus avium* L.) is an important fruit crop in China. With the continuous expansion in cultivation area and production, fungal pathogens have become a prevalent challenge in fruit production and quality. This study aims to understand the disease occurrence and to identify main disease and pathogens associated with major sweet cherry producing regions in China. Diseases investigations were carried out in Beijing, Guizhou, Hebei, Liaoning, Shandong, Sichuan and Zhejiang in China. The putative pathogens were identified through morphology and multi-gene phylogenetic analysis, with pathogenicity tests. Cherry leaf spot and gummosis are dominant diseases in the open field, while stem canker and gray mold are major concern in greenhouse. Wood rot, plaster disease, root rot and brown rot are also common fungal diseases of cherry. From leaf samples, 550 isolates were identified as 21 genera with *Alternaria* (31.8%), *Colletotrichum* (27.3%), *Cladosporium* (7.1%), *Phoma* (6.2%), *Fusarium* (5.3%) as top five genera. From wood samples, 255 isolates were identified as fifteen genera, with *Alternaria* (28.9%), *Cladosporium* (25.1%), *Fusarium* (11.9%), *Botryosphaeria* (9.8%) and *Nothophoma* (8.2%) as top five genera. Pathogenicity of *Colletotrichum*, *Botryosphaeria*, *Diaporthe* and *Fusarium* species were confirmed in detached cherry leaves and shoots, while remaining species are still under investigation. Based on our observations with the expansion of the cultivation area and the growth of tree age, the occurrence disease and diversity of pathogens will be varied and complicated. This study will lay a foundation with theoretical basis for the effective diagnosis, as well as green prevention and control of cherry diseases.

Keywords: Cherry disease, fungal pathogen, morphology, phylogeny, pathogenicity

Abstract

The diversity of Xylariales in China

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This work highlights the newly described taxa and expanded range records of Xylariales from China, while resolving nomenclatural inconsistencies through type specimen examinations. This unified taxonomic resource advances both mycological systematics and conservation prioritization by providing: (1) a verified reference baseline for Chinese Xylariales, (2) evidence-based genus delimitation criteria, and (3) documentation of endemic lineages with pharmaceutical potential. Our annotated checklist synthesizes records of 655 Xylariales species across genera reported until August 2025, incorporating taxonomic revisions from historical collections, contemporary field studies, and curated databases including Index Fungorum. Each entry combines morphological descriptions with ecological metadata, molecular phylogenies, geographic distributions, and applied biological data (pathogenicity reports and bioactive compound profiles). We aim to establish a standardized taxonomic framework for Xylariales species documented in China, integrating multidisciplinary data to create a comprehensive species inventory.

Keywords: Xylariales, China, Taxonomy, Phylogeny, Metabolites

Abstract

Studies on Macrofungi Diversity of Nyáng basin in Xizang**Congcong Liu ^{1,2}, * Yonghong Zhou ¹, #**¹*Key Laboratory of Biodiversity and Environment on the Qinghai-Xizang Plateau, Ministry of Education, Xizang University, 850000 Lhasa, China*²*Xizang Museum of Natural Science, 10 Zangda East Road, Lhasa, 850000, Xizang, China**zyh800623@163.com*

Nyáng River, situated in the Nyingchi Prefecture of southeastern Xizang, drains a topographically complex catchment that encompasses a wide spectrum of ecological habitats. This heterogeneity has fostered exceptionally rich assemblages of flora, fauna, and microorganisms, including a highly diverse macrofungal community. Here we present a taxonomically rigorous inventory of macrofungi collected throughout the Nyáng watershed and analyze familial, generic, and species composition to elucidate the mycogeographical affinities of the basin. The principal findings are:

1. A total of 2,236 sporocarp specimens were collected across the Nyáng River watershed and critically identified by integrating detailed morphological assessment with molecular identification, yielding 465 species distributed among 177 genera, 70 families, 21 orders, 7 classes, and 2 phyla, underscoring exceptional macrofungal species richness.
2. Eleven families dominate the flora, accounting for 291 species (62.45 % of the total). The most speciose lineages are Russulaceae, Cortinariaceae and Polyporaceae. At the generic level, 19 genera—led by *Cortinarius*, *Russula* and *Inocybe*—comprise 229 species (49.25 %).
3. Excluding cosmopolitan taxa, the generic flora is predominantly temperate in distribution: 35 genera (20.35 %) exhibit a North-temperate pattern, whereas 24 genera (13.95 %) display a European–North American disjunction. Similarity analyses with adjacent regions indicate that the monsoonal climate and pronounced elevational vegetation belts strongly shape the macrofungal biogeography of the watershed.

The Nyáng basin is ecologically fragile, and construction of the Yaxi hydropower complex has already commenced. Timely, comprehensive biodiversity inventories are therefore urgent. This study provides the first systematic baseline data for macrofungi in the region, furnishing both a scientific framework for conservation prioritization and a reference for sustainable utilization of fungal resources in southeastern Xizang.

Keywords: *Nyáng basin; Macrofungi Species diversity; Mycogeographical affinity*

Abstract

Phylogeny and species diversity of the family Phyllostictaceae in South Africa, America and other countries, with the descriptions of *Neophyllosticta* gen. nov., one epitypification and thirteen new species**Cheng Li (1), George, Carroll (2), Lei Cai (3), Qian Chen (1#)**

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Abstract: Species within the genus *Phyllosticta* (Phyllostictaceae, Botryosphaerales) occurred as pathogens, endophytes and saprobes on a wide range of hosts. Since the resurrection of the *Phyllostictaceae* by Wikee (2013), the *Phyllosticta* has been consistently accommodated within this family. In the present study, we investigated 72 *Phyllosticta*-like isolates from South Africa, America, Canada and other three countries. Through multi-locus phylogenetic analyses using ITS, LSU, *tefl*, *act* and *gpdh* sequences, a distinct lineage sister to *Phyllosticta* was recognized, which represented a novel genus *Neophyllosticta*, comprising two new species and one new combination, and therefore a total of three genera were accepted in Phyllostictaceae, i.e., *Phyllosticta*, *Neophyllosticta* gen. nov. and *Pseudofusicoccum*. The phylogenetic and Maximum Clade Credibility (MCC) analyses of the order Botryosphaerales confirmed the placement of the newly established genus *Neophyllosticta* within the family Phyllostictaceae. Furthermore, an epitype of *P. podocarp*i was designated and 11 new species in *Phyllosticta* were introduced based on the combination of phylogenetic analyses and morphological features.

Key words: Botryosphaerales, novel taxa, *Phyllosticta*, Phylogenetic analyses, Taxonomy

Abstract

Research on *Ganoderma sichuanense* Image Enhancement and Phenotypic Big Data Analysis for Precision Breeding

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In the context of modern industrialized and intelligent edible mushroom cultivation, the reliance on machine vision technology enables efficient collection and analysis of phenotypic data throughout the growth cycle of *Ganoderma sichuanense* cultivation. In the *Ganoderma* industry, precision breeding, yield prediction, and quality control are realized, thanks to the application of core technologies such as big data and artificial intelligence. However, non-ideal lighting conditions such as low light, overexposure, and shadows are common in the greenhouse environment for *Ganoderma sichuanense* cultivation, leading to image degradation issues such as blurred edges, texture loss, and color distortion. These issues decrease the robustness of target detection models, causing missed detections, false detections, and localization errors, which limit the reliability and intelligent management of phenotypic data analysis.

This study focuses on the quality optimization of *Ganoderma sichuanense* images under abnormal lighting conditions, aiming to enhance image lighting robustness and recognition accuracy through technological innovation, providing key technical support for precision breeding and industrial upgrading of *Ganoderma sichuanense*.

In collaboration with Zhejiang Shouxiangu Pharmaceutical Co., Ltd., we have constructed a phenotypic data collection system based on our independently developed *Ganoderma sichuanense* image collection equipment. This system systematically collects phenotypic image data throughout the entire growth cycle of *Ganoderma sichuanense*, establishing an integrated "*Ganoderma sichuanense* Phenotypic Image Data Collection and Analysis System" that provides a high-quality data foundation for subsequent image processing and model training.

In the development of the quantitative exposure correction model, a local-global feature fusion exposure correction AI model is proposed. Through multi-scale detail fusion and global feature enhancement mechanisms, it adaptively recovers the color and texture information of *Ganoderma sichuanense* images under abnormal lighting conditions. The model adopts a lightweight structure design, with a total of only 71,826 parameters, significantly lower than existing complex models. It balances accuracy and computational efficiency, adapting to the needs of edge computing scenarios in greenhouses.

Experimental results show that the proposed model significantly improves image quality. In terms of Peak Signal-to-Noise Ratio (PSNR), its performance has increased by 0.74 dB compared to the baseline model. This effectively enhances image detail recovery and noise suppression capabilities, laying a high-quality visual foundation for subsequent phenotypic feature extraction and thereby enhancing the robustness of the detection model. After processing with this model, the detection

accuracy of YOLOv11 for *Ganoderma sichuanense* fruiting bodies increased by 8.3%, directly alleviating the problems of missed and false detections caused by non-ideal lighting, validating the positive gain of image enhancement technology on target recognition performance.

This study has overcome the technical issues associated with phenotypic data collection in traditional cultivation of *Ganoderma sichuanense*, which are affected by lighting disturbances. It has developed a lightweight, high-precision image enhancement model, providing a new paradigm for real-time processing of low-quality images in agricultural scenarios. Through the analysis of growth phenotypic feature differences, it assists in selecting high-yield and disease-resistant superior *Ganoderma sichuanense* varieties, promoting the process of precision breeding. It provides reliable phenotypic data support for the intelligent cultivation of *Ganoderma sichuanense*, aiding the industry's shift from experiential management to data-driven transformation, and collectively enhancing the quality and efficiency of the *Ganoderma* industry. The outcomes of this research offer a scalable technical framework for the intelligent breeding and cultivation of edible fungi.

Keywords: Artificial intelligence; Fungal phenomics; Intelligent breeding

Abstract

Elucidate the molecular mechanism by which the MAPK kinases *Fus3/kss1* in *Ganoderma lucidum* positively regulate MeJA-induced triterpenoid biosynthesis through phosphorylation of *bHLH5*.

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Ganoderma triterpenoids are the main pharmacologically active components of *Ganoderma lucidum*. However, their natural content is relatively low, which severely restricts their application. Methyl jasmonate (MeJA), as a highly effective elicitor, can significantly increase the content of *Ganoderma* triterpenoids. Therefore, this study focuses on the analysis of the signaling pathway induced by MeJA. The research found that the *bHLH5* transcription factor can be significantly up-regulated in response to MeJA induction. Further functional studies showed that the triterpenoid contents in three *GlbHLH5* over-expression strains increased significantly ($P < 0.05$) by 45%, 37%, and 21% respectively, while those in three *GlbHLH5* silenced strains decreased significantly ($P < 0.05$) by approximately 24%, 31%, and 19% respectively, confirming its core regulatory role. At the molecular mechanism level, yeast two-hybrid, BiFC, and GST pull-down experiments verified that the MAPK kinases *Fus3/Kss1* (key factors in the fungal exogenous hormone response) interact with and phosphorylate *bHLH5*. After activation, through molecular docking, yeast one-hybrid, and EMSA, it was confirmed that *bHLH5* can specifically bind to the promoters of key genes (*SE*, *LS*, *PMK*) involved in triterpenoid synthesis and drive their expression. The elucidation of the *Fus3/Kss1* - *bHLH5* - *SE/LS/PMK* pathway comprehensively reveals the molecular mechanism network of MEJA-induced triterpenoid synthesis at both the functional and mechanistic levels, providing a solid theoretical basis and key targets for enhancing the yield of *Ganoderma* triterpenoids through targeted regulation of *bHLH5*.

Keywords: *Ganoderma* triterpenoids, functional verification, phosphorylation, target gene regulation

Abstract

Phylogeny and Toxin Profile of Microfungi associated with Chili peppers in Guangzhou

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Chili peppers (*Capsicum annuum*) are among the most widely consumed spices worldwide and constitute a vital agricultural commodity in China, where annual production exceeds 18 million tons. Beyond their culinary and economic importance, peppers contribute to rural livelihoods and global trade. However, their cultivation and postharvest handling are increasingly threatened by fungal pathogens and associated mycotoxins, which compromise both yield and food safety. Dried peppers are particularly vulnerable to contamination due to extended storage under warm and humid conditions, yet systematic comparisons of fungal communities and toxin burdens between fresh and dried peppers remain scarce. To address this gap, extensive market surveys were conducted between 2022 and 2023 in Guangzhou. More than 200 symptomatic fresh chili samples and dried chili samples were collected and subjected to fungal isolation, morphological characterization, and multilocus sequencing (ITS, TEF, RPB2, TUB2, CMD). Phylogenetic analyses using Bayesian inference and maximum-likelihood methods enabled precise taxonomic identification, while LC-MS/MS assays quantified key mycotoxins. The study uncovered a diverse fungal community dominated by *Fusarium* and *Penicillium*. Identified *Fusarium* species included *F. annulatum*, *F. commune*, *F. concentricum*, *F. fujikuroi*, *F. verticillioides*, *F. ramsdenii*, and notably *F. tardichlamydosporum*, which was reported for the first time from chili peppers, expanding its known host range beyond bananas. Other isolates included *Penicillium citrinum* and *P. steckii*. Mycotoxin profiling detected ochratoxin A (OTA), deoxynivalenol (DON), aflatoxin B1 (AFB1), zearalenone (ZEN), fumonisin B1 (FB1), and citrinin. Dried peppers consistently harbored higher toxin concentrations than fresh samples, with AFB1 frequently exceeding international safety limits. The frequent co-occurrence of multiple toxins in single samples poses an elevated public health risk due to potential additive or synergistic toxic effects. This work highlights chili peppers as a high-risk crop for fungal colonization and mycotoxin contamination. The emergence of *F. tardichlamydosporum* underscores the dynamic ecology of pathogens under global trade and

climate pressures. Effective mitigation requires integrated strategies, including resistant cultivars, optimized drying and storage, biological control, and HACCP-based monitoring. Collectively, these findings provide essential insights for ensuring food safety, sustainable agriculture, and public health protection.

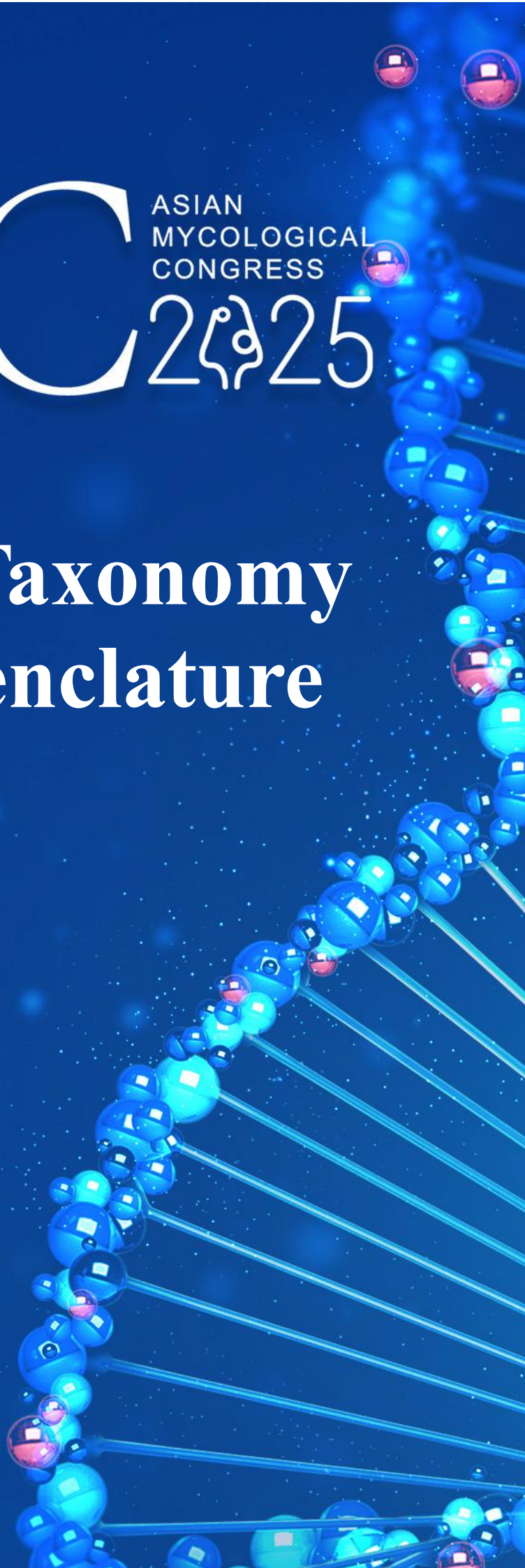
Keywords: *Capsicum annuum*, *Fusarium* spp, Mycotoxin, *Penicillium* spp.

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Session 1 Taxonomy and Nomenclature



Abstract

Linking Historical Types with the Current Fungal Taxonomy

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Fungi are among the most widely distributed organisms on our planet and play irreplaceable roles in the earth ecosystems. It is generally accepted that more than 90% of fungal species still remain unknown to science. Thus, identifying and naming fungi is huge amount of work and an arduous task. With molecular techniques, more and more taxa can be recognized and delimited relatively quickly and easily compared with the classification of the last centuries. However, many (probably) common species were described in the past and those potential new taxa on the molecular tree may have already been described. In many groups of fungi, species complexes with diverse similar taxa, are present. Without studying the types of the described species, it is often impossible to determine the species at hand. For nomenclatural stability, mycologists should try their best to link historical types with the current taxonomy and to avoid publication of invalid, unnecessary and illegitimate names, while curators should encourage mycologists to study the types housed in their fungaria, including allowing the generation of DNA sequences from types specimens. DNA sequences from the type species, as a “standard references,” can provide unambiguous information for the delimitation and identification of fungal species. Although herbaria and fungaria house a huge number of type specimens worldwide, types of numerous species lack DNA sequence data. To obtain high-quality DNA from historical types is often a challenging task due to DNA degradation in type specimens. With the continuous development of next-generation sequencing technology, sufficiently long DNA sequences have been successfully extracted from many historical specimens using techniques such as low-cost genome skimming. There are numerous single-collection-based new species in the literature. To understand the range of variation a of species correctly and precisely, and to avoid superfluous names, population concepts should be applied in fungal taxonomy rather than to individual collections.

Keywords: DNA sequences, genome skimming, nomenclature stability, population concepts

Abstract

Re-evaluation of *Diaporthe* and Clarifying the Distinctive Boundaries between Species and Species complexes.

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Diaporthe is an important genus of plant pathogens that also contains saprobes and endophytes. It was discovered that many *Diaporthe* species with similar morphologies were genetically different. Taxonomy of *Diaporthe* mostly relies on host relationships, morphological characteristics, and multi gene phylogeny, which is flawed and may overestimate or underestimate the number of species in this significant pathogenic genus. Prior to this study, there were no established boundaries for the recognized species. Thus, single gene phylogenies (ITS, *tef*, *tub*, *cal*, and *his*), multi-gene phylogeny, and coalescence-based models (PTP – Poisson Tree Processes and mPTP – multi-rate Poisson Tree Processes) are used to reorganize the species of *Diaporthe*. Multi-gene phylogenies are justified by applying GCPSR (Genealogical Concordance Phylogenetic Species Recognition) methodology. Based on all available type isolates, *Diaporthe* is divided into seven sections, with boundaries proposed for 13 species and 15 species-complexes. This re-evaluation of the genus was supported by an analysis of eighty-two *Diaporthe* isolates obtained from woody hosts in Guizhou Province, China. The findings revealed the existence of 17 previously identified species in addition to two new species. The synonyms for thirty-one species have been established based on DNA data and morphological research. By splitting *Diaporthe* into multiple sections based on phylogenetic analyses, it would be able to avoid producing long phylogenetic trees of the entire genus in future taxonomic research. In other words, when performing research connected to *Diaporthe*, only species from the relevant section should be selected for phylogenetic analysis.

Keywords: Coalescence-based models • GCPSR • PTP/mPTP • Species/species-complexes • species boundaries

Abstract

An Updated Molecular Phylogeny and Chinese Species Diversity of Tricholomataceae (Agaricales)

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Fungi of the family *Tricholomataceae* are of great ecological and economic importance. Most species in this family are ectomycorrhizal fungi that play a crucial role in tree growth. Many species, such as *Tricholoma caligatum* and *T. matsutake*, are highly regarded delicacies, while others, like *T. highlandense* and *T. pardinum*, are poisonous to humans. However, limited taxon sampling and insufficient molecular data have hindered our understanding of the species diversity of *Tricholomataceae* in many regions, including China. In this study, 276 specimens of *Tricholomataceae* from China were analyzed using integrated approaches, including phylogenomic data, multi-gene phylogenetic evidence, morphological characteristics, and ecological features. Phylogenetic analysis of the concatenated dataset of nrLSU/ITS/nrSSU/RPB2 revealed that *Tricholomataceae* s. str. forms a monophyletic group, comprising 11 genus (*Albomagister*, *Corneriella*, *Dennisiomyces*, *Dermoloma*, *Leucopaxillus*, *Neodermoloma*, *Porpoloma*, *Pseudobaeospora*, *Pseudoporpoloma*, *Pseudotricholoma* and *Tricholoma*). Among these, *Tricholoma* is the largest genus, accounting for about 90% of the species within the family. Phylogenomic analysis utilizing 1,477 single-copy orthologous genes indicated that *Tricholoma* comprises five subgenera (Subgen. *Tricholoma*, Subgen. *Pardinicutis*, Subgen. *Sericeicutis*, Subgen. *Terrea*, and *Melleum* clade) and 15 section-level clades (Sect. *Tricholoma*, Sect. *Fucata*, Sect. *Genuina*, Sect. *Matsutake*, Sect. *Muscaria*, Sect. *Rigida*, Sect. *Pardinicutis*, Sect. *Lasciva*, Sect. *Sericella*, Sect. *Atrosquamosa*, Sect. *Terrea*, Clades A, B, and C, and *Melleum* clade), with phylogenetic relationships among these clades fully resolved. Detailed morphological studies allowed for the identification of possibly synapomorphies of *Tricholoma* and diagnostic characteristics for each subgenus and section. From China, 82 species of *Tricholomataceae* were documented, including 24 novel species and 58 known species from five genera. These findings were based on multi-gene analysis (ITS/RPB2/MCM7/EF1- α /mtSSU), ITS phylogenetic inference, morphological characteristics, and ecological data. Among the 82 *Tricholoma* species recognized in China, 52 were originally described from East Asia, while 30 originated from Europe or North America, suggesting that over 60% of the family's species are likely endemic to (East) Asia.

Keywords: morphological characteristics, single-copy orthologous genes, species diversity, tricholomatoid fungi

Abstract

Mushroom diversity in rehabilitated mine and community forests of Mae Sot District, Thailand

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This study investigated mushroom diversity in the Phadang Mine rehabilitation area and adjacent community forests in Mae Sot District, Tak Province, Thailand. Field surveys were conducted during the rainy seasons of 2024–2025 across nine forest plots representing mixed deciduous, dry evergreen, and dipterocarp ecosystems. A total of 156 specimens were collected and identified as 59 species from 47 genera through a combination of morphological and molecular analyses. The findings revealed a rich fungal community, including 33 edible, 10 medicinal, and 16 ecologically or economically important species. Notable taxa included *Amanita hemibapha*, *Astraeus odoratus*, *A. sirindhorniae*, *Pleurotus djamor*, *Pleurotus pulmonarius*, *Russula sribuabanensis*, *R. pseudomodesta*, *Schizophyllum commune*, *Termitomyces entolomoides*, *T. fuliginosus*, and *T. microcarpus*, while three novel *Termitomyces* species were discovered. The project also established a digital database of mushroom diversity to support taxonomy, conservation, and community education. Beyond scientific documentation, the results demonstrate the role of fungal resources in advancing Thailand's bio-circular-green (BCG) economy through conservation, sustainable harvesting, and potential development of mushroom-based products. These findings highlight the importance of rehabilitated ecosystems as reservoirs of biodiversity and as drivers of local bioeconomic opportunities.

Keywords: BCG economy, community forest, mushroom diversity, rehabilitated mine

Abstract

Recent advances in the Taxonomy, Systematics and Nomenclature of *Trichoderma*: A Pan-Asian Perspective**Entaj Tarafder**^{1*#}

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Trichoderma species (Hypocreaceae, Sordariomycetes) represent a globally important genus of Ascomycetes fungi, widely recognized for their ecological versatility and industrial applications in biocontrol, enzyme production, and plant growth promotion. Due to its high biodiversity, varied environments, and increasing molecular studies, Asia has become a hotspot for the discovery of new *Trichoderma* taxa in recent decades. The aim of this work is to summarize recent systematic and taxonomic developments of *Trichoderma* in various Asian environments. We used an integrative method that included phylogenetic analyses based on ITS, *tefl*- α , and *rpb2* data with conventional morphological characteristics. Resolving taxonomic difficulties within species complexes like *T. harzianum*, *T. virens*, and *T. koningii* was given particular attention. Our findings demonstrate the difficulties in species delimitation based on by morphological plasticity, cryptic diversity, and possible horizontal gene transfer. Species boundaries and evolutionary relationships have been further revealed using genome-level data and ecological data. Notably, biogeographic analysis suggests region-specific clades and endemism in tropical forests, mangroves, and agroecosystems across Southeast and South Asia. This study underscores the critical need for regional collaboration, robust culture repositories, and standardized taxonomic frameworks to support the accurate identification, sustainable application, and conservation of this critical fungal genus.

Keywords: *Trichoderma*, integrative taxonomy, phylogeny, Asia mycobiota, species delimitation

Abstract

gcFungi: a data portal for erasing taxonomic gaps and advancing fungal genomics research**Junmin Liang^{*,1}, Qinglan Sun², Linhuan Wu^{#,2}, Lei Cai^{#,1}***Affiliation1: State Key Laboratory of Microbial Diversity and Innovative Utilization, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, China**Affiliation2: Microbial Resource and Big Data Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China**Email of the corresponding author cail@im.ac.cn; wulh@im.ac.cn*

The Global Catalogue of Fungal Genomes (gcFungi, <https://nmdc.cn/gcfungi/>) now unites 20,278 public genomes (spanning 5,230 named species) with 1,168 newly sequenced type-derived genomes generated by the Chinese Microbial Type-strain Genome (CMTG) project. This release closes genomic representation gaps for 2 classes, 18 orders, 64 families, and 195 genera that previously lacked any reference sequence. Every genome has been uniformly de-contaminated and comprehensively functionally annotated, enabling systematic surveys of current sequencing efforts, in-depth genomic and pan-genomic analyses across phylogenetically and ecologically diverse lineages, and direct access to integrated bioinformatics tools for comparative and functional studies.

Keywords: Annotation pipeline, Fungal tree of life, Genome statistics, Type-derived genomes

Abstract

Inferring Host Preferences of Palm Fungi: Insights from PalmFungi.org and the Palm Fungus Diversity Survey

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Palms (*Arecaceae*) are of broad economic and ecological importance and are widely distributed across tropical and subtropical regions. The fungal communities associated with palm trees, often referred to as "palm fungi," were first documented by Kunze and Schmidt in 1817. Currently, over 9,000 fungal records exist in approximately 100 palm genera. These fungi span nearly all major fungal taxa, reflecting the remarkable diversity of palm-associated fungi. Despite this, research on palm fungi remains fragmented and lacks a centralized, up-to-date resource for precise documentation. To address these challenges, we have launched palmfungi.org, an interactive online platform designed to serve mycologists and palm researchers worldwide. This website provides curated data and tools for exploring the diversity of fungi associated with palm trees in different regions. Furthermore, combined with fresh samples collected in southern China and southern Thailand between 2021 and 2025, host information and phylogenetic data from these fungi revealed several distinct palm host-preferential genera among palm fungi. Our research aims to provide a global alliance and sustainable information hub to support all aspects of palm fungal research and shed new light on fungal host preferences.

Keywords: Fungal database, Palm fungi, Phylogenetics

Abstract

Biosurveillance of invasive southern corn rust: insights into recent migration patterns and virulence variation

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Emerging pathogen races spreading via long-distance migration increasingly threaten global agricultural ecosystems. Understanding how pathogens migrate and adapt to new hosts via virulence evolution is crucial for developing strategies to mitigate future crop damage. Here we performed biosurveillance of *P. polysora*, a global fungal pathogen causing southern corn rust, across China, Thailand and the Philippines. By analyzing 193 field transcriptomic data, we detected both epidemic and endemic lineages co-circulating in each country, and elucidated the crucial role of host selection in driving the diversification of endemic lineages. Gene flow assessments and trajectory tracking indicated that the SCR infection source in northern China is likely of domestic origin and pathogen migration from the Philippines/Thailand into China is restricted to Hainan, coastal Guangdong and southern Yunnan. We detected country-specific variants in 32 effector genes, with *AvrRppC* exhibiting the strongest positive selection. A phylogenetically distinct Luzon Island lineage (the Philippines), carrying a novel *AvrRppC* allele capable of overcoming *RppC*-mediated resistance, and represents a potentially invasive threat. Finally, we reviewed the global migration history of *P. polysora* in light of our findings. Our work represents the first step towards establishing an international surveillance network for *P. polysora*, and emphasized a comprehensive control strategy integrating local governance and invasion prevention of international races. This study was financially supported by the Key Collaborative Research Program of the Alliance of National and International Science Organizations for the Belt and Road Regions (Grant No. ANSO-CR-KP-202207).

Keywords: Effector variation, Host selection, Population transcriptomics, Trajectory tracking

Abstract

Unrecorded Species of *Pezizomycotina* Isolated from Freshwater in Korea

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Pezizomycotina is the largest group in the *Ascomycota* phylum, occupying diverse ecological niches including symbiotic relationships, organic matter decomposition and pathogenic interactions with plants and animals. During a survey of fungal diversity in freshwater environments in Korea, nine new records were isolated from freshwater samples collected in Cheongyang-gun, Gumi-si, Gwangju, Gimje-si, Gochang-gun, Jinan-gun, Suncheon-si. Based on morphological characteristics and multi-loci (ITS, LSU rDNA, *BenA*, *CaM*, *RPB2* and *TEF1-α*), the isolates were identified as *Anteaglonium queenslandicum*, *Cladosporium guizhouense*, *Didymella aquatica*, *Fusarium meitneriae*, *Monochaetia hanzhongensis*, *Nothophoma pruni*, *Plectosphaerella endophytica*, *Talaromyces novojersensis*, *Talaromyces udagawae*. These findings contribute to the enhancement of the mycological inventory of Korea and to improving our understanding of fungal diversity in freshwater. Detailed morphological descriptions and phylogenetic trees of these strains will be presented in the poster.

Keywords: New record, Phylogeny, Taxonomy

Abstract

Re-identification of *Penicillium* section *Aspergilloides* in Korean Agricultural Culture Collection (KACC) and Description of Three novel species**Daseul Lee*, Anbazhagan Mageswari, Jae Sung Lee, Donghun Kang, Le Dinh Thao and Seung-Beom Hong#***Korean Agricultural Culture Collection, Agricultural Microbiology Division, National Institute of Agricultural Sciences, RDA, Wanju, Republic of Korea**corresponding author: funguy@korea.kr*

Penicillium section *Aspergilloides* has a worldwide distribution and is commonly found in diverse environments. Taxonomically, section *Aspergilloides* is subdivided into 12 clades comprising 52 species. Since 1995, 34 strains have been collected from different substrates and locations in Korea and deposited in the Korean Agricultural Culture Collection (KACC), National Institute of Agricultural Sciences. These fungal isolates were previously identified mainly based on morphological characteristics, with limited molecular data available. To confirm their taxonomic placement within section *Aspergilloides*, we analyzed DNA sequences of four loci—nuclear ribosomal internal transcribed spacers (ITS), β -tubulin (BenA), calmodulin (CaM), and RNA polymerase II second largest subunit (RPB2)—from the 34 isolates. Molecular phylogenetic analyses revealed that they represent 14 different species: *P. jejuense* (8 isolates), *P. montanense* (1 isolate), *P. kananaskense* (1 isolate), *P. glabrum* (7 isolates), *P. grevilleicola* (1 isolate), *P. austroafricanum* (1 isolate), *P. aurantioviolaceum* (5 isolates), *P. frequentans* (1 isolate), *P. jinfoshanicum* (1 isolate), *P. valentinum* (1 isolate), *P. jiangsuense* (1 isolate), and three novel species (6 isolates). We describe here the distinguishing morphological and molecular features of these three novel species.

Keywords: *Aspergilloides*, KACC, *Penicillium*, Re-identification

Abstract

Hidden diversity uncovered: three new powdery mildew species parasitizing *Juglans regia* in Xizang, China**Li Liu^{1,2}, Shu-Yan Liu^{1,2,#}, Mu Wang³, Qu-Cuo Zhuoma³***1 College of Plant Protection, Jilin Agricultural University, Changchun, China**2 Engineering Research Center of Edible and Medicinal Fungi, Ministry of Education, Jilin Agricultural University, Changchun, China**3 Plant Pathology and Microbiology Laboratory, College of Plant Science, Xizang Agricultural and Animal Husbandry University, Xizang, China**Email: 1459526728@qq.com (L. Liu), liussyan@163.com (S. Y. Liu), wm1252354663@163.com (M. Wang), 563065652@qq.com (Q. C Zhuoma)*

The walnut tree (*Juglans regia*) is native to an area from West Asia to the western Himalayas, and has been introduced to East Asia, South Asia, Central Asia, North Africa, and Europe. To date, two *Erysiphe* species (*Erysiphe juglandis* and *Erysiphe juglandis-nigrae*) and three *Phyllactinia* species (*Phyllactinia juglandis*, *Phyllactinia juglandis-mandshuricae*, and *Phyllactinia himalayensis*) have been documented on *J. regia*. This study collected powdery mildew samples from *J. regia* in the Xizang Autonomous Region of China for molecular and morphological analyses. Besides the known species, two new *Erysiphe* species (*Erysiphe lhokaensis* sp. nov. and *Erysiphe juglandicola* sp. nov.) and one new *Phyllactinia* species (*Phyllactinia qamdoensis* sp. nov.) were identified and described with illustrations. *Erysiphe lhokaensis* and *Erysiphe juglandicola* formed a large clade with *E. juglandis*, suggesting common ancestry. *Erysiphe lhokaensis* differs from *E. juglandis* in having smaller chasmothecia (75.5–110.9 vs. 60–135), fewer terminal branches of appendages (1–2 vs. 1–4), and fewer asci (3–7 vs. 2–12). *Erysiphe juglandicola* is distinguished from *E. juglandis* by its smaller chasmothecia (75.3–111.3 vs. 60–135), fewer appendages (3–10 vs. (2–)3–17), and a reduced number of asci (3–5 vs. 2–12). The main difference between *Erysiphe lhokaensis* and *Erysiphe juglandicola* lies in the length/width ratio of the asci (1.05–1.77 vs. 1.00–1.28). *E. lhokaensis* is restricted to cities of Lhoka and Nyingchi of Xizang, while *Erysiphe juglandicola* was widely distributed in Qamdo (Xizang), Beijing, and the provinces of Shaanxi, Shanxi, Hebei, and Shandong (through the examination of powdery mildew samples from *J. regia* in other provinces), suggesting that the unique geographical environment of Xizang and its isolation from surrounding regions may have played a key role in species differentiation. The *Phyllactinia qamdoensis* collected from walnuts in Qamdo City, Xizang, differed from known species and formed a distinct phylogenetic clade. *Phyllactinia qamdoensis* can be separated from *P. juglandis* based on its fewer appendages (6–14 vs. 5–22), fewer asci (11–20 vs. 6–33), smaller asci (55.3–75.9 × 28.3–40.0 vs. 50–100 × 25–55), longer conidiophores (91.3–265.7 vs. 150), and larger conidia (57.6–185.7 vs. 100). The phylogeny and taxonomy of *Erysiphe* and *Phyllactinia* species on *Juglans* are discussed in detail, and a key to relevant species is provided. By reporting three new species parasitic on *J. regia* in Xizang for the first time, this study significantly expands the understanding of the diversity and phylogeny of powdery mildew on walnuts. It not only provides critical data for research on fungal diversity in the Xizang but also offers important insights into the evolutionary and

geographical distribution patterns of powdery mildew across Eurasia, underscoring the importance of Xizang as a treasure trove of biodiversity and a center of species differentiation.

Keywords: *Erysiphaceae*; *Juglandaceae*; *Juglans*; taxonomic novelties; 3 new taxa

Acknowledgments: This research was supported by the National Natural Science Foundation of China (No. U21A20177).

Abstract

Ramichloridium saxicola (Dissoconiaceae, Mycosphaerellales), A New Rock-Inhabiting Fungus from China

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Ramichloridium is a fungal genus in the family *Dissoconiaceae* (Mycosphaerellales, Dothideomycetes), encompassing diverse morphological traits. Members of this genus have been reported to have various lifestyles, such as phytopathogens, endophytes, lichenicolous, and soil fungi. However, its occurrence as a rock-inhabiting fungus has not been documented. Here, we describe a novel species, *Ramichloridium saxicola*, isolated from rock samples collected from the rocky shore of Huizhou City, Guangdong Province, China. Species delineation was supported by macro- and micro-morphological characteristics, as well as multigene phylogenetic analyses combining the internal transcribed spacer (ITS), large subunit ribosomal RNA (LSU), and translation elongation factor 1- α (*tef1- α*) genes. Pairwise nucleotide comparisons further confirmed species novelty, revealing 7.65% (42/549 bp, including gaps) divergence in ITS and 0.97% (8/822 bp, including gaps) in LSU from the closest relative, *R. eucleae*. This study provides the first report of a rock-inhabiting species in *Ramichloridium*, broadening our understanding of the ecological diversity within this genus.

Keywords: Asexual morph, Dothideomycetes, New taxa, Taxonomy

Abstract

Geographical distribution, host associations and some interesting additions to Aigialaceae, Astrosphaeriellaceae, and Pseudoastrosphaeriellaceae (Pleosporales, Dothideomycetes)**Danushka Sandaruwan Tennakoon^{1*}, Nimali Indeewari de Silva¹, Ning Xie¹, Sinang Hongsanan^{1#}**¹ *Shenzhen Key Laboratory of Microbial Genetic Engineering, College of Life Science and Oceanography, Shenzhen University, Shenzhen 518060, China**Corresponding author: Sinang Hongsanan (E-mail address: sinang333@gmail.com)**Danushka Sandaruwan Tennakoon: danushkasandaruwanatm@gmail.com**Nimali Indeewari de Silva: nimalindeewari@gmail.com**Ning Xie: ning.xie@szu.edu.cn*

China is a global hotspot for fungal diversity, harbors an extensive array of species due to its diverse ecosystems, which range from tropical rainforests to alpine forests with favorable climates. During a survey of plant litter-associated microfungi in Guangdong Province, China, several specimens that have carbonaceous ascomata were collected. Morphological characteristics combined with multi-gene (LSU, SSU, and *tefl-α*) phylogeny revealed that they belong to the Aigialaceae, Astrosphaeriellaceae, and Pseudoastrosphaeriellaceae families. Phylogenetic analyses were conducted using Maximum Likelihood (ML) and Bayesian Inference (BI) approaches. *Caryospora pruni* and *Pseudoastrosphaeriella zingiberacearum* are introduced as new species from *Prunus persica* and *Hedychium coronarium*, respectively. Two new host records, *Astrosphaeriella bambusae* and *Neoastrosphaeriella aquatica*, are also revealed from *Metroxylon sagu* and *Phoenix paludosa*, respectively. All the newly introduced species tally well with their respective generic concepts and can be distinguished from closely related species in their morphology and DNA molecular data. The new host records also provide similar morphological characteristics with their respective type species, and multi-gene phylogeny analyses also offer evidence for their placements. In addition, we compiled geographical distribution and host associations of all the recorded Aigialaceae, Astrosphaeriellaceae, and Pseudoastrosphaeriellaceae species. It provides a database for future studies to understand the ecological interactions and geographical variations.

Key words: Check list, multi-gene phylogeny, new species, new host records, saprobes

Abstract

Exploring Fungal Diversity of the South China Sea Coast

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Coastal regions encompass some of the most biodiverse and unique ecosystems on Earth. China is one of the countries possess numerous sea resources with vast maritime region. The South China Sea is the largest marginal sea in the western Pacific, covering ~3.5 million km² along the continental margin of East Asia and averaging over 2,000 m in depth. The South China coastline harbours different ecosystem types, i.e., coral reefs, seagrasses, mangroves, wetlands, bays, and coastal vegetation. The largest mainland coastline in South China sea can be seen in Guangdong Province which spans along in 14 coastal cities. The persistence of many fungal species relies upon diverse macro and micro habitats in coastal areas. Despite the availability of abundant ecological niches, research on the fungi inhabiting coastal South China region remains limited. Therefore, as a research team at the College of Life Science and Oceanography, Shenzhen University, we investigate fungal diversity from various coastal substrates, including dead plants, soil, and rocks. Currently we have observed numerous plant specimens and revealed 35 new fungal species, 40 new host records, and new collections from Dothideomycetes and Sordariomycetes (Ascomycota). We identified new plant pathogens records and confirmed their pathogenicity via Koch's postulates. Exploration of soil fungal diversity in natural and agricultural areas is another research goal in our team. Our findings show that majority of new fungal taxa and new records of soil inhabitant fungi belongs to Ascomycota. We studied these beneficial soil fungi to promote plant growth and soil health. The coastal environment is one of the extreme environments for rock inhabiting fungi since they are exposed to stresses, such as solar radiation, desiccation and rehydration, and temperature fluctuations. The peculiar morphological features based on melanisation and slow growth of rock inhabiting fungi are important in ecology, and potential in biotechnology. Thus, we conduct research to achieve a better understanding of the hidden biodiversity and their salt tolerant abilities and gene regulation.

Keywords: Plant pathogens, rock inhabiting fungi, saprobes, soil fungi, taxonomy

Abstract

Diversity and Taxonomy of Mucorales (Mucoromycota) in Korea

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Mucoromycota represents one of the most ancient lineages of terrestrial fungi, known for colonizing a wide range of organic and inorganic substrates. Among them, the order *Mucorales* is the largest, comprising over 400 species. Members of *Mucorales* are commonly found in environments such as soil, dung, seeds, and decaying fruits. Many species of them are important in biotechnological areas, such as production of enzymes, lipids and antifungal proteins and can cause life-threatening infections. This study aimed to investigate the species diversity of a basal fungal lineage of *Mucorales* in soil, freshwater and invertebrates samples in Korea. Fungal strains were isolated using dilution plating, baiting, and direct incubation techniques on different culture media. Species identification was performed using a polyphasic approach including phylogenetic analysis of ITS, LSU, *TEF-1α*, *RPB1*, *MCM7*, and *argA* gene sequences, and morphological analyses. A total of 38 species of *Mucorales* were identified, representing the genera *Absidia*, *Backusella*, *Circinella*, *Cunninghamella*, *Lichtheimia*, *Mucor*, *Neofennellomyces*, *Protomycocladus*, *Rhizopus*, and *Saksenaea*. Among these, 15 species are newly recorded in Korea, while 23 are new to science. Notably, our results showed that species diversity is higher in invertebrate samples compared to those from soil and freshwater. Detailed descriptions and illustrations are provided for the newly discovered species.

Keywords: *Mucorales*, Identification, Taxonomy.

Abstract

New Fungal Discoveries from Guangdong, China: *Heterohelminthosporium* gen. nov. (Massarinaceae) and *Zasmidium hydei* sp. nov. (Mycosphaerellaceae)

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Fungi associate with plants through a variety of lifestyles, including as phytopathogens, endophytes, and saprotrophs, highlighting their essential roles in maintaining plant health, facilitating nutrient cycling, and supporting ecosystem stability. This study focused on isolating fungi associated with plants collected from Guangdong Province, China. Two plants, Ti plant showing leaf spot symptoms (*Cordyline fruticosa*, *Asparagaceae*) and screw pine (*Pandanus tectorius*, *Pandanaceae*), were collected from Zhuhai City and Huizhou City, Guangdong Province, China, respectively. From the Ti plant, four fungal strains (MBSZU 25–011 to MBSZU 25–014) were isolated and found to be associated with leaf spot. Based on morphological characteristics and multi-gene phylogenetic analyses of the 28S large subunit (LSU), internal transcribed spacer (ITS), RNA polymerase II second largest subunit (*rpb2*), and actin (*act*) genes, these strains were identified as a new species, *Zasmidium hydei*. Two fungal strains (MBSZU 25–015 and MBSZU 25–016) were recovered as endophytes from screw pine leaves. These strains represented a new genus, *Heterohelminthosporium*, typified by *Heterohelminthosporium hydei* sp. nov. The sexual morph of this genus is characterized by pale brown to brown, fusoid, 1-sepate ascospores lacking a mucilaginous sheath. Phylogenetic analyses using a combination of 18S small subunit (SSU), LSU, ITS, translation elongation factor 1- α (*tefl- α*), and *rpb2* genes revealed that *H. hydei* forms an independent lineage within *Massarinaceae*, sister to the genus *Massarina* (*M. tangneyae* and *M. pandanicola*) with high statistical support. These findings expand our understanding of fungal diversity and evolution, revealing new taxa with important ecological roles in plant-fungus interactions.

Keywords: Fungal identification, Fungal isolation, New taxa, Taxonomy

Abstract

Ecological and Geographical Patterns of Fungal Diversity in Pakistan: Unravelling Habitat-Specific Distribution**Mubashar Raza^{1,*,#}, Rahila Hafeez², Nalin N. Wijayawardene³, Yushanjiang Maimaiti^{1,#}**¹*Key Laboratory of Integrated Pest Management on Crops in Northwestern Oasis, Ministry of Agriculture and Rural Affairs, Institute of Plant Protection, Xinjiang Academy of Agricultural Sciences, Urumqi 830091, China*²*College of Life Sciences and Oceanography, Shenzhen University, Shenzhen 518055, China*³*Center for Yunnan Plateau Biological Resources, Protection and Utilization & Yunnan International Joint Laboratory of Fungal Sustainable Utilization in South and Southeast Asia, College of Biology and Food Engineering, Qujing Normal University, Qujing 655011, China.**Email of the corresponding author: mubasharraza73@yahoo.com*

Fungi play a crucial role in balancing nutritional uptake through various pathways, with their abundance, spatial distribution, and population dynamics being essential components of their ecology. However, the ecology and distribution of fungal species in Pakistan remain underexplored. This study investigates the ecological and geographical variation of fungal species reported from Pakistan, focusing on the hypothesis that fungal species diversity is primarily influenced by differences in habitat conditions. Our analysis revealed that the fungal species in Pakistan belong to 11 phyla, with the majority falling under Ascomycota and Basidiomycota. Some fungi exhibited wide geographic distributions, but species indicator analyses showed that habitat characteristics were key factors in species occurrence. Principal component analysis (PCA) demonstrated that geological location and substrate type (ecological class) explained 72.79% (Eigenvalue = 48.66) and 79.63% (Eigenvalue = 42.62) of the variance, respectively, in PC1 (axis one). These patterns were further confirmed through Nonmetric Multidimensional Scaling (NMDS), which displayed the data in two dimensions. Multivariate analyses consistently linked fungal genera to geological and ecological variables. Fungi associated with rock, air, and water habitats showed low specificity, while significant variation was observed in species inhabiting plants and soil. These results suggest that fungal occurrences are more strongly influenced by habitat-specific conditions than by broad geographic distribution. This study also compiled an updated list of 2,947 fungal species reported from various habitats across Pakistan, accessible through the online portal www.fungiofpakistan.com. The database includes one fungus-like organism, 135 ambiguous species, 597 novel species, and 125 species identified through sequencing data.

Keywords: Biodiversity, Fungal Species, Fungal Habitat Specialization, Geographical Distribution, Principal Component Analysis (PCA).

Abstract

An Updated Molecular Phylogeny and Chinese Species Diversity of Tricholomataceae (Agaricales)

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Fungi of the family Tricholomataceae are of great ecological and economic importance. Most species in this family are ectomycorrhizal fungi that play a crucial role in tree growth. Many species, such as *Tricholoma caligatum* and *T. matsutake*, are highly regarded delicacies, while others, like *T. highlandense* and *T. pardinum*, are poisonous to humans. However, limited taxon sampling and insufficient molecular data have hindered our understanding of the species diversity of Tricholomataceae in many regions, including China. In this study, 276 specimens of Tricholomataceae from China were analyzed using integrated approaches, including phylogenomic data, multi-gene phylogenetic evidence, morphological characteristics, and ecological features. Phylogenetic analysis of the concatenated dataset of nrLSU/ITS/nrSSU/RPB2 revealed that Tricholomataceae s. str. forms a monophyletic group, comprising 11 genus (*Albomagister*, *Corneriella*, *Dennisiomyces*, *Dermoloma*, *Leucopaxillus*, *Neodermoloma*, *Porpoloma*, *Pseudobaeospora*, *Pseudoporpoloma*, *Pseudotricholoma* and *Tricholoma*). Among these, *Tricholoma* is the largest genus, accounting for about 90% of the species within the family. Phylogenomic analysis utilizing 1,477 single-copy orthologous genes indicated that *Tricholoma* comprises five subgenera (Subgen. *Tricholoma*, Subgen. *Pardinicutis*, Subgen. *Sericeicutis*, Subgen. *Terrea*, and *Melleum* clade) and 15 section-level clades (Sect. *Tricholoma*, Sect. *Fucata*, Sect. *Genuina*, Sect. *Matsutake*, Sect. *Muscaria*, Sect. *Rigida*, Sect. *Pardinicutis*, Sect. *Lasciva*, Sect. *Sericella*, Sect. *Atrosquamosa*, Sect. *Terrea*, Clades A, B, and C, and *Melleum* clade), with phylogenetic relationships among these clades fully resolved. Detailed morphological studies allowed for the identification of possibly synapomorphies of *Tricholoma* and diagnostic characteristics for each subgenus and section. From China, 82 species of Tricholomataceae were documented, including 24 novel species and 58 known species from five genera. These findings were based on multi-gene analysis (ITS/RPB2/MCM7/EF1- α /mtSSU), ITS phylogenetic inference, morphological characteristics, and ecological data. Among the 82 *Tricholoma* species recognized in China, 52 were originally described from East Asia, while 30 originated from Europe or North America, suggesting that over 60% of the family's species are likely endemic to (East) Asia.

Keywords: morphological characteristics, single-copy orthologous genes, species diversity, tricholomatoid fungi

Abstract

Re-identification of *Penicillium* section *Aspergilloides* in Korean Agricultural Culture Collection (KACC) and Description of Three novel species**Daseul Lee*, Anbazhagan Mageswari, Jae Sung Lee, Donghun Kang, Le Dinh Thao and Seung-Beom Hong#***Korean Agricultural Culture Collection, Agricultural Microbiology Division, National Institute of Agricultural Sciences, RDA, Wanju, Republic of Korea**corresponding author: funguy@korea.kr*

Penicillium section *Aspergilloides* has a worldwide distribution and is commonly found in diverse environments. Taxonomically, section *Aspergilloides* is subdivided into 12 clades comprising 52 species. Since 1995, 34 strains have been collected from different substrates and locations in Korea and deposited in the Korean Agricultural Culture Collection (KACC), National Institute of Agricultural Sciences. These fungal isolates were previously identified mainly based on morphological characteristics, with limited molecular data available. To confirm their taxonomic placement within section *Aspergilloides*, we analyzed DNA sequences of four loci—nuclear ribosomal internal transcribed spacers (ITS), β -tubulin (BenA), calmodulin (CaM), and RNA polymerase II second largest subunit (RPB2)—from the 34 isolates. Molecular phylogenetic analyses revealed that they represent 14 different species: *P. jejuense* (8 isolates), *P. montanense* (1 isolate), *P. kananaskense* (1 isolate), *P. glabrum* (7 isolates), *P. grevilleicola* (1 isolate), *P. austroafricanum* (1 isolate), *P. aurantioviolaceum* (5 isolates), *P. frequentans* (1 isolate), *P. jinfoshanicum* (1 isolate), *P. valentinum* (1 isolate), *P. jiangsuense* (1 isolate), and three novel species (6 isolates). We describe here the distinguishing morphological and molecular features of these three novel species.

Keywords: *Aspergilloides*, KACC, *Penicillium*, Re-identification

Abstract

Mushroom diversity in rehabilitated mine and community forests of Mae Sot District, Thailand

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This study investigated mushroom diversity in the Phadang Mine rehabilitation area and adjacent community forests in Mae Sot District, Tak Province, Thailand. Field surveys were conducted during the rainy seasons of 2024–2025 across nine forest plots representing mixed deciduous, dry evergreen, and dipterocarp ecosystems. A total of 156 specimens were collected and identified as 59 species from 47 genera through a combination of morphological and molecular analyses. The findings revealed a rich fungal community, including 33 edible, 10 medicinal, and 16 ecologically or economically important species. Notable taxa included *Amanita hemibapha*, *Astraeus odoratus*, *A. sirindhorniae*, *Pleurotus djamor*, *Pleurotus pulmonarius*, *Russula sribuabanensis*, *R. pseudomodesta*, *Schizophyllum commune*, *Termitomyces entolomoides*, *T. fuliginosus*, and *T. microcarpus*, while three novel *Termitomyces* species were discovered. The project also established a digital database of mushroom diversity to support taxonomy, conservation, and community education. Beyond scientific documentation, the results demonstrate the role of fungal resources in advancing Thailand's bio-circular-green (BCG) economy through conservation, sustainable harvesting, and potential development of mushroom-based products. These findings highlight the importance of rehabilitated ecosystems as reservoirs of biodiversity and as drivers of local bioeconomic opportunities.

Keywords: BCG economy, community forest, mushroom diversity, rehabilitated mine

Abstract

Species Diversity of Yeast from Freshwater and Soil Habitats in Korea

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Yeasts are an ecologically and biotechnologically significant group of fungi, yet their diversity across various habitats remains unexplored. During a survey of yeast diversity in soil and freshwater samples collected from different locations in Korea, 19 isolates were obtained using the dilution plating method. Based on morphology and molecular phylogenetic analyses using the ITS region and the D1/D2 domain of the large subunit (LSU) rRNA gene, a total of 13 species were identified. These species belonged to ten genera, including *Apiotrichum*, *Candida*, *Debaryomyces*, *Filobasidium*, *Galactomyces*, *Geotrichum*, *Pichia*, *Rhodotorula*, *Saccharomycopsis*, and *Wickerhamomyces*. Among them, three species in the genera *Debaryomyces*, *Saccharomycopsis*, and *Wickerhamomyces* are proposed as new species. Additionally, three species *Apiotrichum loubieri*, *A. sporotrichoides*, and *Geotrichum sinense* are new records for Korea. This poster provides comprehensive descriptions of the morphological, physiological, and cultural characteristics, as well as the phylogenetic status of representative strains of each species.

Keywords: Diversity, Taxonomy, Yeast

Abstract

***Blumeria xizangensis* sp. nov., a new powdery mildew species on *Roegneria* sp. and *Anthoxanthum odoratum* from Xizang (Tibet) Autonomous Region, China**

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The genus *Blumeria*, containing important cereals and grasses powdery mildew pathogens, has been shown to comprise multiple species through integrated morphological and phylogenetic studies. However, its diversity on grasses in biodiversity hotspots like the Xizang Autonomous Region remains poorly explored. Five powdery mildew specimens on *Roegneria* sp. and *Anthoxanthum odoratum* were collected in Xizang in 2023. Morphological observation revealed that their asexual characteristics are similar to *B. graminicola* in having colourless hyphae. However, multiloci molecular phylogenetic analyses (ITS+28S+IGS+CHS1+Bgt-1929+Bgt-4572) placed them in a clade with three other species: *B. avenae*, *B. dactylidis*, and *B. americana* with strong bootstrap (BS) support (MP/ML = 100%). A common feature among the latter three species is the presence of pigmented mycelium. So, the five specimens represent a novel species, *Blumeria xizangensis* sp. nov., and is described and illustrated based on distinct morphological traits and robust multi-locus phylogenetic analysis. Its hyphal appressoria exhibits greater diversity. They are not strictly nipple-shaped, but more variable, nipple-shaped, somewhat curved, slightly sinuous to somewhat lobate. This discovery not only enriches the known species diversity of powdery mildews on Poaceae in the Tibet region but also provides new taxonomic insights for understanding the evolution and host adaptation of the genus *Blumeria*.

Keywords: *Erysiphaceae*; *Poaceae*; *Blumeria*; Xizang Autonomous Region; new taxa

Funding: This research was supported by the National Natural Science Foundation of China--Regional Joint Fund for Innovation and Development (No. U21A20177).

Abstract

New Species and New Records of *Dothideomycetes* from Korea

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Dothideomycetes is the largest class of *Ascomycota* and found worldwide in terrestrial, freshwater, and marine habitats. During a survey of fungal species diversity in *Dothideomycetes* from soil and freshwater sample in Korea, nine fungal strains were isolated. These strains were identified based on phylogenetic analyses of DNA sequences obtained from the internal transcribed spacer (ITS) region, large ribosomal subunit (28S rDNA), RNA polymerase II second largest subunit (*RPB2*) and the translation elongation factor 1- α gene (*TEF-1 α*), in combination with morphological characteristics. The results revealed seven species belonging to the genera *Anteaglonium*, *Cladosporium*, *Didymella*, *Letendraea*, *Lorrainsmithia*, *Nigrograna*, and *Nothophoma*. Among these, one species belonging to genus *Letendraea* is proposed as new species. In addition, six species, *Anteaglonium queenslandicum*, *Cladosporium guizhouense*, *Didymella aquatica*, *Lorrainsmithia pennsylvanica*, *Nigrograna locuta-pollinis*, and *Nothophoma pruni* are reported for the first time in Korea. Detailed morphological descriptions and phylogenetic trees of these strains will be presented in the poster.

Keywords: *Dothideomycetes*, Diversity, Phylogeny, Taxonomy

Abstract

Antifungal Potential of Endophytic *Diaporthe* spp. from the Medicinal Plant *Pereskia bleo* (Kunth) DC**Ling Yang^{1*}, Chiawei Phan^{1,2#}, Yeeshin Tan^{2,3} and Jaya Seelan Sathiya Seelan⁴**¹Department of Pharmaceutical Life Sciences, Faculty of Pharmacy, Universiti Malaya, 50603 Kuala Lumpur, Malaysia²Mushroom Research Centre, Universiti Malaya, 50603 Kuala Lumpur, Malaysia³Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia⁴Institute of Tropical Biology and Conservation, Universiti Malaya Sabah, 88400 Kota Kinabalu, Sabah, MalaysiaCorresponding author: Chiawei Phan, Email: phanew@um.edu.myYeeshin Tan, Email: tanyeeshin@um.edu.my

Background: Malaysia's remarkable biodiversity provides a rich yet underexplored reservoir of microbial life, particularly endophytic fungi, which hold great promise as natural sources of bioactive compounds. However, research on Malaysian endophytes remains scarce. With the rising global incidence of invasive fungal infections and the limitations of existing antifungal drugs—marked by resistance and toxicity—there is an urgent need to discover novel antifungal agents from natural origins. Motivated by this scientific gap and ecological responsibility, the present study aimed to isolate and identify endophytic fungi from *Pereskia bleo* (Kunth) DC, a medicinal plant widely used in Malaysia, and to evaluate their antifungal potential against opportunistic pathogens *Penicillium sumatrense* and *P. citrinum*.

Methods: Healthy tissues of *Pereskia bleo* were dynamically sampled over a two-month period to isolate endophytic fungi. Six isolates belonging to the genus *Diaporthe* were identified through morphological characterisation and ITS rDNA sequencing, and subsequently selected for functional assessment. Their antagonistic activity against the opportunistic pathogens *Penicillium sumatrense* and *P. citrinum* was evaluated using dual-culture assays, where inhibition rates were quantified and hyphal interaction patterns examined microscopically. In addition, the culture filtrate of the most active strain, *D. biconispora* PBS24-3, was tested for antifungal efficacy, and its secondary metabolites were analysed using UHPLC-Orbitrap HRMS/MS.

Results: A total of 127 endophytic fungal isolates were obtained from *P. bleo*, including *Colletotrichum*, *Diaporthe*, and *Fusarium*. Most *Diaporthe* isolates exhibited strong antagonistic effects against both pathogenic *Penicillium sumatrense* and *P. citrinum*. Notably, *Diaporthe* sp. PBS30-7 and *D. biconispora* PBS24-3 achieved inhibition rates exceeding 65%. The culture filtrate of *D. biconispora* PBS24-3 showed potent antifungal activity, suppressing the growth of *P. sumatrense* by 50.13%, comparable to the positive control itraconazole (54.78%). UHPLC-Orbitrap HRMS metabolomic analysis revealed the presence of multiple diketopiperazine alkaloids—well-recognised antifungal compounds—confirming *D. biconispora* PBS24-3 as a metabolically active endophyte with significant antifungal potential.

Conclusion: This study represents the first comprehensive investigation of *Diaporthe* species isolated from *Pereskia bleo* and demonstrates that *D. biconispora* PBS24-3 produces bioactive diketopiperazine derivatives not characterised by the *Diaporthe* genus. These findings underscore the immense potential of Malaysian endophytic fungi as promising sources of new antifungal compounds and deepen our understanding of the ecological and pharmacological significance of *Diaporthe* species.

Keywords: *Pereskia bleo* (Kunth) DC, Endophytic fungi, *Diaporthe*, Antifungal activity, Diketopiperazine metabolites

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Session 2 Fungal Metabolites and Bioactive Compounds



Abstract

Genome Mining of Phytopathogenic Fungi for Pharmacological Products

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Since 2014, Tom Hsiang of the University of Guelph (Ontario, Canada) has been cooperating with scientists at ECUST (East China University of Science and Technology, Shanghai, China). The expertise of the collaborators at ECUST includes drug discovery from microbials. For example, they have worked with actinomycetes (deep sea, high mountains) for novel natural products. They started genome mining actinomycetes (over 12,000 in mixed batches) for potential pharmacological products. In 2014, they attended a seminar given by Tom Hsiang on fungal genome sequencing, and they became interested in mining fungal genomes. In 2015, T. Hsiang shared the draft genome sequences of two dozen phytopathogenic fungi with them initially, and they found some biosynthetic gene clusters (BGCs) of interest. The researchers discussed further, and the over 450 draft phytopathogenic fungal genomes were shared. Phytopathogenic fungi are in a constant state of warfare with their hosts, and the numerous secondary metabolites they produce, such as terpenoids, may have potent anti-microbial and anti-cancer activity. This presentation details some of the methods and major findings from that work starting with fungal genome sequencing and assembly, and highlighting the novel compounds which may be of pharmacological interest from a wide range of phytopathogenic fungi.

Keywords: fungi, genome mining, pharmaceuticals

Desired Presentation Type: Oral

Submission Topic: 2. Fungal Metabolites and Bioactive Compounds

Abstract

Studies On the Chemical Constituents, Quality Control and Metabolism of *Ganoderma lucidum***Chun-Ru Cheng*, Ting-Chi Wen #**

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Ganoderma lucidum, which has been used in clinic in eastern countries for thousands of years, is an important traditional Chinese medicine. In present study, the chemical constituents, qualitative analysis and *in vivo* metabolism of *G. lucidum* were studied.

Using various chromatographic methods and identification technologies, totally 54 compounds were separated and characterized, including 47 triterpenoids, 4 sterols, and 3 fatty acids. The separated triterpenoids were assayed for their inhibitory activities against human HeLa cervical cancer cell lines using MTT method. Most of the compounds exhibited strong or moderate cytotoxicity, and their structure-activity relationships were discussed.

The fragmentation pathways of triterpenoids from *G. lucidum* were systematically studied using a combination of multistage tandem mass spectrometry experiments and high resolution mass spectrometry. The results indicated that the fragmentation pathways of triterpenoid acids were rather characteristic. The cleavage modes were highly dependent on the position of the carbonyl and hydroxyl groups in the tetracyclic skeleton. Different substitutions on the ring skeleton would result in different fragmentation pathways to form stable ions with highly conjugated systems. These decomposition features are extremely useful in distinguishing the isomers of the above types of compounds and unknowns. Using the HPLC/MSⁿ method and fragmentation pathways, 73 triterpenoids, including 11 new ones, were identified from the MeOH extract of *G. lucidum*. 44 of them were identified by comparison with the standards. The other unknown compounds were tentatively identified using the fragmentation rules. Further more, the triterpenoid components of *G. lucidum* and *G. sinense* have been compared.

In order to study the metabolism of the extract, we first investigated the metabolism of a representative single compound (ganoderic acid D). The results indicated that the biliary rather than renal clearance was the major route of excretion. Using ultraperformance liquid chromatography/quadrupole time-of-flight mass spectrometry, totally 25 metabolites were identified from the bile sample. The metabolic pathways of ganoderic acid D (GD) were proposed and the metabolic “soft spots” were identified. These results were very important in the further metabolism study of the triterpenoid extract. The present study also exhibits the importance of the high resolution mass spectrometry in metabolic study, especially in the correct interpretation of the fragmentation pathways and identification of the unknowns. A LC/MS/MS method of GD in rat plasma was established, and studies on the absorptive kinetics of GD in rats were performed. The

bioavailabilities between general preparation and nanoparticle preparation of GD in rats were compared. The results indicated that the nanoparticle preparation of GD could increase the concentration and bioavailability.

Based on the study of the metabolism of single compounds, we further investigated the metabolism of the triterpenoid extract. Using ultraperformance liquid chromatography/quadrupole time-of-flight mass spectrometry, totally 57 compounds were identified from the bile sample. The results indicated that most of the triterpenoid acids could be absorbed, and excreted as the parent compounds. The hepatocyte metabolism is the major route of clearance for the triterpenoid acids. After absorbing, the triterpenoids could transform to each other through oxidation, reduction, desaturation. However, the total tendency is that the low polar triterpenoid acids transformed into the high polar triterpenoid acids to excrete out of the organism. Like the metabolism of GD, the other transformation of triterpenoid acids included hydroxylation, sulfatation and glucuronide conjugation. A LC/MS/MS method of ganoderic acid D, A, B and E in rat plasma was established, and studies on the absorptive kinetics of ganoderic acids in rats were performed.

In conclusion, through the studies of chemical constituents, quality control and metabolism of *G. lucidum*, we know more about it. The results are very important in the further pharmacological and clinic studies.

Keywords: *Ganoderma lucidum*, Chemical Constituents, Quality control, Metabolism

Abstract

Fungal Solutions to Fungal Problems: Antifungal Compounds from *Coprinus comatus* Targeting Plant Pathogens

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Plant diseases caused by pathogenic fungi remain a significant challenge to global agriculture and food security. Antimicrobial resistance has been observed against our commercially available synthetic fungicides. Addressing this challenge requires the discovery of natural, eco-friendly alternatives from biological resources like mushrooms. In our study, the culture filtrate of the shaggy ink cap mushroom *Coprinus comatus* was subjected to a bioassay-guided chromatographic fractionation of antifungal compounds, leading to the isolation of a novel tricyclic polyketide with a rare furanone-fused chromene skeleton, and a known compound, orsellinaldehyde. The identity of the novel compound was established through comprehensive spectroscopic analyses, including high-resolution mass spectrometry, nuclear magnetic resonance, optical rotation, and computational methods, and was named coprinolide. The inhibitory and fungicidal activity of coprinolide and orsellinaldehyde were evaluated against various economically important plant pathogenic fungi. Coprinolide demonstrated inhibitory activity against five economically important plant pathogenic fungi, with the lowest IC₅₀ value of 7.1 ppm. Orsellinaldehyde exhibited potent *in-vitro* inhibitory activity against different *Colletotrichum* species, with the lowest IC₅₀ value of 0.9 ppm. It also showed *in-vivo* efficacy by suppressing anthracnose lesion symptoms on mango and cucumber fruits caused by *C. gloeosporioides* and *C. orbiculare*, respectively, indicating its potential as a natural fungicide against anthracnose-causing fungi. To investigate their possible mode of action, fungal conidia viability staining assay was assessed using fluorescein diacetate and propidium iodide. The results suggest that both compounds may potentially target the fungal cell membrane. These findings highlight the potential of *C. comatus* as a promising natural source of antifungal agents for sustainable agriculture. The discovery of coprinolide and the demonstrated efficacy of orsellinaldehyde provide valuable leads for the development of environmentally friendly fungicides with promising modes of action.

Keywords: Antifungal compounds, *Coprinus comatus*, Mushroom, Plant pathogenic fungi

Abstract

Evolution of Fungal Fatty Acid Synthases from Primary to Secondary Metabolism

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Fatty acid synthases (FASs) are essential multi-domain enzymes that catalyze fatty acid production in all living organisms. In fungi, distinct FASs not only fulfill primary metabolic roles, producing membrane lipids, but also participate in secondary metabolism (SM), supplying specific acyl-CoA precursors for diverse natural products such as aflatoxins, HC-toxin, and sporothriolide. These SM FASs are typically co-localized with biosynthetic genes in secondary metabolite biosynthetic gene clusters (BGCs). The evolutionary origin of these specialized FASs and their recruitment into SM BGCs remain largely unexplored. In this study, we investigated fungal FAS evolution and functional diversification by combining phylogenetics and comparative genomics across 1,324 Pezizomycotina genomes. Our analyses reveal distinct evolutionary lineages corresponding to primary and secondary metabolism, highlighting gene duplication and co-evolution with associated biosynthetic enzymes as key drivers of functional specialization. This work sheds light on how fungi expand the chemical diversity of their secondary metabolites and provides a framework to understand the evolutionary mechanisms underlying the organization and diversification of SM biosynthetic pathways. Ultimately, insights into FAS recruitment may inform synthetic biology approaches to harness fungal pathways for novel natural products

Keywords: evolution history, phylogenetic analysis, genomic comparison, duplication events, fungal natural products

Abstract

Mechanism of High Polysaccharide Production in *Tremella fuciformis* Spores and the Correlation Between Monosaccharide Composition and *In Vitro* Bioactivities

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Background: *Tremella fuciformis* polysaccharides have attracted considerable attention due to their diverse bioactivities and favorable physicochemical properties; however, their biosynthetic mechanisms and structure–activity relationships remain unclear, which constrains the screening of highly active polysaccharides and the breeding of superior strains. **Methods:** three *T. fuciformis* spores (TYH-SD1, TWW01-AX, and TWW02-TMS) were selected in this study as research objects to analyze their growth characteristics, polysaccharide biosynthetic and metabolic mechanisms, and the relationship between monosaccharide composition and *in vitro* activities. **Results:** both biomass and yields of intracellular and extracellular polysaccharides (IPs and EPs) in TWW01-AX and TWW02-TMS were significantly higher than those in TYH-SD1, reflecting differences in polysaccharides biosynthetic capacity among the spores. Targeted sugar metabolomics revealed the accumulation of monosaccharides associated with polysaccharides biosynthesis in TYH-SD1, which suggested a relatively low metabolic conversion efficiency. Transcriptomic analysis further demonstrated that TWW01-AX and TWW02-TMS were more active in polysaccharide precursor synthesis and carbohydrate metabolism pathways, with significantly higher expression levels of the key enzyme genes UTP-glucose-1-phosphate uridylyltransferase (TF1G000973) and GTP-mannose-1-phosphate guanylyltransferase (TF3G001229) compared with TYH-SD1, which may represent the molecular basis of their high polysaccharide yield. The IPs and EPs from different *T. fuciformis* spores were all composed of mannose (Man), glucuronic acid (GlcA), glucose (Glc), galactose (Gal), xylose (Xyl), and fucose (Fuc), but their molar ratios differed substantially: IPs were Glc-rich (>60 mol%), whereas EPs were Man-rich (>64 mol%). *In vitro* activities evaluation indicated that EPs exhibited stronger DPPH and hydroxyl radical scavenging activities, higher cupric ion-reducing antioxidant capacity (CUPRAC), and superior hypoglycemic activity, while IPs showed higher ABTS radical scavenging activity and ferric ion-reducing power (FRAP). Ridge regression analysis further confirmed that variations in the molar ratios of key monosaccharides had significant effects on the *in vitro* activities of polysaccharides, providing a quantitative basis for elucidating structure–activity relationships. **Conclusion:** this study systematically revealed the metabolic and molecular basis underlying yield differences of *T. fuciformis* spore polysaccharides, identified critical monosaccharide determinants affecting structure–activity relationships, and provided theoretical support for improving polysaccharide yield and quality as well as for the efficient screening of superior strains.

Keywords: *Tremella fuciformis* spore polysaccharides, High-yield mechanism, Monosaccharide profile–*in vitro* activity correlation

Abstract

Quercetin-mediated modulation of host-pathogen interactions in *Aspergillus fumigatus* infection through integrated HPPIN modeling and molecular docking analysis

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Aspergillus fumigatus infection represents a significant threat to immunocompromised individuals, often leading to severe complications, high morbidity, and mortality rates, and imposing a considerable economic burden on healthcare systems. This study aims to explore the therapeutic potential of quercetin, an active compound derived from *Rosa chinensis* Jacq, in combating *A.fumigatus* infection. Employing an integrated approach that combines High-Performance Protein-Protein Interaction Network (HPPIN) modeling, molecular docking studies, cross-species mapping, and molecular dynamics simulations, we identified critical molecular targets and assessed the binding affinity of quercetin. HPPIN analysis revealed key proteins, including Ras homolog gene family member A and guanine nucleotide-binding protein G(i) subunit alpha, as potential therapeutic targets. Molecular docking results demonstrated stable interactions between quercetin and host targets such as the IL-6 receptor and STAT3, indicating its anti-inflammatory and anticancer properties. Cross-species mapping highlighted conserved targets, including SAICAR synthase, elucidating evolutionary relevance. Molecular dynamics simulations indicated that quercetin effectively inhibits SAICAR synthase, revealing its potential in disrupting purine metabolism in fungal pathogens. Furthermore, functional equivalence mapping suggested glutathione reductase *glr1* as a compensatory target for His72Phe mutations in *CatA*, providing insights into overcoming drug resistance. While limitations such as the need for in vitro validation and small sample sizes were acknowledged, our findings underscore quercetin's potential in developing innovative therapeutic strategies against *Aspergillus fumigatus* infections. Future research should focus on clinical validation and the exploration of quercetin derivatives to enhance therapeutic efficacy.

Keywords:Host-pathogen interaction; *Aspergillus fumigatus*; network pharmacology; quercetin; *Rosa chinensis* Jacq

Abstract

Enhanced Antibacterial Activity of Ultrasonic-extracted *Auricularia* sp.: Mycochemistry and Toxicity

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The escalating threat of fatal bacterial infections underscores the urgent need for new antibiotics that combine potency with efficient production. *Auricularia* sp., an edible mushroom valued in traditional medicine, contains diverse secondary metabolites with notable pharmacological properties. However, its antibacterial potential under ultrasound-assisted extraction (UAE) remains largely unexplored. This study aimed to investigate the antibacterial activity of UAE-derived *Auricularia* sp. ethanolic extracts (AEE) against *Staphylococcus aureus* and *Escherichia coli*, compared with maceration-derived extracts. The extracts were also analyzed for molecular spectroscopic characteristics, mycochemical profiles, total phenolic content (TPC), and toxicity. Fruiting bodies were extracted with 95% ethanol via 15-, 30-, and 45-minute ultrasonication (UAE15, UAE30, UAE45) and 72-hour maceration (M72). Antibacterial efficacy was determined using resazurin microplate assays. UV–Vis and Fourier transform infrared (FT–IR) spectroscopy provided molecular spectroscopic data, while thin-layer chromatography (TLC) and TPC analysis were used for mycochemical characterization. Toxicity was assessed using the Brine Shrimp Lethality Assay (BSLA). Among the ultrasonic-extracted AEEs, UAE15 showed the highest antibacterial potency at the shortest extraction time, with minimum inhibitory and bactericidal concentration (MIC/MBC) values of 1.563/3.125 mg/mL for *S. aureus* and 0.195/0.391 mg/mL for *E. coli*, compared with M72 values of 12.50/50.00 mg/mL and 6.250/25.00 mg/mL, respectively. The MIC/MBC ratio of 2 for both pathogens observed in UAE15 indicates strong bactericidal activity. The UV–Vis spectrum of UAE15 exhibited absorption bands between 200 and 700 nm, complementing FT–IR peaks corresponding to hydroxyl (–OH), carbonyl (C=O), aromatic (C=C), amine (C–N), ether (C–O–C), and vinyl/phenyl groups, indicative of polyphenols, flavonoids, tannins, alkaloids, triterpenoids, sterols, and saponins as confirmed by TLC. TPC analysis showed a slightly higher value in UAE15 (228.420 mg GAE/g) than M72 (213.590 mg GAE/g), suggesting that its enhanced antibacterial activity may be due to additional bioactive constituents beyond polyphenols. The BSLA of UAE15 yielded a median lethal concentration (LC₅₀) of 104.573 µg/mL, indicating mild toxicity. Overall, these results demonstrate the superior antibacterial activity of ultrasonically extracted *Auricularia* compounds, with mild toxicity indicating strong bioactivity and potential for optimal antibacterial applications.

Keywords: Antibacterial Activity, *Auricularia* sp., Mycochemical profiling, Toxicity, Ultrasound-assisted Extraction

Abstract

Melatonin-Induced Reprogramming Enhances *Brassica juncea* Plant Defense and Reduces Phytopathogen *Alternaria brassicae* Virulence

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Brassica juncea (Indian mustard, brown mustard, or leaf mustard) is a vital oilseed and vegetable crop belonging to the family Brassicaceae. It is widely cultivated in South Asia, including India, as well as in parts of China and Europe. In India, it is the second most important oilseed crop after groundnut, contributing significantly to edible oil production. Its productivity is severely constrained by *Alternaria brassicae*, a major fungal phytopathogen responsible for significant yield losses worldwide. In this study, we attempted to elucidate the protective role of exogenous melatonin (MT) in reprogramming defense responses of *Brassica juncea* plants, with a particular focus on mitigating the virulence of the fungal phytopathogen *Alternaria brassicae*. The exogenous application of melatonin (200 μ M) markedly reduced disease severity in *B. juncea* by up to 75%. MT treatment delayed infection onset, minimized necrotic lesion development, and strengthened plant defense responses through enhanced chlorophyll and proline accumulation, improved water retention, and reduced oxidative stress.

Biochemical analyses further revealed lower levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content, alongside elevated activities of antioxidant enzymes, confirming MT's role in alleviating oxidative damage. In addition, HRMS-based metabolite profiling of *A. brassicae* grown in the presence of MT demonstrated extensive reprogramming of fungal metabolism, including increased production of antifungal metabolites and reduced levels of antioxidant and indole derivatives, indicating weakened fungal defense system and reduced pathogenicity.

Collectively, these findings provide clear evidence that melatonin exerts contrasting effects on host and pathogen by simultaneously reprogramming plant defense mechanisms and disrupting fungal metabolism. This work establishes melatonin as a promising eco-friendly antifungal molecule, offering a sustainable alternative to chemical fungicides for protecting *B. juncea* and potentially other crops.

Keywords: *Alternaria brassicae*; *Brassica juncea*; Melatonin; Oxidative stress

Abstract

Quercetin-mediated modulation of host-pathogen interactions in *Aspergillus fumigatus* infection through integrated HPPIN modeling and molecular docking analysis**Xiaoyu Gao, Zixuan Wei, Yan MA *#***Department of Dermatology Department, Second Hospital of Shanxi Medical University , Taiyuan ,China**Email of the corresponding author: mayan197522@163.com*

Aspergillus fumigatus infection represents a significant threat to immunocompromised individuals, often leading to severe complications, high morbidity, and mortality rates, and imposing a considerable economic burden on healthcare systems. This study aims to explore the therapeutic potential of quercetin, an active compound derived from *Rosa chinensis* Jacq, in combating *A.fumigatus* infection. Employing an integrated approach that combines High-Performance Protein-Protein Interaction Network (HPPIN) modeling, molecular docking studies, cross-species mapping, and molecular dynamics simulations, we identified critical molecular targets and assessed the binding affinity of quercetin. HPPIN analysis revealed key proteins, including Ras homolog gene family member A and guanine nucleotide-binding protein G(i) subunit alpha, as potential therapeutic targets. Molecular docking results demonstrated stable interactions between quercetin and host targets such as the IL-6 receptor and STAT3, indicating its anti-inflammatory and anticancer properties. Cross-species mapping highlighted conserved targets, including SAICAR synthase, elucidating evolutionary relevance. Molecular dynamics simulations indicated that quercetin effectively inhibits SAICAR synthase, revealing its potential in disrupting purine metabolism in fungal pathogens. Furthermore, functional equivalence mapping suggested glutathione reductase *glr1* as a compensatory target for His72Phe mutations in *CatA*, providing insights into overcoming drug resistance. While limitations such as the need for in vitro validation and small sample sizes were acknowledged, our findings underscore quercetin's potential in developing innovative therapeutic strategies against *Aspergillus fumigatus* infections. Future research should focus on clinical validation and the exploration of quercetin derivatives to enhance therapeutic efficacy.

Keywords: Host-pathogen interaction; *Aspergillus fumigatus*; network pharmacology; quercetin; *Rosa chinensis* Jacq

Abstract

Improved Saccharification and Fermentative Bioethanol production from Waste paper using the White-rot fungus

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White-rot fungi possess both plant cell wall-degrading enzyme activity and ethanol fermentation ability, making them attractive for consolidated bioprocessing. *Schizophyllum commune* NBRC 4928 has been shown to exhibit high ethanol productivity and efficiently convert various monosaccharides, especially glucose. As the biomass feedstock, waste paper was selected because lignin has been largely removed. This study examined a physical pretreatment method to promote saccharification and improve ethanol yield. Waste paper substrates, specifically office paper, newspaper, cardboard, and thermal paper, were used as feedstocks. To enhance ethanol yield, supportive pretreatment strategies were introduced prior to fermentation, involving enzymatic hydrolysis and physical pretreatment such as ball milling to improve saccharification efficiency. *S. commune* NBRC 4928, pre-cultured on PDA medium, was then inoculated into liquid media containing the pretreated substrates and incubated anaerobically. Sugar and ethanol concentrations in the culture supernatants were determined using HPLC. Comparison showed that enzymatic treatment significantly improved saccharification efficiency, resulting in higher ethanol yields than untreated substrates. Ethanol production was achieved from all types of waste paper tested, including office paper, newspaper, cardboard, and thermal paper, and the ethanol yields were comparable among these paper types. These findings indicated that waste paper is a suitable feedstock for consolidated bioethanol production using white-rot fungi. The comparable ethanol yields across different paper types suggest its broad applicability, while the use of waste paper helps overcome the limitations imposed by lignin in lignocellulosic biomass.

Keywords: Bioethanol, white-rot fungus, waste paper, saccharification, fermentation

Abstract

Direct bioethanol fermentation of citrus peel polysaccharides by the white-rot fungus *Schizophyllum commune* NBRC4928

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The White-rot fungus *Schizophyllum commune* NBRC4928 strain exhibits rapid growth, salt tolerance, and strong ethanol fermentation capability. Furthermore, as a white rot fungus possessing lignin-degrading ability, it has the potential to contribute to the entire biomass conversion process—including delignification, saccharification, and fermentation—thereby facilitating the realization of an integrated bioprocess (CBP). In this study, the fermentation performance was evaluated using citrus peel waste, including *Citrus tamurana*, *C. maxima*, *C. sinensis*, *C. paradisi*, and *C. junos*, which are presumed to contain high levels of hemicellulose. The peels were crushed using a cutting mill, and both the peel material and liquid medium were sterilized separately by autoclaving before being combined in glass flasks. Mycelial discs of *S. commune* NBRC4928, pre-cultured on PDA medium, were inoculated into the flasks and incubated under anaerobic conditions with rotary shaking for three weeks. Sugar and ethanol concentrations in the liquid medium were quantified by HPLC, and fermentation efficiency was assessed. Additionally, ethanol fermentation experiments were conducted to enhance sugar utilization by adding polysaccharide-degrading enzymes. *S. commune* NBRC4928 produced ethanol from both polysaccharides and monosaccharides in citrus peels. However, in most cases, the addition of polysaccharide-degrading enzymes decreased ethanol yield compared to fermentations without enzyme supplementation. These findings demonstrate the potential of *S. commune* NBRC 4928 to ferment citrus peels into ethanol, suggesting a promising approach for sustainable bioethanol production. The reduced ethanol yield observed with enzyme addition may be attributable to suboptimal enzymatic treatment conditions, such as pH and medium composition, which require further optimization.

Keywords: *Schizophyllum commune*, Bioethanol fermentation, White rot fungus, Citrus peels

Abstract

From Color to Power: Elucidating and Engineering Fungal Blue Pigment Xylindein for Sustainable Production

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Xylindein is a blue-green pigment produced by *Chlorociboria aeruginascens* and *Chlorociboria aeruginosa*. Its striking colour has long been valued for traditional wood staining, while its remarkable optoelectronic properties enable its use as a natural semiconductor with potential applications in organic solar cells and other sustainable electronic devices. However, production from native cultures is severely limited by the very slow growth of *Chlorociboria* species and the poor solubility of the pigment. To address these challenges, we re-sequenced the genomes of both species and employed genome mining, phylogenetic dereplication, and comparative genomics, combined with RNA sequencing during pigment formation, to identify a candidate biosynthetic gene cluster (BGC). This BGC harbours a non-reducing polyketide synthase (*XLNpks*) co-regulated with eight neighbouring genes, including two fatty acid synthases (*XLNfas1* and *XLNfas2*) likely involved in providing the starter unit. Heterologous expression attempts in *Aspergillus oryzae* using the closely related viriditoxin nrPKS produced expected intermediates, supporting the proposed biosynthetic model. We further aim to dissect the roles of tailoring enzymes to uncover the unique dimerisation mechanism of xylindein and to engineer or combine pathways to generate novel pigments and bioactive metabolites. These results provide the first insights into the biosynthetic route of xylindein and establish a foundation for pathway engineering. Beyond enabling sustainable production of this unique pigment, our work highlights the broader potential of fungi to expand chemical diversity and to deliver new bioactive compounds with applications spanning biotechnology, medicine, and advanced materials.

Keywords: fungal pigment, genome mining, RNA sequencing, heterologous expression, biosynthetic pathway

Abstract

Astromycological insights from deep Red Sea fungi: taxonomic characterisation and silver nanoparticles mycosynthesis under simulated microgravity

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The Red Sea offers a unique environment characterised by extreme salinity, high temperature, hydrothermal vents, and brine pools enriched with heavy metals. These conditions make it an exceptional terrestrial analogue for extraterrestrial settings, such as the Mars and the subsurface oceans of icy moons. Despite their potential, fungal communities from this ecosystem remain underexplored. These fungi are expected to exhibit remarkable adaptations, including novel metabolic pathways and stress-resistance mechanisms, enabling survival under multiple extremes. Characterising them will not only reveal hidden fungal diversity but also provide valuable bioactive metabolites, enzymes, and biomolecules with potential applications in biotechnology and nanotechnology. Importantly, investigating these extremophilic fungi can also advance astrobiological models by offering insights into how life may persist in similarly extreme environments beyond Earth. This study aimed at obtaining fungal axenic cultures, characterising and describing them through morphological and molecular analyses, and examining their potential for silver nanoparticles (AgNPs) production under simulated microgravity (S μ M).

Samples were collected from various sites at Hatiba Mons, located in the Central Red Sea Rift, during the M194 scientific expedition (10th October-7th November, 2023). Gravity coring with a 3-m core barrel was used to retrieve soil samples from the hydrothermal mounds. Subsamples were taken from unexposed areas of the gravity cores at room temperature using sterile, disposable plastic syringes or spatulas to prevent contamination. Fungal isolation was done using three culture media: potato dextrose agar (PDA), malt extract agar (MEA), and malt extract broth (MEB) at varying salinities (1.5% and 2.5%) supplemented with streptomycin (200 μ g/ml) and kanamycin (50 μ g/ml).

Twenty-two fungal strains were isolated and characterised (colony characteristics, growth rate, stereomicroscopy and optical microscopy analyses). Ultimately, 21 strains were identified as belonging to the genus *Aspergillus* and one strain to the genus *Talaromyces*. Further studies are underway to identify these strains at species level, and to explore and characterise their AgNPs production under S μ M. Our findings contribute to our understanding of fungal diversity in extreme marine environments and highlight the relevance of astromycology in identifying microbial candidates for space biotechnology.

Keywords: Aspergilli, Astromycology, Halophiles, Life limits, Mycosynthesis

Abstract

Antioxidant Activities of Pigment-Producing Endolichenic Fungi Inhabiting the Lichens *Usnea* and *Parmelia*

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Oxidative stress driven by reactive oxygen species (ROS) plays a central role in the pathogenesis of chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions. Due to safety concerns associated with synthetic antioxidants, natural sources—particularly pigment-producing endolichenic fungi (ELF)—are being explored as promising alternatives for their bioactive potential, particularly antioxidants. These ELF, which inhabit lichen thalli, produce secondary metabolites with notable antioxidant properties. In this study, six pigment-producing ELF—*Xylaria psidii*, *Nemania diffusa*, *Hypoxyton haematostroma*, *Hypoxyton trugodes*, and *Xylaria* sp.—were isolated from the fruticose lichen *Usnea* and the foliose lichen *Parmelia* and identified through morpho-cultural and molecular methods. Cultivated in potato dextrose broth, their crude culture extracts and extracellular pigments were evaluated for antioxidant activity using DPPH radical scavenging (RSA), ferric reducing antioxidant power (FRAP), and total phenolic/flavonoid content (TPC) assays. Ethyl acetate extracts of red pigment-producing *X. psidii* consistently showed the highest antioxidant activity (RSA = 88%, FRAP = 3.7 Fe²⁺/mg extract), along with the highest phenolic (10.6 mg GAE/g extract) and flavonoid (5.66 mg QE/g extract) contents. Ethyl acetate extracts outperformed aqueous ones across most assays. Given its consistently high antioxidant activity, the red pigment of *X. psidii* was further characterized—an aspect not previously reported in the literature. The pigment exhibited a peak absorbance at 530 nm and remained visibly stable across highly acidic to moderately basic pH environments. Additionally, potato dextrose broth was identified as the most favorable medium for supporting its pigment production. Our findings highlight the potential of pigment-producing endolichenic fungi for therapeutic applications.

Keywords: antioxidants, natural products, DPPH assay, FRAP assay, fungal pigments

Abstract

Optimization of Submerged Fermentation for Enhanced Adenosine Production in *Cordyceps militaris***Noppasorn Chuenprasert^{1,2,5*}, Natthawut Yodsuwan^{1,2}, Tao Wei^{3,4}, Sinang Hongsanant⁵ & Sunita Chamyuang^{1,2#}**¹*School of Science, Mae Fah Luang University, Tha-Sut, Muang, Chiang Rai, 57100 Thailand*²*Microbial Products and Innovation Research Group, Mae Fah Luang University, Chiang Rai, 57100 Thailand*³*College of Food Science & Institute of Food Biotechnology, South China Agricultural University, Guangzhou, 510640, China*⁴*Research Center for Micro-Ecological Agent Engineering and Technology of Guangdong Province, Guangzhou, 510640, China*⁵*College of Life Science and Oceanography, Shenzhen University, Shenzhen, China**Email of the corresponding author: sunita@mfu.ac.th*

Cordyceps militaris, a medicinal fungus prominent in traditional East Asian medicine, is a valuable source of adenosine, a bioactive compound with known anti-aging, skin-regenerating, and hair growth-stimulating properties. This study aimed to optimize adenosine production from *C. militaris* in submerged fermentation using the Taguchi experimental design. Four key variables were systematically evaluated: *C. militaris* strain (SH01, ATCC 34165, and a hybrid), glucose concentration (20–60 g/L), yeast extract concentration (5–20 g/L), and initial pH (4.0–7.0). Among the strains tested, *C. militaris* SH01 was the most productive. The optimal conditions were determined to be 45 g/L glucose, 25 g/L yeast extract, and an initial pH of 4.5. Under these conditions, the maximum adenosine yield reached 8.662 ± 0.269 mg/g with a productivity of 2.495 ± 0.077 mg/g·d, exceeding the predicted value by 44%. However, when scaling up the process to a 5-L shaking flask, productivity decreased to 1.849 ± 0.094 mg/g·d, indicating challenges in maintaining efficiency at larger volumes. These findings demonstrate the efficacy of the Taguchi method for optimizing fermentation parameters and highlight the potential of *C. militaris* as a promising source of adenosine for applications in healthcare, functional food, and cosmetic industries. Further work is needed to address the challenges of industrial scale-up.

Keywords: Adenosine, *Cordyceps militaris*, Process Optimization, Submerged Fermentation, Taguchi Method

Abstract

Epigenetic activation of latent secondary metabolites in *Xylaria papulis*: Insights from metabolomic profiling post UNC1999 treatment

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The endophytic fungus *Xylaria papulis* was isolated from *Andrographis paniculata* Willd. stem, commonly known as green chiretta, native to India and Sri Lanka. Until now, no study has reported on the epigenetic modulation of *X. papulis* using histone methyltransferase inhibitor UNC-1999; therefore, this study was conducted to investigate the effects of these probes individually. The fungal cultures were evaluated for enzymatic activity, antioxidant (DPPH, ABTS radical scavenging assays, and nitric oxide), and antibacterial potential against five human pathogens: *S. aureus*, MRSA (MU50), *S. boydii*, *K. pneumoniae* (ATCC 700603), and *E. coli* (ATCC 25922). The crude extract recovered from the samples treated with 250 nM of UNC1999 exhibited enhanced enzymatic activity, antioxidant, and increased antibacterial efficacy against *S. aureus* and MRSA, compared to the control. The chemical profiles of crude extracts, both treated and untreated, were analyzed using GC-MS and UHPLC. The UNC1999-treated extract induced the production of phthalic acid (antithyroid activity), 9-octadecenoic acid (antibacterial activity), undecanoic acid (anticancer activity), 1-heptatriacotanol (anticancer and antioxidant activity), 2H-pyran (antifungal activity), and octasiloxane (antimicrobial activity). These results highlight the capacity of UNC1999 to modulate the synthesis of cryptic metabolites in *X. papulis*, displaying antimicrobial and anticancer potential. This study underlines the importance of further research into the molecular mechanisms regulating these epigenetically modulated metabolic pathways.

Keywords: Anticancer, Antimicrobial, Endophytes, Epigenetic modulation, Histone methyltransferase inhibitors,

Abstract

Studies On the Chemical Constituents, Quality Control and Metabolism of *Ganoderma lucidum***Chun-Ru Cheng*, Ting-Chi Wen #**

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Ganoderma lucidum, which has been used in clinic in eastern countries for thousands of years, is an important traditional Chinese medicine. In present study, the chemical constituents, qualitative analysis and *in vivo* metabolism of *G. lucidum* were studied.

Using various chromatographic methods and identification technologies, totally 54 compounds were separated and characterized, including 47 triterpenoids, 4 sterols, and 3 fatty acids. The separated triterpenoids were assayed for their inhibitory activities against human HeLa cervical cancer cell lines using MTT method. Most of the compounds exhibited strong or moderate cytotoxicity, and their structure-activity relationships were discussed.

The fragmentation pathways of triterpenoids from *G. lucidum* were systematically studied using a combination of multistage tandem mass spectrometry experiments and high resolution mass spectrometry. The results indicated that the fragmentation pathways of triterpenoid acids were rather characteristic. The cleavage modes were highly dependent on the position of the carbonyl and hydroxyl groups in the tetracyclic skeleton. Different substitutions on the ring skeleton would result in different fragmentation pathways to form stable ions with highly conjugated systems. These decomposition features are extremely useful in distinguishing the isomers of the above types of compounds and unknowns. Using the HPLC/MSⁿ method and fragmentation pathways, 73 triterpenoids, including 11 new ones, were identified from the MeOH extract of *G. lucidum*. 44 of them were identified by comparison with the standards. The other unknown compounds were tentatively identified using the fragmentation rules. Further more, the triterpenoid components of *G. lucidum* and *G. sinense* have been compared.

In order to study the metabolism of the extract, we first investigated the metabolism of a representative single compound (ganoderic acid D). The results indicated that the biliary rather than renal clearance was the major route of excretion. Using ultraperformance liquid chromatography/quadrupole time-of-flight mass spectrometry, totally 25 metabolites were identified from the bile sample. The metabolic pathways of ganoderic acid D (GD) were proposed and the metabolic “soft spots” were identified. These results were very important in the further metabolism study of the triterpenoid extract. The present study also exhibits the importance of the high resolution mass spectrometry in metabolic study, especially in the correct interpretation of the fragmentation pathways and identification of the unknowns. A LC/MS/MS method of GD in rat plasma was established, and studies on the absorptive kinetics of GD in rats were performed. The

bioavailabilities between general preparation and nanoparticle preparation of GD in rats were compared. The results indicated that the nanoparticle preparation of GD could increase the concentration and bioavailability.

Based on the study of the metabolism of single compounds, we further investigated the metabolism of the triterpenoid extract. Using ultraperformance liquid chromatography/quadrupole time-of-flight mass spectrometry, totally 57 compounds were identified from the bile sample. The results indicated that most of the triterpenoid acids could be absorbed, and excreted as the parent compounds. The hepatocyte metabolism is the major route of clearance for the triterpenoid acids. After absorbing, the triterpenoids could transform to each other through oxidation, reduction, desaturation. However, the total tendency is that the low polar triterpenoid acids transformed into the high polar triterpenoid acids to excrete out of the organism. Like the metabolism of GD, the other transformation of triterpenoid acids included hydroxylation, sulfatation and glucuronide conjugation. A LC/MS/MS method of ganoderic acid D, A, B and E in rat plasma was established, and studies on the absorptive kinetics of ganoderic acids in rats were performed.

In conclusion, through the studies of chemical constituents, quality control and metabolism of *G. lucidum*, we know more about it. The results are very important in the further pharmacological and clinic studies.

Keywords: *Ganoderma lucidum*, Chemical Constituents, Quality control, Metabolism

Abstract

Screening of Beta-Glucan Production from Insect-Pathogenic Fungi for Potential Cosmeceutical Applications

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Beta-glucans are polymers of D-glucose that are essential components of the cell walls of bacteria, plants, and fungi. In fungi, beta-glucans have been reported to provide numerous benefits for human nutrition and health, including anticancer, antimicrobial, immune-modulating, antioxidant, and blood glucose/lipid-regulating activities. In this study, 73 strains of insect-pathogenic fungi (IPF) were screened for beta-glucan production in two liquid media (PDB, and M102) for potential cosmeceutical applications. Forty-three strains produced more than 1 g/L of beta-glucan; in PDB, 15 strains produced more than 1.5 g/L and 23 strains more than 2.1 g/L in M102. Cytotoxicity tests on human dermal fibroblasts (HDFn) and mouse lung fibroblasts (NCTC clone 929) revealed that 68 strains in PDB and 69 strains in M102 showed no effect on HDFn cells, and all 73 strains showed no effect on NCTC clone 929 cells. Among the screened isolates, *Polycephalomyces phaothaiensis* BCC 84546, *Akanthomyces pistillariiformis* BCC 79835, *Cordyceps lepidopterorum* BCC 79842, and *Polycephalomyces* sp. BCC 85057 produced over 1 g/L of beta-glucan and exhibited molecular weights within the specified criteria. These strains will be further evaluated for cosmeceutical activity and scaled-up fermentation.

Keywords: Beta-glucans, Insect-pathogenic fungi, Cosmeceutical Applications

Abstract

Mechanism of High Polysaccharide Production in *Tremella fuciformis* Spores and the Correlation Between Monosaccharide Composition and *In Vitro* Bioactivities

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Background: *Tremella fuciformis* polysaccharides have attracted considerable attention due to their diverse bioactivities and favorable physicochemical properties; however, their biosynthetic mechanisms and structure–activity relationships remain unclear, which constrains the screening of highly active polysaccharides and the breeding of superior strains. **Methods:** three *T. fuciformis* spores (TYH-SD1, TWW01-AX, and TWW02-TMS) were selected in this study as research objects to analyze their growth characteristics, polysaccharide biosynthetic and metabolic mechanisms, and the relationship between monosaccharide composition and *in vitro* activities. **Results:** both biomass and yields of intracellular and extracellular polysaccharides (IPs and EPs) in TWW01-AX and TWW02-TMS were significantly higher than those in TYH-SD1, reflecting differences in polysaccharides biosynthetic capacity among the spores. Targeted sugar metabolomics revealed the accumulation of monosaccharides associated with polysaccharides biosynthesis in TYH-SD1, which suggested a relatively low metabolic conversion efficiency. Transcriptomic analysis further demonstrated that TWW01-AX and TWW02-TMS were more active in polysaccharide precursor synthesis and carbohydrate metabolism pathways, with significantly higher expression levels of the key enzyme genes UTP-glucose-1-phosphate uridylyltransferase (TF1G000973) and GTP-mannose-1-phosphate guanylyltransferase (TF3G001229) compared with TYH-SD1, which may represent the molecular basis of their high polysaccharide yield. The IPs and EPs from different *T. fuciformis* spores were all composed of mannose (Man), glucuronic acid (GlcA), glucose (Glc), galactose (Gal), xylose (Xyl), and fucose (Fuc), but their molar ratios differed substantially: IPs were Glc-rich (>60 mol%), whereas EPs were Man-rich (>64 mol%). *In vitro* activities evaluation indicated that EPs exhibited stronger DPPH and hydroxyl radical scavenging activities, higher cupric ion-reducing antioxidant capacity (CUPRAC), and superior hypoglycemic activity, while IPs showed higher ABTS radical scavenging activity and ferric ion-reducing power (FRAP). Ridge regression analysis further confirmed that variations in the molar ratios of key monosaccharides had significant effects on the *in vitro* activities of polysaccharides, providing a quantitative basis for elucidating structure–activity relationships. **Conclusion:** this study systematically revealed the metabolic and molecular basis underlying yield differences of *T. fuciformis* spore polysaccharides, identified critical monosaccharide determinants affecting structure–activity relationships, and provided theoretical support for improving polysaccharide yield and quality as well as for the efficient screening of superior strains.

Keywords: *Tremella fuciformis* spore polysaccharides, High-yield mechanism, Monosaccharide profile–*in vitro* activity correlation

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Abstract

Antifungal Susceptibility Profile and Resistance Mechanisms of *Microsporium canis* in China

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Background: This study aimed to investigate the extracellular enzyme activities (including keratinase, phospholipase, lipase, hyaluronidase, and urease) and environmental adaptability of *Microsporium canis* from clinical, animal, and environmental sources. It also sought to characterize the antifungal susceptibility profiles and spatiotemporal distribution of *M. canis* in mainland China, correlating in vitro data with clinical outcomes. Furthermore, the study intended to establish epidemiological cut-off values (ECOFFs) for common antifungal drugs and explore underlying resistance mechanisms.

Methods: A total of 348 *M. canis* isolates were analyzed. Extracellular enzyme activities and stress tolerance were assessed. Antifungal susceptibility testing against 11 drugs was performed according to CLSI M38-A3 guidelines. Clinical efficacy was evaluated in 54 tinea capitis cases. ECOFFs for seven antifungals were established through a multi-center analysis. Resistance mechanisms were investigated via Sanger sequencing of the SQLE and CYP51 genes, complemented by molecular docking and dynamics simulations.

Results: Significant physiological differences were observed among isolates from different sources. Animal-derived strains showed the highest keratinase activity. Lipase and phospholipase were critical for early infection. Temperature exerted the strongest inhibitory effect on growth, though clinical strains demonstrated thermal adaptability. Minimum inhibitory concentrations (MICs) for most antifungals were generally low. Fluconazole had the highest geometric mean MIC, while orlofin showed potent activity. Resistance was detected to griseofulvin, fluconazole, and terbinafine in a small number of strains. Terbinafine remained a common first-line treatment, but clinical breakpoints need refinement for better correlation with outcomes. ECOFFs were successfully established. Elevated terbinafine MICs were linked to specific mutations (L391F, F395I, F395L) in the SQLE gene. Structural modeling suggested these mutations alter the drug-binding site, potentially conferring resistance.

Conclusions: *M. canis* modulates its enzyme expression dynamically to adapt to environmental changes. While most Chinese isolates remain susceptible to common antifungals, emerging resistance to terbinafine, associated with SQLE mutations, warrants attention. Griseofulvin and itraconazole continue to show good clinical efficacy. This study provides essential ECOFFs and insights into resistance mechanisms, aiding in the monitoring and treatment of this dermatophyte.

Keywords: *Microsporium canis*, antifungal susceptibility, Terbinafine resistance, SQLE gene mutation

Abstract

Tracking the origins and spread of *Candidozyma auris* through phylogenomics**Yue Wang, Jianping Xu***Department of Biology, McMaster University, Canada**jpxu@mcmaster.ca*

Understanding the origins and dispersals of fungal pathogens can have significant implications for developing both the short-term and long-term public health strategies. *Candidozyma auris* is a recently emerged multidrug-resistant human fungal pathogen that have dispersed through many countries on all continents, creating numerous outbreaks. However, our understanding of its global dispersal patterns remained limited. Using phylogenomic analyses of over 20,000 *C. auris* strains, we are estimating the routes and frequencies of strain dispersals, with a focus on those between countries. In this presentation, we will describe our approach and preliminary results of this large-scale phylogeographic analyses of *C. auris*.

Keywords: Phylogenomics, phylogeography, international dispersal

Abstract

Global Emergence and Rapid Detection of *Trichophyton mentagrophytes* genotype VIII a Highly Recalcitrant Lineage Within the *T. mentagrophytes* Complex

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Background

Trichophyton indotineae has spread rapidly across Asia and into other regions, presenting a significant clinical challenge due to its recalcitrant nature and frequent resistance to terbinafine. Standard laboratory methods struggle to differentiate this pathogen from other members of the *T. mentagrophytes* complex, creating an urgent need for affordable, rapid diagnostic tools in high-burden areas.

Methods

We compiled 4,357 ITS sequences (3,774 high-quality) from public repositories to map the global distribution of *T. indotineae*. Comparative genomic analysis of 21 *Trichophyton* genomes identified single-copy loci unique to *T. indotineae*, which we used to develop an affordable duplex PCR assay

(targeting species-specific marker OG7443 plus an ITS control). We validated this assay using 107 isolates. For clinical validation, we tested skin scrapings from 41 consecutive patients in China with a *T. indotineae*-specific TaqMan real-time PCR (qPCR). We further evaluated 90 clinical isolates from two centres—Radboud University Medical Center in the Netherlands and Peking Union Medical College Hospital in China. The primary endpoints were sensitivity, specificity, positive predictive value, and negative predictive value against bidirectional ITS sequencing. We also determined the limit of detection and assay reproducibility.

Results

Database analysis revealed 1,100 documented *T. indotineae* cases spanning 28 countries, with the highest burden in India (n=511) and Iran (n=333). The duplex PCR correctly identified all *T. indotineae* isolates without cross-reactivity to other complex members (107 isolates: sensitivity 100% [46/46; 95% CI 92.3–100], specificity 100% [61/61; 95% CI 94.1–100]). Direct testing of clinical specimens (n=41) yielded perfect performance for the TaqMan qPCR: sensitivity 100% (8/8; 95% CI 63.1–100) and specificity 100% (33/33; 95% CI 89.4–100). In the isolate validation panel (20 *T. indotineae*, 70 non-*T. indotineae*), the TaqMan qPCR detected all true positives (sensitivity 100%, 95% CI 83.2–100). One false positive occurred with *T. tonsurans*, resulting in a specificity of 98.6% (69/70; 95% CI 92.3–99.96).

Conclusion

Trichophyton indotineae has achieved global distribution but remains concentrated in settings where advanced diagnostic platforms are often unavailable. The gel-based duplex PCR and species-specific TaqMan qPCR provide accurate, same-day identification and can be readily implemented in resource-constrained laboratories. Widespread adoption of these assays could strengthen epidemiological surveillance and reduce delays in initiating appropriate antifungal therapy. Multicentre implementation studies examining the impact of rapid diagnosis on patient outcomes are now needed.

Keywords: Epidemiology, *Trichophyton mentagrophytes*, duplex PCR, TaqMan real time PCR, Whole genome sequences

Abstract

Concurrent chromoblastomycosis and eumycetoma: a unique case of dual neglected tropical fungal diseases in Asia

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Chromoblastomycosis (CBM) and mycetoma, as implantation mycoses, have been listed as neglected tropical diseases (NTDs) by the World Health Organization. The concurrent occurrence of these two NTDs in a single patient is extremely rare. A 69-year-old female patient presented with papules on the dorsum of her left hand for over 5 months and nodules on the left lower limb accompanied by ulceration and pain for 20 days. Histopathological examination of the papule on the dorsum of the left hand revealed muriform cells and fungal culture of the tissue identified *Fonsecaea monophora*. Microscopic examination of the purulent secretion from the ulcer on the left lower calf revealed the presence of grains, and the tissue culture result was *Scedosporium apiosperma* complex, with metagenomic next-generation sequencing further identifying *S. dehoogii* as the predominant pathogen. The clinical diagnosis was CBM caused by *F. monophora* combined with eumycetoma due to *S. dehoogii*. The patient was treated with voriconazole at a dosage of 200 mg twice daily for 4 weeks, after which the papules on the dorsum of the left hand and the ulcer on the left lower calf showed gradual improvement. This case represents the first reported instance of concurrent CBM caused by *F. monophora* and eumycetoma due to *S. dehoogii*, providing a novel perspective on the clinical manifestations and early identification of neglected implantation mycoses.

Keywords: Chromoblastomycosis; Concurrent; *Fonsecaea monophora*; Mycetoma; *Scedosporium dehoogii*

Abstract

Spread of fluconazole-resistant *Candida parapsilosis* isolates associated with increased *ERG11* and *CDR1B* copies

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Candida parapsilosis is one of the most common species associated with candidemia infections globally. Recently, the emergence of fluconazole-resistant (FLU-R) *C. parapsilosis* has become a significant global public health concern. In this study, we investigated the genomic epidemiology and potential mechanisms of antifungal resistance among 51 hospital isolates in Qatar, of which 18 were FLU-R. Whole-genome single nucleotide polymorphism analysis revealed the presence of 5 major genetic clades and evidence for cross-hospital transmission within clade I, II, III. These 18 FLU-R isolates had no missense variants known to be associated with azole resistance in loci such as *ERG11*, *ERG6*, and *TAC1*; however, all FLU-R isolates had increased copy numbers of *ERG11*, ranging from 3-7 copies. In addition, most FLU-R isolates (n=16) had increased *CDR1B* copies (2-8 copies). The clade I isolates may have evolved greater propensity to persist within hospitals for prolonged periods and cause clonal transmission than isolates in other genetic clusters susceptible to fluconazole. In addition, increased *ERG11* copies have been detected in FLU-R *C. parapsilosis* isolates in other countries, that also had no reported mutation in *ERG11*. Copy number variation is an important mechanism linked to increased drug resistance.

Keywords: azole resistance, candidemia, copy number variation, evolution, genomics

Abstract

Study on Hypothetical Protein PAT1LP Regulates the Growth and Virulence of *Fusarium oxysporum***Cong Liu^{1,2*}, Dan He^{2#}, Yanbing Zhao², Li Wang², Huaiqiu Huang¹***1 Department of Dermatology and Venereology, the Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong 510630, China**2 Department of Pathogenobiology, Jilin University Mycology Research Center, College of Basic Medical Sciences, Jilin University, Changchun 130021, China**Email of the corresponding author: hedan@jlu.edu.cn**Email of the presenting author: liuc_0705@163.com**Email of other topgdbing@126.com (Yanbing Zhao), wli99@jlu.edu.cn (Li Wang), hhuaiq@mail.sysu.edu.cn (Huaiqiu Huang)*

Fusarium oxysporum is an important plant pathogen in agriculture and an opportunistic pathogen of humans and animals. Its pathogenic mechanism is still unclear. Fungal growth is thought to be closely related to virulence. Studies have already found that some genes, as well as signaling pathways, regulate growth and virulence. In this study, we used strain growth as the initial screening criterium, a T-DNA random insertion mutant of *F. oxysporum* was obtained by using *Agrobacterium tumefaciens*-mediated transformation (ATMT), which had a severe growth and virulence defect. The T-DNA flanking sequences were amplified using touchdown TAIL-PCR, the T-DNA interrupted gene has been found that it has an unknown function and encodes the hypothetical protein, which is homologues of Topoisomerase II-associated protein, here named as PAT1-like protein (PAT1LP). Compared the DNA sequence and encoded amino acid sequence with those of other *Fusarium* species, we found that this hypothetical protein had high DNA and protein sequence identities. No homologs of the hypothetical protein encoded were identified. The protein sequence comparison indicated that the hypothetical protein encoded might have a topoisomerase II-associated protein (PAT1) functional region, which might involved in glucose metabolism and conserved in other species of *Fusarium*. Therefore, deletion and complementation strains were constructed. The colony morphology indicated that the gene deletion strain is similar to T-DNA random insertion mutant, showing a severe growth defect. The microscopic morphology of mycelia and macroconidia revealed that they were also abnormal. These findings suggest that PAT1LP plays important roles in *F. oxysporum* growth. The contribution of PAT1LP to *F. oxysporum* virulence was evaluated using the *C. elegans* infection model. The deletion of PAT1LP significantly increased the *C. elegans* survival rate, indicating that this gene contributes to *F. oxysporum* virulence. Through qPCR analysis of the genes related to the glucose metabolism pathway and determination of the key metabolic products and enzyme activities in the glycolysis pathway, it was found that the deletion of PAT1LP would lead to significant changes in the gene expression levels (such as the gene of hexokinase, 6-phosphofructokinase, pyruvate kinase, glucose-6-phosphate 1-dehydrogenase, etc.) , key enzyme activities (hexokinase, 6-phosphofructokinase, pyruvate kinase) and metabolic products (pyruvate) contents in glucose metabolism. In summary, hypothetical protein PAT1LP may regulate the growth and virulence of *F.*

oxysporum through glycometabolism. These findings provide a theoretical basis for identifying potential therapeutic targets of fungal infections.

Keywords: *Fusarium oxysporum*, glycometabolism, growth, hypothetical protein, virulence

Abstract

D-lactic acid inhibits *Candida albicans* associated with vulvovaginal candidiasis: in vitro effects and underlying mechanisms

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Background: *Candida albicans* (*C. albicans*) is a major opportunistic fungal pathogen responsible for both invasive and superficial infections, including vulvovaginal candidiasis (VVC), which affects a large proportion of women worldwide and is prone to recurrence. Limitations of conventional antifungal therapies and the emergence of drug resistance highlight the urgent need for novel therapeutic agents. D-lactic acid (D-LA), a chiral organic acid naturally produced by *Lactobacillus spp.*, has been proposed as a potential antifungal candidate. However, its activity against *C. albicans* and underlying mechanisms remain unclear.

Results: We investigated the antifungal properties of D-LA against *C. albicans* strain SC5314 using *in vitro* assays. D-LA inhibited fungal growth, reduced hyphal formation, and impaired biofilm development in a concentration-dependent manner. Crystal violet and XTT assays confirmed substantial suppression of biofilm biomass and metabolic activity. Live/dead staining and trypan blue exclusion indicated increased cell membrane damage after D-LA exposure. Transcriptomic profiling revealed significant changes in genes related to hyphal development, cell wall organization, iron metabolism, acid phosphatase activity, and stress response. qRT-PCR validation demonstrated downregulation of hypha-associated genes (*ECE1*, *TEC1*, *ALS1*) and upregulation of the pH-regulated gene *PHR2*. Cytotoxicity testing on VK2/E6E7 vaginal epithelial cells indicated minimal toxicity at concentrations effective against *C. albicans*.

Conclusion: D-LA exhibits potent antifungal activity against *C. albicans* by disrupting hyphal formation, biofilm development, and cell wall integrity, accompanied by transcriptional reprogramming of virulence-associated pathways. Its low cytotoxicity towards vaginal epithelial cells and mouse vaginal mucosa supports its potential as a safe alternative or adjunctive agent for VVC management.

Keywords: D-lactic acid; *Candida albicans*; vulvovaginal candidiasis; biofilm; hyphal formation

Abstract

Subcutaneous *Thyridium curvata* Infection with Lymphangitic Spread in an Immunosuppressed Patient with PLA2R-Associated Membranous Nephropathy

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Thyridium curvata (previously classified as *Phialemoniopsis curvata*, *Phialemonium curvatum*) is an emerging opportunistic pathogen capable of causing invasive infections, particularly in immunosuppressed individuals. This article presents the first documented case in mainland China of subcutaneous *T. curvata* infection with lymphangitic spread in a patient receiving immunosuppressive therapy for membranous nephropathy. Through systematic review of global cases, we identified significant variability in clinical manifestations and potential nosocomial transmission patterns.

Primary cutaneous *T. curvata* infection should be suspected in patients with trauma history and immunosuppression, as it typically presents without systemic symptoms. Diagnosis requires histopathology, culture, and ITS sequencing due to its sporotrichoid mimicry. Our case achieved complete resolution with voriconazole monotherapy, though combined surgical intervention may be necessary for advanced infections.

This study expands the recognized clinical spectrum of *T. curvata* infections and underscores the importance of molecular diagnostics in guiding appropriate antifungal therapy. These findings address critical gaps in managing rare fungal infections in immunocompromised patients and emphasize the need for heightened clinical awareness of this emerging pathogen.

Keywords: *Thyridium curvata* ; Subcutaneous fungal infection; Immunosuppression; Molecular diagnosis; Fungi

Abstract

Metagenomics approach for the detection of fungal pathogens in clinical microbiology laboratory

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Clinical metagenomics has been an emerging method in the diagnosis of infectious diseases that uses next generation sequencing technology to identify the etiologic agents to allow for more effective and targeted treatment of infectious diseases. Conventional fungal diagnostic methods are mainly based on basic morphology, MALDI-TOF MS and/or ITS rRNA sequence analyses which can be insensitive and/or time consuming. Metagenomic NGS (mNGS) can be performed with only a small amount of nucleic acid from the specimens and not only can the pathogen be identified and characterized. We have performed mNGS on 17 clinical specimens, including one negative control and 16 specimens (mainly blood) collected from patients confirmed with fungal infections. However, 3 samples were failed in sequencing. Out of 13 specimens, mNGS can detect the fungal pathogens among all the samples, indicating very high sensitivity. On one case, mNGS revealed the presence of two *Aspergillus* species in a surgical site infection, indicating the possibility of mixed infections. Our study demonstrated that mNGS can be considered a promising tool for rapid and accurate pathogenic detection in patients with suspected fungal infection.

Keywords: bioinformatics, genomics, medical mycology, *Candida*, *Aspergillus*

Abstract

A Comparative Study on Evaluation of Antioxidant Properties of the Philippine Mushrooms *Pleurotus djamor* (Pink Oyster Mushroom), *Pleurotus citrinopileatus* (Yellow Oyster Mushroom), and *Pleurotus pulmonarius* (Brown Oyster Mushroom)

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This study explores the antioxidant properties of the *Pleurotus djamor* (Pink oyster mushroom), *Pleurotus citrinopileatus* (Yellow oyster mushroom), and *Pleurotus pulmonarius* (Brown oyster mushroom) that was cultivated in the Philippines. Natural resources such as edible mushrooms have a lot of potential as an therapeutic agent, not only could they be consumed safely, but their antioxidant properties can be used to treat and cure some diseases. They are known to contain beneficial compounds, such as phenolics, flavonoids, and polysaccharides, which contribute to their medicinal and health benefits. Using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, the study employed a quantitative research design, and data were analyzed using various statistical tools, including mean, standard deviation, percentage, one-way analysis of variance (ANOVA), Tukey's post hoc test, and p-test. The findings showed that *Pleurotus djamor* showed the highest antioxidant activity at 100% concentration. However, when considering overall potency across concentrations and EC50 values, *Pleurotus citrinopileatus* proved to be the most potent, especially at 75% concentration. Furthermore, the mushroom with the least antioxidant activity was *Pleurotus pulmonarius* at 50% concentration. There is plenty of research internationally proving the presence of antioxidants in *Pleurotus* species, but only a few in the Philippines, which is why the aim of this research is to expand the context of antioxidant activities of Philippine varieties of *Pleurotus* species.

Keywords: antioxidants, *Pleurotus citrinopileatus*, *Pleurotus djamor*, *Pleurotus pulmonarius*, scavenging assay

Abstract

Heparinase gene homologue is associated with capsule formation in *Cryptococcus neoformans*

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Cryptococcus neoformans is known as a major opportunistic pathogen in immunocompromised individuals, patients with AIDS, transplant recipients, and those with lymphoreticular malignancies. These diseases are associated with high morbidity and mortality despite antifungal drug treatment (Meya & Williamson 2024). The characteristic feature of *C. neoformans* is a polysaccharide capsule that surrounds the cells, which is thought to be involved in pathogenicity, and the mechanism of capsule formation has been attracted considerable attention.

Diazo benzoic acid B (DBB) staining method can be used to distinguish basidiomycetous yeast from ascomycetous yeast; it stains only the former ones. All proposed yeast species have been evaluated for DBB staining (Kurtzman et al., 2011). To clarify the mechanism of DBB staining, we screened DBB- negative mutants of the basidiomycetous yeast *C. neoformans*, and found that *CAP64* gene or *PMT2* gene is responsible for DBB staining and capsule formation (Shimizu et al., 2014, Imanishi-Shimizu et al., 2021, Chang et al., 1996). In this study, we isolated a heparinase gene homologue mutant that showed negative for DBB staining, and the cell morphology, and capsule formation were compared between the mutant and the wild type strain.

A heparinase gene homologue disruption strain was constructed using a gene disruption cassette, and then a complementation strain was constructed by re-introducing a wild type copy of the heparinase gene homologue into the mutant. We performed DBB staining using these strains. As a result, the heparinase gene homologue deletion strain was not stained, whereas the wild type and a complementation strain was stained. Next, cell morphology and capsule thickness were compared among these strains. As capsule-inducing medium, 10-fold diluted Sabouraud liquid medium was used. The capsule thickness of the deletion strain was approximately 65% thinner than that of the wild-type strain. After their cultivation in YPD liquid medium until stationary phase, and the cell diameters were measured. The cell diameters of the deletion strain was 1.6 times larger than that of the wild type strain. Furthermore, the deletion strain could not grow on YPD plate containing 0.01% SDS.

To analyze the localization of the heparinase gene homologue protein, we constructed a strain that expresses a His-tagged heparinase gene homologue. After the constructed strain was cultured in PDB liquid medium, a protein sample was prepared from cells or culture supernatant and His-

tagged protein was detected by Western blotting. His-tagged protein was detected from both protein samples, indicating that the heparinase gene homologue protein is secreted extracellularly.

DBB couples with aromatic compounds such as phenols and naphthols to form red diazo compounds, which are used for their detection. This suggests that heparinase gene homologue proteins are secreted extracellularly and acts on capsule or cell wall polysaccharides to produces OH groups within the structure. Then, DBB forms diazo compounds with these structures, turning the color red.

Keywords: *Cryptococcus neoformans*, Diazonium blue B color reaction, heparinase gene homolog, capsule

Abstract

The impact of simulated lunar and Martian conditions on pathogenic fungi

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This project investigates how clinically relevant pathogenic fungi, *Candida albicans*, *C. parapsilosis*, and *C. tropicalis*, and environmental fungi frequently detected in space habitats, *Aspergillus* spp. and *Penicillium* spp., respond to simulated lunar and Martian gravity, as well as exposure to artificial regoliths. Within the growing field of Astromycology, this study intends to explore fungal morphological plasticity, stress adaptation, and virulence modulation under extraterrestrial analogue conditions.

Previous research showed that spaceflight and microgravity environments can significantly impact fungal physiology. For instance, *C. albicans*, when in these conditions, exhibits increased filamentation, biofilm formation, and growth acceleration, all linked to heightened virulence and antifungal resistance. Environmental isolates of *A. fumigatus* and *A. niger* recovered from the International Space Station (ISS) demonstrate molecular adaptations and remarkable spore resistance to space radiation. These traits raise concerns about fungal persistence and material degradation during long-duration missions. Moreover, *Aspergillus*-driven corrosion of spacecraft alloys under simulated microgravity and *Penicillium* spp. involvement in biomineralization of lunar regolith simulants underscore their dual role as both biosafety risks and biotechnological resources.

This study will generate new insights into how dimorphic and filamentous fungi adapt morphologically and genetically to reduced gravity and regolith exposure. It will also examine mitochondrial alterations and stress responses and evaluate pathogenicity using *Galleria mellonella* infection models.

Our results will inform biosafety strategies for crewed space missions, contribute to our understanding of microbial resilience in changing terrestrial ecosystems, and inspire new

biotechnological applications, from bioremediation to sustainable bioengineering, for all future missions and off-Earth habitats.

Keywords: Astromycology, Simulated microgravity, Fungal pathogenicity, Artificial regoliths

Abstract

Tinea capitis in adults: A 17-year retrospective, single-centre study in southern China and Chinese literature review

[LI Meirong*1], [WU Rong2], [LIU Wentao3], [ZHAN Miaorong4], [WEI Ling5], [CHEN Zhirui6], [YIN Songchao7], [LIU Cong8], [HUANG Huaiqiu9], [FENG Peiying#10]

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To raise awareness of adult tinea capitis and reduce misdiagnosis by analyzing the clinical characteristics and etiological agents of adult tinea capitis cases treated at a tertiary hospital in Guangzhou from 2007 to 2024. **Methods** A retrospective analysis was conducted on the clinical and mycological data of 40 adult patients diagnosed with tinea capitis at the Department of Dermatology, Third Affiliated Hospital of Sun Yat-sen University, from January 2007 to December 2024. Data included patient demographics, clinical manifestations, risk factors, dermatoscopic findings, and results of fungal microscopy and culture. **Results** Among the 40 patients, 14 were male (35%) and 26 were female (65%), with an age range of 18 to 72 years. The most common clinical types were grey patch (65%), followed by black dot (17.5%) and tinea kerion (17.5%). No cases of favus were identified. Fungal microscopy was positive in 38 cases (95%), and fungal culture was positive in 24 cases (60%). The predominant pathogens were *Microsporum canis* (22.5%) and *Trichophyton violaceum* (15%), with other pathogens including *Trichophyton mentagrophytes complex* (7.5%), *Trichophyton tonsurans* (7.5%), *Trichophyton rubrum* (5%), and *Nannizzia gypseum* (2.5%). The addition of dermoscopy and fungal fluorescence microscopy significantly increased the detection rate of tinea capitis. Risk factors varied by age group, with pet contact being a major factor in younger patients and menopause-related immune changes in older patients. **Conclusion** Adult tinea capitis is more prevalent in young women and menopausal women, primarily caused by *Microsporum canis* and *Trichophyton violaceum*, respectively. Grey patch is the most common clinical type. The incorporation of dermoscopy and fungal immunofluorescence microscopy can enhance the diagnostic accuracy of adult tinea capitis.

Keywords: adult tinea capitis; dermoscopy; female; *Microsporum canis*; *Trichophyton violaceum*;

Abstract

Metagenomic sequencing of wastewater samples for detection of emerging fungal pathogen

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Wastewater (WW) surveillance of pathogens can provide data on community health non-invasively and inform public health decisions. Metagenomic sequencing allows detection of microbial communities present in complex samples like WW. *Candidozyma auris* (formerly *Candida auris*) is a critical priority pathogen with cases consistently detected in Hong Kong hospitals, making it a prime target for WW epidemiology. The aim of this project was to determine whether *C. auris* was present in Hong Kong WW and if WW surveillance was effective in tracking emerging fungal pathogens. In addition, we validated a pipeline for fungal genome assembly from mixed environmental samples. Samples (n=22) were generated from 14 different WW treatment plants in Hong Kong in 2021. Relative abundance of *C. auris* was calculated by CoverM. The pipeline to generate metagenome assembled genomes (MAGs) consisted of assembly (metaSPAdes), binning (concoct) and detection of eukaryotic genomes (EUKCC). Species were screened using MASH. The pipeline was validated using in-silico reads of *C. auris*. A mixed dataset consisting of *C. auris* and 6 wastewater-associated bacterial species was used to calculate the number of reads per litre required to generate a MAG. Preliminary data indicated that *C. auris* reads can be detected at low levels in all 14 WW treatment plants. Approximately $\geq 25,000$ reads/L (0.3 copies of *C. auris*/L) and ≥ 10 million reads in total (120 copies of *C. auris*) is required for successful speciation of a fungal MAG from WW. These results are a benchmark for fungal metagenomics from mixed samples.

Keywords: bioinformatics, genomics, microbiome, molecular diagnostics, one health

Abstract

The impact of simulated lunar and Martian conditions on pathogenic fungi

Ana Filipa Lenha-Silva^{1,*}, Gabriel Marques de Barros², Alireza Armand¹, Célia Fortuna Rodrigues^{3,4}, Koon Ho Wong⁵, Marta Filipa Simões^{1,6,#}

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biotechnological applications, from bioremediation to sustainable bioengineering, for all future missions and off-Earth habitats.

Keywords: Astromycology, Simulated microgravity, Fungal pathogenicity, Artificial regoliths

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Tinea capitis in adults: A 17-year retrospective, single-centre study in southern China and Chinese literature review

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Keywords: adult tinea capitis; dermoscopy; female; *Microsporum canis*; *Trichophyton violaceum*;

Abstract

Study on Hypothetical Protein PAT1LP Regulates the Growth and Virulence of *Fusarium oxysporum***Cong Liu^{1,2*}, Dan He^{2#}, Yanbing Zhao², Li Wang², Huaiqiu Huang¹***1 Department of Dermatology and Venereology, the Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong 510630, China**2 Department of Pathogenobiology, Jilin University Mycology Research Center, College of Basic Medical Sciences, Jilin University, Changchun 130021, China**Email of the corresponding author: hedan@jlu.edu.cn**Email of the presenting author: liuc_0705@163.com**Email of other topgdbing@126.com (Yanbing Zhao), wli99@jlu.edu.cn (Li Wang), hhuaiq@mail.sysu.edu.cn (Huaiqiu Huang)*

Fusarium oxysporum is an important plant pathogen in agriculture and an opportunistic pathogen of humans and animals. Its pathogenic mechanism is still unclear. Fungal growth is thought to be closely related to virulence. Studies have already found that some genes, as well as signaling pathways, regulate growth and virulence. In this study, we used strain growth as the initial screening criterium, a T-DNA random insertion mutant of *F. oxysporum* was obtained by using *Agrobacterium tumefaciens*-mediated transformation (ATMT), which had a severe growth and virulence defect. The T-DNA flanking sequences were amplified using touchdown TAIL-PCR, the T-DNA interrupted gene has been found that it has an unknown function and encodes the hypothetical protein, which is homologues of Topoisomerase II-associated protein, here named as PAT1-like protein (PAT1LP). Compared the DNA sequence and encoded amino acid sequence with those of other *Fusarium* species, we found that this hypothetical protein had high DNA and protein sequence identities. No homologs of the hypothetical protein encoded were identified. The protein sequence comparison indicated that the hypothetical protein encoded might have a topoisomerase II-associated protein (PAT1) functional region, which might involved in glucose metabolism and conserved in other species of *Fusarium*. Therefore, deletion and complementation strains were constructed. The colony morphology indicated that the gene deletion strain is similar to T-DNA random insertion mutant, showing a severe growth defect. The microscopic morphology of mycelia and macroconidia revealed that they were also abnormal. These findings suggest that PAT1LP plays important roles in *F. oxysporum* growth. The contribution of PAT1LP to *F. oxysporum* virulence was evaluated using the *C. elegans* infection model. The deletion of PAT1LP significantly increased the *C. elegans* survival rate, indicating that this gene contributes to *F. oxysporum* virulence. Through qPCR analysis of the genes related to the glucose metabolism pathway and determination of the key metabolic products and enzyme activities in the glycolysis pathway, it was found that the deletion of PAT1LP would lead to significant changes in the gene expression levels (such as the gene of hexokinase, 6-phosphofructokinase, pyruvate kinase, glucose-6-phosphate 1-dehydrogenase, etc.) , key enzyme activities (hexokinase, 6-phosphofructokinase, pyruvate kinase) and metabolic products (pyruvate) contents in glucose metabolism. In summary, hypothetical protein PAT1LP may regulate the growth and virulence of *F.*

oxysporum through glycometabolism. These findings provide a theoretical basis for identifying potential therapeutic targets of fungal infections.

Keywords: *Fusarium oxysporum*, glycometabolism, growth, hypothetical protein, virulence

Abstract

Antimicrobial Metabolites of *Bacillus velezensis* FZB42 Reshape the Rice Rhizosphere Microbial Community and Enhance Host Resistance to *Rhizoctonia solani*

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Biocontrol agents (BCAs) and their metabolites offer a green and sustainable solution to emerging food production challenges, but knowledge of their role in shaping rhizosphere microbial communities is still lacking. To address this knowledge gap, the current study evaluated the effect of a biocontrol bacterium *Bacillus velezensis* FZB42, its mutants for fengycin AK2 ($\Delta fenA$), surfactin CH01 ($\Delta srfA$) and bacillomycin D AK1 ($\Delta bmyA$) and corresponding pure metabolites fengycin (Fin), surfactin (Sin), and bacillomycin D (Bin) on induced systemic resistance (ISR) in rice against pathogen *Rhizoctonia solani* and rhizosphere microbial communities' composition. The biocontrol agent FZB42, its mutants, as well as their metabolites, strongly inhibited *R. solani* mycelium growth in petri plate assay. The SEM and TEM analyses found deformed and destroyed cells as well as impairing the plasma cell membranes of mycelia growth. The bacterial strains and their crude extracts as well as metabolites showed strong antifungal potential due to the induction of severe oxidative stress in the mycelial cells. The 16S and ITS2-1 rRNA gene sequence data revealed that the application of specific treatments reshaped the rhizosphere microbial communities and significantly altered their diversity. Furthermore, after the application of mutants in the rhizosphere, different bacterial phyla (*Chlamydiae*, *Verrucomicrobia*, *Armatimonadetes*, and *Candidatus saccharibacteria*), and fungal phyla (*Chytridiomycota*, *Aphleidiomycota*, and *Blastocladiomycota*) which were not found in FZB42 and CKH. The application of pure metabolites in the rhizosphere also had various bacterial phyla (*Chlamydiae*, *Armatimonadetes*, and *Planctomycetes*) and fungal phyla (*Basidiobolomycota*, *Blastocladiomycota*, and *Mortierellomycota*) which were not present in control and disease. This means that the metabolites played a crucial role in preventing disease by directly inhibiting growth and reshaping microbial communities. The intra-kingdom network analysis revealed a decreased community number and modularity but an improved number of connections per node. Moreover, FZB42, its mutants, and pure metabolites protected plants by inhibiting the growth of *R. solani* and activating plant-induced systemic resistance by regulating defense genes. Overall, our findings suggest that the FZB42 and their metabolites Fin, Sin, and Bin can be used as BCAs to achieve sustainable disease management

due to their antimicrobial properties, capability to induce systemic resistance, and ability to promote a healthy and green state of the microbial community.

Keywords: *Rhizoctonia solani*, Biocontrol agents, Disease suppression, Induced system resistance, Rhizosphere microbiota.

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Abstract

Genetic mapping and nuclear interactions of an incompatibility response in the button mushroom *Agaricus bisporus*.**K. Scholtmeijer, B. Auxier, Patrick Hendrickx, B Lavrijssen, A.J.M. Debets, JJP. Baars, DK Aanen, A. van Peer.**Pre-publication on this work is available: doi: <https://doi.org/10.1101/2025.08.15.670591>

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During cultivation, mixing of different heterokaryotic individuals of the button mushroom, *Agaricus bisporus*, reduces yield. This phenomenon could be caused by direct antagonistic responses and/or reduced synchronization by not forming a chimeric hyphal network. In other fungi, highly divergent alleles for a set of genes affect successful network formation either by preventing fusion or, more commonly, triggering cell death post-fusion. To understand this process in *A. bisporus*, it is important to identify the variants allowing these fungi to discriminate self from non-self, preventing fusion. We leverage a recently described cell death staining method utilizing Evans Blue to visualize mycelial compatibility. Here, we provide results of a first genetic mapping of incompatibility alleles in *A. bisporus*. Crossing strains between *A. bisporus* var. *bisporus* and *A. bisporus* var. *burnetti* we find segregation ratios of compatible progeny generally consistent with 3 nuclear loci. To identify these regions, we first use a set of single Chromosome Substitution Lines, produced by genotyping non-recombinant progeny. We localize the main effect to be between two and three chromosomes, depending on the common nucleus. Using genome-wide markers for 167 sexual progeny, we identify loci controlling this trait on chromosomes 4, 6 and 7, the same chromosomes as indicated by chromosome substitution lines. Notably, while the choice of a common nucleus seemed to affect the compatibility of CSLs, it did not seem to affect the loci identified in the sexual progeny. The ability to mix different strains of this mushroom-forming fungus could allow additional cultivation approaches, combining strains with complementary characteristics. These results provide a starting point towards understanding the molecular mechanisms underlying this fundamental property of hyphal networks in basidiomycetes.

Abstract

FUNGA: a large language model based phenotype-oriented key gene mining platform for fungi species

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Fungi play pivotal economical and ecological roles in our society and ecosystem. Identification of functional genes controlling important agronomic traits and response to external stresses is one of the most important mission in fungi breeding and genetic studies. Many multi-omics techniques, emphasizing gene sequence variations, expression profile and epigenetic status, facilitates to generate massive potential gene exhibiting statistical significance; however, identification of key functional genes from those gene set is still time-consuming and challenging, largely due to the lack of a utility to mine candidate genes using large-scale gene and phenotype relationship information. Here, we present FUNGA (FUNctional Gene Annotation platform), an innovative tool for key gene mining platform for fungi species. Based on large language models (LLMs) with Retrieval-Augmented Generation (RAG) and Tool Calling technologies, FUNGA integrates multimodal gene and phenotype data, including sequencing data, gene function databases and academic literature, and employs vector embedding techniques to develop a phenotype-oriented gene mining platform. Taking advantage of phenotype-oriented gene mining method, FUNGA could not only comprehensively screen and accurately prioritize candidate functional genes from potential gene sets, but also unravel regulatory network among those genes. The evidence and deducing process for gene mining are illuminated by interactive visualizations and AI-generated summaries. More important, FUNGA exhibits robust expansibility to general fungi taxa, including model species yeast, edible and medicinal mushrooms and ascomycetes pathogens. To summary, as the first large language model based gene analysis utility for fungi species, FUNGA provide a tool for fungi gene function and interaction studies, as well as a phenotype-oriented platform for key gene mining for general fungi species.

Keywords: artificial intelligence; big data; fungi; gene mining; phenotype

Abstract

Gene Editing in *Cordyceps militaris* and Its Application in Germplasm Innovation

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With its well-characterized genetic background and advanced genetic manipulation toolkit, *Cordyceps militaris* has emerged as a model organism for investigating the biology of edible fungi, especially filamentous ascomycetes. In this study, we established a highly efficient, marker-free editing system in *C. militaris* using an autonomously replicating AMA1-based plasmid. This system enabled random knockout of single and double genes as well as precise gene deletions via homologous recombination repair. To date, approximately 50 genes have been successfully disrupted in *C. militaris* with efficiencies ranging from 40% to 90%. Furthermore, we developed a targeted gene integration strategy into genomic “safe harbors,” which, for the first time in *C. militaris*, allowed precise gene complementation. Site-directed mutagenesis and large-fragment deletions were also successfully achieved. These advances have substantially accelerated gene functional studies and genetic research in *C. militaris*.

The AMA1 plasmid replicates extrachromosomally, and the Cas9 protein is degraded after performing its function, leaving no genomic disruption or foreign DNA residues. Consequently, this system offers powerful technical support for the genetic improvement of *C. militaris*. Based on this system, several novel germplasms have already been developed. For instance, disruption of the ustiloxin gene cluster generated strains incapable of producing ustiloxin, while retaining normal fruiting body development and other agronomic traits. Genome resequencing confirmed the absence of off-target effects and foreign DNA integration. In addition, site-directed insertion enabled overexpression of a hydrophobin gene, resulting in strains with enhanced resistance to white mold disease. Editing of the pigment biosynthesis gene *Cmpks1* produced albino strains, whereas overexpression of the morphogenesis-related gene *Cmare2* yielded *C. militaris* strains with improved genetic stability.

Keywords: AMA1; Breeding; *Cordyceps militaris*; Crispr/Cas9

Abstract

Exploring the Interaction Network of PcTYR: Unveiling the Genetic Mechanisms Behind Cap Color Formation in *Pleurotus cornucopiae*

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The cap color of *Pleurotus cornucopiae* is a quantitative trait controlled by a complex genetic network. While the tyrosinase-encoding gene PcTYR plays a crucial role in regulating melanin-mediated cap pigmentation, the regulatory components interacting with PcTYR remain poorly characterized. In this study, we identified 43 proteins interacting with PcTYR through yeast two-hybrid (Y2H) screening.

Integrated previous QTL results and transcriptomic analyses prioritized two candidate regulators, PcMFS (a major facilitator superfamily transporter) and PcPKc (a protein kinase), which exhibited differential expression between dark- and light-colored caps. Protein-protein interactions between PcTYR and these candidates were rigorously validated by pairwise Y2H, bimolecular fluorescence complementation (BiFC), and in vitro pull-down assays. Functional validations using exogenous addition of tyrosinase inhibitors and RNA interference (RNAi) demonstrated that both PcMFS and PcPKc positively regulate cap pigmentation in *P. cornucopiae*. RNAi-mediated knockdown strains showed significantly lighter cap color phenotypes. Phylogenetic analysis further indicated the conserved roles of these genes in fungal melanin biosynthesis. This study elucidates a novel regulatory module involving PcTYR interactors and provides molecular targets for breeding oyster mushrooms with desirable cap color. Our findings also advance the understanding of melanin biosynthesis in basidiomycetes.

Keywords: *Pleurotus cornucopiae*, cap color, melanin biosynthesis, protein-protein interaction, PcTYR, PcMFS, PcPKc, yeast-two hybrid, RNA interference

Abstract

Transcriptional Regulatory Mechanism of a Zn(II)₂Cys₆ Transcription Factor PoZCP26 in Fruiting Body Development of *Pleurotus ostreatus***Xiangli Wu^{*#}, Zhihao Hou, Jingqi Pei, Chenyang Huang***Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China**Email of the corresponding author: wuxiangli@caas.cn*

Pleurotus ostreatus is one of the most cultivated edible mushrooms worldwide, of which the fruiting body development is a highly complex process involving precise genetic regulatory networks. Transcription factors are well-recognized key factors among these networks. In this study, one Zn(II)₂Cys₆ transcription factor named *PoZCP26* was up-regulated in primordium and fruiting body compared to mycelium stage. Thus, the overexpression and RNA interference transformants of *PoZCP26* were constructed for *P. ostreatus* and the roles of *PoZCP26* on mycelia growth and fruiting body development were examined. The results showed that *PoZCP26* promoted the mycelial growth rate, the primordial differentiation and fruiting body formation. To further detect the binding motifs and potential target genes of *PoZCP26*, we performed DNA-affinity purification sequencing (DAP-seq) analysis *in vitro*. In combination of RNA-seq and DAP-seq results together, we subsequently identified several target genes of *PoZCP26* that might be involved in fruiting body development. Among them, an uncharacterized protein encoding gene *g10029* were selected out for in-depth study. EMSA (Electrophoretic Mobility Shift Assay) and Dual-Luciferase Reporter Assay showed that *PoZCP26* directly bound to the motifs in the promoters of *g10029* and negatively regulated its transcription. Overexpression and RNA interference transformants of *g10029* were constructed and its roles in growth and development of *P. ostreatus* were also studied. It was found that the *g10029* inhibited the growth of *P. ostreatus* mycelium on PDA medium, delayed the primordium formation, and slowed the fruiting body development. This study revealed the promotive roles of transcription factor *PoZCP26* in fruiting body development of *P. ostreatus* and clarified its gene regulation pathway during the process. The downstream regulatory network and target genes of *PoZCP26*, providing insights into the molecular network involved in fruiting body development of mushrooms.

Keywords: Fruiting body development, Transcription factor, Transcriptional regulatory mechanism, *Pleurotus ostreatus*

Abstract

Exploring the polymorphism and origins of accessory chromosomes in *Tremella fuciformis*: insights into genome divergence and structural variation**Author(s)****Jinxiang Zhang¹, Qianwen Tong¹, Fengjiao Lin¹, Baogui Xie^{1#}, Ray Ming^{2,3#}, Youjin Deng^{1#}**¹College of Life Science, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China.²Department of Plant Biology, University of Illinois at Urbana-Champaign, 1201 W. Gregory Drive, Urbana, IL 61801, USA.³College of Future Technology, Fujian Agriculture and Forestry University, Fuzhou 350002, China.

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Accessory chromosomes, while not essential for normal growth and development, have been extensively studied in Ascomycota fungi, but their presence in basidiomycetes is less documented. In this study, whole-genome sequencing was conducted on 16 strains of *Tremella fuciformis* from diverse sources, resulting in 27 complete haplotype genome sequences for comparative analysis. These genomes showed significant size variation, exceeding one-third, mainly due to accessory chromosomes and repetitive sequences in core chromosomes. They contain 8 to 10 core chromosomes, and their polymorphism is mainly caused by the fusion or separation of chromosomes. Each genome contained between 2 to 10 accessory chromosomes, totaling 107, with their distribution reflecting phylogenetic relationships and mating specificity with *Annulohypoxylon stygium*. Accessory chromosomes were small, rich in transposons or repetitive sequences, and had lower gene densities, yet their homology similarity was notably higher than that of core chromosomes. They exhibited rapid and diverse structural variations (SVs), resulting in a lower proportion of shared genes within each branch compared to core chromosomes. Both accessory and core chromosomes showed a high frequency of chromosomal copy number variation during the growth and transformation of the two morphological cell types. Most genes on the accessory chromosomes were not homologous to those on the core chromosomes, and the proteins they encode lacked matching sequences in the NCBI nr database. This research is the first to reveal the diversity of accessory chromosomes in Basidiomycota, providing new insights into their origin, as they are believed to have originated from unexplored species before the speciation of *T. fuciformis*.

Key words: Genome plasticity, Accessory chromosome, Rapid structural variation

Abstract

Genomic signatures of climate-driven reticulate evolution, hybrid origins, adaptive evolution, and domestication in button mushroom**Zhilin Ling^{1*}, Guotao Chen^{1,2}, Ruilin Zhao^{1,2#}**

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The evolutionary and domestication history of mushroom-forming fungi remains poorly understood despite their ecological and economic significance. Here, we present the first large-scale population genomic study of the button mushroom [*Agaricus bisporus* (J. E. Lange) Imbach], the world's most cultivated edible mushroom, integrating resequencing data from 483 wild and commercial strains. Our analyses reveal a complex reticulate evolutionary history shaped by Quaternary glacial cycles. Ice age-induced bottlenecks drove population differentiation into North American and European lineages, while interglacial expansions facilitated hybridization, yielding seven admixed populations. Demographic reconstructions trace cultivated strains to hybrid origins between European and North American populations, resolving their domestication lineage. Through genome-wide association studies (GWAS), transcriptomics, and transgenic validation, we identify *AbPPO1* as a key domestication gene, with its mutant haplotype "Hap_2" underpinning the white cap trait—a hallmark of commercial strains. Unlike albinism in other fungi, white caps in *A. bisporus* arise from polygenic interactions involving melanin synthesis pathways, environmental adaptation, and growth regulation, reflecting a quantitative trait under artificial selection. By bridging evolutionary genomics with domestication genetics, this work provides foundational insights into fungal adaptation and offers a genomic toolkit for enhancing agronomic traits in economically vital species. Our findings redefine the genetic complexity of the button mushroom domestication and underscore the role of historical climate dynamics in shaping fungal biodiversity.

Keywords: Demographic history; Domestication gene; Edible mushroom; Population genomics; Reticulate Evolution

Abstract

Innovative Breeding of Mushroom via BioDesign

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Macrofungi play a crucial role as 'scavengers' and 'recyclers' in Earth's ecosystems. Wood-decaying fungi, in particular, decompose recalcitrant lignocellulosic biomass from agricultural and forestry waste, breaking down complex organic matter into simple inorganic compounds. This process releases elements like carbon, nitrogen, and phosphorus, which are then reutilized to synthesize the fungi's own biomass. Annually, plants fix approximately 200 billion tons of carbon through photosynthesis, with about 70% converted into lignocellulose. In the field of synthetic biology, developing robust macrofungal 'chassis cells' capable of efficiently utilizing lignocellulose holds revolutionary significance for advancing green biomanufacturing based on agricultural circular economy principles. This represents a key technology for promoting a lignocellulose-based bioeconomy that utilizes all components and for achieving sustainable development goals. This study selected and bred strains with fast growth, simple nutritional requirements, clear genetic backgrounds, and suitability for large-scale cultivation to serve as universal chassis. An efficient gene editing system was constructed to enhance substrate-utilizing enzyme systems and optimize the chassis cells. Subsequently, innovative breeding of these mycelial chassis cells was conducted through rational biodesign. This primarily involved designing metabolic pathways and cell wall structures to establish cellular factories for producing macrofungal natural compounds, fungal proteins, and mycelium leather. The research outcomes hold strong prospects as a powerful engine driving future sustainable development.

Keywords: Chassis cells, Macrofungi, Mycoprotein, Mycelium leather, Natural products

Abstract

Genomic Insights and Mating Systems of *Oudemansiella apalosarca*: A Promising Edible Mushroom for Industrial Cultivation

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Oudemansiella apalosarca is a rare edible mushroom highly regarded for its suitability for tourism picking and its nutritional benefits. Compared to other species within the *Oudemansiella* genus, *O. apalosarca* offers a shorter production cycle and does not require soil covering, making it particularly promising for industrial cultivation. However, its complex life cycle and unclear hybrid compatibility among progeny have significantly hindered hybrid breeding efforts. This study focuses on the *O. apalosarca* strain BIPP21200004. By isolating single spores, the homokaryotic strains K72 and G13 were obtained. Genomic analysis revealed that each homokaryon consists of 12 chromosomes. Annotation of mating type genes identified 13 homeodomain (HD) genes, 9 pheromone receptor (RCB) genes, and 5 pheromone precursor (phb) genes on the G13 genome, while the K72 genome contained 14 HD genes, 8 RCB genes, and 5 phb genes. The A and B mating type loci for both strains were located on chromosomes 4 and 12. Specific molecular markers were developed based on polymorphisms in mating type genes. Analysis of 150 F₂ single spore strains revealed a 4:1 ratio of homokaryons to heterokaryons, with four distinct mating types observed: A1B1, A2B2, A1B2, and A2B1. Spore nuclear analysis and hybridization experiments suggest that *O. apalosarca* exhibits both homothallic and heterothallic mating systems. In the F₂ progeny, heterothallic mating offspring were more prevalent. The presence of A1B2 and A2B1 mating types indicates a tetrapolar mating system.

Keywords: *Oudemansiella apalosarca*, homokaryons, heterokaryons, mating types

Abstract

Creation and application of intraspecific cytoplasmic hybrids in *Lentinula edodes*

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In the face of the current situation that nuclear source of *Lentinula edodes* cultivars cannot be easily replaced in China, our team has created a breeding technique of intraspecific cytoplasmic hybrids simply replacing cytoplasmic source. This study provides a detailed introduction to the background, development, cultivation, and molecular mechanism insights of this technique, aiming to facilitate its promotion and adoption. Firstly, through the study of the diversity of nuclear ITS1-5.8S-ITS2 and the mitochondrial genome, it was found that the nuclear and mitochondrial genotypes of most cultivars were single, while those of the wild strains were diverse, and molecular markers capable of identifying each nuclear and mitochondrial typing were screened out. Secondly, the maternal inheritance of cytoplasm of *L. edodes* was verified. The technique of intraspecific cytoplasmic hybrid was created and strains with different cytoplasmic sources were obtained based on the multiple steps of isolation and regeneration of haploid protoplasts and mon-mon crossing. Thirdly, after many years of cultivation and application, it has been confirmed that this technology has the advantages of stabilizing fruiting capacity, clearing nuclear and cytoplasm sources and changing reproduction rate. Finally, through the study of the relevant analysis between the nuclear and mitochondrial transcriptomes, it was found that the replacement of cytoplasm mainly affected the expression level of genes related to browning processes, especially the metabolism and secretion of small molecules, such as melanin, thereby significantly altering the browning processes and changing the number of fruiting bodies per bag.

Keywords: *Lentinula edodes*, cytoplasmic replacement, breeding, browning process, reproduction rate

Abstract

Breeding of a high-lysine yellow *Flammulina filiformis* new cultivar ‘Shangyan Jin 31’

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Flammulina filiformis are favored by consumers for their nickname ‘Smartness Mushroom’. Among them, the yellow cultivar exhibits a crispy texture when eaten, but due to issues such as low yield, high cultivation technology requirements, and insufficient market recognition, its market promotion process is fraught with difficulties. but their promotion process is limited by multiple factors such as low yield, high cultivation technology requirements, and insufficient market recognition. The localization analysis of the genes in the candidate region revealed that when the amplification product at the SNP site 2028634 is C, it can be identified as a high lysine-producing strain. Based on the amino acid detection results of 405 strains, selected extreme strains with high and low lysine content as parents to prepare spore hybrid offspring. From the offspring, 15 strains with low and high lysine content were respectively selected, and after bulked segregant analysis (BSA) localization analysis, 9 candidate regions were obtained. This research used the high-yielding variety ‘Shangyan 1’ and the thick-pileus and sturdy -stipe variety ‘J4137’ as parents. After protoplast mononuclearization, hybrid strains were obtained through single-cross. The hybrid strains were followed by preliminary screening, small and medium re-screening, and demonstration cultivation, as well as identified by molecular markers at high lysine loci, a new cultivar ‘Shangyan Jin31’ that combines high yield, thick pileus, sturdy stipe, and high lysine content. Under the industrial-bottle cultivation mode, the average yield of ‘Shangyan Jin 31’ reached 459.82 g/bottle, an increase of 88.40% compared to the parent ‘J4137’ (244.06 g/bottle). The average number of effective stipes is 1025 (counted based on more than 1/3 of the stipe). The primordial formation time is comparable to that of the parent ‘J4137’; the fruiting body growth time is 25 days, 2 days earlier than that of the parents. The total amino acid content is higher than that of the two parents, which lysine is increased by 40% and 7.69% respectively. The new cultivar ‘Shangyan Jin31’ has the characteristics of high yield, excellent commercial traits, and high lysine content, which is suitable for industrialized cultivation and consumers’ pursuit of healthy diets.

Keywords: *Flammulina filiformis*; lysine; molecular marker; hybridization; agronomic traits

Abstract

Breeding and Promotion of 'Shenxiu Series' New Varieties of *Pleurotus pulmonarius* for Industrial Production

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Pleurotus pulmonarius, commonly known as Phoenix oyster mushroom, is highly valued for its appealing morphology, tender texture, and crisp flavour. With advancements in industrial cultivation facilities and equipment, industrialized production has become the mainstream method for its cultivation. According to statistics, the national production of *P. pulmonarius* reached 692,000 tons in 2023, reflecting a remarkable growth of 242.36% over the past decade. To meet the urgent demand for high-quality strains suited to industrial cultivation, this study conducted systematic breeding and nutritional evaluation. 'Shenxiu No. 1' was selected from a self-breed population of 318 strains. It exhibits rapid mycelial growth (5.3 ± 0.2 mm/d at 23°C on PDA), high yield, and excellent morphological characteristics, including a distinct white-center and gray-margin pileus that is resistant to breakage, and a firm stipe (diameter: 11 ± 0.13 mm; length: 56 ± 0.49 mm). 'Shenxiu No. 2' was developed through the domestication of wild germplasm collected from Tibet. Its mycelial growth rate is 4.23 ± 0.03 mm/d at 25°C on PDA, with no aerial mycelium observed. The fruiting bodies are light brown, grow in clusters, and can be harvested as whole clusters. The pileus is funnel-shaped (length: 6.47 ± 0.21 cm; width: 6.07 ± 0.08 cm), and the stipe is white (length: 4.74 ± 0.04 cm; diameter: 1.17 ± 0.27 cm). ISSR molecular marker analysis confirmed its distinct genetic profile. An integrated "Elite Seed-Elite Method" cultivation approach was developed, enabling year-round production of these varieties. They have been successfully demonstrated and promoted in multiple provinces, including Shanghai and Fujian, with outstanding performance. Nutritional evaluation of 12 *P. pulmonarius* samples from different regions revealed that all samples were rich in amino acids. A total of 15 amino acids were detected, with glutamic acid being the most abundant. The total amino acid (TAA) content ranged from 16.43 to 39.67 mg/g, showing significant variation. The ratios of essential amino acids to total amino acids (IAA/TAA) and essential to non-essential amino acids (IAA/NIAA) ranged from 16.49% to 30.12% and 19.75% to 43.11%, respectively. Methionine + cysteine was identified as the first limiting amino acid. Cluster analysis classified the 12 samples into four distinct categories. These findings provide critical data for quality breeding and advanced product development based on nutritional characteristics.

Keywords: *Pleurotus pulmonarius*; industrial cultivation; variety breeding; domestication; nutritional evaluation

Abstract

Identification of the regulatory factors in sexual reproduction signaling of *Pleurotus eryngii*

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Pleurotus eryngii is an important commercially cultivated edible mushroom in Asia. Research on its sexual reproduction and fruiting body development contributes to variety improvement through molecular genetic breeding. By introducing compatible mating-type genes into the monokaryotic strain of *P. eryngii*, we observed the formation of clamp connections and the development of fruiting bodies. Further transcriptome sequencing of the monokaryotic transformants revealed that the expression of certain genes was strongly induced by the introduced mating-type genes. Using experimental methods such as yeast two-hybrid screening, gene overexpression, and RNAi, we conducted further studies on *MRP1*, an HMG-box transcription factor gene, and *PRK1*, a serine-threonine kinase gene, both of which are induced by the mating-type genes. The results showed that *MRP1* interacts with homologs of the proteins regulating cell polarity growth and sexual reproduction in yeast. Overexpression of *MRP1* promoted mycelial growth and enhanced antioxidant enzyme activity, while RNAi transformants of *MRP1* exhibited slowed mycelial growth. Yeast two-hybrid results for *PRK1* indicated that its interacting proteins are primarily enriched in pathways such as oxidative phosphorylation, MAPK signaling, and protein kinase signaling. RNAi transformants of *PRK1* showed prolonged primordium formation and produced small fruiting bodies with abnormal cap development. Additionally, downregulation of *PRK1* transcript levels in the RNAi transformants led to a decrease in *MRP1* expression, suggesting that *PRK1* acts upstream of *MRP1* in the signaling pathway. These findings suggest that *MRP1* and *PRK1* are regulators induced by mating-type genes in the sexual reproduction signaling process in *P. eryngii*.

Keywords: *Pleurotus eryngii*, Regulatory factors, Sexual reproduction signaling

Abstract

Genomic Insight and Light-Regulated Transcriptomics of Fruiting Body Development in *Oudemansiella apalosarca*

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The Short production cycle and lack of a casing layer requirement of *Oudemansiella apalosarca* carry significant implications for commercial cultivation as a newly identified edible and medicinal fungus in the family *Physalacriaceae*. Varietal development and genetic improvement have been significantly hindered by the shortage of genomic data resource of *O. apalosarca*. This study aimed to sequence the first genome of *O. apalosarca* and to gain a better understanding of transcriptome regulation of fruiting body development under different light conditions. Whole-genome sequencing was performed to assemble and annotate the genome of *O. apalosarca* and phylogenetic analysis was conducted using single-copy orthologous proteins from 25 fungal genomes. Cultivation experiments were conducted under light, dark, light-to-dark, and dark-to-light conditions, followed by a comparative transcriptome analysis of caps and stipes. Differentially expressed genes and enriched pathways were found using Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Set Enrichment Analysis (GSEA). The *O. apalosarca* genome was assembled into 27 scaffolds (total 53.13 Mb) with 14,650 predicted protein-coding genes, achieving higher scaffold N50 and BUSCO completeness than other *Oudemansiella* genomes. Phylogenetic analysis revealed close relationships with *Oudemansiella raphanipes* and *Mucidula mucidula*, with strong protein collinearity within the family *Physalacriaceae*. Dark cultivation produced pure white, small-cap and long-stipe fruiting bodies. Transcriptome analysis indicated a significant downregulation of 10 cell cycles and mismatch repair genes in caps under darkness, while genes involved in cell wall synthesis and carbon metabolism were upregulated in stipes, encouraging elongation. Results showed that tyrosine metabolism influenced cap pigmentation. Phylogenetic analysis revealed distinct evolutionary origins for the seven multicopy tyrosinase genes. Two tyrosinase-related genes (A6_A10477 and A6_A09603) were overexpressed under light, indicating their potential roles in the production of melanin. This study provides the first genomic resource for *O. apalosarca*, elucidating the molecular mechanisms underlying light-regulated fruiting body development and establishing a foundation for further research and commercial applications.

Keywords: Fruiting body development, Genome, Light-regulation, *Oudemansiella apalosarca*, Transcriptome

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Abstract

Transcriptional Regulatory Mechanism of a Zn(II)₂Cys₆ Transcription Factor PoZCP26 in Fruiting Body Development of *Pleurotus ostreatus*

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Pleurotus ostreatus is one of the most cultivated edible mushrooms worldwide, of which the fruiting body development is a highly complex process involving precise genetic regulatory networks. Transcription factors are well-recognized key factors among these networks. In this study, one Zn(II)₂Cys₆ transcription factor named PoZCP26 was up-regulated in primordium and fruiting body compared to mycelium stage. Thus, the overexpression and RNA interference transformants of PoZCP26 were constructed for *P. ostreatus* and the roles of PoZCP26 on mycelia growth and fruiting body development were examined. The results showed that PoZCP26 promoted the mycelial growth rate, the primordial differentiation and fruiting body formation. To further detect the binding motifs and potential target genes of PoZCP26, we performed DNA-affinity purification sequencing (DAP-seq) analysis in vitro. In combination of RNA-seq and DAP-seq results together, we subsequently identified several target genes of PoZCP26 that might be involved in fruiting body development. Among them, an uncharacterized protein encoding gene g10029 were selected out for in-depth study. EMSA (Electrophoretic Mobility Shift Assay) and Dual-Luciferase Reporter Assay showed that PoZCP26 directly bound to the motifs in the promoters of g10029 and negatively regulated its transcription. Overexpression and RNA interference transformants of g10029 were constructed and its roles in growth and development of *P. ostreatus* were also studied. It was found that the g10029 inhibited the growth of *P. ostreatus* mycelium on PDA medium, delayed the primordium formation, and slowed the fruiting body development. This study revealed the promotive roles of transcription factor PoZCP26 in fruiting body development of *P. ostreatus* and clarified its gene regulation pathway during the process. The downstream regulatory network and target genes of PoZCP26, providing insights into the molecular network involved in fruiting body development of mushrooms.

Keywords: Fruiting body development, Transcription factor, Transcriptional regulatory mechanism, *Pleurotus ostreatus*

Abstract

Creation and application of intraspecific cytoplasmic hybrids in *Lentinula edodes*

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In the face of the current situation that nuclear source of *Lentinula edodes* cultivars cannot be easily replaced in China, our team has created a breeding technique of intraspecific cytoplasmic hybrids simply replacing cytoplasmic source. This study provides a detailed introduction to the background, development, cultivation, and molecular mechanism insights of this technique, aiming to facilitate its promotion and adoption. Firstly, through the study of the diversity of nuclear ITS1-5.8S-ITS2 and the mitochondrial genome, it was found that the nuclear and mitochondrial genotypes of most cultivars were single, while those of the wild strains were diverse, and molecular markers capable of identifying each nuclear and mitochondrial typing were screened out. Secondly, the maternal inheritance of cytoplasm of *L. edodes* was verified. The technique of intraspecific cytoplasmic hybrid was created and strains with different cytoplasmic sources were obtained based on the multiple steps of isolation and regeneration of haploid protoplasts and mon-mon crossing. Thirdly, after many years of cultivation and application, it has been confirmed that this technology has the advantages of stabilizing fruiting capacity, clearing nuclear and cytoplasm sources and changing reproduction rate. Finally, through the study of the relevant analysis between the nuclear and mitochondrial transcriptomes, it was found that the replacement of cytoplasm mainly affected the expression level of genes related to browning processes, especially the metabolism and secretion of small molecules, such as melanin, thereby significantly altering the browning processes and changing the number of fruiting bodies per bag.

Keywords: *Lentinula edodes*, cytoplasmic replacement, breeding, browning process, reproduction rate

Abstract

Multi-omics Analysis of Pathogenic Mechanisms and Disease-resistant Breeding of Yellow-root Disease in *Ganoderma lingzhi*

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Background: Yellow-root disease of *Ganoderma lingzhi* represents a critical constraint to the development of the *Ganoderma* industry, resulting in deterioration of fruiting body quality and yield losses of 30-50%. However, the causative pathogen and pathogenic mechanisms remain unclear, severely impeding effective disease control and breeding of resistant cultivars. This study aimed to elucidate the molecular mechanisms underlying this disease through multi-omics approaches and provide a foundation for disease-resistant breeding.

Methods: ITS amplicon sequencing and pathogen isolation were conducted on diseased fruiting bodies and cultivation substrates collected from major *G. lingzhi* production areas in China, followed by pathogenicity verification through Koch's postulates. The genome of *Xylogone ganodermophthora* was sequenced and assembled using next-generation sequencing technology, and virulence factor genes were predicted based on the PHI-base database with stringent criteria (alignment length ≥ 80 amino acids/240 nucleotides, E-value $< 1e-30$, identity $\geq 96\%$). Metabolomic profiles of healthy and diseased fruiting bodies were systematically analyzed using UPLC-MS/MS-based untargeted metabolomics. Additionally, disease-resistant mutants were screened through chemical mutagenesis combined with ARTP (atmospheric and room temperature plasma) mutagenesis. Growth performance of the mutant and parental strains was compared on media supplemented with pathogen metabolites, and the molecular mechanisms underlying stress resistance in the mutant strain were elucidated through integrated transcriptomic and metabolomic analyses.

Results: ITS amplicon sequencing revealed that the relative abundance of *Xylogone ganodermophthora* reached 60-70% in both diseased fruiting bodies and cultivation substrates. Combined with back-inoculation verification and re-isolation results, *X. ganodermophthora* was identified as the causative agent of yellow-root disease in *G. lingzhi* for the first time. Genome-wide analysis identified six high-confidence candidate virulence factor genes with phenotypes including reduced virulence, loss of pathogenicity, chemistry target: resistance to chemical, lethal, and unaffected pathogenicity. Metabolomic analysis revealed 24 significantly differential metabolites in diseased fruiting bodies compared to healthy tissues, predominantly alkaloids, terpenoids, phenolic acids, and flavonoids, which were likely induced by pathogen infection or produced as stress responses in *G. lingzhi*.

One disease-resistant mutant strain was successfully obtained, exhibiting significantly faster mycelial growth than the wild-type strain under pathogen metabolite stress. Transcriptomic and metabolomic analyses revealed that the mutant and wild-type strains shared 473 core stress-responsive genes and 624 metabolites. The mutant strain specifically up-regulated 142 genes and 289 metabolites under stress conditions, constitutively expressed 13 genes and 151 metabolites that remained up-regulated under stress after mutagenesis, and possessed 117 unique stress-responsive genes and 113 unique metabolites. In-depth data mining identified significant associations between 69 mutant-specific genes and 72 metabolites, which were functionally enriched in carbohydrate metabolism and lipid metabolism pathways, indicating that the mutant strain enhanced stress resistance primarily through strengthened carbohydrate and fatty acid metabolism.

Conclusion: This study identified the causative pathogen of yellow-root disease in *G. lingzhi* and systematically elucidated the molecular mechanisms of pathogenesis and disease resistance at the genomic, transcriptomic, and metabolomic levels. The disease-resistant germplasm resources generated provide important theoretical foundations and superior genetic materials for green prevention and control of yellow-root disease and breeding of resistant *G. lingzhi* cultivars.

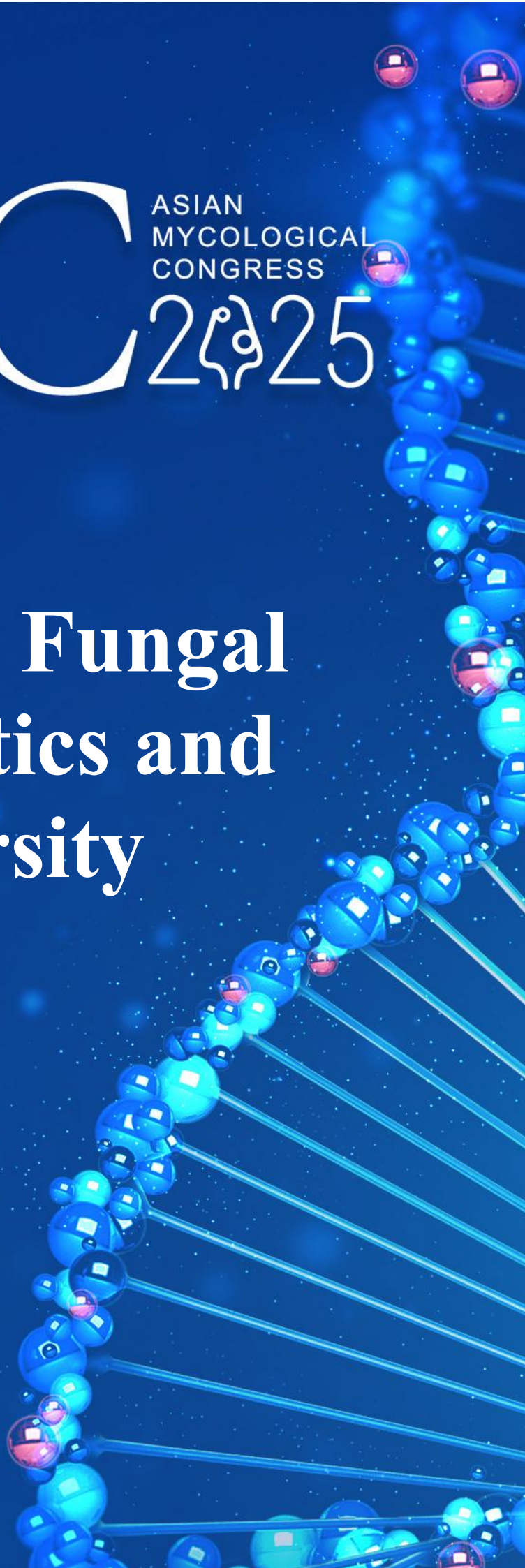
Keywords: *Ganoderma lingzhi*; yellow-root disease; *Xylogone ganodermophthora*; multi-omics; pathogenic mechanism; disease-resistant breeding

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Abstract

Speciation and evolutionary mechanisms of *Colletotrichum***Fang Liu^{1,2*#}, Ziyang Ma^{1,2}, Lei Cai^{1,2#}**¹*State Key Laboratory of Microbial Diversity and Innovative Utilization, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, P. R. China.*²*University of Chinese Academy of Sciences, Beijing, 100049, P. R. China.**Fang Liu: liufang@im.ac.cn, Ziyang Ma: ziyangss@163.com, Lei Cai: cail@im.ac.cn*

Understanding speciation in plant pathogens is crucial for predicting the emergence of novel virulent strains and comprehending biodiversity. This study investigates the evolutionary history and mechanisms driving diversification in *Colletotrichum*, a globally significant plant-pathogenic fungal genus. Our divergence dating analysis indicates an origin in the middle Eocene (~47.57 Mya) with an ancestral broad host range, followed by host specialization towards monocots and legumes during the Eocene-Oligocene transition (~32–39 Mya), a period coinciding with global cooling and aridification. Accelerated diversification, particularly within the *C. gloeosporioides* species complex (CGSC), occurred from the late Miocene onward, likely influenced by Pliocene vegetation shifts and recent human-mediated crop domestication. Through the analysis of 49 CGSC genomes, we reveal that transposable element expansion, especially long terminal repeat retrotransposons, significantly contributes to genome size and genetic variation. Furthermore, our findings suggest that chromosomal rearrangements, mediated by repetitive sequences, may act as a primary driver of speciation in CGSC, generating highly dynamic lineage-specific genomic regions. Overall, our work demonstrates how ancient climatic shifts, host adaptation, genomic plasticity, and anthropogenic factors interactively shape *Colletotrichum* speciation and adaptive radiation.

Keywords: Adaptive radiation, Chromosomal rearrangement, Diversification rate, Genome evolution, Host range shift

Abstract

Phylogenetic Analysis Reveals Unaccounted Sequence Diversity in *Stipitochaete* (Hymenochaetales, Basidiomycota)**[Jiří Kout 1*#], [Josef Vlasák 2]**

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The genus *Stipitochaete* was established for two stipitate *Hymenochaete* species growing in South America, *S. damicornis* and *S. reniformis*. Even if it has not been confirmed by multigenic phylogeny as separate from *Hymenochaete*, we use this accustomed genus name for better orientation. Noting remarkable morphological variability of *Stipitochaete* in nature, we have performed sequence analysis of GenBank ITS sequences as well as of our own collections to confirm/question two *Stipitochaete* species. Extensive sampling of *Stipitochaete* sp. was carried out in Central and South America (Belize, Costa Rica, French Guiana, Ecuador, Peru) in years (2006) 2015–2025, DNA isolated and ITS gene sequenced. Sequence analysis was performed by standard methods. Our broad sampling of *Stipitochaete* in Central- and South American countries and ITS-based molecular analysis reveal surprisingly high sequence variability within the genus, suggesting perhaps five species, which have to be confirmed by multigenic phylogeny and morphologically defined.

Keywords: *Hymenochaetaceae*, Neotropical Region, phylogeny

Abstract

Investigation of Novel Follicolous Fungi Associated with Medicinal Plants for Their Antimicrobial Potential

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Recent discoveries of new asexual fungi with distinctive morphology and phylogeny have led to the establishment of several novel genera, including *Marcstadlera*, *Neocercospora*, *Neoclypeosphaerella*, *Neohyalokamallomyces*, *Phaeokamallomyces*, and *Uwebrauniomyces*. These fungi, primarily occurring on living leaves of various medicinal plants, reveal previously unexplored taxonomic diversity within the *Mycosphaerellaceae* family.

Phylogenetic analyses using multigene markers (LSU, *RPB2*, ITS) have identified species that fall outside any previously established lineages, necessitating the creation of new genera and species. For instance, *Neocercospora* and *Uwebrauniomyces*—found on *Peristrophe bicalyculata* and *Tinospora cardifolia*, respectively—are distinguished by unique conidiogenous structures with conical loci bearing a small rim-like depression, a feature absent in other members of the family. Despite superficial similarities to *Passalora*, these morphological traits provide strong taxonomic justification for their separation.

A particularly notable addition is *Marcstadlera*, proposed for a fungus isolated from *Mallotus philippensis* in India. This genus, characterized by cylindrical, truncate conidiogenous loci and a narrow, obconically truncate conidial base, is phylogenetically related to cercosporoid fungi but sufficiently distinct in both morphology and genetic makeup to warrant its own genus. The introduction of *Marcstadlera* prompted the reclassification of *Clypeosphaerella sticheri* into the newly described *Neoclypeosphaerella*. Additionally, *Neohyalokamallomyces* and *Phaeokamallomyces*—reported on *Holoptelea* spp.—further expand the genus-level diversity of the family.

Beyond their taxonomic significance, these fungi possess notable biotechnological potential. Secondary metabolites produced by these novel genera show promising activity against multidrug-resistant bacteria, making them potential sources of antimicrobial compounds. Investigating these metabolites not only enhances our understanding of plant–fungus interactions but also opens avenues for the discovery of novel pharmaceutical agents that could serve as alternatives to conventional medicines.

Keywords: Novel fungal strains, Antimicrobial, New taxa

Abstract

Phylogenomics, outline, and database of macrofungi

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Owing to their irreplaceable ecological functions and considerable potential in food, medicine, and other applications, macrofungi have become a central focus of contemporary scientific research and resource utilization. In recent years, rapid advances in molecular systematics have greatly promoted fungal taxonomy, leading to continuous refinement of classification systems. Against this backdrop, a comprehensive, accurate, and regularly updated taxonomic database of macrofungi is critically needed for mycological and related studies. To address the current absence of a dedicated classification framework, we compiled a hierarchical classification of all macrofungal genera up to the phylum level, based on the most recent literature and a phylogenomic tree generated in this study. As of the current update, we document 1,982 macrofungal genera distributed across two phyla, 15 classes, 49 orders, and 247 families. Accordingly, we established the online database MTSEM (Macrofungal Taxonomic System and Economic Mushrooms; <https://nmde.cn/macromungi/>). MTSEM is built on the taxonomic framework of the *Outline of Fungi and fungus-like taxa* and integrates systematic information across all taxonomic ranks (phylum, class, order, family, genus, and species), together with genomic data, specimen and strain records from global herbaria and culture collections, as well as related publications and patents. In addition, three specialized modules—edible, medicinal, and poisonous mushrooms—offer up-to-date insights into research and applications. Currently, MTSEM incorporates more than 9,900 genera, 142,300 species, 20,100 genomes, 101,400 specimens, 213,100 strains, and 808,300 publications and patents, and features a sustainable, dynamic update mechanism to ensure its long-term value as an authoritative data resource for research, education, and industry.

Keywords: Ascomycota – Basidiomycota – classification – mushroom – website

Abstract

Polyphasic Taxonomy of Fungi Associated with Coffee in Yunnan (China) and Chiang Rai (Thailand) provinces**Li Lu^{1,2,3,4}, Samantha C. Karunarathna², Ruvishika S. Jayawardena^{3,4}, Kevin D. Hyde^{3,4*}, Saowaluck Tibpromma^{2*}**¹ *Bioengineering and Technological Research Center for Edible and Medicinal Fungi, Jiangxi Agricultural University, Nanchang 330045, P.R. China*² *Center for Yunnan Plateau Biological Resources Protection and Utilization & Yunnan International Joint Laboratory of Fungal Sustainable Utilization in South and Southeast Asia, College of Biology and Food Engineering, Qujing Normal University, Qujing 655099, P.R. China*³ *Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand*⁴ *School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand**Email of the corresponding author: kdhyde3@gmail.com and saowaluckfai@gmail.com*

Yunnan, China, and Chiang Rai, Thailand, are located in the northern part of the Greater Mekong Subregion (GMS) and are characterized by their warm climate and rich ecological diversity, offering a unique habitat for diverse fungal communities. These environmental conditions not only support the growth of *Coffea arabica*, one of the region's most economically important cash crops, but also favor the proliferation of fungi, increasing the crop's vulnerability to fungal infections during both cultivation and post-harvest periods. Thus, a comprehensive study was conducted to investigate the fungi associated with the economic crop coffee in Yunnan, China, and Chiang Rai, Thailand.

A total of 360 saprobic fungal collections, showcasing the rich diversity of the region, were obtained from eight locations—Baoshan, Dali, Dehong, Jinghong, Lincang, Nujiang, and Pu'er in Yunnan, China, and Chiang Rai, Thailand. Initial ITS analyses revealed that these collections represent seven classes, 37 orders, 83 families, and 137 genera. The life modes of these taxa were interpreted based on previously published studies and were compared and discussed. From the total collections, 139 well-preserved samples with abundant fruiting bodies and mature morphological structures were selected for detailed morphological and multi-locus phylogenetic analyses. These collections span four classes, 27 orders, 69 families, and 87 genera, resulting in the identification of six new genera, 77 new species, one novel DNA sequence, 47 new records, and four additional collections. Notably, four species (*Acrocalymma daliense*, *Pseudohelminthosporium clematidis*, *Tubeufia coffeae*, and *Menisporopsis dinemasporioides*) were documented in both their sexual and asexual morphs on natural substrates.

In addition, 235 fungal endophytes were isolated from 270 leaf segments of *Coffea arabica* collected from four coffee plantations in Yunnan. All isolates were identified through ITS sequencing, revealing their classification within Ascomycota, distributed across two classes, 10 orders, and 17 families. To assess their potential for biological control, 61 endophytes were

evaluated in dual-culture assays against four known plant pathogens. Notably, *Nigrospora* sp. XCE-7 and *Daldinia* sp. ME-9 inhibited *Alternaria alternata* with over 70% growth suppression. Likewise, *Daldinia* sp. ME-9, *Nigrospora* sp. T5E-7, and *Daldinia* sp. T5E1-3 effectively inhibited *Penicillium digitatum*, also exceeding 70% inhibition rates.

Besides, a new species of sooty mold, *Trichomerium puerense*, was also described and confirmed through pathogenicity testing as a pathogen of *Coffea arabica* in Yunnan.

Overall, this work reveals the extensive diversity of microfungi associated with coffee plants in southern China and northern Thailand. The discovery of new taxa, which significantly expands our understanding of the microfungi diversity, and geographic records enhances biodiversity inventories, while the identification of bioactive endophytes offers promising avenues for environmentally friendly disease management strategies. These findings not only support sustainable agricultural practices and promote biodiversity conservation but also inspire and motivate by contributing to innovation in plant health management.

Keywords: 77 New Species, 6 New Genera, Endophytic, Saprobic, Sooty Mold Fungi

Abstract

Revision of acremonium-like fungi in *Hypocreales*: A taxonomic and phylogenetic perspective

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Acremonium-like fungi represent a highly diverse group that occur as saprobes, pathogens, and endophytes in a wide range of habitats, including soil, plants, marine organisms, sediments, and air. Their ecological versatility is reflected in their ability to produce a broad spectrum of secondary metabolites with potential applications in medicine, agriculture, and biotechnology. However, the genus *Acremonium* has long been regarded as one of the most morphologically reduced groups among filamentous asexual fungi, and its simple morphology combined with overlapping species characteristics has greatly complicated its taxonomy. Recent integrative studies combining morphology, phylogeny, and ecological traits have revealed that acremonium-like fungi are distributed across several orders, including *Cephalothecales*, *Coniochaetales*, *Glomerellales*, and *Hypocreales*, with *Acremonium* sensu stricto now restricted to *Bionectriaceae*. In this study, we examined 402 acremonium-like strains from the CBS collection, including many that had not been previously subjected to molecular analysis. Morphological characterisation was combined with phylogenetic analyses using DNA sequence data from the nuclear ribosomal internal transcribed spacer (ITS), partial 28S large subunit (LSU) rDNA, partial RNA polymerase II second largest subunit (*RPB2*), and partial translation elongation factor 1-alpha (*TEF1*). Our results show that the analysed strains belong to two major lineages, *Hypocreales* and *Trichosphaeriales*, encompassing 17 families and 148 taxa. The most represented families are *Bionectriaceae*, *Sarocladiaceae*, and *Trichosphaeriaceae*. Furthermore, this study establishes two new families, seven new genera, three new combinations, and 34 novel species. A robust phylogenetic framework for *Hypocreales* is also provided, resolving 29 families, including two newly introduced families. Together, these findings refine the taxonomy of acremonium-like fungi and provide a foundation for future studies on their ecology, diversity, and biotechnological potential.

Keywords: acremonium-like, *Bionectriaceae*, *Hypocreales*, multi-locus, new taxa, phylogeny, taxonomy

Abstract

Exploring Macrofungal Biodiversity and Distribution on Kyodong Island, Republic of Korea**Hannah Suh^{1,*}, Abel Severin Lupala^{1,2}, Hae Jin Cho³, Sumin Jo¹, Jiyun Choi¹ and Young Woon Lim^{1,#}**¹*School of biological Sciences and institute of biodiversity, Seoul National University, Seoul, Republic of Korea*²*Department of Microbiology, Parasitology and biotechnology, Sokoine University of Agriculture, Morogoro, Tanzania*³*Division of Microbiology, Honam National institute of biological Resources, Mokpo-si, Republic of Korea*

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Macrofungi are essential components of forest ecosystems, functioning as decomposers, symbiotic partners, pathogens, and indicators of biodiversity. Kyodong Island, located near the Korean Demilitarized Zone (DMZ), has experienced limited human disturbance, providing a unique setting for investigating fungal diversity. In recent years, however, the island has been subjected to rapid development. This study was to assess the macrofungal diversity of Kyodong Island and investigate how ongoing anthropogenic and natural environmental changes influence macrofungal communities. From March to September 2024, a total of 226 specimens were collected and classified into 99 species through both morphological and molecular approaches. Among them, six species were newly recorded for Korea. The community was dominated by saprotrophic fungi (79.8%), with ectomycorrhizal fungi (19.19%) and parasitic fungi (1.01%). Genera such as *Amanita* and *Collybiopsis* were especially abundant. Interestingly, several taxa typically associated with tropical regions were also identified, suggesting a potential northward expansion of fungal distributions linked to climate change. These results underscore the role of Kyodong Island as an important reservoir of fungal diversity and highlight the need for ongoing monitoring. Furthermore, our results establish a valuable baseline for evaluating shifts in fungal communities in response to continuing and future development.

Keywords: Biogeography; Climate change; Conservation; Macrofungi; Military Demarcation line (MDL)

Abstract

Study on diversity, distribution and secondary metabolites of endophytic fungi of *Camphora* Fabr. with citral chemotype

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High-throughput sequencing, manual strain isolation, and gas chromatography-mass spectrometry technology were used to study the diversity, distribution characteristics and secondary metabolites of endophytic fungi of three *Camphora* Fabr. with citral chemotype. The results showed that: (1) The endophytic fungi of citral *Camphora* were mainly Ascomycota, Basidiomycota and Glomeromycota. The diversity of endophytic fungi was different among *Camphora*. (2) Distribution characteristics of endophytic fungi was affected by tree species, tissue sites and host secondary metabolites. The endophytic fungal clusters of *Camphora bodinieri* and *Camphora officinarum* were closer than those of *Camphora parthenoxylon*; and the endophytic fungi of leaves and stems were closer than those of roots. It was worth noting that *Alternaria/Sordariales* was significantly related to host's neral/geranial and safrole, respectively. (3) Strain BO16 was *Alternaria*, isolated from leaves, contained citral (relative content 3.32%), linalool (8.25%) and 2,6-octadiene-1-ol (0.52%), which compositions were also present in the host.

In this study, the association between endophytic fungi and host essential oil components was clarified, and potential functional strains were screened. It provided a new source for the development of endophytic fungi with high value-added natural products, and laied the foundation for the precise interaction pathway of "microbial-plant secondary metabolism" of *Camphora* with citral chemotype.

Keywords: *Camphora* Fabr.; citral chemotype; endophytic fungi; secondary metabolites; *Alternaria*

Abstract

Study on the Species Diversity of Pathogenic Fungi Affecting Chinese Walnuts and Their Biological Control

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Walnuts are a key "woody grain and oil" strategic tree crop in China, with the country ranking first globally in both walnut production and cultivation area. However, extensive walnut cultivation in recent years has led to an increase in fungal diseases affecting walnut fruits, leaves, and branches, significantly impacting both yield and quality, and resulting in substantial economic losses. This study systematically investigates the species diversity of pathogenic fungi affecting Chinese walnuts. Disease samples were extensively collected from major walnut-growing regions in China and identified using integrated morphological and molecular systematic approaches. Pathogenicity was confirmed based on Koch's postulates, yielding the following findings. A total of 827 specimens of fungal diseases affecting walnuts were collected from nine provinces (municipalities and autonomous regions), including Beijing, Gansu, Hebei, Shandong, Shanxi, Shaanxi, Sichuan, Yunnan, and Xinjiang. These comprised 359 fruit disease samples, 192 leaf disease samples, and 276 branch disease samples, from which 1,882 fungal strains were isolated. Through morphological observation and identification of the strains, coupled with multi-gene sequence analysis using ITS, *tefl-α*, *tub2*, *RPB1*, *RPB2*, *CAL*, *HIS*, and *LSU*, and validation based on Koch's postulates, 77 species of fungi under 23 genera, 12 families, and six orders were identified as pathogenic to walnuts, including 26 newly described species. The dominant groups isolated were *Colletotrichum* (23.6%), *Botryosphaeriaceae* (17.2%), *Diaporthe* (16.2%), *Didymellaceae* (11.7%), *Alternaria* (8.6%), and *Fusarium* (7.8%). Among these, *Colletotrichum* and *Alternaria* primarily affected fruits and leaves, *Diaporthe* caused branch diseases, while *Botryosphaeriaceae*, *Didymellaceae*, and *Fusarium* were pathogenic to fruits, leaves, and branches. Additionally, the study isolated and screened four promising biocontrol microorganisms, which included one fungal species, *Granulobasidium vellereum*, and three bacterial species: *Bacillus amyloliquefaciens*, *B. safensis*, and *B. velezensis*. Validation was conducted through dual-culture assays on agar plates, in vitro tests, and branch inoculation experiments. It was found that *G. vellereum* effectively controlled multiple fungal diseases, with pre-inoculation of the biocontrol strain demonstrating superior efficacy compared to simultaneous or post-inoculation. The three bacterial strains exhibited antagonistic effects against nine species of anthracnose fungi in the dual-culture assays, with *Bacillus velezensis* showing the strongest inhibitory effect. This research clarifies the diversity of pathogenic fungi associated with walnut diseases in China and provides theoretical guidance and practical significance for the biological control of fungal diseases in walnuts.

Keywords: Biocontrol, Koch's Postulates Test, Pathogenic Fungi, Walnut

Abstract

The diversity of Xylariales in China

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This work highlights the newly described taxa and expanded range records of Xylariales from China, while resolving nomenclatural inconsistencies through type specimen examinations. This unified taxonomic resource advances both mycological systematics and conservation prioritization by providing: (1) a verified reference baseline for Chinese Xylariales, (2) evidence-based genus delimitation criteria, and (3) documentation of endemic lineages with pharmaceutical potential. Our annotated checklist synthesizes records of 655 Xylariales species across genera reported until August 2025, incorporating taxonomic revisions from historical collections, contemporary field studies, and curated databases including Index Fungorum. Each entry combines morphological descriptions with ecological metadata, molecular phylogenies, geographic distributions, and applied biological data (pathogenicity reports and bioactive compound profiles). We aim to establish a standardized taxonomic framework for Xylariales species documented in China, integrating multidisciplinary data to create a comprehensive species inventory.

Keywords: Xylariales, China, Taxonomy, Phylogeny, Metabolites

Abstract

Fungal Diversity Across Tropical and Non-Tropical Rainforests Through Edna Metabarcoding

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Our assessment of fungal communities across tropical (Hainan, China) and non-tropical (Guizhou, China) rainforests via eDNA metabarcoding of 97 samples revealed stark biogeographical contrasts, demonstrating a profound tropical vulnerability where mixed land use (MIX) caused a complete diversity collapse (Shannon = 0.00) in Hainan, while Guizhou retained residual diversity (Shannon = 0.28), with disturbance reducing diversity by 90% in the tropics versus 64% in the non-tropics. This heightened sensitivity was linked to extreme habitat specialization, as organic matter (OM) habitats sustained peak diversity in Hainan (Shannon = 3.73), a finding supported by a GLM showing OM had the strongest positive effect (estimate = 3.815, $p < 0.001$), with Hainan harboring 5.3× more habitat-specialist OTUs (64 vs. 12) and Soil/Tree (ST) habitats acting as critical refugia (34 indicator OTUs, association strength = 0.966). We further found a temporal asymmetry, where, despite fluctuations, Hainan maintained stable regional diversity (interannual $\Delta = -1.45\%$, $p = 0.653$), whereas 33% of Guizhou samples showed persistent zero diversity across years, indicating the presence of extinction debts. PERMANOVA confirmed habitat type was the primary driver of community structure ($R^2 = 0.301$, $p = 0.001$), with MIX causing an 87% diversity loss in the tropics versus non-significant effects in Guizhou ($p = 0.207$), leading to the conservation imperative that

tropical fungi are facing a catastrophic collapse under land-use change due to niche specialization, demanding urgent protection of OM-rich habitats, while non-tropical systems require restoration to counter volatility from dispersal limitation.

Keywords: Environmental DNA, Ethanol preserved, Guizhou Province, Habitats, Hainan Province, Metabarcoding, α -diversity

Abstract

Systematics of Tropical Ant Nests-Associated Chaetothyriales**[G.P.G. Isuri Thakshila^{1*}], [Fuqiang Yu^{1#}], [Indunil Chinthani Senanayake^{1#}]**

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Interactions between ants and fungi have been documented since last century. Chaetothyriales is an overriding fungal order that creates a symbiosis with ants residing in the ant nests. The oligotrophic lifestyle, melanized cell wall, and vast diversity of enzyme production fit chaetothyrialean species to survive in this unique niche. These enzymes are utilized in various industries, such as detergent production, food processing, bioremediation, and the breakdown of polymers. They also produce a range of bioactive compounds with potential applications in developing new drugs. Melanin pigments in the cell wall are used for cosmetics, food coloring, and as a protective coating for sensitive materials. Therefore, chaetothyrialean species are proposed for use not only in ecological aspects but also in the exploitation of novel biotechnological processes and biomolecules. The literature reports biodegradation is a significant character of most chaetothyrialean fungi, and ant nests are rich in hydrocarbons and volatile compounds released by ants and plants. Scientists paid less attention to the diversity of chaetothyrialean taxa and their utilization in this under-studied niche. Poor sample collection, unstable phylogeny in different scenarios, undetermined isolates in GenBank, and unavailability of protein-coding gene sequences for many species create incorrect classification and identification. A broad sample collection and multigene phylogenies with protein-coding genes are required for accurate delimitation of species and generic boundaries. In this ongoing study, we collected and isolated ant nests-associated fungal isolates from southwest China; and identified them by morphology and multigene phylogeny. This study will be found more species and novel species will be introduced, and the systematics of some incertae sedis taxa will be determined. The physiological performance of chaetothyrialean species will be determined.

Keywords: Bioremediation, Extremophiles, Fungal diversity, Symbiosis, Taxonomy

Abstract

Speciation and evolutionary mechanisms of *Colletotrichum***Fang Liu^{1,2*#}, Ziyang Ma^{1,2}, Lei Cai^{1,2#}**¹*State Key Laboratory of Microbial Diversity and Innovative Utilization, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, P. R. China.*²*University of Chinese Academy of Sciences, Beijing, 100049, P. R. China.**Fang Liu: liufang@im.ac.cn, Ziyang Ma: ziyangss@163.com, Lei Cai: cail@im.ac.cn*

Understanding speciation in plant pathogens is crucial for predicting the emergence of novel virulent strains and comprehending biodiversity. This study investigates the evolutionary history and mechanisms driving diversification in *Colletotrichum*, a globally significant plant-pathogenic fungal genus. Our divergence dating analysis indicates an origin in the middle Eocene (~47.57 Mya) with an ancestral broad host range, followed by host specialization towards monocots and legumes during the Eocene-Oligocene transition (~32–39 Mya), a period coinciding with global cooling and aridification. Accelerated diversification, particularly within the *C. gloeosporioides* species complex (CGSC), occurred from the late Miocene onward, likely influenced by Pliocene vegetation shifts and recent human-mediated crop domestication. Through the analysis of 49 CGSC genomes, we reveal that transposable element expansion, especially long terminal repeat retrotransposons, significantly contributes to genome size and genetic variation. Furthermore, our findings suggest that chromosomal rearrangements, mediated by repetitive sequences, may act as a primary driver of speciation in CGSC, generating highly dynamic lineage-specific genomic regions. Overall, our work demonstrates how ancient climatic shifts, host adaptation, genomic plasticity, and anthropogenic factors interactively shape *Colletotrichum* speciation and adaptive radiation.

Keywords: Adaptive radiation, Chromosomal rearrangement, Diversification rate, Genome evolution, Host range shift

Abstract

Advances in the Taxonomy of the Genus *Mutinus* (Phallaceae)**Ting Li, Taihui Li, Wangqiu Deng[#]**

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Mutinus is an important group within the phylum Basidiomycota. The genus *Mutinus* Fries belongs to the family Phallaceae Corda of the order Phallales Fischer, and it is characterized by an immature basidioma that is globose to ovoid, a pseudostipe that is cylindrical to fusiform and hollow, a mucilaginous gleba covering the apical portion of the pseudostipe, and basidiospores that are elliptical and smooth. Currently, there are numerous issues regarding taxonomic identification and phylogeny between the genus *Mutinus* and its related genera, with deficiencies in scientific rigor, completeness, and systematicity. We summarized 36 worldwide distribution of known species of *Mutinus*, among which five species are found in China, namely *M. bambusinus* (Zoll.) E. Fischer, *M. caninus* (Huds.) Fries, *M. elegans* (Mont.) E. Fischer, *M. fleischeri* Penzig, and *M. taishanensis* P.M. Wang & Z.L. Zhou. Recent surveys have identified an additional three putative new species distributed in Guangdong and Fujian Provinces, designated as *Mutinus* sp. 1, *Mutinus* sp. 2, and *Mutinus* sp. 3. Specifically, *Mutinus* sp. 1 is characterized by a yellow-green fertile part and a pseudostipe with a vertical color gradient from yellow-green at the top to white at the base. In contrast, *Mutinus* sp. 2 has a brownish-green fertile part and a pale yellow pseudostipe, while *Mutinus* sp. 3 possesses an olive-green fertile part and a white pseudostipe. The molecular sequences of all three species differ from those of other known *Mutinus* species. Additionally, we compiled a morphological identification key for 22 *Mutinus* species. This study provides scientific data for relevant research in China.

Keywords:(Morphology; Gasteromycetes; Molecular phylogenetics; *Mutinus*)

Abstract

FIRST RECORDS OF RARE LINGZHI RACES IN THE NORTH VIETNAM, ADDED TO THE TOMOPHAGUS COLOSSUS COMPLEX WITH SUPPLEMENT MULTIGENES - GENOME SEQUENCING DATA**Tran Dong Anh¹, JM. Moncalvo², B. Alexander³, Bryn M. Dentinger^{3*}, Le Xuan Tham^{4*}***¹University of Agriculture, Hanoi**²University of Toronto, Canada**³University of Utah, USA**⁴Van Lang University, HoChiMinh City – COSARI - Faculty of Applied Technology;***Corresponding tham.lexuan@vlu.edu.vn; bryn.dentinger@gmail.com*

Studies on some rare races of the type *Tomophagus colossus* Murr. – an YellowGolden Lingzhi medicinal belonging to the family Ganodermataceae Donk, incl. first collections recently found in North Vietnam for the first time to note their changeabilities in morphological (fruitbodies and spores) and even molecular characters, particularly multigenes have been determined. Their similarities and speciations in molecular analysis sophisticatedly with the 5 regions of nuclear Small Subunit (SSU) ribosomal DNA, Large Subunit (LSU) ribosomal DNA, intergenic spacer (ITS1,2) and mitochondrial Small Subunit (SSU) ribosomal DNA and pairwise identity using BLASTN, even whole genome are undergoing on for complete segregation with largest length primary >115-120Mbp from the pivotal genera on the world, such as *Ganoderma* s.l. (8 species already sequenced) and 2 species of *Sanguinoderma* (*Amauroderma* s.l.) just determined. The morphological characters and molecular data would lead to new insights into *Tomophagus* genus complex in the Ganodermataceae Donk, that complex would be original from Southeastern Asia and perfectly cultivated for medicinal potentials of utilization in Vietnam.

KeyWords: YellowGolden Lingzhi - Hoangkimzhi *Tomophagus colossus* complex, Multigenes Sequencing, Genome, Phylogeny, Cultivations

Abstract

Speciation and evolutionary mechanisms of *Colletotrichum***Fang Liu^{1,2*#}, Ziyang Ma^{1,2}, Lei Cai^{1,2#}**¹*State Key Laboratory of Microbial Diversity and Innovative Utilization, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, P. R. China.*²*University of Chinese Academy of Sciences, Beijing, 100049, P. R. China.**Fang Liu: liufang@im.ac.cn, Ziyang Ma: ziyangss@163.com, Lei Cai: cail@im.ac.cn*

Understanding speciation in plant pathogens is crucial for predicting the emergence of novel virulent strains and comprehending biodiversity. This study investigates the evolutionary history and mechanisms driving diversification in *Colletotrichum*, a globally significant plant-pathogenic fungal genus. Our divergence dating analysis indicates an origin in the middle Eocene (~47.57 Mya) with an ancestral broad host range, followed by host specialization towards monocots and legumes during the Eocene-Oligocene transition (~32–39 Mya), a period coinciding with global cooling and aridification. Accelerated diversification, particularly within the *C. gloeosporioides* species complex (CGSC), occurred from the late Miocene onward, likely influenced by Pliocene vegetation shifts and recent human-mediated crop domestication. Through the analysis of 49 CGSC genomes, we reveal that transposable element expansion, especially long terminal repeat retrotransposons, significantly contributes to genome size and genetic variation. Furthermore, our findings suggest that chromosomal rearrangements, mediated by repetitive sequences, may act as a primary driver of speciation in CGSC, generating highly dynamic lineage-specific genomic regions. Overall, our work demonstrates how ancient climatic shifts, host adaptation, genomic plasticity, and anthropogenic factors interactively shape *Colletotrichum* speciation and adaptive radiation.

Keywords: Adaptive radiation, Chromosomal rearrangement, Diversification rate, Genome evolution, Host range shift

Abstract

Morphology and Phylogeny of *Meliolaceae***Feng Yu-Zhe*, Zeng Xiang-Yu#***Department of Plant Pathology, College of Agriculture, Guizhou University, Guiyang 550025, China**Email of the corresponding author xyzeng3@gzu.edu.cn*

The family *Meliolaceae* is one of the most species-rich groups within the fungal kingdom, with over 2,400 described species globally. However, due to its obligate biotrophic lifestyle, these fungi are unable to cultivate, and direct DNA extraction is often contaminated by surface epibionts or cohabiting microbes, resulting in fewer than 50 reliably sequenced taxa in GenBank—a severe limitation for molecular phylogenetic studies. Traditionally, the family *Meliolaceae* has been considered an obligate parasitic fungal group, with species identification primarily relying on morphological characteristics such as host-plant associations, the position and length of setae, the size and arrangement of appressoria and phialides, and the dimensions of ascomata. However, asexual reproductive structures in this family have been rarely reported. Furthermore, phylogenetic analyses reveal that the type genus *Meliola* of the family exhibits polyphyly, highlighting inconsistencies in the current taxonomic framework and underscoring the need for further systematic revision.

In this study, we conducted a systematic survey of *Meliolaceae* in tropical and subtropical regions of China. Through morphological examination, host-association analysis, and multi-gene phylogenetics (ITS, LSU, SSU), we examined 36 specimens, obtaining 9 ITS, 16 LSU, and 13 SSU sequences. Our work revealed six new species and documented putative conidial structures in multiple taxa. Notably, we observed multiple *Meliola* species coexisting on the same host plant and a single *Meliola* species infecting hosts from different plant families, challenging the traditional view of strict obligate parasitism. These findings provide new insights for refining the taxonomic boundaries of *Meliolaceae* and offer valuable insights into their evolutionary history, host adaptation, and life-history strategies.

Keywords: *Sordariomycetes*, species diversity, conidia, obligate parasitism, phylogeny

Abstract

Speciation and evolutionary mechanisms of *Colletotrichum***Fang Liu^{1,2*#}, Ziyang Ma^{1,2}, Lei Cai^{1,2#}**¹*State Key Laboratory of Microbial Diversity and Innovative Utilization, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, P. R. China.*²*University of Chinese Academy of Sciences, Beijing, 100049, P. R. China.**Fang Liu: liufang@im.ac.cn, Ziyang Ma: ziyangss@163.com, Lei Cai: cail@im.ac.cn*

Understanding speciation in plant pathogens is crucial for predicting the emergence of novel virulent strains and comprehending biodiversity. This study investigates the evolutionary history and mechanisms driving diversification in *Colletotrichum*, a globally significant plant-pathogenic fungal genus. Our divergence dating analysis indicates an origin in the middle Eocene (~47.57 Mya) with an ancestral broad host range, followed by host specialization towards monocots and legumes during the Eocene-Oligocene transition (~32–39 Mya), a period coinciding with global cooling and aridification. Accelerated diversification, particularly within the *C. gloeosporioides* species complex (CGSC), occurred from the late Miocene onward, likely influenced by Pliocene vegetation shifts and recent human-mediated crop domestication. Through the analysis of 49 CGSC genomes, we reveal that transposable element expansion, especially long terminal repeat retrotransposons, significantly contributes to genome size and genetic variation. Furthermore, our findings suggest that chromosomal rearrangements, mediated by repetitive sequences, may act as a primary driver of speciation in CGSC, generating highly dynamic lineage-specific genomic regions. Overall, our work demonstrates how ancient climatic shifts, host adaptation, genomic plasticity, and anthropogenic factors interactively shape *Colletotrichum* speciation and adaptive radiation.

Keywords: Adaptive radiation, Chromosomal rearrangement, Diversification rate, Genome evolution, Host range shift

Abstract

Polyphasic Taxonomy of Fungi Associated with Coffee in Yunnan (China) and Chiang Rai (Thailand) provinces**Li Lu^{1,2,3,4}, Samantha C. Karunarathna², Ruvishika S. Jayawardena^{3,4}, Kevin D. Hyde^{3,4*}, Saowaluck Tibpromma^{2*}**¹ *Bioengineering and Technological Research Center for Edible and Medicinal Fungi, Jiangxi Agricultural University, Nanchang 330045, P.R. China*² *Center for Yunnan Plateau Biological Resources Protection and Utilization & Yunnan International Joint Laboratory of Fungal Sustainable Utilization in South and Southeast Asia, College of Biology and Food Engineering, Qujing Normal University, Qujing 655099, P.R. China*³ *Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand*⁴ *School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand**Email of the corresponding author: kdhyde3@gmail.com and saowaluckfai@gmail.com*

Yunnan, China, and Chiang Rai, Thailand, are located in the northern part of the Greater Mekong Subregion (GMS) and are characterized by their warm climate and rich ecological diversity, offering a unique habitat for diverse fungal communities. These environmental conditions not only support the growth of *Coffea arabica*, one of the region's most economically important cash crops, but also favor the proliferation of fungi, increasing the crop's vulnerability to fungal infections during both cultivation and post-harvest periods. Thus, a comprehensive study was conducted to investigate the fungi associated with the economic crop coffee in Yunnan, China, and Chiang Rai, Thailand.

A total of 360 saprobic fungal collections, showcasing the rich diversity of the region, were obtained from eight locations—Baoshan, Dali, Dehong, Jinghong, Lincang, Nujiang, and Pu'er in Yunnan, China, and Chiang Rai, Thailand. Initial ITS analyses revealed that these collections represent seven classes, 37 orders, 83 families, and 137 genera. The life modes of these taxa were interpreted based on previously published studies and were compared and discussed. From the total collections, 139 well-preserved samples with abundant fruiting bodies and mature morphological structures were selected for detailed morphological and multi-locus phylogenetic analyses. These collections span four classes, 27 orders, 69 families, and 87 genera, resulting in the identification of six new genera, 77 new species, one novel DNA sequence, 47 new records, and four additional collections. Notably, four species (*Acrocalymma daliense*, *Pseudohelminthosporium clematidis*, *Tubeufia coffeae*, and *Menisporopsis dinemasporioides*) were documented in both their sexual and asexual morphs on natural substrates.

In addition, 235 fungal endophytes were isolated from 270 leaf segments of *Coffea arabica* collected from four coffee plantations in Yunnan. All isolates were identified through ITS sequencing, revealing their classification within Ascomycota, distributed across two classes, 10 orders, and 17 families. To assess their potential for biological control, 61 endophytes were evaluated in dual-culture assays against four known plant pathogens. Notably, *Nigrospora* sp. XCE-7 and *Daldinia* sp. ME-9 inhibited *Alternaria alternata* with over 70% growth suppression. Likewise, *Daldinia* sp. ME-9, *Nigrospora*

sp. T5E-7, and *Daldinia* sp. T5E1-3 effectively inhibited *Penicillium digitatum*, also exceeding 70% inhibition rates.

Besides, a new species of sooty mold, *Trichomerium puerense*, was also described and confirmed through pathogenicity testing as a pathogen of *Coffea arabica* in Yunnan.

Overall, this work reveals the extensive diversity of microfungi associated with coffee plants in southern China and northern Thailand. The discovery of new taxa, which significantly expands our understanding of the microfungi diversity, and geographic records enhances biodiversity inventories, while the identification of bioactive endophytes offers promising avenues for environmentally friendly disease management strategies. These findings not only support sustainable agricultural practices and promote biodiversity conservation but also inspire and motivate by contributing to innovation in plant health management.

Keywords: 77 New Species, 6 New Genera, Endophytic, Saprobic, Sooty Mold Fungi

Abstract

Taxonomy Based on a Culture of a Species of the Genus *Pyxidiophora* (Laboulbeniomycetes) Isolated from Animal Cadaver

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The class Laboulbeniomycetes, including the orders Laboulbeniales, Herpomyces and Pyxidiophorales, are biotrophic parasites associated with arthropods and fungi. The Laboulbeniomycetous fungi except Pyxidiophorales are typically characterized by the loss of hyphae, entire life cycle on a host animal, and lacking asexual reproduction. On the other hand, the Pyxidiophorales is known to produce several different shapes of anamorphs: dispersal anamorphs and hyphal anamorphs. Its teleomorph has been discovered from various habitats like dung, submerged wood, rotting mushroom, and bark beetle galleries etc. It is believed that when a small animal like mite walks around perithecium, ascospores released from perithecium might attach to its surface. Then, ascospores directly divide into a few-celled thalli of dispersal anamorph which produce conidia. Pyxidiophorales which has intermediate life cycle between Laboulbeniales and other typical hyphal forming ascomycetes, has been considered as a missing link between them, but its morphological and ecological information has been poorly known. Therefore, establishing culture is indispensable for advanced studies for clarifying characters of the missing link such as mechanisms of attachment to their host animals. In the aid of bathing methods using animal cadavers (e.g., bones of pig and fish), perithecioid fungus was collected, which was identified as a fungus of the genus *Pyxidiophora* due to a flask-shaped perithecium with oval to irregularly angular cells and long necks constructed of parallel, narrow, and elongated cells. Phylogenetic analyses using a concatenated SSU + ITS + LSU rDNA region show that this fungus is involved in Pyxidiophorales, though we could not identify the species because of the lack of reference molecular data of the species of the order. In addition, no morphologically matching described species exists, and this is the first case of *Pyxidiophora* fungus detected from animal cadavers. Based on these facts, we concluded the present fungus is an undescribed species. When a single ascospore and a single hyphal anamorph conidium were isolated on CMA and Sh3A (0.3% shrimp powder agar), both of the spores germinated to be short hphyae (or small conidia like *Thaxteriola*) on both of the media, but these hyphae stopped their growth. In the end, we succeeded in establishing dual culture by inoculating these short hyphae (and small codinida) with *Fusarium solani* which was isolated from the same habitat of the present fungus, which indicates the present fungus is a mycoparasite. Under the dual culture, the present fungus vigorously produced “*Gabarnardia*” type hyphal arthroconidia within 3-4 days, and also perithecia with mature ascospores within 5-7days, which proved the species to be homothallic. This is the first report of homothallic *Pyxidiophora* species whose teleomorph can be easily induced under culture, which can be an ideal material to analyze the evolutionary history of the Laboulbeniomycetes.

Keywords: Dual culture; *Fusarium solani*; mycoparasite; one new species; *Pyxidiophora*.

Abstract

Progress in the Systematic Taxonomy of *Capnodiaceae* (Sooty Moulds)

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As important sooty moulds, fungi of *Capnodiaceae* are widely distributed in tropical and subtropical regions worldwide. Studies on their species diversity and taxonomy are of great significance for understanding material cycles in ecosystems and plant-microbe interactions. However, the current taxonomic system of this family has several limitations: the delimitation of some genera and species relies on single morphological characters, lacking molecular data support; incomplete information on host ranges and distributions hinders in-depth analysis of their phylogenetic relationships. In this study, we adopted an integrative taxonomic approach combining morphology and molecular systematics. Phylogenetic trees were constructed based on four gene sequences (ITS, LSU, *tef1*, and *rpb2*) for multi-gene combined analysis of *Capnodiaceae* species, with comparative studies on key morphological characters such as conidiomatal structure and sporulation mode. One new genus (*Hyphopolychaeton*), eight new species, and three new record species were discovered, and morphological data of one species were supplemented. Molecular phylogenetic analyses clarified the independent evolutionary status of *Hyphopolychaeton*, confirming that the taxonomy of *Capnodiaceae* should be based primarily on asexual morphological characters, particularly the position of the swollen part of conidiomata: the swollen part is located in the upper part in *Conidiocarpus*, lower part in *Capnodium*, and apical part in *Leptoxypodium*. The study also revealed that intraspecific minor morphological variations are insufficient for species delimitation. Additionally, the overlapping genetic distances observed between some species indicate that in-depth studies incorporating more gene fragments and ecological data are required. In the future, supplementing molecular evidence and ecological data is expected to further clarify the phylogenetic relationships within *Capnodiaceae*, laying a foundation for constructing a more natural global classification system.

Keywords: Fungal taxonomy, molecular systematics, morphology, sooty moulds

Abstract

Taxonomy, enzymatic activity, and pathogenicity of entomopathogenic fungi isolated from various insect cadavers in Northern Thailand

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Owing to their ecological significance, commercial application for controlling insect pests, and unique infection mechanisms, studies on entomopathogenic fungi (EPF) show growing research interest. In the present study, we conducted a survey at a mountain forest in Chiang Mai Province, Northern Thailand, and collected mycosed insect cadavers and isolated. Morphological characteristics and multi-locus phylogenetic analysis using ITS, LSU, rpb1, rpb2, TEF, and TUB gene sequences were conducted for proper identification of the species. Fungal isolates were further screened for proteolytic, chitinolytic, and lipolytic activity (in triplicate for each) using an agar plate assay. The larval mortality bioassay was conducted by placing ten larvae of *Tenebrio molitor* in a PDA plate that had been grown with each fungal strain. Three replicates for each fungal strain were arranged here as well. In the field, seven insect cadavers were collected with infections of five different species of EPF. The collected isolations were identified as *Beauveria asiatica*, *B. bassiana*, *Clonostachys rogersoniana*, *Cordyceps blackwelliae*, *Isaria tenuipes* (two strains), and *Purpureocillium takamizusanense*. According to the enzymatic assay, *I. tenuipes* strain 82 showed the overall highest total enzymatic activity index, followed by *P. takamizusanense*, *C. rogersoniana*, *I. tenuipes* strain 81, *B. asiatica*, *B. bassiana*, and *C. blackwelliae*. Based on the larval cumulative mortality percentage (at the 10th day), *B. bassiana* (100%) was the most virulent species, followed by *B. asiatica* plus *C. blackwelliae* (86.66% each), *I. tenuipes* strain 82 (63.33%), *P. takamizusanense* (33.33%), *C. rogersoniana* (30%), and *Isaria tenuipes* strain 81 (23.33%). In conclusion, our study has demonstrated the potential for a higher diversity of EPF in the study area and shown that the isolated fungi release key enzymes, such as protease, chitinase, and lipase, which are crucial in breaking the insect integument and advancing the infection. Despite the *B.*

bassiana, no isolated strains indicated effective larvicidal activity. Thus, *B. bassiana* could be a potential candidate for inclusion in biocontrol formulations in future studies.

Keywords: *Beauveria bassiana*, Diversity, *Isaria tenuipes*, Protease, Virulence

Abstract

Species Diversity of Poisonous Mushrooms in South China**Zhengjie Sun ^{1,2*}, Wangqiu Deng ^{1#}**

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South China is characterized by a high species richness of poisonous mushrooms, making it one of the high-risk areas for mushroom poisoning incidents in China. Systematic analyses of poisonous mushrooms from South China were conducted through field investigation and literature review. There are 243 species of poisonous mushrooms in South China, belonging to 2 phyla, 5 classes, 12 orders, 40 families, and 89 genera. The top five families with the number of poisonous species are *Amanitaceae* (38), *Boletaceae* (26), *Russulaceae* (23), *Agaricaceae* (18), and *Hymenogastraceae* (15). The top five genera are *Amanita* (38), *Russula* (11), *Lactarius* (9), *Inocybe* (8), and *Panaeolus* (7). Common poisonous mushroom species include *Amanita exitialis*, *Chlorophyllum molybdites*, and *Entoloma omiense*. Additionally, 20 putative new species of poisonous mushrooms were discovered and await further investigation. The clinical classification of the poisonous mushrooms in South China includes acute liver failure (16), acute kidney injury (7), rhabdomyolysis (1), gastroenteritis (148), psycho-neurological disorders (68), hemolysis (2), and photosensitive dermatitis (1). Research on the toxins of poisonous mushrooms remains insufficient, and many mushroom toxins need further exploration, especially the newly discovered poisonous mushroom toxins. This study clarifies the species diversity and toxicity of poisonous mushrooms in south China, which is of great significance for risk assessments, precise prevention and control, and reduction of poisoning and death of mushroom poisoning.

Keywords: Mushroom toxin; *Amanita*; Poisoning types

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Abstract

Phylogeny and Taxonomy of the genera *Cantharellus* and *Craterellus* (Hydnaceae, Basidiomycota) in China**Ming Zhang^{1*}, Yi-Xiu Huang¹, Wen-Xiao Xia¹, Tai-Hui Li¹, Ping Zhou³, Chao-Qun Wang^{2#}, Wang-Qiu Deng^{1#}**¹ Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, State Key Laboratory of Applied Microbiology Southern China, Guangdong Institute of Microbiology, Guangdong Academy of Sciences, Guangzhou 510070, Guangdong, China² College of Biology and Food Engineering, Guangdong University of Education, Guangzhou 510303, China³ Guangdong Nanling Forest Ecosystem National Observation and Research Station

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Members of *Cantharellus* and *Craterellus* are usually called chanterelles. They are widely distributed and have significant ecological and economic values. Although there have been several studies especially in recent years focusing on the chanterelle mushrooms in China, the research on their species diversity and phylogenetic relationships is still far from sufficient. In this study, taxonomic and phylogenetic analyses on the species diversity, distribution patterns, and interspecific relationships of *Cantharellus* and *Craterellus* in China were conducted through morphological examination and molecular phylogenetic analyses of multiple loci, including the internal transcribed spacer regions (ITS), the large subunit of nuclear ribosomal RNA gene (nLSU), the second largest subunit of RNA polymerase II (rpb2), the translation elongation factor 1 gene (tefl), and the small subunit of mitochondrial ribosomal RNA gene (mtSSU). In total, 68 chanterelle species are recognized in China, including 42 species of *Cantharellus* and 26 species of *Craterellus*. Among them, there are 20 new species of *Cantharellus* and *Craterellus* were firstly reported in China. Besides, 6 new combination names were proposed. Additionally, one ambiguous taxon was tentatively named as *Craterellus* sp., and five synonyms were redefined. Among the accepted species, 36 known species were described in detail based on observations, and the other 9 species were reviewed. Keys to the accepted species of *Cantharellus* and *Craterellus* in China were provided.

Keywords: chanterelle, Cantharellales, edible fungi, novel taxa, phylogenetics

Abstract

Screening and Identification of Phosphate-Solubilizing Fungi from Agricultural Soils in Guangdong Province, China

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Phosphorus is a critical macro-element required for nearly all major metabolic activities in plants. However, 95–99% of the phosphorus in soil typically exists in insoluble mineral forms and therefore cannot be directly utilized by plants. Consequently, several soil fungi, particularly phosphate-solubilizing fungi, play a vital role in converting insoluble phosphorus into soluble forms that can be readily assimilated by plants. The present study aimed to isolate and identify phosphate-solubilizing fungi from agricultural soils in Guangdong Province, China. Fungi were isolated using the serial-dilution spread plate technique on Pikovskaya's agar. A total of nine positive fungal strains (MBSZU 25-065 to MBSZU 25-073) were obtained. These strains were initially identified using internal transcribed spacer (ITS) rDNA regions, and their ability to solubilize insoluble phosphate [$\text{Ca}_3(\text{PO}_4)_2$] was further evaluated using the agar plate assay. The strains were classified into two genera *Aspergillus* and *Penicillium*, and identified as three species including *Aspergillus neoniger* (MBSZU 25-065, MBSZU 25-066, and MBSZU 25-071), *A. trinidadensis* (MBSZU 25-067), and *Penicillium oxalicum* (MBSZU 25-068, MBSZU 25-069, MBSZU 25-070, MBSZU 25-072, and MBSZU 25-073). All fungal strains were able to solubilize insoluble phosphate, with phosphate solubilization index values ranging from 2.1 to 2.3. This study enhances Guangdong's phosphate-solubilizing fungi resources, and the identified high-efficiency strains show promise for localized use in biofertilizers to improve soil phosphorus availability, reduce chemical fertilizer use, and support sustainable subtropical agriculture.

Keywords: Fungal identification, Fungal isolation, Phosphate solubilization, Soil fungi

Abstract

FIRST REPORT OF ENDOPHYTIC MICROFUNGI FROM BEGONIA SPP. IN INDONESIA

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Begonia is a plant with the potential to be an ornamental plant with high economic value. Biological control and increasing quality resistance in Begonia collection are needed for recovery and disease control using endophytic fungi. This research aims to identify endophytic fungi and their potential in controlling anthracnose pathogens by *Colletotrichum* sp. in the Begonia collection at the Green House of Begonia Bogor Botanical Gardens. The identification methods used morphological and molecular approaches. Of the 14 strains, 7 genera of endophytic fungi were identified, including Absidia, Colletotrichum, Diaporthe, Humicola, Penicillium, Phyllosticta, and Trichoderma. This research can enrich knowledge of the diversity of endophytic fungi in Begonia using specific ITS genes, thus providing more effective clues in controlling pathogens as biocontrol agents.

Keywords:Diversity, ITS, molecular phylogenetic, morphology, Systematics

Abstract

Fungal Diversity Across Tropical and Non-Tropical Rainforests Through Edna Metabarcoding

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Our assessment of fungal communities across tropical (Hainan, China) and non-tropical (Guizhou, China) rainforests via eDNA metabarcoding of 97 samples revealed stark biogeographical contrasts, demonstrating a profound tropical vulnerability where mixed land use (MIX) caused a complete diversity collapse (Shannon = 0.00) in Hainan, while Guizhou retained residual diversity (Shannon = 0.28), with disturbance reducing diversity by 90% in the tropics versus 64% in the non-tropics. This heightened sensitivity was linked to extreme habitat specialization, as organic matter (OM) habitats sustained peak diversity in Hainan (Shannon = 3.73), a finding supported by a GLM showing OM had the strongest positive effect (estimate = 3.815, $p < 0.001$), with Hainan harboring 5.3× more habitat-specialist OTUs (64 vs. 12) and Soil/Tree (ST) habitats acting as critical refugia (34 indicator OTUs, association strength = 0.966). We further found a temporal asymmetry, where, despite fluctuations, Hainan maintained stable regional diversity (interannual $\Delta = -1.45\%$, $p = 0.653$), whereas 33% of Guizhou samples showed persistent zero diversity across years, indicating the presence of extinction debts. PERMANOVA confirmed habitat type was the primary driver of community structure ($R^2 = 0.301$, $p = 0.001$), with MIX causing an 87% diversity loss in the tropics versus non-significant effects in Guizhou ($p = 0.207$), leading to the conservation imperative that

tropical fungi are facing a catastrophic collapse under land-use change due to niche specialization, demanding urgent protection of OM-rich habitats, while non-tropical systems require restoration to counter volatility from dispersal limitation.

Keywords: Environmental DNA, Ethanol preserved, Guizhou Province, Habitats, Hainan Province, Metabarcoding, α -diversity

Abstract

gcFungi: a data portal for erasing taxonomic gaps and advancing fungal genomics research**Junmin Liang^{*,1}, Qinglan Sun², Linhuan Wu^{#,2}, Lei Cai^{#,1}***Affiliation1: State Key Laboratory of Microbial Diversity and Innovative Utilization, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, China**Affiliation2: Microbial Resource and Big Data Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China**Email of the corresponding author cail@im.ac.cn; wulh@im.ac.cn*

The Global Catalogue of Fungal Genomes (gcFungi, <https://nmdc.cn/gcfungi/>) now unites 20,278 public genomes (spanning 5,230 named species) with 1,168 newly sequenced type-derived genomes generated by the Chinese Microbial Type-strain Genome (CMTG) project. This release closes genomic representation gaps for 2 classes, 18 orders, 64 families, and 195 genera that previously lacked any reference sequence. Every genome has been uniformly de-contaminated and comprehensively functionally annotated, enabling systematic surveys of current sequencing efforts, in-depth genomic and pan-genomic analyses across phylogenetically and ecologically diverse lineages, and direct access to integrated bioinformatics tools for comparative and functional studies.

Keywords: Annotation pipeline, Fungal tree of life, Genome statistics, Type-derived genomes

Abstract

Biosurveillance of invasive southern corn rust: insights into recent migration patterns and virulence variation

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Emerging pathogen races spreading via long-distance migration increasingly threaten global agricultural ecosystems. Understanding how pathogens migrate and adapt to new hosts via virulence evolution is crucial for developing strategies to mitigate future crop damage. Here we performed biosurveillance of *P. polysora*, a global fungal pathogen causing southern corn rust, across China, Thailand and the Philippines. By analyzing 193 field transcriptomic data, we detected both epidemic and endemic lineages co-circulating in each country, and elucidated the crucial role of host selection in driving the diversification of endemic lineages. Gene flow assessments and trajectory tracking indicated that the SCR infection source in northern China is likely of domestic origin and pathogen migration from the Philippines/Thailand into China is restricted to Hainan, coastal Guangdong and southern Yunnan. We detected country-specific variants in 32 effector genes, with *AvrRppC* exhibiting the strongest positive selection. A phylogenetically distinct Luzon Island lineage (the Philippines), carrying a novel *AvrRppC* allele capable of overcoming *RppC*-mediated resistance, and represents a potentially invasive threat. Finally, we reviewed the global migration history of *P. polysora* in light of our findings. Our work represents the first step towards establishing an international surveillance network for *P. polysora*, and emphasized a comprehensive control strategy integrating local governance and invasion prevention of international races. This study was financially supported by the Key Collaborative Research Program of the Alliance of National and International Science Organizations for the Belt and Road Regions (Grant No. ANSO-CR-KP-202207).

Keywords: Effector variation, Host selection, Population transcriptomics, Trajectory tracking

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Session 6 Mycotoxins and Toxicogenic Fungi



Abstract

Investigating fungicide resistance and genetic diversity in peanut-associated *Aspergillus flavus* with comparative genomics.

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In 2019, a severe outbreak of aflatoxin contamination in peanuts in the Southeastern US occurred resulting in significant financial losses for the US peanut industry. In early 2020, seed lots produced in the previous season faced seed and seedling rots due to *Aspergillus flavus* infections. Subsequent analyses of 78 isolates from these seed lots found that many of the isolates were resistant to the QoI fungicide azoxystrobin, a major chemistry used in peanut seed treatments. In addition, resistance was also observed, though at lesser frequencies, to other fungicides including prothioconazole, fludioxonil, thiabendazole, and fluopyram. Here we investigated the genetic diversity of this collection of 78 isolates using whole genome re-sequencing. Variant calling was performed against the *A. flavus* AF13 reference genome and a NRRL3357 mitogenome resulting in 14,692 and 24 SNPs, respectively. Isolate sensitivity (EC₅₀) to the previously mentioned fungicides was used for genome wide association analysis (GWAS) resulting in the identification of SNPs associated with fludioxonil and prothioconazole resistance localized in amino acid transport and G-protein signaling genes, respectively. Population structure analysis showed two distinct subpopulations roughly aligning with isolate mating type. Population 1 showed higher incidences of fungicide resistance markers compared to Population 2, specifically for azoxystrobin, prothioconazole, and thiabendazole. Genetic markers identified here will be used in continuing surveys of *A. flavus* populations in peanut production in the Southeastern US to predict the prevalence of fungicide resistances and inform future seed treatment fungicide recommendations.

KeyWords: *Aspergillus flavus*, fungicide resistance, genomics

Abstract

Microbial Diversity and Mycotoxin Distribution Patterns in Wheat Seeds from Major Wheat-Producing Areas of China

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Wheat (*Triticum aestivum* L.) is a globally significant cereal crop that plays a vital role in ensuring food security, making its quantity, quality, and safety of paramount importance. In China, despite the widespread cultivation of wheat, a comprehensive understanding of the wheat microbiome's diversity, composition, and its relationship with disease incidence across diverse geographical environments remains limited. To address this knowledge gap, we conducted a comprehensive survey across China's major wheat-producing regions, collecting 485 samples from 53 cities in 13 provinces. Our sample set included newly harvested wheat seeds and stored seeds from warehouses, representing storage durations of one, two, and three years.

Our results demonstrated that newly harvested wheat seeds exhibited greater microbial diversity in southern China compared to those from northern China. In stored seeds, the influence of geographical location on microbial diversity diminished with prolonged storage, while moisture content and climatic factors exerted a more pronounced effect, leading to the formation of diversity hotspots. Furthermore, the community structures of newly harvested and stored seeds differed significantly, with geographical factors, climatic conditions, and storage practices having a marked influence. Distance-decay relationship (DDR) analysis revealed that microbial community similarity decreased with increasing geographical distance and longer storage durations. Core species analysis identified 28 families within Actinobacteriota, Firmicutes, and Proteobacteria. Subsequent testing of core species revealed that *Streptomyces* ZxBac193 exhibited strong antagonistic activity against toxin-producing fungi, achieving a 98% inhibition rate. Through high-resolution mass spectrometry and gene knockout verification, bafilomycin was identified as a key antifungal substance produced by this strain. Finally, coating wheat seeds with *Streptomyces* ZxBac193 demonstrated the strain's ability to colonize wheat roots and significantly inhibit the occurrence of wheat crown rot.

In conclusion, this study provides valuable insights into the distribution of the microbiome and mycotoxins in Chinese wheat seeds, highlighting the significant roles of geographical location, storage time, and environmental factors in shaping microbial community structure. By characterizing the microbial diversity, identifying key influencing factors, and discovering biocontrol agents, this research lays a foundation for the development of environmentally sustainable strategies for preventing and controlling wheat diseases.

Keywords: Wheat microbiome, Fungal diversity, Toxin-producing fungi, Wheat crown rot

Abstract

Epigenetic regulation of secondary metabolism and metabolic trade-offs by HosA in *Aspergillus fumigatus***Zhengyu Zhou¹, Yuqi Zhu¹, Renwei Gao¹, Yamei Wang¹, Yin Chen², Ling Lu^{1#}, Yuanwei Zhang^{1*#}**¹Jiangsu Key Laboratory for Pathogens and Ecosystems, Jiangsu Engineering and Technology Research Centre for Microbiology; College of Life Sciences, Nanjing Normal University, Nanjing, China²Department of Pharmacology and Toxicology, R. Ken Coit College of Pharmacy, University of Arizona, Tucson, AZ, USAEmail of the corresponding author: linglu@njnu.edu.cn or ywzhang@njnu.edu.cn

Aspergillus fumigatus is a ubiquitous saprophytic fungus whose remarkable environmental adaptability is considered a key factor underlying its strong virulence. During growth, *A. fumigatus* produces a wide range of secondary metabolites that enhance its competitiveness through microbial antagonism and evasion of host immunity. However, the mechanisms by which this pathogen coordinates the trade-off between growth and secondary metabolism in response to environmental signals remain largely unknown. In this study, we systematically identified histone deacetylases (HDACs) in *A. fumigatus* and revealed that HosA functions as a bifunctional epigenetic regulator. HosA promotes fungal development and virulence while repressing the biosynthesis of secondary metabolites. Mechanistically, HosA targets the *fma*/*pso* supercluster and key developmental regulators to coordinate secondary metabolism and morphogenesis. Moreover, its regulatory activity appears to be environmentally responsive, enabling *A. fumigatus* to adjust metabolic priorities according to external or host-related cues. These findings identify HosA as a central metabolic switch that dynamically balances growth, virulence, and secondary metabolism under changing environmental conditions, providing new insights into the epigenetic control of fungal pathogenicity and secondary metabolite production.

Keywords: *Aspergillus fumigatus*; epigenetic regulation; histone deacetylase; secondary metabolism; virulence

Abstract

Chitosan-Enriched Antifungal Formulation Against The Growth of Food Spoilage Molds and Aflatoxin B₁ Contamination

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Molds contamination is one of the major causes of agri-food spoilage during storage, especially in tropical and sub-tropical countries. Among all known mycotoxin, aflatoxin B₁ (AFB₁) produced by the *Aspergillus flavus* has been paid significant attention to agri-food industries world-wide, due to its carcinogenic, mutagenic, teratogenic, and immunosuppressive effects. International Agency for Research on Cancer, recognized AFB₁ as a most resilient and class 1 category human carcinogen.

The study reports the antifungal efficacy of nanofabricated plant-based formulation Ne-CIM (a synergistic combination of essential oil of *Carum carvi*, *Illicium verum*, and methyl anthranilate using mathematical modelling) inside the chitosan biopolymer against the growth of selected molds (*A. flavus*, *A. niger*, *Penicillium glaucum*, and *Fusarium graminearum*) and aflatoxin B₁ (AFB₁) production. The prepared nanoformulation was characterized by Dynamic Light Scattering, Fourier Transform Infrared Spectroscopy and X-ray diffraction.

The results revealed the distantly improved antifungal efficacy of Ne-CIM ranged between (0.2 to 0.04 µl/ml) against selected molds, and AFB₁ inhibition (0.03 µl/ml). In addition, Ne-CIM disrupted ergosterol biosynthesis, membrane integrity, mitochondrial potential, and altered redox homeostasis. Transcriptomic and biochemical results reveal a coordinated suppression of glycolysis, Krebs cycle, ATP synthesis, oxidative damage, and aflatoxin B₁ biosynthesis through transcriptional silencing and interference with key enzymatic targets at sub-lethal concentrations. Furthermore, during the *in-situ* trial the Ne-CIM preserve the overall nutritional and organoleptic properties of model food.

The findings demonstrated the potential of developed nanoformulation as a promising novel alternative to synthetic chemicals for sustainable food protection against food spoilage molds and aflatoxin B₁ mediated toxicity.

Keywords: Aflatoxin B₁; Chitosan nanoparticles; plant-based antifungal formulation; transcriptomic approaches

Abstract

Aflatoxin Reduction by *Aspergillus oryzae* Strain from the Korean Meju

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Doenjang is a Korean traditional soybean paste which is very important for Korean cuisine. The soybean brick, Meju, is a starting material of the soybean paste yet is sometimes vulnerable for mycotoxin contamination due to the inaccurate control of fungal fermentation. To secure the safety against mycotoxin contamination of traditional meju produced by natural fermentation, a metagenomic analysis of meju was performed, and filamentous fungal strains were isolated from meju to confirm their characteristics. A total of 30 species filamentous fungi in 7 genera, including *Aspergilli*, *Penicilli*, *Cladosporia*, and *Mucor*, were isolated and identified, and the production of mycotoxins was confirmed. The results of metagenome analysis showed that the distribution and abundance of fungi were very diverse according to the difference in production regions and fermentation conditions of meju. The activity of peptidase, amylase, and lipase was investigated in nonaflatoxigenic *Aspergillus flavus/oryzae* among strains isolated from meju, and this activity was compared with commercially available yellow-koji strains for meju production. Nonaflatoxigenic *A. oryzae* strain AO3222 showed more suitable enzyme activity for fermentation of meju than conventional yellow-koji. Also, result of whole genome analysis, it was found that all yellow-koji strains were clustered in very similar close relationships, thus they had almost identical genomic characteristics. However, strain AO3222 was clustered into *A. oryzae* group different from yellow-koji. To analyze the possibility of using the AO3222 strain as a new koji for the production of soybean meju, the AO3222 strain was inoculated and fermented simultaneously with strains such as *A. flavus*, *Mucor racemosus*, and *Rhizopus arrhizus*. Through this, the ability to reduce the production of aflatoxin in AO3222 strains was analyzed, and the distribution pattern of each strain inoculated in meju was analyzed using the metagenomic method. This research was supported by a grant (21162MFDS028) from Ministry of Food and Drug Safety in 2022.

Keywords: Aflatoxin, *Aspergillus flavus*, *Aspergillus oryzae*, Meju, Yellow Koji,

Abstract

Diethyl aminoethyl hexanoate inhibits *Aspergillus flavus* growth and aflatoxin biosynthesis by disrupting cell structure and redox homeostasis

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Aflatoxin B₁ (AFB₁), mainly produced by *Aspergillus flavus*, is one of the most potent naturally occurring carcinogens, posing severe threats to food safety and human health. Here, we report for the first time that the plant growth regulator diethyl aminoethyl hexanoate (DA-6) exhibits strong antifungal activity against *A. flavus* and effectively suppresses AFB₁ biosynthesis, with potent inhibitory effects on fungal colonization in stored rice bran and maize. DA-6 treatment inhibited *A. flavus* mycelial growth, conidial germination and sclerotia formation in a dose-dependent manner. Microscopic and biochemical analyses revealed that DA-6 disrupted plasma membrane integrity, leading to cytoplasmic leakage and hyphae collapse. Further analysis demonstrated that DA-6 induced reactive oxygen species (ROS) accumulation and impaired antioxidant defenses, resulting in mitochondrial dysfunction characterized by reduced membrane potential and ATP content. These physiological alterations were accompanied by nuclear damage and activation of autophagy. Transcriptomic profiling revealed that DA-6 caused extensive repression of developmental regulators and the velvet complex genes (*laeA* and *veA*), as well as down-regulation of numerous genes involved in membrane structure. More importantly, the key cluster regulators *aflR* and *aflS* were strongly down-regulated, leading to the near-complete silencing of the entire biosynthetic gene cluster and consequent loss of AFB₁ production. Collectively, this study provides new insights into the inhibitory mechanism of DA-6 against *A. flavus*, thereby supporting its application as a novel strategy to mitigate aflatoxin risks in food storage.

Keywords: Diethyl aminoethyl hexanoate; *Aspergillus flavus*; Aflatoxin; Antifungal activity; Reactive oxygen species

Abstract

Histone methyltransferase Bre2 regulates fungal virulence via chromatin remodeling factor Arp9 in *Aspergillus flavus*

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Aspergillus flavus, along with its notorious secondary metabolite aflatoxin B1 (AFB1), endangers human health around the world. We previously revealed that histone methyltransferase complex COMPASS plays a crucial role in regulating various important biological processes of *A. flavus*, but its further mechanism is not clear. Here, we obtained the deletion and complementary mutant strains of *bre2*, a key subunit of COMPASS methyltransferase, and following invading experiments showed that Bre2 plays a vital role in fungal virulence to crop kernels and the model insect silkworm through the methylation of H3K4. ChIP-seq analysis indicated that the chromatin remodeling factor Arp9 is directly targeted by Bre2 for epigenetic regulation, which was further confirmed by ChIP-qPCR. ATAC assay reveals that Arp9 deeply participates in fungal pathogenicity through regulating a series of morphogenesis, secondary metabolism and virulence associated regulators, including M2DH, SMAP, PIK2, PIKA and PSD, by modulating the chromatin conformation. The study revealed an epigenetic signaling pathway from COMPASS complex to Arp9 mediating chromatin remodeling for fungal secondary metabolism and virulence, which provides potential strategy for pathogenic fungi and mycotoxins control.

Keywords: *Aspergillus flavus*, Arp9, Bre2, chromatin remodeling factor, histone methyltransferase

Abstract

Enhanced Antibacterial Activity of Ultrasonic-extracted *Auricularia* sp.: Mycochemistry and Toxicity

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The escalating threat of fatal bacterial infections underscores the urgent need for new antibiotics that combine potency with efficient production. *Auricularia* sp., an edible mushroom valued in traditional medicine, contains diverse secondary metabolites with notable pharmacological properties. However, its antibacterial potential under ultrasound-assisted extraction (UAE) remains largely unexplored. This study aimed to investigate the antibacterial activity of UAE-derived *Auricularia* sp. ethanolic extracts (AEE) against *Staphylococcus aureus* and *Escherichia coli*, compared with maceration-derived extracts. The extracts were also analyzed for molecular spectroscopic characteristics, mycochemical profiles, total phenolic content (TPC), and toxicity. Fruiting bodies were extracted with 95% ethanol via 15-, 30-, and 45-minute ultrasonication (UAE15, UAE30, UAE45) and 72-hour maceration (M72). Antibacterial efficacy was determined using resazurin microplate assays. UV–Vis and Fourier transform infrared (FT–IR) spectroscopy provided molecular spectroscopic data, while thin-layer chromatography (TLC) and TPC analysis were used for mycochemical characterization. Toxicity was assessed using the Brine Shrimp Lethality Assay (BSLA). Among the ultrasonic-extracted AEEs, UAE15 showed the highest antibacterial potency at the shortest extraction time, with minimum inhibitory and bactericidal concentration (MIC/MBC) values of 1.563/3.125 mg/mL for *S. aureus* and 0.195/0.391 mg/mL for *E. coli*, compared with M72 values of 12.50/50.00 mg/mL and 6.250/25.00 mg/mL, respectively. The MIC/MBC ratio of 2 for both pathogens observed in UAE15 indicates strong bactericidal activity. The UV–Vis spectrum of UAE15 exhibited absorption bands between 200 and 700 nm, complementing FT–IR peaks corresponding to hydroxyl (–OH), carbonyl (C=O), aromatic (C=C), amine (C–N), ether (C–O–C), and vinyl/phenyl groups, indicative of polyphenols, flavonoids, tannins, alkaloids, triterpenoids, sterols, and saponins as confirmed by TLC. TPC analysis showed a slightly higher value in UAE15 (228.420 mg GAE/g) than M72 (213.590 mg GAE/g), suggesting that its enhanced antibacterial activity may be due to additional bioactive constituents beyond polyphenols. The BSLA of UAE15 yielded a median lethal concentration (LC₅₀) of 104.573 µg/mL, indicating mild toxicity. Overall, these results demonstrate the superior antibacterial activity of ultrasonically extracted *Auricularia* compounds, with mild toxicity indicating strong bioactivity and potential for optimal antibacterial applications.

Keywords: Antibacterial Activity, *Auricularia* sp., Mycochemical profiling, Toxicity, Ultrasound-assisted Extraction

Abstract

Microbial Diversity and Mycotoxin Distribution Patterns in Wheat Seeds from Major Wheat-Producing Areas of China**Xin Zhou¹*, Da Li^{1,2}, Qinni Yang³, Zhihui Qi⁴, Fang Tang⁴, Fang Liu^{1#}, Lei Cai^{1#}**¹ *State Key Laboratory of Microbial Diversity and Innovative Utilization, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China.*² *College of Life Science, University of Chinese Academy of Sciences, Beijing 100049, P. R. China.*³ *College of Life Science, Northeast Forestry University.*⁴ *Academy of National Food and Strategic Reserves Administration, Beijing 100037, P. R. China.*
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Wheat (*Triticum aestivum* L.) is a globally significant cereal crop that plays a vital role in ensuring food security, making its quantity, quality, and safety of paramount importance. In China, despite the widespread cultivation of wheat, a comprehensive understanding of the wheat microbiome's diversity, composition, and its relationship with disease incidence across diverse geographical environments remains limited. To address this knowledge gap, we conducted a comprehensive survey across China's major wheat-producing regions, collecting 485 samples from 53 cities in 13 provinces. Our sample set included newly harvested wheat seeds and stored seeds from warehouses, representing storage durations of one, two, and three years.

Our results demonstrated that newly harvested wheat seeds exhibited greater microbial diversity in southern China compared to those from northern China. In stored seeds, the influence of geographical location on microbial diversity diminished with prolonged storage, while moisture content and climatic factors exerted a more pronounced effect, leading to the formation of diversity hotspots. Furthermore, the community structures of newly harvested and stored seeds differed significantly, with geographical factors, climatic conditions, and storage practices having a marked influence. Distance-decay relationship (DDR) analysis revealed that microbial community similarity decreased with increasing geographical distance and longer storage durations. Core species analysis identified 28 families within Actinobacteriota, Firmicutes, and Proteobacteria. Subsequent testing of core species revealed that *Streptomyces* ZxBac193 exhibited strong antagonistic activity against toxin-producing fungi, achieving a 98% inhibition rate. Through high-resolution mass spectrometry and gene knockout verification, bafilomycin was identified as a key antifungal substance produced by this strain. Finally, coating wheat seeds with *Streptomyces* ZxBac193 demonstrated the strain's ability to colonize wheat roots and significantly inhibit the occurrence of wheat crown rot.

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Keywords: Wheat microbiome, Fungal diversity, Toxin-producing fungi, Wheat crown rot

Epigenetic regulation of secondary metabolism and metabolic trade-offs by HosA in *Aspergillus fumigatus*

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Keywords: *Aspergillus fumigatus*; epigenetic regulation; histone deacetylase; secondary metabolism; virulence

Abstract

GAPDH-mediated glycolysis provides energy for effector secretion in *Magnaporthe oryzae*

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Rice blast, caused by the fungus *Magnaporthe oryzae*, is a devastating fungal disease of rice. During the early stages of the interaction between *M. oryzae* and rice, the pathogen synthesizes effectors to suppress the rice reactive oxygen species (ROS) immune response. However, how these newly synthesized effectors are promptly secreted into rice cells remains unclear. To investigate the secretion mechanism of *M. oryzae* effectors under ROS stress, we analyzed the vesicular transport rate during the early interaction stage and found that effectors entered rice cells more rapidly via accelerated vesicular transport. This acceleration requires energy. By using glycolytic inhibitors and the mitochondrial inhibitor oligomycin, we discovered that inhibiting glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity suppressed the vesicular transport rate, indicating that glycolysis serves as the energy source for accelerating vesicular transport under ROS stress. Further research revealed that ROS inhibits the palmitoylation of GAPDH, leading to the release of more active GAPDH to the vesicle surface. By purifying *M. oryzae* vesicles and adding glycolytic substrates, we found that vesicles under ROS stress could produce more ATP, suggesting that GAPDH accumulated on the vesicle surface provides energy for the acceleration of vesicular transport. These findings highlight the importance of glycolysis-mediated vesicular transport in *M. oryzae* and provide insights for designing efficient and environmentally friendly disease control strategies.

Keywords: *Magnaporthe oryzae*, vesicular transport, glycolysis, reactive oxygen species (ROS) immunity

Abstract

Microbial Diversity and Mycotoxin Distribution Patterns in Wheat Seeds from Major Wheat-Producing Areas of China**Xin Zhou¹*, Da Li^{1,2}, Qinni Yang³, Zhihui Qi⁴, Fang Tang⁴, Fang Liu^{1#}, Lei Cai^{1#}**¹ *State Key Laboratory of Microbial Diversity and Innovative Utilization, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China.*² *College of Life Science, University of Chinese Academy of Sciences, Beijing 100049, P. R. China.*³ *College of Life Science, Northeast Forestry University.*⁴ *Academy of National Food and Strategic Reserves Administration, Beijing 100037, P. R. China.*

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Keywords: Wheat microbiome, Fungal diversity, Toxin-producing fungi, Wheat crown rot

Abstract

Phylogeny and Toxin profile of Microfungi associated with Chili peppers in Guangzhou

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Chili peppers (*Capsicum annuum*) are among the most widely consumed spices worldwide and constitute a vital agricultural commodity in China, where annual production exceeds 18 million tons. Beyond their culinary and economic importance, peppers contribute to rural livelihoods and global trade. However, their cultivation and postharvest handling are increasingly threatened by fungal pathogens and associated mycotoxins, which compromise both yield and food safety. Dried peppers are particularly vulnerable to contamination due to extended storage under warm and humid conditions, yet systematic comparisons of fungal communities and toxin burdens between fresh and dried peppers remain scarce. To address this gap, extensive market surveys were conducted between 2022 and 2023 in Guangzhou. More than 200 symptomatic fresh chili samples and dried chili samples were collected and subjected to fungal isolation, morphological characterization, and multilocus sequencing (ITS, TEF, RPB2, TUB2, CMD). Phylogenetic analyses using Bayesian inference and maximum-likelihood methods enabled precise taxonomic identification, while LC-MS/MS assays quantified key mycotoxins. The study uncovered a diverse fungal community dominated by *Fusarium* and *Penicillium*. Identified *Fusarium* species included *F. annulatum*, *F. commune*, *F. concentricum*, *F. fujikuroi*, *F. verticillioides*, *F. ramsdenii*, and notably *F. tardichlamydosporum*, which was reported for the first time from chili peppers, expanding its known host range beyond bananas. Other isolates included *Penicillium citrinum* and *P. steckii*. Mycotoxin profiling detected ochratoxin A (OTA), deoxynivalenol (DON), aflatoxin B1 (AFB1), zearalenone (ZEN), fumonisin B1 (FB1), and citrinin. Dried peppers consistently harbored higher toxin concentrations than fresh samples, with AFB1 frequently exceeding international safety limits. The frequent co-occurrence of multiple toxins in single samples poses an elevated public health risk due to potential additive or synergistic toxic effects. This work highlights chili peppers as a high-risk crop for fungal colonization and mycotoxin contamination. The emergence of *F.*

tardichlamydosporum underscores the dynamic ecology of pathogens under global trade and climate pressures. Effective mitigation requires integrated strategies, including resistant cultivars, optimized drying and storage, biological control, and HACCP-based monitoring. Collectively, these findings provide essential insights for ensuring food safety, sustainable agriculture, and public health protection.

Keywords: *Capsicum annuum*, *Fusarium* spp.; *Penicillium* spp.; Mycotoxin

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Session 7 Recent Advancements in Fungi-like taxa



Abstract

Lichen Diversity in Qinghai-Tibetan Plateau and the Response to Climate Change

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The Qinghai-Tibet Plateau (QTP) is the highest and largest plateau in the world, encompassing a wide range of habitats—from evergreen broad-leaved forests to alpine scree. However, due to the lack of systematic taxonomic studies in this region, its lichen diversity remains largely unknown. Recent field surveys have resulted in the collection of over 30,000 lichen specimens, revealing numerous previously unknown species in what is often called “the Roof of the World.” This rapid increase in recorded species is largely due to detailed floristic surveys and the application of molecular techniques, which have uncovered a vast number of cryptic species. Additionally, many lichen species were documented in this region over a century ago, but due to the absence of molecular data, species boundaries have remained unclear. Through comprehensive morphological, chemical, and molecular analyses, we have discovered hidden species diversity in this region and redefined species concepts using more precise morphological features, chemical profiles, and genetic data.

The QTP is also a globally significant region for climate change research, with its dramatic elevation gradients providing ideal conditions for such studies. Lichens in this region may respond to climate changes by altering their photobionts, growth patterns, or reproductive strategies. Using the common shrubby lichen *Dolichousnea* as a model, we conducted transplantation experiments to investigate its response to climate change. The results indicated that increased temperature and humidity can induce stress, leading to reduced thallus mass, increase of apothecia production, and a shift in photobiont association from the main stem to fibrils. These findings suggest that QTP lichens are highly sensitive to climate change. Detrimental impacts could reduce the range and biomass of *D. longissima*, thereby decreasing food availability for the endangered lichen-feeding monkey *Rhinopithecus bieti*. Conservation efforts are urgently needed to protect these lichens.

This study highlights that the QTP region harbours a unique lichen flora with exceptionally high species diversity. However, due to their sensitivity to environmental change, some species may disappear before they are even documented. Therefore, targeted conservation strategies are urgently needed to protect the lichen biodiversity of this region.

Keywords: Conservation Strategies, Hidden Diversity, Lichenized Fungi, Species Recognition.

Abstract

Diversity and Distribution of Myxomycetes in Recreational Parks of Albay, Philippines

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Myxomycetes, also known as slime molds, are amoeboid, fungus-like organisms that are well-documented in natural and undisturbed habitats such as tropical forests, but are limitedly studied in urban green spaces. In this study, we assessed the occurrence, distribution and diversity of myxomycetes in relation to their microhabitats in recreational areas in Albay, Philippines. We hypothesized that human transient activities in these recreational parks may influence their occurrence and diversity. Ground leaf litter and twigs were collected from two recreational parks [Farm Plate (FP), Quituinan Hill Ranch (QH)], two eco-nature parks [Kawa-Kawa (KW), Lignon Hill (LH)], and a historic-cultural park [Cagsawa Park (CP)]. We used moist chamber (MC) culture techniques to evaluate myxomycetes. A total of 120 records of myxomycetes were collected from 150 moist chambers, resulting in 80% MC productivity. The collected myxomycetes were identified as belonging to 23 species and 13 genera. Among these, *Arcyria cinerea*, *Comatricha nigra*, *Diderma hemisphaericum*, and *Ophiotheca chrysosperma* occurred in all sampling sites. Taxonomic diversity index (TDI) was highest in the recreational parks, FP and QH (1.4) than the eco-nature (KW=1.3, LH=1.2) and historic-cultural (CP=1.1) parks. FP also had the highest species diversity (FAI = 10.03, H = 2.18, SDI = 0.86) among all parks. This park is known for many human-transient activities like picnics and other family-oriented activities. The comparison of our study with nearby forested areas showed a lower species diversity, indicating that recreational activities may have influenced the composition and diversity of myxomycetes. Nevertheless, the presence of myxomycetes in many man-made parks highlights their resilience against anthropogenic impacts.

Keywords: diversity, man-made parks, occurrence, slime molds, urban ecology

Abstract

Utilizing antiviral medicine to control lichen biological disease of stone cultural relics

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World heritage sites such as rock caves, stone carvings, and the Great Wall have long been threatened by biodeterioration caused by lichen colonization. Conventional methods like physical removal and biocide spraying often lead to damage of the relic substrates or induce microbial resistance. In this study, metagenomic analysis was conducted on saxicolous lichens from cultural heritage sites including the Niujaiozhai Grottoes in Sichuan Province, leading to the discovery of a novel double-stranded RNA virus, Pyxine petricola partitivirus 1 (PpPV1). Transmission electron microscopy and immunofluorescence assays confirmed that this virus specifically infects the lichen symbiotic fungi. Screening of 118 lichen samples from global sources revealed a high infection rate of 70.2% in saxicolous lichens, with widespread presence at renowned world cultural heritage sites such as Angkor Wat in Cambodia and the Great Wall of China. Through the application of the antiviral drug oseltamivir, targeted inhibition of the lichen symbiotic virus and suppression of the photosynthetic activity of symbiotic algae were achieved, thereby reducing the overall viability of the lichen. This treatment resulted in a 32% reduction in lichen coverage. Based on these findings, a “virus inhibition–algae inactivation” cascade model was established, enabling synergistic effects between biofilm removal and the prevention of secondary corrosion on relic surfaces. This approach offers a “kill two birds with one stone” mechanism for the conservation of stone cultural relics.

Keywords: Alphapartitivirus, biological disease, cultural relics conservation, lichen, oseltamivir,

Abstract

Updates on Myxomycetes from Varied Ecosystems in Luzon Island, Philippines

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Luzon - the largest and most populous island in the Philippine archipelago - lies in the country's north. Its complex topography, from coastal plains and river valleys to volcanic plateaus and rugged mountain ranges like the Cordillera, Sierra Madre, Bicol highlands, creates a diverse and complex habitats that support rich microbial biota, including the fungal-like organisms, myxomycetes, also known as slime molds. These organisms thrive on decaying plant substrates like leaf litter and twigs. Herein, we present the first island-wide synthesis of myxomycete diversity for Luzon Island, spanning 38 provinces across the Ilocos, the Cagayan Valley, Cordillera and Central Luzon regions, and from Metro Manila (NCR) to the Southern Luzon and Bicol regions. About 160 species belonging to 32 genera were recorded on Luzon Island. Of these, over 40 represent new records for the country since Reynolds' annotated list (1981). Based on published records and new data from our field surveys and moist-chamber cultures, we consolidate species occurrence, update the provincial and regional checklists, and characterize assemblage patterns across major ecosystems - lowland dipterocarp forests, montane or mossy forests, grassland, mangroves, wetlands, and other coastal or beach forest sites. Species richness across regions is summarized as follows: Ilocos (39 species), Cagayan Valley (22), CAR (6), Central Luzon (97), NCR (23), Southern Luzon (99), Mindoro and Palawan Islands (74), and Bicol Peninsula (78). In relation to substrate and habitat associations, Luzon myxomycetes tend to grow on twigs, followed by aerial leaf litter, and other forest substrates. Many species are broadly distributed across varied ecosystems highlighting their occurrence in diverse habitats. This account provides a baseline data for understanding the biogeography and conservation value of myxomycetes in Luzon's varied ecosystems, while also contributing to broader biodiversity assessments across the Philippines.

Keywords: amoebozoa, biodiversity, biogeography, Philippines, slime molds

Abstract

Mechanisms of microbial interactions within the lichen holobiont**Yan-Yan Wang***School of Agriculture and Biotechnology, Sun Yat-Sen University, ShenZhen, China**Email: wangyan@im.ac.cn*

Lichens are symbiotic associations composed of fungi and algae or cyanobacteria, are dominant species in extreme habitats, and cover 8% of the land surface. Lichens have important ecological functions, they are key initiators and drivers of element cycling during the early stages of primary succession in ecosystems. Lichens represent an untapped treasure chest of natural compounds, yielding numerous uniquely structured secondary metabolites with potent biological activity and medicinal potential. All these biological characteristics are based on the symbiotic nature of lichens. However, due to their slow growth rate, artificially reconstructing lichen symbiotic system is extremely difficult to achieve, which limits the development and utilization of lichen resources. Therefore, elucidating the interactions among the microorganisms that compose lichens is an essential scientific challenge that demands urgent resolution. In our recent studies, we have established a research platform for lichen-forming fungal-algal interactions using the *Umbilicaria muhlenbergii* and *Trebouxia jamesii*. We have characterized the morphological and genetic hallmarks of initial fungal-algal interactions, successfully cultivated primary lichen structure under habitat-mimicking conditions, and discovered that the cAMP-PKA and MAPK signalling pathways regulated lichen morphogenesis. Beyond lichen-forming fungi and photobiont, lichen thalli harbor numerous endophytic microbes with unknown functions. Recent studies reveal that an endophytic bacterium can rapidly induce morphological changes in lichen-forming fungi, we have also uncovered complex interactions between fungi-algae and bacteria. These discoveries provide a foundation for deepening understanding of lichen symbiosis, thereby advancing the development and utilization of lichen resources.

Keywords: Lichen, symbiosis, signalling pathway, fungal-algal interaction

Abstract

Lichen Derived Metabolites from *Lobaria* spp. Exhibit Dual Therapeutic Efficacy Against Neurodegenerative Diseases

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Background: The aggregation of amyloid proteins, particularly amyloid- β (A β) in Alzheimer's disease (AD) and α -synuclein (α S) in Parkinson's disease (PD), is a key factor in neurodegenerative diseases. Natural products offer a valuable resource for identifying multi-target inhibitors against these pathological processes. Our study explores the therapeutic potential of depsides and depsidones isolated from the lichen *Lobaria* spp., including stictic acid, norstictic acid, and gyrophoric acid, in addressing the core pathologies of both AD and PD.

Methods: We utilized an integrated approach that combined biological and computational techniques with both *in vitro* and *in vivo* models. The inhibitory effects on protein aggregation were measured by using Thioflavin T fluorescence assays and atomic force microscopy. Molecular dynamics simulations provided insights into the interactions between the compounds and proteins. In parallel, the neuroprotective effects and transcriptome analysis were conducted in MES23.5 dopaminergic neurons and PC12 cells exposed to toxic α S or A β 42 species. *In vivo* validation was performed using an MPTP-induced mouse model of PD, where we assessed motor function and neuronal survival.

Results: The depsides and depsidones of *Lobaria* demonstrated strong dual inhibitory effects. All compounds significantly protected neuronal cells from cytotoxicity induced by both α S and A β 42, restored cell viability, and promoted neurite outgrowth. Gyrophoric acid was particularly effective, inhibiting A β 42 fibrillation and disaggregating mature fibrils. Stictic acid showed the highest efficacy against PD pathologies, inhibiting α S fibrillation by up to 85% and destabilizing preformed fibrils, surpassing the reference compound EGCG. In cellular models, stictic acid restored neuronal viability by 30% and reversed α S-induced dysregulation of immune and inflammatory pathways. Concurrently, in PD mice, treatment with stictic acid (10 mg/kg/day) significantly improved motor function and reduced neuronal loss.

Conclusion: This study is the first report to reveal the dual neuroprotective effects of metabolites from *Lobaria* spp., demonstrating their capacity to target the core amyloid aggregation pathologies of both AD and PD. Our findings highlight these lichen-derived natural products, particularly

gyrophoric acid and stictic acid, as promising multi-target agents for developing novel disease-modifying therapies for neurodegenerative disorders.

Keywords: Lichen metabolites, Neuroprotection, Alpha-synuclein, Amyloid-beta, Therapeutic efficacy

Abstract

Diversity and Distribution of Myxomycetes in Recreational Parks of Albay, Philippines

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Myxomycetes, also known as slime molds, are amoeboid, fungus-like organisms that are well-documented in natural and undisturbed habitats such as tropical forests, but are limitedly studied in urban green spaces. In this study, we assessed the occurrence, distribution and diversity of myxomycetes in relation to their microhabitats in recreational areas in Albay, Philippines. We hypothesized that human transient activities in these recreational parks may influence their occurrence and diversity. Ground leaf litter and twigs were collected from two recreational parks [Farm Plate (FP), Quituinan Hill Ranch (QH)], two eco-nature parks [Kawa-Kawa (KW), Lignon Hill (LH)], and a historic-cultural park [Cagsawa Park (CP)]. We used moist chamber (MC) culture techniques to evaluate myxomycetes. A total of 120 records of myxomycetes were collected from 150 moist chambers, resulting in 80% MC productivity. The collected myxomycetes were identified as belonging to 23 species and 13 genera. Among these, *Arcyria cinerea*, *Comatricha nigra*, *Diderma hemisphaericum*, and *Ophiotheca chrysosperma* occurred in all sampling sites. Taxonomic diversity index (TDI) was highest in the recreational parks, FP and QH (1.4) than the eco-nature (KW=1.3, LH=1.2) and historic-cultural (CP=1.1) parks. FP also had the highest species diversity (FAI = 10.03, H = 2.18, SDI = 0.86) among all parks. This park is known for many human-transient activities like picnics and other family-oriented activities. The comparison of our study with nearby forested areas showed a lower species diversity, indicating that recreational activities may have influenced the composition and diversity of myxomycetes. Nevertheless, the presence of myxomycetes in many man-made parks highlights their resilience against anthropogenic impacts.

Keywords: diversity, man-made parks, occurrence, slime molds, urban ecology

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Session 8 Edible Fungal Resources and Cultivation



Carbon Neutrality Potential in China's Edible Mushroom Bases

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Using life cycle assessment (LCA) to evaluate the environmental impacts of combined mushroom production with bioethanol (FBBE), material (FBBC), and methane (FBBM) production enables an in-depth understanding of the practical environmental advantages of utilizing the three-substance circulation for converting biomass resources. The results of LCA indicate that the greenhouse gas (GHG) emission potential of producing only mushroom is 4.55 kg CO₂ eq, accounting for 36.55% (FBBE), 92.11% (FBBC), and 78.45% (FBBM) of the respective combined processes. In the FBBE process, the chemical materials required for bioethanol production and the energy consumed during the heating process are the main contributing factors to the GHG emission potential. The results of functional unit allocation in the LCA show that raw material consumption, transportation, electricity use, and diesel use consistently have a relatively high impact on the environmental load across all circulation technologies. For the FBBE process, effective reduction of GHG emission potential cannot be achieved without implementing guiding or advanced strategies and incorporating carbon transfer. For the FBBC process, effective reduction of phosphorus use requires the implementation of advanced strategies combined with increased production. For the FBBM process, effective reduction of land use necessitates advanced strategies and reduced electricity consumption. Technological advancements implemented on farms related to mushroom and by-product production have the potential to lower labor costs and mitigate the environmental impacts of inputs. The findings of this chapter demonstrate the environmental sustainability of biomass degradation through the three-substance circulation involving mushrooms. When operated as a year-round circular production process, it provides a supplementary value of -1.24×10^8 t CO₂ eq for agricultural carbon neutrality, equivalent to protecting 1.09×10^5 ha of forest area in China. The results indicate that integrating the three-substance circulation with mushrooms into the production of agricultural food and related by-products can effectively promote the achievement of agricultural carbon neutrality.

Abstract

The mechanism of efficient metabolism of plant photosynthetic products by fungi

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Lignin, a main product of plant photosynthesis, is the second most abundant renewable bioresource in nature and the most difficult component to degrade in plant cell walls. White-rot fungi are the only group of organisms in nature capable of completely degrading lignin. However, the mechanism by which they respond to different lignin signals and efficiently degrade lignin remains unclear. We constructed a lignin degradation-inactivated degenerate strain using the *Trametes hirsuta* AH28-2 strain. Through phenotypic and transcriptome analysis, it was found that the key gene *Thipk1* in the polyphosphate inositol pathway could respond to different lignin monomer signals and regulate the coordinated expression of downstream lignin-degrading enzymes. Meanwhile, 5mC DNA methylation, as an epigenetic regulatory strategy, participated in the signal transduction process of *ThIPK1* and its downstream transcription factors, ultimately mediating the efficient degradation of lignin.

Sucrose, another product of plant photosynthesis, serves as an important bridge for carbon exchange between plants and microorganisms. However, most beneficial fungi cannot directly utilize plant sucrose but instead rely on its hydrolysis by the plant cell wall invertase. In recent years, studies have found that some beneficial fungi also have the ability to absorb sucrose directly, but the molecular mechanism and ecological function of this are still unclear. We discovered that the invertase *GspINV* and the sugar transporter *GspSUT1* from *Gongronella* sp. w5 play crucial roles in the interaction between w5 and plants. W5 can directly obtain sucrose from plants through the high-affinity sugar transporter *GspSUT1* and convert a portion of it into usable monosaccharides, and simultaneously release them into the rhizosphere. This process not only promotes the growth of nitrogen-fixing bacteria but also enhances the plant's absorption of nitrogen, thereby establishing a three-way interaction mechanism of "plant provides carbon - fungus distributes carbon - bacteria returns nitrogen". *GspSUT1* regulates the distribution of carbon, alters the structure of the rhizosphere microbial community, improves the availability of soil nutrients, and promotes plant growth in natural environments.

Abstract

Elucidation of mushroom derived bioactive compound biosynthesis via an *aspergillus oryzae* heterologous expression platform

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Mushrooms are an abundant source of structurally unique and pharmacologically valuable natural products that play crucial roles in food, medicine, and health industries. However, understanding their biosynthetic pathways has been hindered by the genetic complexity and slow growth of basidiomycetes. In this study, we established the filamentous fungus *Aspergillus oryzae* as an efficient heterologous host for expressing and characterizing mushroom derived biosynthetic genes. This system enables precise transcription and translation of intron-rich genes, providing a reliable platform for functional studies. Using *Hericium erinaceus* as a model, we reconstructed the biosynthetic pathway of the neuroactive compounds erinacines[1] and hericenones[2], which are known for their nerve growth factor-inducing activity. Functional analysis revealed that the non-reducing polyketide synthase HerA catalyzes the formation of the core polyketide scaffold, while the prenyltransferase HePT8 introduces geranyl modifications that diversify molecular structures. Structural modeling and mutagenesis identified several key amino acid residues essential for HePT8 catalytic efficiency. In addition, a rapid screening approach for mushroom sesquiterpene synthases was developed using the *A. oryzae* expression system, exemplified by studies in *Flammulina velutipes*[3].

These results demonstrate that *A. oryzae* serves as a versatile and powerful tool for elucidating mushroom natural product biosynthesis, paving the way for the discovery and engineering of novel bioactive metabolites with promising industrial applications.

Keywords: Mushroom; Natural products; *Aspergillus oryzae*; Heterologous expression

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Abstract

Genomic Insights and Mating Systems of *Oudemansiella apalosarca*: A Promising Edible Mushroom for Industrial Cultivation

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Oudemansiella apalosarca is a rare edible mushroom highly regarded for its suitability for tourism picking and its nutritional benefits. Compared to other species within the *Oudemansiella* genus, *O. apalosarca* offers a shorter production cycle and does not require soil covering, making it particularly promising for industrial cultivation. However, its complex life cycle and unclear hybrid compatibility among progeny have significantly hindered hybrid breeding efforts. This study focuses on the *O. apalosarca* strain BIPP21200004. By isolating single spores, the homokaryotic strains K72 and G13 were obtained. Genomic analysis revealed that each homokaryon consists of 12 chromosomes. Annotation of mating type genes identified 13 homeodomain (HD) genes, 9 pheromone receptor (RCB) genes, and 5 pheromone precursor (phb) genes on the G13 genome, while the K72 genome contained 14 HD genes, 8 RCB genes, and 5 phb genes. The A and B mating type loci for both strains were located on chromosomes 4 and 12. Specific molecular markers were developed based on polymorphisms in mating type genes. Analysis of 150 F₂ single spore strains revealed a 4:1 ratio of homokaryons to heterokaryons, with four distinct mating types observed: A1B1, A2B2, A1B2, and A2B1. Spore nuclear analysis and hybridization experiments suggest that *O. apalosarca* exhibits both homothallic and heterothallic mating systems. In the F₂ progeny, heterothallic mating offspring were more prevalent. The presence of A1B2 and A2B1 mating types indicates a tetrapolar mating system.

Keywords: *Oudemansiella apalosarca*, homokaryons, heterokaryons, mating types

Abstract

From Spore to Spread: Green Mold Contamination in Grey Oyster Mushroom Cultivation**Yee Shin Tan^{1,2*}, Ana Hazirah Ajis¹, Lay Ching Chai³**¹*Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.*²*Mushroom Research Centre, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.*³*Department of Biological Sciences, School of Medical and Life Sciences, Sunway University, Bandar Sunway, 47500 Selangor, Malaysia.*

Edible mushrooms are categorised as one of the high-value commodities under Malaysia's National Agrofood Policy 2.0 (2021-2030). *Pleurotus pulmonarius* is the most popular and widely cultivated oyster mushroom species which contained numerous nutrients content also as source of wealth for farmers. However, recent outbreaks of green mold contamination (GMC) caused by *Trichoderma* spp. have severely affected the oyster mushroom farms, resulting in significant production losses. This study aimed to identify the causal agents of green mold contamination and to determine the critical points in the mushroom cultivation process that contribute to the occurrence of green mold contamination. Samples were collected from different stages of mushroom cultivation process. The results revealed that *Trichoderma pleuroti* is the predominant species responsible for GMC, with *T. harzianum* and *T. ghanense* were identified as major green mold contaminants, which attributes to the suppression of *Pleurotus* growth during different cultivation stages and eventually colonized the mushroom substrates and causing severe GMC. This study enhances understanding of the GMC etiology and provides a basis for developing strategies to mitigate the green mold contamination in oyster mushroom cultivation.

Keywords: Green mold contamination; Grey oyster mushroom; *Trichoderma*

Abstract

Identification of the regulatory factors in sexual reproduction signaling of *Pleurotus eryngii*

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Pleurotus eryngii is an important commercially cultivated edible mushroom in Asia. Research on its sexual reproduction and fruiting body development contributes to variety improvement through molecular genetic breeding. By introducing compatible mating-type genes into the monokaryotic strain of *P. eryngii*, we observed the formation of clamp connections and the development of fruiting bodies. Further transcriptome sequencing of the monokaryotic transformants revealed that the expression of certain genes was strongly induced by the introduced mating-type genes. Using experimental methods such as yeast two-hybrid screening, gene overexpression, and RNAi, we conducted further studies on *MRP1*, an HMG-box transcription factor gene, and *PRK1*, a serine-threonine kinase gene, both of which are induced by the mating-type genes. The results showed that *MRP1* interacts with homologs of the proteins regulating cell polarity growth and sexual reproduction in yeast. Overexpression of *MRP1* promoted mycelial growth and enhanced antioxidant enzyme activity, while RNAi transformants of *MRP1* exhibited slowed mycelial growth. Yeast two-hybrid results for *PRK1* indicated that its interacting proteins are primarily enriched in pathways such as oxidative phosphorylation, MAPK signaling, and protein kinase signaling. RNAi transformants of *PRK1* showed prolonged primordium formation and produced small fruiting bodies with abnormal cap development. Additionally, downregulation of *PRK1* transcript levels in the RNAi transformants led to a decrease in *MRP1* expression, suggesting that *PRK1* acts upstream of *MRP1* in the signaling pathway. These findings suggest that *MRP1* and *PRK1* are regulators induced by mating-type genes in the sexual reproduction signaling process in *P. eryngii*.

Keywords: *Pleurotus eryngii*, Regulatory factors, Sexual reproduction signaling

Abstract

Comprehensive flavoromics and DFT calculations reveal the influence of flavor formation in *Auricularia heimuer*

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Flavor quality is a critical determinant of consumer acceptance in edible fungi, especially *Auricularia heimuer*, a mushroom deeply rooted in Chinese culinary tradition. Despite its cultural significance, flavor has often been overlooked in favor of yield and disease resistance in modern cultivation. This study explores how different cultivation methods—traditional log-based and industrialized bag-based—affect the flavor profile of *A. heimuer* after stir-frying, a cooking method known for intensifying umami and aroma. Employing integrated metabolomics approaches (GC-MS, UHPLC-MS/MS in untargeted and targeted modes), combined with electronic nose and tongue analyses, and density functional theory (DFT) simulations, we conducted a comprehensive analysis of the volatile and non-volatile compounds responsible for flavor. The results revealed that log-cultivated *A. heimuer* contained higher levels of fatty acids such as arachidonic acid and more complex aroma-contributing volatiles, while both types exhibited distinct but overlapping profiles of taste-related amino acids. DFT simulations further confirmed that oil-phase Maillard reactions facilitate the formation of thermodynamically stable flavor compounds with high selectivity. This work underscores the critical influence of cultivation practices on the flavor chemistry of *A. heimuer*, providing valuable insights for optimizing both traditional and modern mushroom production systems toward improved sensory quality.

Keywords: *Auricularia heimuer*, Cultivation methods, Flavor compounds, Maillard reaction, Metabolomics

Abstract

***Stropharia rugosoannulata* Cultivation as Biological Preprocessing of Agricultural and Forestry Wastes Lignocellulose Degradation Pattern, Structural Changes and Enzymes Secretion and Expression**

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Stropharia rugosoannulata demonstrated the capability to degrade various types of agricultural and forestry wastes, including cherry wood (CW), corn cob (CC), and rape straw (RS). Significant differences were observed in the secretion and expression profiles of lignocellulolytic enzymes during the degradation process. The lignin degradation rates reached 65.07%, 78.88%, and 81.64% for CW, CC, and RS, respectively, during mycelial growth after 45 days of solid-state fermentation incubation, and the degradation of cellulose and hemicellulose was largely driven by lignin degradation. Fourier-transform infrared spectroscopy and X-ray diffraction analyses indicated more extensive lignin removal in RS, while the crystallinity of both CW and RS was effectively reduced, with a particularly notable decrease in CW. Among the key lignocellulolytic enzymes, laccase exhibited the highest activity in cultures supplemented with CW. The highest lytic polysaccharide monooxygenase (LPMO) activity was detected in RS medium, reaching 436.10 U/g. Furthermore, cytochrome P450 gene expression was significantly upregulated in wood-based substrates, indicating a substrate-specific response. Acetyl xylan esterases displayed broad substrate adaptability. Notably, the LPMO gene family was identified to play a central role in lignocellulose deconstruction. These findings highlight the efficiency of *S. rugosoannulata* in transforming lignocellulosic biomass, suggesting its potential for applications in enzyme production and biorefinery processes.

Keywords: *Stropharia rugosoannulata*; Lignocellulose degradation; Lytic polysaccharide monooxygenase (LPMO); Agricultural waste; Enzyme activity

Abstract

A Comparative Study on Evaluation of Antioxidant Properties of the Philippine Mushrooms *Pleurotus djamor* (Pink Oyster Mushroom), *Pleurotus citrinopileatus* (Yellow Oyster Mushroom), and *Pleurotus pulmonarius* (Brown Oyster Mushroom)

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This study explores the antioxidant properties of the *Pleurotus djamor* (Pink oyster mushroom), *Pleurotus citrinopileatus* (Yellow oyster mushroom), and *Pleurotus pulmonarius* (Brown oyster mushroom) that was cultivated in the Philippines. Natural resources such as edible mushrooms have a lot of potential as an therapeutic agent, not only could they be consumed safely, but their antioxidant properties can be used to treat and cure some diseases. They are known to contain beneficial compounds, such as phenolics, flavonoids, and polysaccharides, which contribute to their medicinal and health benefits. Using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, the study employed a quantitative research design, and data were analyzed using various statistical tools, including mean, standard deviation, percentage, one-way analysis of variance (ANOVA), Tukey's post hoc test, and p-test. The findings showed that *Pleurotus djamor* showed the highest antioxidant activity at 100% concentration. However, when considering overall potency across concentrations and EC50 values, *Pleurotus citrinopileatus* proved to be the most potent, especially at 75% concentration. Furthermore, the mushroom with the least antioxidant activity was *Pleurotus pulmonarius* at 50% concentration. There is plenty of research internationally proving the presence of antioxidants in *Pleurotus* species, but only a few in the Philippines, which is why the aim of this research is to expand the context of antioxidant activities of Philippine varieties of *Pleurotus* species.

Keywords: antioxidants, *Pleurotus citrinopileatus*, *Pleurotus djamor*, *Pleurotus pulmonarius*, scavenging assay

Abstract

Systematic knockout efforts highlight conserved transcription factors regulating the initiation of mushroom formation in *Coprinopsis cinerea*

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Mushroom development starts with the formation of hyphal knots (HKs), which represent the transition from vegetative to reproductive growth, and is one of the most complex morphogenetic processes in fungi. To investigate the gene regulatory networks (GRN) underlying HK formation, we systematically knocked out 37 conserved transcription factors (TFs) in the *Coprinopsis cinerea* AmutBmut #326 strain. Most of these TFs are up-regulated during mushroom initiation. The mutants' phenotypes range from the lack of HK formation to arrest at diverse developmental stages, or the production of dense HKs and primordia. Among them, six TFs' mutants showed healthy vegetative mycelia but no HK formation. To further test their function, we examined genetic interactions between six shortlisted TFs. Constitutive expression of one TF (by the *gpd* promoter), referred to as *fluffy1*, in the wild-type strain could produce HK rings precociously, without starvation induction. Overexpression of two other TFs, *Nhk1* and *Nhk2* (no hyphal knot 1 and 2), in other mutants partially or completely restored HK formation, suggesting that these TFs play a key role in the pathway. We also identified two apparent repressors of HK formation. Knockout mutants of one of these, called *Dpr1* (dense primordia 1), could produce dense HKs and primordia without light induction, even under the suboptimal temperature conditions (37 °C), at which the WT does not. Therefore, *Dpr1* may act as a key upstream repressor to regulate HK formation in suitable environments. Combined with RNA-seq data, we inferred a gene regulatory network underlying mushroom initiation in Agaricomycetes. Our work provided new insights into mushroom development at the molecular level and also provides potential benefits for genetic modification and strain breeding.

Keywords: Basidiomycota, CRISPR/Cas9, Evolution, Morphogenesis

Abstract

Integrated Assessment of Growth and Protein Content in Basidiomycetous Fungi for Mycoprotein Production

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The rising demand for sustainable and health-conscious protein sources has driven interest in fungal-derived mycoprotein as an alternative to conventional meat products. While commercial mycoprotein production predominantly utilizes *Fusarium venenatum*, concerns over mycotoxin potential and limited strain diversity indicate the need to explore safer and edible basidiomycetes. In this study, 28 species across four taxonomic orders within Agaricomycetes were screened for their potential as mycoprotein sources. Hyphal growth dynamics were measured on potato dextrose agar, and crude protein content was quantified from submerged mycelial cultures using the Kjeldahl method. Results revealed significant inter-order variation: *Polyporales* exhibited the fastest radial growth, while *Agaricales* grew the slowest. Highest crude protein levels were observed in *Inonotus obliquus* (41.98%), *Neolentinus lepideus* (40.27%), and *Bjerkandera adusta* (39.15%). The dual assessment of growth kinetics and nutritional value identified strains from *Gloeophyllales*, *Hymenochaetales*, and *Polyporales* as promising candidates for scalable mycoprotein development. These findings show the potential of basidiomycetous fungi as safe and effective sources of mycoprotein and provide a framework for future fermentation optimization and functional food innovation.

Keywords: Biotechnology, crude protein, submerged fermentation

Abstract

Beyond Birch: Enhanced Growth of Pakuri (*Inonotus obliquus*) on Alder-Derived Substrates

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The cultivation of specialty fungi has gained increasing attention among forest owners in Finland over the past decade. Among these, *Inonotus obliquus* (Pakuri) — valued for its use in functional beverages and dietary supplements — is emerging as a high-potential non-timber forest product. Despite strong commercial demand, scientific knowledge of optimal cultivation practices, particularly regarding strain and substrate selection, remains limited.

This study evaluated the mycelial growth of geographically distinct *I. obliquus* strains when cultured on agar media supplemented with sawdust from either sapwood or heartwood of *Betula pendula* and *Alnus incana*. Growth rates were compared across strain–substrate combinations under controlled laboratory conditions to identify optimal pairings.

Significant variation in growth rates was observed, driven by the interaction between fungal strain origin, host tree species, and wood tissue type. The most vigorous growth occurred on sapwood derived *A. incana* substrates inoculated with strains originating from northern and eastern Finland.

These findings challenge the prevailing association of *I. obliquus* cultivation with birch species and highlight *A. incana* as a promising alternative substrate in industrial valorisation of forestry side streams and production of Pakuri as non-timber forest product. Adoption of such optimised strain–substrate combinations could improve Pakuri production efficiency and diversify its cultivation potential in boreal forestry systems and in industrial applications.

Keywords: *Inonotus obliquus*, Pakuri cultivation, substrate optimisation, *Alnus incana*, edible fungi

Abstract

Sustainable Development in Fungal Protein Biorefineries: Life Cycle Assessment of Plastic Recycling Strategies

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Plastic waste recycling and sustainable management constitute critical challenges in global environmental governance. As the world's largest fungal protein producer, China generates approximately 1.2 million tonnes of agricultural plastic waste annually from fungal biorefineries, predominantly managed through landfilling and open-air incineration—practices that necessitate transformative recycling solutions. Life Cycle Assessment (LCA), a robust screening tool for identifying environmental hotspots across complex value chains, was integrated with Techno-Economic Analysis (TEA) to holistically evaluate fungal production plastics. Results demonstrate mechanical recycling (MR) as the societally optimal method under Chinese operational contexts, exhibiting favorable environmental-economic tradeoffs (GWP: 61.86 kg CO₂ eq; NPV: 88.63 CNY per tonne of plastic processed for fungal synthesis). This study establishes a multidimensional assessment framework for fungal plastic waste valorization, providing empirical decision-making support for closed-loop agricultural plastic management in developing economies.

Keywords: Agricultural plastics, Life cycle assessment, Middle point impact, End point damage, Sustainable management, Techno-Economic Analysis

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Abstract

Exploration of important genetic resources and germplasm Innovation of Edible Fungi based on large-scale omics data

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Germplasm innovation in edible fungi serves as a core driver for industry development. To systematically explore and utilize their important genetic resources, this study integrates large-scale multi-omics data and employs comprehensive strategies including population genetic diversity analysis, pan-genome construction, genome-wide association studies (GWAS), and weighted gene co-expression network analysis (WGCNA). The aim is to establish precise associations between phenotypes and genotypes, and to identify key candidate genes for functional validation. Recently, using *Flammulina filiformis* and *Agaricus bisporus* as models, we have deciphered the genetic basis underlying cap color variation, uncovering crucial regulatory genes and their molecular mechanisms. These findings provide a solid molecular basis and novel theoretical insights for the targeted breeding of new edible fungus germplasms with superior traits.

Keywords: Edible mushroom genetics, function gene, genomics, molecular breeding

Abstract

Novel Genomic Insights into Drug Resistance Evolution and Morphological Adaptation of *Candida tropicalis* from Clinical-Environmental Isolates in Hainan, China

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Candida tropicalis is an emerging fungal pathogen with dual clinical and environmental relevance, posing threats to public health and exhibiting environmental persistence in tropical/subtropical regions like Hainan, China. To investigate the genetic basis of phenotypic diversity and drug resistance in this species, we performed a comprehensive population genomic analysis of 274 clinical and environment isolates collected from Hainan, China. Whole-genome sequencing (WGS) was performed to identify single nucleotide polymorphisms (SNPs), copy number variations (CNVs), and loss of heterozygosity (LOH). Population structure was explored via K-means clustering and principal component analysis (PCA), and evolutionary relationships via a maximum-likelihood phylogenetic tree. Genome-wide association studies (GWAS) with a multi-locus mixed model (MLMM) identified genetic variants associated with resistance to voriconazole/amphotericin B and colony morphology (smooth/wrinkled/rough). We identified distinct genetic clusters between clinical and environmental isolates, revealing novel SNPs associated with drug resistance and morphological adaptation—key for understanding its ecological persistence and developing sustainable control strategies. For voriconazole, two highly significant missense mutations were detected in genes encoding a histidinol dehydrogenase (CTRG_00122, p.Arg417His) and a hypothetical protein (CTRG_00150, p.Asp1205Asn). For amphotericin B, associations were found near genes involved in S-adenosylmethionine synthesis and other hypothetical proteins. Additionally, we identified SNPs associated with colony morphology, including one near a magnesium ion transmembrane transporter gene (CTRG_02912). We also observed extensive LOH patterns across the genome, which may play a role in the rapid adaptation to environmental stressors such as antifungal drugs. This study provides a foundational genomic framework for understanding the population structure and evolutionary dynamics of *C. tropicalis* in Hainan. The identified genetic variants provide targets for developing eco-friendly antifungal therapies (e.g., precision medicine to reduce drug overuse) and regional surveillance systems, supporting sustainable management of fungal diseases in tropical/subtropical ecosystems.

Keywords: *Candida tropicalis*; Drug resistance; Genomics; GWAS; Morphological variation

Abstract

Population genomics unveils the evolution of adaptation in *Fusarium* head blight pathogens in response to agricultural practices and human migration

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Fusarium head blight (FHB) is one of the most destructive wheat diseases worldwide. To understand the impact of human migration and changes in agricultural practices on crop pathogens, here population genomic analysis with 245 representative strains from a collection of 4,427 field isolates of *Fusarium asiaticum*, the causal agent of FHB in Southern China is conducted. Three populations with distinct evolution trajectories are identified over the last 10,000 years that can be correlated with historically documented changes in agricultural practices due to human migration caused by the Southern Expeditions during the Jin Dynasty. The gradual decrease of 3ADON-producing isolates from north to south along with the population structure and spore dispersal patterns shows the long-distance (>250km) dispersal of *F. asiaticum*. These insights into population dynamics and evolutionary history of FHB pathogens are corroborated by a genome-wide analysis with strains originating from Japan, South America, and the USA, confirming the adaptation of FHB pathogens to cropping systems and human migration.

Keywords: *Fusarium* head blight (FHB), adaptation, migration, population genomics

Abstract

Population genetics and phylogeography of endophytic *Phyllosticta capitalensis* in Indonesia based on MIG-seq genome-wide SNPs**Indriati Ramadhani^{a,b,#}, Yoshiaki Tsuda^c, Kaoru Yamaguchi^d, Izumi Okane^e**^a*Graduate School of Science and Technology, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan. Email: indriatiramadhani@gmail.com*^b*Research Center for Biosystematics and Evolution, Research Organization for Life Sciences and Environment, National Research and Innovation Agency (BRIN), Cibinong 16911, West Java, Indonesia*^c*Sugadaira Research Station, Mountain Science Center, University of Tsukuba, 1278-294 Sugadairakogen, Ueda, Nagano 386-2204, Japan. Email: tsuda.yoshiaki.ge@u.tsukuba.ac.jp*^d*Biological Resource Center (NBRC), National Institute of Technology and Evaluation, 2-5-8 Katusakamatari, Kisarazu, Chiba 292-0818, Japan. Email: yamaguchi-kaoru@nite.go.jp*^e*Institute of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan. Email: okane.izumi.fw@u.tsukuba.ac.jp**Email: indriatiramadhani@gmail.com*

Reduced genome sequencing approaches, such as MIG-seq, enable genome-wide single nucleotide polymorphism (SNP) discovery in non-model organisms, offering detailed insights into population structure and evolutionary dynamics. This is particularly valuable for mycological science in Indonesia, where studies on fungal endophytes remain limited despite the expectation that tropical regions harbor greater genetic diversity. In this study, we applied MIG-seq to examine the genetic diversity, population structure, and reproductive strategy of the widespread endophyte *Phyllosticta capitalensis*. We genotyped 714 isolates, including both monokaryotic and heterokaryotic strains, from Indonesia and previously collected samples from Japan. After filtering, 403 SNP loci were retained. The results revealed clear differences in genetic diversity and structure among regions. Indonesian populations exhibited the highest nucleotide diversity, allelic richness, and private alleles, highlighting the role of tropical regions as reservoirs of genetic variation. By contrast, isolates from Iriomote and Ishigaki islands in southern Japan showed reduced diversity and formed distinct clusters, consistent with founder effects and geographic isolation. Although overall differentiation was weak, population structure analyses revealed consistent separation among tropical Indonesia, subtropical–tropical southern Japan, and temperate mainland Japan. Analyses of fixation indices indicated uniformly positive values higher than 0.9, pointing to a predominantly homothallic reproductive system. Elevated values also suggest a Wahlund effect, reflecting substructure, as supported by the admixture analysis. Moderate gene flow among regions suggests potential connectivity through natural dispersal as well as human-mediated movement of host plants. This study shows that *P. capitalensis* maintains broad host associations and genetic cohesion through reproductive assurance, while also exhibiting subtle geographic structuring shaped by climate and isolation. These findings advance our understanding of how generalist

fungal endophytes persist across diverse hosts and environments, offering a broader perspective on fungal diversity across tropical, subtropical, and temperate ecosystems.

Keywords: climatic gradients, gene flow, genetic diversity, population structure, reproductive strategy

Abstract

Genomes and population genomics of *Cenococcum geophilum* highlight the genetic diversity, evolution, and stress responses of ectomycorrhizal fungi

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Cenococcum geophilum is among the most globally abundant ectomycorrhizal (ECM) fungi and often dominates the root systems of woody plants, particularly in extreme environments. Therefore, *C. geophilum* is an ideal model for studying genetic diversity and adaptation of ectomycorrhizal symbiosis. In this study, we constructed a *C. geophilum* pan-genome using six high-quality whole-genome sequences representing the global diversity and found that dispensable genes are important for its environmental adaptation. We identified millions of single-nucleotide polymorphisms, hundreds of thousands of small insertions/deletions, 0.04–0.2% structural variation, and 21 horizontally transferred genes from non-host plants. Based on resequencing data from 304 global isolates, we investigated the geographic structure of *C. geophilum* populations. Genome-wide association and transcriptome studies identified 161 genomic regions significantly associated with nine biological and environmental adaption characteristics, including 2738 candidate genes such as EVM0002574 for cadmium, salt, and high temperature stresses. These reference genomes and diversity datasets provide resources and a comparative genomic platform for the study of ectomycorrhizal symbioses.

Keywords: *Cenococcum geophilum*; Genetic diversity; Mycorrhiza; Pan-genome; GWAS; Population structure

Abstract

Genomic and Phenotypic Basis for the Clinical Dominance of *Cryptococcus neoformans* VN1a-5 in Asia**Nan Hong^{1,2}, Wanqing Liao³, Jianping Xu^{4,*}, Min Chen^{2,5*} and Matthew Fisher^{6,*}**¹*Department of Dermatology, Jinling Hospital, Affiliated Hospital of Medical School, Nanjing University, Nanjing, China*²*Department of Dermatology, Shanghai University of Medicine & Health Sciences Affiliated Zhoupu Hospital, Shanghai, China*³*Shanghai Key Laboratory of Molecular Medical Mycology, Changzheng Hospital, Shanghai, China*⁴*Department of Biology, McMaster University, Hamilton, Canada*⁶*Department of Infectious Disease Epidemiology, MRC Centre for Global Infectious Disease Analysis, School of Public Health, Imperial College, London, UK*

Cryptococcus neoformans is a globally distributed fungal pathogen that predominantly causes life-threatening infections in immunocompromised individuals. In East Asia, however, a clonal subclade, VN1a-5, accounts for approximately 80% of clinical cases and is disproportionately found in immunocompetent patients, suggesting a distinct pathogenic profile. Here, we conduct a population genomic study of clinical and environmental isolates from China and find that the clinical predominance of VN1a-5 is not due to environmental prevalence but rather intrinsic virulence traits. Environmental isolates are dominated by a clinically rare subclade, VN1a-31, providing an appropriate comparator for dissecting the features of VN1a-5 that are associated with virulence. Comparative genomic and transcriptomic analyses identify signatures of selection and differences in gene-expression in molecular pathways related to extracellular vesicle production, mitochondrial function, and glucose sensing. Notably, we identified the Snf1 pathway—central to the glucose starvation response—as both upregulated and under a selective sweep in VN1a-5. Accompanying phenotypic assays demonstrate that VN1a-5 exhibits enhanced melanin biosynthesis, increased production of small extracellular vesicles, and elevated mitochondrial tubularization under oxidative and glucose-limited conditions. Notably, remodelling of mitochondrial morphology in VN1a-5 under low-glucose with oxidative stress mirror those mitochondrial traits observed in the hypervirulent Vancouver Island outbreak genotype of *C. gattii*. These findings indicate that metabolic adaptation to nutrient stress underlies the enhanced virulence of VN1a-5 and provide a mechanistic basis for its clinical predominance in non-HIV-infected individuals.

Abstract

An evolutionarily ancient transcription factor drives spore morphogenesis in mushroom-forming fungi

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Sporulation is the most widespread means of reproduction and dispersal in fungi and, at the same time, an industrially important trait in crop mushrooms. In the Basidiomycota, sexual spores are produced on specialised cells known as basidia, from which they are discharged forcibly by the highest known acceleration in nature. However, the genetics of sporulation remains poorly known. Here, we build on the discovery of a new, highly conserved transcription factor, Sporulation-Related Regulator 1 (*srr1*), to systematically address the genetics of spore formation for the first time in the Basidiomycota. We show that *Srr1* regulates postmeiotic spore morphogenesis but not other aspects of fruiting body development or meiosis, and its role is conserved in the phylogenetically distant, but industrially important *Pleurotus* spp (oyster mushrooms) too. We used RNA-Seq to understand genes directly or indirectly regulated by *Srr1* and identified a strongly supported binding motif for the protein. Using an inferred network of putative target genes regulated by *Srr1* and comparative genomics, we searched for genes linked to ballistospority. Overall, our study offers systematic insights into the genetics of spore morphogenesis in the Basidiomycota.

Keywords: mushroom-forming fungi, regulatory network, sporulation, transcription factor

Abstract

Omics Approaches for Understanding the Genetics of Fruiting Body Development in the Agaricomycetes

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Mushroom-forming fungi (Agaricomycetes) produce some of the most striking fruiting bodies in the fungal kingdom, and hold significant economic value, particularly in the food industry. Although interest in the genetics of fruiting body development has recently grown, our understanding of its fundamental principles remains largely incomplete.

In this presentation, I will summarize recent findings obtained through a combination of omics data, post-genomics, and genetics, all aimed at unraveling the genetic basis of fruiting body development. Comparative transcriptomic analyses have helped identify a core, conserved gene set that is developmentally regulated, offering new insights into the cellular processes active within fruiting bodies. This approach also aided in shortlisting regulatory genes for in-depth investigation in *Coprinopsis cinerea*, a model mushroom well-suited for experimental studies.

Our analyses of these genes, coupled with a developing knockout collection of over 100 regulatory genes in *C. cinerea*, have uncovered transcriptional networks involved in key morphogenetic steps, including the initiation of fruiting body development, the regulation of fruiting body numbers, cap morphogenesis, and meiotic spore formation.

Finally, I will outline future directions and emphasize the necessity of developing post-genomic techniques and datasets to bring mushroom-forming fungi up to par with the most extensively researched filamentous fungal model organisms. I will argue that this will unlock a wealth of exciting new biological knowledge about these important organisms.

Keywords: mushrooms, fruiting body development, post-genomics, transcriptomics, evolution

Abstract

Tracking plant pathogen emergence using thousand-genome panels**Daniel Croll***Institute of Biology, University of Neuchâtel, Switzerland**daniel.croll@unine.ch*

Adaptation of plant pathogens proceeds at speeds that easily overwhelm the rate of resistant cultivar deployment and fungicide development. Low cultivar diversity and the application of single fungicides can exacerbate these dynamics. Understanding the molecular basis of pathogen adaptation is critical to define more sustainable containment strategies. Emerging traits such as virulence on resistant cultivars or fungicide resistance often appear in a geographically structured manner. Such geographic mosaics can be determined by regional variation in selection pressures or as a consequence of the pathogen's population history. I will introduce the use of very large panels of pathogen genomes from the same species. Such genome panels can recapitulate historic colonization patterns and reveal constraints on pathogen genetic diversity. In combination with phenotyping assays, large genome panels enable the tracking of fungicide resistance mutations across continents and reveal how effector gene loci are undergoing adaptive sequence rearrangements. Recent research has pointed to selfish elements (i.e. transposable elements) as key factors in pathogen evolution, facilitating adaptation to biotic and abiotic factors in their environment. Selfish elements, by nature, can also impact the integrity of genomes and lead to deleterious dynamics for the pathogen. In conclusion, large-scale genomic investigations of individual pathogen species unravel essential mechanisms of pathogen adaptation.

Keywords: Population genomics, crop pathogens, wheat disease

Abstract

Genomic signatures of climate-driven evolution, hybrid origins, adaptive evolution, and domestication in button mushroom**Zhilin Ling^{1*}, Guotao Chen^{1,2}, Ruilin Zhao^{1,2#}**¹*State Key Laboratory of Microbial Diversity and Innovative Utilization, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China.*²*College of Life Sciences, University of Chinese Academy of Sciences, Beijing 101408, China.**Zhilin Ling email: lingzl@im.ac.cn; Guotao Chen email: chengt@im.ac.cn; Ruilin Zhao email: zhaorl@im.ac.cn*

The evolutionary and domestication history of mushroom-forming fungi remains poorly understood despite their ecological and economic significance. Here, we present the first large-scale population genomic study of the button mushroom *Agaricus bisporus*, the world's most cultivated edible mushroom, integrating resequencing data from 483 wild and commercial strains. Our analyses reveal a complex admixture evolutionary history shaped by Quaternary glacial cycles. Ice age-induced bottlenecks drove population differentiation into North American and European lineages, while interglacial expansions facilitated gene flow, yielding seven admixed populations. Our contribution here also includes the successful connection between the domestication trait and its gene function. Through selective sweeps, genome-wide association studies (GWAS), transcriptomics, and transgenic validation, we identify *AbPPO1* as a key domestication gene, with its mutant haplotype "Hap_2" underpinning the white cap trait—a hallmark of commercial strains. By bridging evolutionary genomics with domestication genetics, this work provides foundational insights into fungal adaptation and offers a genomic toolkit for enhancing agronomic traits in economically vital species. Our findings redefine the genetic complexity of the button mushroom domestication and underscore the role of historical climate dynamics in shaping fungal biodiversity.

Keywords: Demographic history; Domestication gene; Edible mushroom; Population genomics

Abstract

Pan-mitogenomic analysis of *Ganoderma lingzhi* reveals intron evolution as a driver of genomic diversity and a source for species authentication.**Jingling Li¹, Chang Zhang¹, Lei Sun², Xian-hao Cheng^{2#}, Chang Liu^{1#*}.**¹*Center for Bioinformatics, Institute of Medicinal Plant Development, PeKing Union Medical College, Chinese Academy of Medical Sciences.* ²*School of horticulture, Ludong University.*Chang Liu: cliu@implad.ac.cn; Xian-hao Cheng: chengxianhao@sohu.com

Background: *Ganoderma lingzhi* is a traditional medicinal fungus widely cultivated for harvesting its fruiting bodies or spores, which are then used to extract active components. However, there is currently a significant issue of species origin confusion in the market, where different species are often misused interchangeably, seriously affecting medicinal safety and efficacy. Due to similar morphological characteristics and the limitations of traditional identification methods, accurately distinguishing *G. Lingzhi* species is challenging. In recent years, mitochondrial DNA has gradually become an important molecular marker for fungal species identification due to its moderate evolutionary rate, high copy number, and structural conservation. **Methods:** We collected cultivated strains of *G. Lingzhi* from production areas in Shandong, Henan, Fujian, Zhejiang, Sichuan, Anhui, Jilin, Guangdong, and other regions of China. Additionally, we obtained standard strains from multiple microbial culture preservation centers, totaling 151 strains. Whole-genome DNA was extracted for the construction of genomic libraries. Based on the Illumina platform, whole-genome sequencing (WGS) was performed to obtain high-throughput data. Using GetOrganelle software with the author-recommended default parameters, we assembled the mitochondrial genomes of all 151 strains. Annotation of the mitochondrial genomes was completed using the online tool Mfannot. We also downloaded 48 available mitochondrial genomes of *Ganoderma* species from the NCBI public database, along with two outgroups, resulting in a total of 201 mitochondrial genomes for phylogenetic analysis. Using PhyloSuite, we extracted common mitochondrial genes, aligned the sequences with MAFFT, and constructed a phylogenetic tree with IQ-TREE 2 based on the aligned sequence matrix. **Results:** Based on phylogenetic analysis, among the 151 *Ganoderma* strains we collected, 127 belonged to *G. lingzhi*, 3 were *G. sinense*, 1 was *G. resinaceum*, 10 were *G. oregonense*, 1 was *G. subamboinense*, 2 were *G. steyaertianum*, and 7 strains have not yet been identified to species level. The analysis revealed that 3 strains collected from farmers and 2 from culture preservation centers were inconsistent with their claimed identities. The 127 *G. Lingzhi* strains clustered into two main branches, indicating that these strains can be divided into two major groups. Comparative analysis of the mitochondrial genomes revealed widespread intron variation within the *Ganoderma* genus, and the loss and gain of introns also contributed to differences in genome size. **Conclusion:** We conducted the first pan-mitogenomic study of *G. lingzhi*. The observed intron dynamics further elucidate the genomic diversity within *Ganoderma*, providing valuable insights for future research on mitochondrial evolution and species differentiation. Our research also underscores the critical role of mitochondrial genomes as reliable molecular markers for authenticating *G. Lingzhi* strains.

Keywords: *Ganoderma Lingzhi*; Intron evolution; Mitochondrial genome; Pan-mitogenome

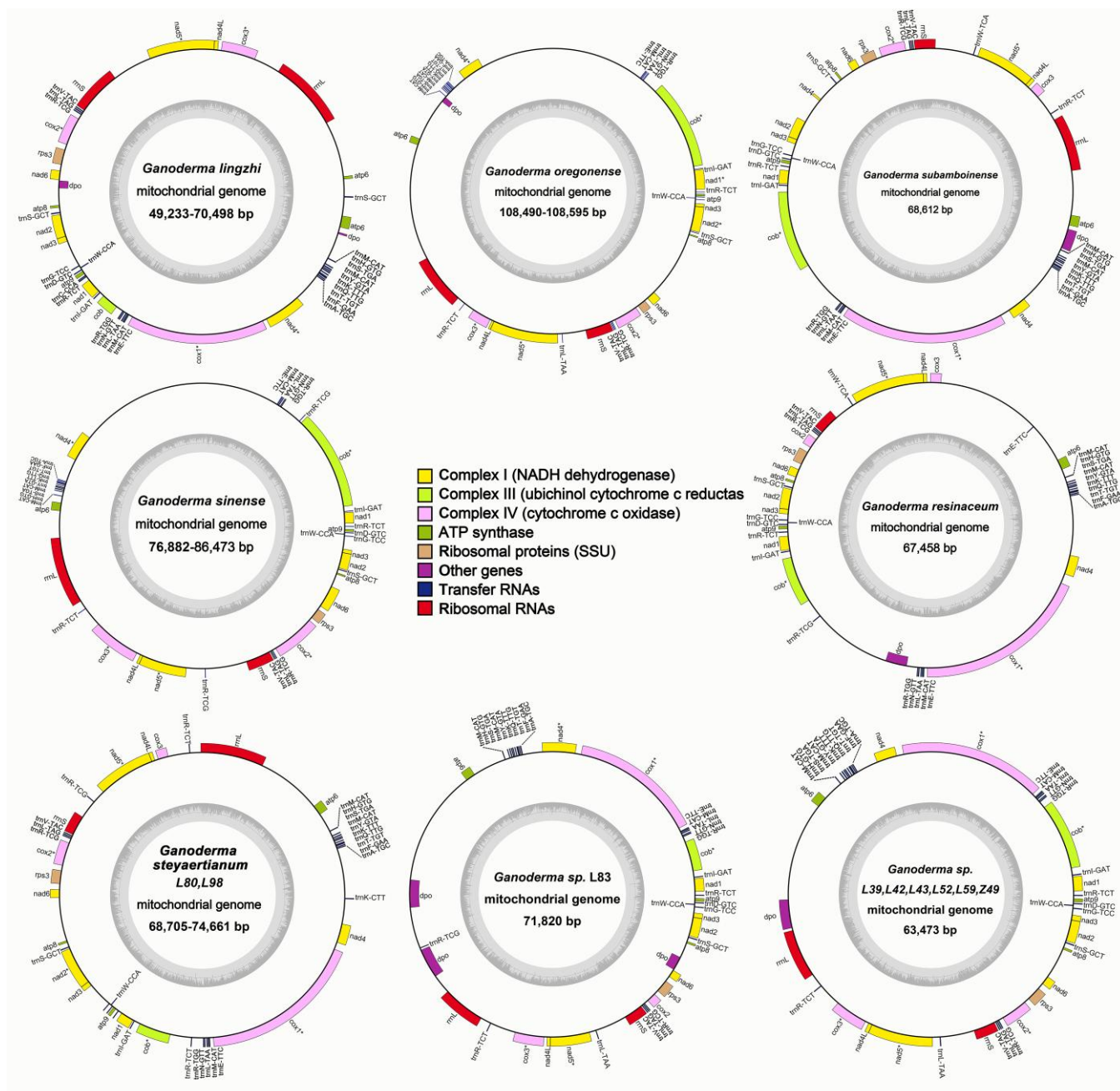


Figure 1. Circular maps of mitogenomes of *Ganoderma* species. Genes are represented with different colour blocks. Strand orientation is indicated by block position: genes on the direct strand are represented by colour blocks outside the ring, while genes on the reverse strand are denoted by colour blocks inside the ring.

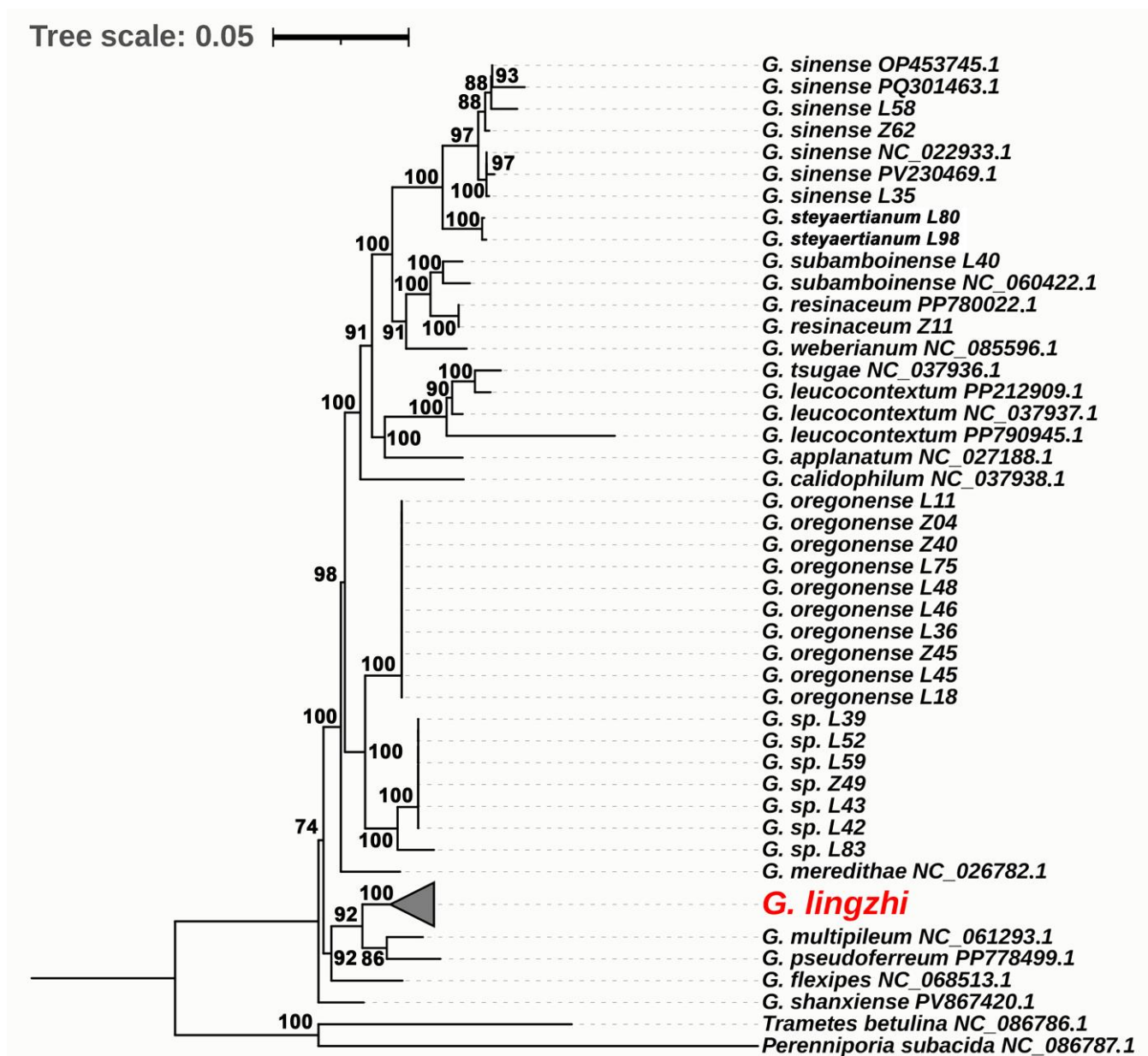


Figure 2. Phylogenetic relationships of *Ganoderma* species, based on 15 common genes (*atp6*, *atp8*, *atp9*, *cob*, *cox1*, *cox2*, *cox3*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6*, *rps3*) using Maximum Likelihood methods implement in IQtree2.

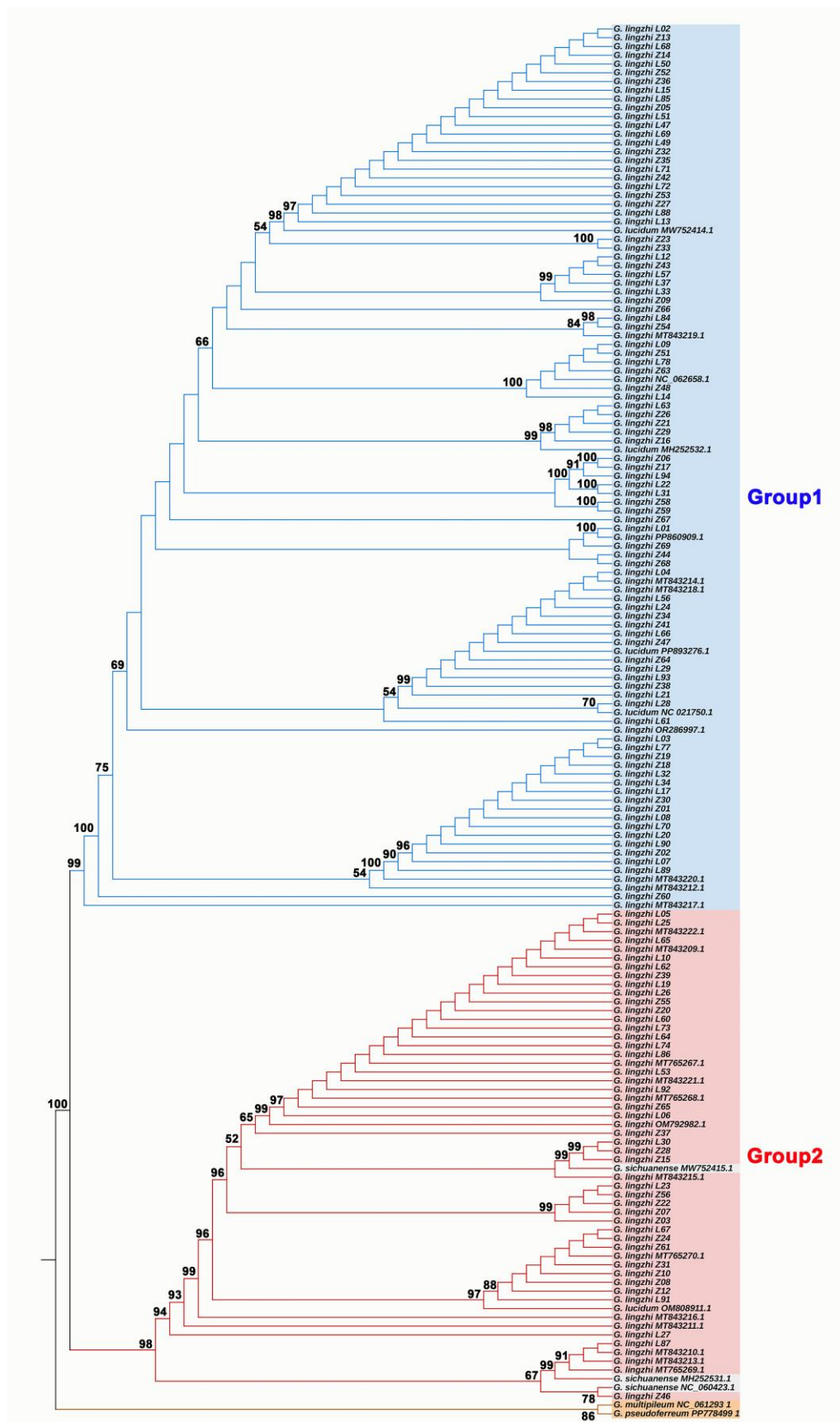


Figure 3. Phylogenetic analysis within *Ganoderma lingzhi*. *G. lingzhi* can be divided into two major clades, with species *G. sichuanense* nested within one of them, indicating close phylogenetic relationships.

Abstract

Title: High-Quality Genomic Resources of Soybean Powdery Mildew (*Erysiphe diffusa*) Were Deciphered Using Nanopore and Illumina

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Soybean powdery mildew is one of the major diseases affecting soybean leaves, which can cause yield losses of up to 25%. However, effective prevention and control of this disease cannot be implemented due to a lack of genome-level understanding of the pathogenic mechanisms of the causative fungus, *Erysiphe diffusa* (*Erysiphe*, *Erysiphaceae*). As an obligate fungal pathogen, obtaining pure DNA materials for genome sequencing is extremely challenging. In this study, single spores were inoculated onto excised leaves placed on water agar medium. After three generations of purification, the cultures were propagated to obtain pure DNA and used to obtain high-quality genomic sequences using third-generation (long-read) and second-generation (short-read) sequencing technologies. The resulting genome was 82.5 Mb in size, comprised of 47 scaffolds with an N50 length of 3.15 Mb. Completeness of 93.3% was confirmed based on benchmarking universal single-copy orthologs (BUSCOs). A high transposable elements (TEs) sequences content (64.8%) was found in the genome. A combination of ab initio, transcriptome-based, and homology-based predictions for *Erysiphe diffusa* yielded 6,961 genes with an average gene length of 1,461 bp, an average CDS length of 1,307 bp, and an average 2.83 exons per gene, an average exon length of 462 bp, and an average intron length of 84 bp. Additionally, non-coding RNAs (ncRNAs) were predicted across the genome: 93 ribosomal RNAs (rRNAs), 53 small nuclear RNAs (snRNA), 2 Regulatory, and 296 tRNA. Gene function information, motifs and domains of their proteins were assigned by comparing with public databases. Annotated genes and their percent out of total were as follows: SwissProt (4,263, 61.2%), NR (6,488, 93.2%), KEGG (3,065, 44%), KOG (1,824, 26.2%), and Gene Ontology (3,480, 49.9%). These results showed that 6496 genes could be annotated into with at least one public database, and this was 93.3% of the total number of predicted genes. To date, this represents the second high-quality genome of the genus *Erysiphe*. Overall, this genomic resource for *E. diffusa* will facilitate future studies aiming to unravel the genetic and molecular mechanisms underlying the interactions between powdery

mildews and their hosts. It will also provide a valuable dataset for further genomics, transcriptomics, and evolutionary research on powdery mildews.

Funding: This research was supported by the National Natural Science Foundation of China (No. U21A20177).

Keywords: Soybean powdery mildew, Comparative genomics, Host adaption

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Abstract

Temperature regulated blast resistance in rice

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As global climate change exacerbates extreme heat events, the interplay between heat stress and blast disease resistance in rice remains poorly understood. In this study, through integrated transcriptome profiling and systematic phenotyping of mutants in several thermosensory pathways, we identified *HsfA1* as a positive regulator of heat priming-enhanced blast resistance in rice. Systematic analysis of miRNA dynamics, bioinformatics prediction, and RNA pull-down experiments revealed that *miR444b.2*, a temperature-responsive miRNA, directly suppresses the expression of *HsfA1* by targeting the second exon of *HsfA1* mRNA. Genetic analyses demonstrated that heat stress-mediated suppression of *miR444b.2* expression relieves the repression of *HsfA1*, thereby enhancing blast resistance in rice. Furthermore, *HsfA1* directly binds to the promoter of *AOC1*, a key jasmonic acid (JA) biosynthesis gene, to activate its expression. Knockout of *HsfA1* or *AOC1* abolishes heat priming-enhanced JA accumulation and blast disease resistance, and the phenotypes are largely restored via *AOC1* overexpression and MeJA treatment. Further identification of *HsfA1* natural variants and generation of the *HsfA1* uORF-edited lines with improved blast resistance offer potential strategies for breeding disease-resistant rice varieties. This study elucidates the *miR444b.2*-*HsfA1*-*AOC1* module that linking thermal sensing to JA-mediated blast resistance, providing a molecular blueprint for engineering climate-resilient crops with concurrent biotic-abiotic stress tolerance.

Keywords: Rice blast; high temperature; disease resistance; *HsfA*

Abstract

A fungal serine protease compromises maize immunity through the degradation of chitinase ZmChn1.

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#These authors contributed equally to this work.

Fusarium verticillioides is a major fungal pathogen responsible for maize ear and stalk rot. Beyond its impact on yield, it produces carcinogenic fumonisins, posing a severe threat to global food safety. A key virulence strategy of *F. verticillioides* involves the secretion of effector proteins to manipulate host immunity. However, the specific mechanisms of these effectors in maize remain largely unknown. In this study, we identified a novel secreted effector, FvAP3, from the apoplastic fluid of *F. verticillioides*-infected maize. Although FvAP3 deletion did not alter fungal growth *in vitro*, it significantly attenuated fungal virulence in maize. Conversely, heterologous expression of FvAP3 in *Nicotiana benthamiana* and maize plants induced intense cell death, suggesting a role in triggering plant immune responses. Using a combination of yeast two-hybrid screening, bimolecular fluorescence complementation (BiFC), and co-immunoprecipitation (Co-IP) assays, we demonstrated that FvAP3 directly interacts with maize chitinase ZmChn1, a key enzyme in chitin-triggered immunity. Critically, *in vitro* protease assays revealed that FvAP3 is a functional serine protease and cleaves ZmChn1 in a protease-dependent manner. Consistent with this finding, transgenic maize lines overexpressing ZmChn1 exhibited enhanced resistance to *F. verticillioides*. This resistance was characterized by pattern-triggered immunity (PTI) responses, including accelerated and stronger reactive oxygen species (ROS) bursts and upregulation of defense-related genes upon infection. Collectively, our results elucidate a novel virulence mechanism in which *F. verticillioides* employs the serine protease effector FvAP3 to degrade ZmChn1, thereby suppressing chitin-triggered immunity and facilitating infection. This study not only advances our understanding of host-pathogen interactions in maize but also identifies ZmChn1 as a promising target for engineering resistance against *Fusarium* ear and stalk rot of maize.

Keywords: *Fusarium verticillioides*, Serine protease, Chitinase, Maize, Plant Immunity

Abstract

Towards the Origin of Genetic Variation in *Fusarium graminearum*

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Fusarium graminearum, a homothallic plant pathogen, exhibits high genetic diversity in natural populations despite its self-fertile life cycle, which limits opportunities for meiotic recombination. This study investigates the origin of genetic variation in this species. Through tetrad sequencing and mutation profiling, we demonstrate that self-fertilization is intrinsically mutagenic, with 70% of tetrads carrying *de novo* mutations. The sexual mutation rate is significantly higher than the asexual rate and comparable to that under forced outcrossing. Mutagenesis occurs independently of meiotic recombination, as evidenced by mutations in crossover-deficient regions, variant ratios indicative of premeiotic origin, and unchanged mutation rates in recombination-deficient mutants. Field-collected ascospores show a similar mutation rate, and the mutation spectrum is dominated by C-to-T/G-to-A transitions, enriched in genomic regions with high natural variation. These findings reveal that *de novo* mutagenesis during sexual reproduction, rather than recombination, is a key driver of genetic diversity in *F. graminearum*, resolving the long-standing paradox of adaptive evolution in homothallic fungi.

Keywords: Homothallic fungi, sexual reproduction, genetic variation

Abstract

GAPDH-mediated glycolysis provides energy for effector secretion in *Magnaporthe oryzae*

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Rice blast, caused by the fungus *Magnaporthe oryzae*, is a devastating fungal disease of rice. During the early stages of the interaction between *M. oryzae* and rice, the pathogen synthesizes effectors to suppress the rice reactive oxygen species (ROS) immune response. However, how these newly synthesized effectors are promptly secreted into rice cells remains unclear. To investigate the secretion mechanism of *M. oryzae* effectors under ROS stress, we analyzed the vesicular transport rate during the early interaction stage and found that effectors entered rice cells more rapidly via accelerated vesicular transport. This acceleration requires energy. By using glycolytic inhibitors and the mitochondrial inhibitor oligomycin, we discovered that inhibiting glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity suppressed the vesicular transport rate, indicating that glycolysis serves as the energy source for accelerating vesicular transport under ROS stress. Further research revealed that ROS inhibits the palmitoylation of GAPDH, leading to the release of more active GAPDH to the vesicle surface. By purifying *M. oryzae* vesicles and adding glycolytic substrates, we found that vesicles under ROS stress could produce more ATP, suggesting that GAPDH accumulated on the vesicle surface provides energy for the acceleration of vesicular transport. These findings highlight the importance of glycolysis-mediated vesicular transport in *M. oryzae* and provide insights for designing efficient and environmentally friendly disease control strategies.

Keywords: *Magnaporthe oryzae*, vesicular transport, glycolysis, reactive oxygen species (ROS) immunity

Abstract

Unraveling *Fusarium* Diversity and Virulence in High-Value Chinese Fruit

Crops Ishara S. Manawasinghe*, Yueyan Zhou, Pranami D. Abewickrama, Chen Chao, Wei Zhang, Pengzhao Chen, Jiye Yan#

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Fusarium (Nectriaceae, Hypocreales) represents one of the most destructive fungal pathogen groups, causing diseases ranked among the global top ten. Taxonomically, the genus is complex, comprising at least 20 species complexes and over 1,860 epithets listed in Index Fungorum, while 21 genera within Nectriaceae produce fusarioid asexual morphs. Despite the application of *tefl-α* as a universal barcode, species delimitation remains problematic, and questions of diversity, evolution, and host specificity remain unresolved. In China, high-end fruit crops such as blueberry, grape, strawberry, and cherry are economically and culturally important, yet increasingly threatened by *Fusarium* diseases, including root, crown, stem, and fruit rots, as well as leaf spots. To investigate, we surveyed 13 provinces and the Beijing administrative region, recovering more than 300 isolates. Grapes yielded 110+ isolates (17 species, seven complexes), dominated by *F. nirenbergiae*; cherries 70 isolates (12 species), mainly *F. lateritium*; blueberries 30 isolates (nine species), mainly *F. oxysporum*; and strawberries over 100 isolates (12 species, including novel taxa), dominated by *F. cugenangense*. *Fusarium annulatum* was the only species shared across all hosts. Pathogenicity assays on grape seedlings using 17 species revealed substantial variation in virulence, with differential effects on symptom development, dry weight, and biomass after nine months. These results highlight the potential for cross-pathogenicity and the importance of host-pathogen interactions in fruit crops. This study provides one of the most comprehensive assessments of *Fusarium* diversity in Chinese fruit crops to date. Ongoing phylogenomic analyses aim to resolve taxonomic uncertainties and identify truly novel species, thereby supporting improved disease management strategies in high-value fruit production systems.

Keywords: Cross-pathogenicity, Diversity, *Fusarium*-like, Species Delineation

Abstract

***Trichoderma* isolates from freshwater environment as Dual Agents for Biocontrol and Growth Promotion in red pepper**

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This study evaluated the biological control potential of seven *Trichoderma* strains isolated from freshwater environments. These strains demonstrated strong antagonistic activity against plant pathogenic fungi *Fusarium solani* and *Phytophthora capsici*, with over 50% inhibition in dual culture assays. Additionally, they exhibited significant plant growth-promoting traits, including phosphate solubilization and siderophore production, with over 60% activity on PVK and CAS media. Enzymatic assays revealed that FBCC-F1634 showed chitinase, glycosidase, and amylase activity, while FBCC-F1547 displayed high glycosidase and amylase activity. All strains showed antifungal activity against six pathogens (*Colletotrichum acutatum*, *C. siamense*, *C. fructicola*, *C. gloeosporioides*, *Fusarium oxysporum*, and *P. capsici*), with inhibition rates exceeding 80% for *Colletotrichum* spp. and complete inhibition of *P. capsici* by FBCC-F1645. Volatile organic compound (VOC) assays confirmed broad-spectrum antifungal effects, with FBCC-F1514 showing the highest inhibition against multiple pathogens. In vivo tests demonstrated effective suppression of chili anthracnose (*C. acutatum*) and wilt disease (*F. oxysporum*), with FBCC-F1547 achieving 100% wilt prevention. Growth promotion assays in chili seedlings revealed enhanced shoot length, stem diameter, and flowering, particularly with FBCC-F1688 and FBCC-F1514. Overall, these freshwater-derived *Trichoderma* strains exhibit promising potential as biocontrol agents and biofertilizers for sustainable agriculture.

Keywords: *Trichoderma*, freshwater fungi, biocontrol, plant growth promotion

Abstract

Isolation, identification of the pathogen causing root rot of *Dioscorea opposita* Thunb. in Shuangfeng, Hunan Province

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The aim of this study was to deeply explore the pathogenic characteristics of the root rot disease of *yam* in Loudi, Hunan Province and its impact on local agricultural production. By using plant tissue isolation techniques, combined with morphological features, ITS, *TEF1-a* and *RPB2* multigene phylogenetic analysis, and pathogenicity tests. The pathogens causing root rot of *yam* were identified as *Fusarium*. 14 pathogenic strains were obtained in total, including 9 strains of *Fusarium oxysporum*, 3 strains of *Fusarium solani* and 2 strains of *Fusarium graminearum*. The result showed the main pathogens causing root rot of *yam* in Shuangfeng were *Fusarium oxysporum*, *Fusarium solani* and *Fusarium graminearum*, *Fusarium oxysporum* is the main pathogen species and has strong pathogenicity. The information on identification of this fungus may be helpful to the control and prevention of the disease.

Key words: *Yam*; Root rot; identification; *Fusarium oxysporum*; *Fusarium solani*; *Fusarium graminearum*

¹ Funding: Hunan Provincial Natural Science Foundation(2025JJ70341)

Abstract

Large-scale analysis of maize holobionts deciphering disease resistance of stem core microbiome in China

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The mutualistic symbiosis between plants and their core microbiota plays a crucial role in maintaining plant health and enhancing disease resistance. However, the specific functions and mechanisms through which the phyllosphere microbial community confers protection against pathogen infection remain poorly understood. In this study, we collected 1,410 samples from multiple compartments (including surrounding soil, rhizosphere, roots, stems, and seeds) of healthy and stalk rot diseased maize plants across a continental-scale transect spanning 3,317 kilometers in China. Amplicon and metagenomic data analyses revealed that stalk rot disease significantly disrupted the composition of the maize microbiota. Compared to other plant tissues, the integrity and function of the highly conserved stem microbiota were more severely compromised by the pathogen. Continental-scale sample analysis identified 14 bacterial and 26 fungal core taxa within the maize stem microbiota. Inoculation with a synthetic microbial community (SynCom) composed of six core bacterial and six core fungal strains demonstrated that these stem-endophytic microbes effectively alleviated pathogen infection. Strain removal experiments within the SynCom identified the fungus *Penicillium fructuariae-cellae* as a key contributor to disease resistance in the stem microbiota. *P. fructuariae-cellae* broadly inhibits multiple pathogenic fungi through the production of small molecular compounds. This study highlights the untapped potential of often-overlooked stem-endophytic core microbes in enhancing disease resistance and provides a promising strategy for mitigating globally prevalent stalk rot in maize.

Keywords: Stalk rot disease, community assembly, synthetic community, fungal metabolites, plant health

Abstract

Ferroptosis as a Conserved Pathogenicity Switch in Plant-Pathogenic *Colletotrichum* spp.

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Background: Anthracnose, caused by hemibiotrophic fungi of the genus *Colletotrichum*, is a globally prevalent and destructive plant disease. The appressorium, a specialized infection structure derived from the conidium, is essential for host penetration.

Methods: We investigated the role of ferroptosis in the pathogenicity of *Colletotrichum higginsianum*. Matrix-assisted laser desorption/ionization time-of-flight imaging mass spectrometry (MALDI-TOF IMS) identified pyochelin as the antifungal compound secreted by the biocontrol bacterium BX1. Scanning electron microscopy (SEM), live-cell staining, and live-cell fluorescence imaging were used to monitor conidial programmed cell death during appressorium maturation. Calcein-AM and C11-BODIPY^{581/591} fluorescent probes were employed to visualize intracellular ferric ions and lipid peroxides, while transmission electron microscopy (TEM) was used to assess mitochondrial ultrastructure, confirming ferroptosis. Functional analyses of iron chelation, chemical ferroptosis inhibition, and *ChNOX2* gene deletion were performed, and pathogenicity was evaluated using detached leaf and potted plant assays.

Results: Conidial cell death began in cells farthest from the appressorium and progressed sequentially. This ferroptotic process depended on ferric ions and lipid peroxidation. Iron chelation or ferroptosis inhibition preserved conidial viability, impaired appressorial invasion, and significantly reduced pathogenicity. *ChNOX2* deletion reduced lipid peroxidation, inhibited ferroptosis, lowered turgor pressure, and nearly abolished pathogenicity. Similar ferroptotic patterns were detected in other pathogenic *Colletotrichum* species, indicating genus-wide conservation.

Conclusion: Our findings first identify ferroptosis as a conserved pathogenicity switch in *Colletotrichum* spp., reveal the antifungal mechanism of a pyochelin-producing biocontrol bacterium, and highlight ferroptosis inhibitors as potential broad-spectrum fungicides.

Keywords: conidial cell death, ferroptosis, *Colletotrichum higginsianum*, NOX, pathogenicity

Abstract

Comparative Genomics and Phylogeny of the Elsinoaceae and Allied Families**Jiyu-Su^{1*}, Shujun-Zhang¹, Jie-Yang¹, Qian-Lu¹, Zonghua-Wang^{2#}, Hongli-Hu^{1#}***1. College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou, China**2. Fuzhou Institute of Oceanography, Minjiang University, Fuzhou, China**Jiyu-Su, E-mail: 916378474@qq.com**Shujun-Zhang, E-mail: 1452608807@qq.com**Jie-Yang, E-mail: 3080320019@qq.com**Qian-Lu, E-mail: 2271565676@qq.com**Zonghua-Wang, E-mail: wangzh@fafu.edu.cn**Hongli-Hu, E-mail: huhongli7905@gmail.com*

The fungal family *Elsinoaceae* comprises important pathogens infecting over 400 plant species, including economically significant crops, causing severe diseases such as scab and spot anthracnose. However, studies on their molecular phylogeny and genomic evolution remain limited. In this study, we isolated 383 strains from 101 diseased samples collected from 45 host plants across 11 provinces in China. Based on morphological characteristics and multigene phylogenetic analyses (LSU, ITS, *rpb2*, and *tef1-α*), we identified 29 species, including 14 new species, 3 new combinations, 34 new host records, and 6 new geographical distribution records and proposed one new genus and two new families.

Using a hybrid sequencing strategy combining PacBio long-read and Illumina short-read technologies, we sequenced the whole genomes of 27 species from four genera across three families within *Elsinoaceae*. A total of 43 high-quality genomes were assembled, 14 of which reached near-chromosome level quality with BUSCO completeness exceeding 97%. Through comparative genomic analysis, the key findings are as follows: 1. Unique Genomic Expansion in *Elsinoe* Clade I: Comparative genomics revealed that this clade exhibits significantly enlarged genomes (>30 Mb), reduced GC content (<50%), and exceptionally high repetitive sequence content (up to 31.36%), distinguishing it from other lineages within *Elsinoaceae* and related families; 2. Transposable Element (TE) Proliferation as the Driving Force: The genomic expansion in *Elsinoe* Clade I was primarily driven by the explosive amplification of Gypsy/DIRS1-class LTR retrotransposons; 3. Phylogeny and Evolutionary Trends: Molecular clock analysis indicated that *Elsinoaceae* and *Pustulaceae* originated during the Paleogene period, with *Elsinoe* diverging earlier than *Neoelsinoe*. Gene contraction was the dominant evolutionary trend across the phylogeny; 4. Novel Insights into Multi-Host Adaptation: All analyzed multi-host strains lacked introns in, suggesting that intron loss may play a critical role in facilitating broad host adaptability in *Elsinoaceae*; 5. Differential Evolutionary Rates: The mitochondrial genome evolved significantly slower than the nuclear genome.

This study systematically elucidates the unique evolutionary trajectory of *Elsinoaceae*, particularly *Elsinoe* Clade I, where Gypsy/DIRS1 transposon expansion drove substantial genomic restructuring. The findings reveal the Paleogene origin of this family, its predominant gene

contraction trend, and the potential link between intron loss and multi-host adaptation. These results provide a critical genomic foundation for understanding the evolutionary dynamics, environmental adaptation, and pathogenic mechanisms of these important plant pathogens.

Keywords: Elsinoaceae, Genomics, Mitochondrial Genome, Phylogenetic, Transposable Element

Abstract

Insight into the Etiology of Cherry Diseases in China

Yueyan Zhou*, Wei Zhang, Pengzhao Chen, Ishara S. Manawasinghe, Pranami D. Abeywickrama, Jiye Yan[#]

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Sweet cherry (*Prunus avium* L.) is an important fruit crop in China. With the continuous expansion in cultivation area and production, fungal pathogens have become a prevalent challenge in fruit production and quality. This study aims to understand the disease occurrence and to identify main disease and pathogens associated with major sweet cherry producing regions in China. Diseases investigations were carried out in Beijing, Guizhou, Hebei, Liaoning, Shandong, Sichuan and Zhejiang in China. The putative pathogens were identified through morphology and multi-gene phylogenetic analysis, with pathogenicity tests. Cherry leaf spot and gummosis are dominant diseases in the open field, while stem canker and gray mold are major concern in greenhouse. Wood rot, plaster disease, root rot and brown rot are also common fungal diseases of cherry. From leaf samples, 550 isolates were identified as 21 genera with *Alternaria* (31.8%), *Colletotrichum* (27.3%), *Cladosporium* (7.1%), *Phoma* (6.2%), *Fusarium* (5.3%) as top five genera. From wood samples, 255 isolates were identified as fifteen genera, with *Alternaria* (28.9%), *Cladosporium* (25.1%), *Fusarium* (11.9%), *Botryosphaeria* (9.8%) and *Nothophoma* (8.2%) as top five genera. Pathogenicity of *Colletotrichum*, *Botryosphaeria*, *Diaporthe* and *Fusarium* species were confirmed in detached cherry leaves and shoots, while remaining species are still under investigation. Based on our observations with the expansion of the cultivation area and the growth of tree age, the occurrence disease and diversity of pathogens will be varied and complicated. This study will lay a foundation with theoretical basis for the effective diagnosis, as well as green prevention and control of cherry diseases.

Keywords: Cherry disease, fungal pathogen, morphology, phylogeny, pathogenicity

Abstract

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Key Laboratory of Environment Friendly Management on Fruit and Vegetable Pests in North China (Co-construction by Ministry and Province), Ministry of Agriculture and Rural Affairs, Institute of Plant Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China.

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Keywords: Cherry disease, fungal pathogen, morphology, phylogeny, pathogenicity

Abstract

What is *Fusarium oxysporum* f.sp. *cubense*?

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Panama disease, caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*), leads to substantial losses in global commercial banana production and is a major quarantine target in banana-growing regions worldwide. Recent advances in molecular phylogenetics and pathogenicity tests indicate that *Foc* is not a monophyletic group. However, comprehensive studies within China have remained limited. In this study, we collected symptomatic banana samples from 14 locations across China and isolated 91 *Fusarium* strains. Through morphological characterization and multi-locus phylogenetic analysis, these isolates were identified as 19 distinct species spanning four *Fusarium* species complexes. By integrating our data with published multi-locus sequences of strains historically identified as *Foc*, we demonstrate that these representative strains form a polyphyletic group encompassing at least 23 species, with *F. odoratissimum*, *F. kalimantanense* and *F. sangayamense* exhibiting highest occurrence frequencies. Furthermore, 86% of the infected samples in this study showed co-infection by multiple *Fusarium* species. For instance, from a single diseased banana plant, we isolated and identified two phylogenetically distant species, *F. gros-michelii* and *F. odoratissimum*, both previously reported as individual causal agents of Panama disease. Our findings indicate that “*Foc*” represents for multiple phylogenetically distinct *Fusarium* species. We therefore recommend moving beyond the broad “*Foc*” concept in future research and quarantine protocols, adopting instead precise and validated species names to prevent misidentification.

Keywords: Diversity, *Forma specialis*, Pathobiome, Pathogenicity, Phylogeny

Abstract

Identification and Biocontrol Potential of Cacao (*Theobroma cacao* L.) Pod Rot Pathogens

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Cacao (*Theobroma cacao* L.) is a vital crop in the global chocolate industry and a significant contributor to the local economies of cacao-producing regions, such as the Philippines. However, cacao production is severely threatened by pod rot, a significant disease caused by diverse fungal pathogens. This study aimed to isolate and characterize the fungal pathogens associated with cacao pod rot, and to evaluate the efficacy of selected biocontrol agents against these pathogens using *in vitro* and *in vivo* approaches. A total of 20 fungal isolates were obtained from infected cacao pods and identified through morphological and molecular methods as *Colletotrichum* spp., *Lasiodiplodia* spp., *Fusarium* spp., and *Diaporthe* spp. Pathogenicity tests confirmed their ability to induce symptoms on unwounded cacao pods, validating their pathogenic potential. To evaluate sustainable control strategies, four candidate biocontrol agents: *Trichoderma* spp., *Aspergillus* spp., *Penicillium* spp., and *Pseudomonas aeruginosa* were tested using dual culture assays, metabolite bioassays, and *in vivo* pod inoculations. *Trichoderma* spp. consistently demonstrated the most potent antagonistic activity, significantly suppressing mycelial growth and reducing disease incidence, particularly against *Fusarium* and *Lasiodiplodia* spp. Non-volatile metabolites from *Trichoderma* spp. and *P. aeruginosa* also showed marked inhibitory effects, while volatile metabolites from *Penicillium* spp. showed moderate suppression of pathogen growth. These findings highlight the diversity of causal fungi and demonstrate the potential of biocontrol agents, especially *Trichoderma* spp., as eco-friendly alternatives to chemical fungicides. By advancing sustainable disease management strategies, this study provides critical insights for safeguarding cacao production, protecting farmer livelihoods, and ensuring the long-term quality of cacao-based products.

Keywords: Biocontrol agents, Cacao pod rot, Fungal pathogens, Sustainable disease management, *Trichoderma* spp.

Abstract

Isolation, Preliminary Screening, and Characterization of Fungi from Mining Soil in Hinatuan Island, Tagana-an, Surigao del Norte, Philippines

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Cabbage *Fusarium* Wilt (CFW) and Gray Tea Blight (GTB), caused by *Fusarium oxysporum* f. sp. *conglutinans* (FOC) and *Pseudopestalotiopsis theae*, respectively, pose serious threats to agricultural productivity. To address these challenges, this study aimed to isolate and identify potential fungal biocontrol agents from mining soil and assess their antagonistic activity against FOC and *P. theae* using dual culture assays. To confirm fungal identity, the D1-D2 region of the 28S rRNA gene was sequenced and analyzed using phylogenetic reconstruction under the Kimura 2-parameter (K80) model with Bayesian inference. The highest inhibition against FOC was exhibited by isolates B1 ($64.38\% \pm 7.24$), C1 ($64.24\% \pm 4.94$), and D1 ($62.80\% \pm 2.81$), while isolates A10 ($67.98\% \pm 5.76$) and D6 ($67.84\% \pm 10.22$) were most effective against *P. theae*. Molecular characterization revealed isolate A7 as *Simplicillium lepidopterorum*, A10 as *Trichoderma afroharzianum*, and isolates B1, C1, D1, D4, and D6 as *Lasiodiplodia theobromae*. These findings underscore the potential of soil-derived fungal antagonists as sustainable alternatives to chemical fungicides. Further biochemical characterization and validation in screenhouse and semi-field conditions are essential to optimize their application in integrated disease management strategies.

Keywords: Biological control, dual culture assay, 28S rRNA gene

Abstract

Pathogens Associated with Post-Harvest Diseases of Potato from a Public Market in Quezon City, Philippines

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Potato (*Solanum tuberosum* L.) is a globally important crop, and in the Philippines, it represents a key component of the agricultural sector. However, its yield and storability are severely impacted by post-harvest diseases, which pose a major challenge to food security. This study aimed to identify the primary pathogens causing post-harvest diseases in potato tubers sold commercially in a high-traffic public market. Diseased potato tubers were collected from Litex Market, Quezon City, and pathogens were isolated from lesion tissues onto Potato Dextrose Agar (PDA). Isolates were then identified based on macroscopic colony characteristics and microscopic examination of their structures. For definitive confirmation, genomic DNA was extracted from pure cultures for Internal Transcribed Spacer (ITS) sequence analysis. To fulfill Koch's postulates, pathogenicity tests were conducted on healthy potato tubers. The pathogens were successfully re-isolated from the inoculated tubers after they developed disease symptoms identical to the source material, confirming their role as causal agents. Three agronomically significant pathogens were identified: *Colletotrichum* sp., *Rhizoctonia* sp., and an oomycete. The presence of these pathogens in potatoes available to consumers highlights a significant risk for post-harvest economic losses and underscores a potential gap in disease management from farm to market. This report provides crucial baseline data on the specific causal agents of potato tuber diseases in the local supply chain, which is essential for developing targeted disease management strategies.

Keywords: Fungal pathogens, ITS sequencing, Oomycete, Post-harvest diseases, *Solanum tuberosum*

Abstract

Identification of physiological race and mating type of *Exserohilum turcicum* in Shanxi, China

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Northern corn leaf blight, caused by the fungus *Exserohilum turcicum*, is one of the most significant fungal diseases affecting corn in China. *Exserohilum turcicum* exhibits notable physiological specialization. To date, 16 physiological races—0, 1, 2, 3, N, 12, 13, 1N, 23, 2N, 3N, 123, 12N, 13N, 23N, and 123N—have been identified in China. As a heterothallic fungus, *Exserohilum turcicum* has three main mating types: A, a, and Aa. Under suitable conditions, the mating of two sexually compatible strains from different races can lead to the combination of new virulence genes, potentially enhancing the pathogenicity and host range of new strains and further complicating the differentiation of physiological races. Shanxi Province's unique geographical location and complex topography make it a "microcosm" of corn production regions in Northern China. It encompasses three major national corn production areas: the Northern Spring Corn Region-Jinzhong, Xinding & Shangdang Regions, the Huang-Huai-Hai Summer Corn Region-Jinnan Region, and the Northwest Irrigated Corn Region-Jinbei Region. Therefore, clarifying the composition of physiological races and mating types of *Exserohilum turcicum* across different years in the province is crucial for understanding the potential for sexual reproduction and the epidemic patterns of the pathogen. An analysis of the physiological races and mating types of 403 *Exserohilum turcicum* isolates collected from 2017 to 2024 across these three corn production areas in Shanxi Province revealed the presence of all 16 known physiological races. Among them, race 123N was the most predominant, with a isolate frequency of 17.4%. The combined frequency of races 123N, N, 13N, 23N, and 3N accounted for 55.6% of the total, identifying them as the main pathogenic race population responsible for northern corn leaf blight in Shanxi. Mating type analysis of the 403 isolates identified all three types—A, a, and Aa—with proportions of 25.3%, 68.2%, and 6.5%, respectively. The a mating type has consistently been the dominant type among *Exserohilum turcicum* populations in Shanxi over the years. Notably, in the "Northwest Irrigated Corn Region" (i.e., the Northern Shanxi area), the primary mating types were A and Aa, and this region exhibited a high diversity of 15 physiological races. Therefore, enhancing the monitoring of physiological race differentiation and mating type distribution of *Exserohilum turcicum* across different regions is essential for understanding its genetic dynamics and, consequently, for implementing effective disease control strategies.

Keywords: *Exserohilum turcicum*, mating type, Northern corn leaf blight, physiological race

Abstract

GAPDH-mediated glycolysis provides energy for effector secretion in *Magnaporthe oryzae*

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Rice blast, caused by the fungus *Magnaporthe oryzae*, is a devastating fungal disease of rice. During the early stages of the interaction between *M. oryzae* and rice, the pathogen synthesizes effectors to suppress the rice reactive oxygen species (ROS) immune response. However, how these newly synthesized effectors are promptly secreted into rice cells remains unclear. To investigate the secretion mechanism of *M. oryzae* effectors under ROS stress, we analyzed the vesicular transport rate during the early interaction stage and found that effectors entered rice cells more rapidly via accelerated vesicular transport. This acceleration requires energy. By using glycolytic inhibitors and the mitochondrial inhibitor oligomycin, we discovered that inhibiting glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity suppressed the vesicular transport rate, indicating that glycolysis serves as the energy source for accelerating vesicular transport under ROS stress. Further research revealed that ROS inhibits the palmitoylation of GAPDH, leading to the release of more active GAPDH to the vesicle surface. By purifying *M. oryzae* vesicles and adding glycolytic substrates, we found that vesicles under ROS stress could produce more ATP, suggesting that GAPDH accumulated on the vesicle surface provides energy for the acceleration of vesicular transport. These findings highlight the importance of glycolysis-mediated vesicular transport in *M. oryzae* and provide insights for designing efficient and environmentally friendly disease control strategies.

Keywords: *Magnaporthe oryzae*, vesicular transport, glycolysis, reactive oxygen species (ROS) immunity

Abstract

Functional Analysis of Polyamines Biosynthesis, Oxidation and Transporter Genes in *Sporisorium scitamineum*

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Abstract: Sugarcane smut, caused by the fungus *Sporisorium scitamineum*, significantly reduces both sugarcane yield and quality. The pathogenic process of *S. scitamineum* depends critically on sexual mating/filamentation and teliospore formation, yet the regulatory mechanisms underlying these steps remain poorly understood. Polyamines—biologically active, aliphatic nitrogen-containing bases—are ubiquitous in living organisms and play vital roles in various biological processes. Although intracellular polyamine levels are regulated through de novo biosynthesis, oxidation, and active transport, their functional roles and mechanisms in *S. scitamineum* have not been elucidated. This study investigates the roles of polyamines biosynthesis (*SsAGA*, *SsODC*, *SsSAMDC*), oxidation (*SsPAO*, *SsCuAO1*), and transport (*SsTPO1*) genes in fungal pathogenicity, including sexual mating/filamentation and host invasion, using reverse genetics, transcriptomic analysis, polyamines quantification, and pathogenicity assays. Our results indicate that polyamines contribute significantly to pathogenic development, likely through collaborative regulation of intracellular redox homeostasis with the cAMP-PKA signaling pathway. Moreover, *SsPAO* and *SsCuAO1* affect polyamines homeostasis, while pH modulates polyamines transport mediated by *SsTPO1*, collectively influencing fungal growth, development, mating, filamentation, and pathogenicity. In summary, this study elucidates the functions of polyamines in growth, development, mating, stress resistance, autophagy, and virulence of *S. scitamineum*, providing new insights into the molecular mechanisms of polyamines metabolism that may inform novel strategies for controlling sugarcane smut.

Keywords: Autophagy; cAMP-PKA signaling pathway; Polyamines; ROS; *Sporisorium scitamineum*

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Abstract

Oceanic Fungal Blueprint: Oceanic Organic Matter Degradation in 3D

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Pelagic fungi are starting to be recognized as key contributors to marine organic matter recycling, yet the environmental drivers that shape their functional niche remain largely unresolved. To generate a three-dimensional map of fungal ecological function, we employed metagenomic and metatranscriptomic analyses across a latitudinal gradient (from the subtropical Atlantic to the Southern Ocean) and a depth gradient (comparing epipelagic and mesopelagic zones globally). Our findings reveal that the functional role of pelagic fungi is not static but fundamentally shifts along these two key environmental axes, revealing a complex 3D biogeography. Fungi contributed significantly to eukaryotic carbohydrate-active enzyme (CAZyme) transcripts (>3%), underscoring a specialized role in carbohydrate degradation, while their minimal contribution to protease transcription (<0.5%) highlights a distinct metabolic niche. We discovered a pronounced taxonomic specificity in CAZyme and peptidase utilization, linked to substrate availability and indicative of distinct carbohydrate and protein niche segregation between polar and non-polar regions. Polar communities exhibited a preference for chitinases (GH18) and binding modules (CBM1), while non-polar systems were enriched in xylanases (CBM13) and glucan-utilizing enzymes (GH45, GH128). This functional partitioning was more pronounced across latitudes and depths than between size fractions. Temperature emerged as a primary driver of total fungal CAZyme activity, indicating a direct susceptibility of this 3D fungal metabolic framework to ocean warming. Collectively, our study provides the first integrated three-dimensional blueprint of the oceanic mycobiome, revealing its previously underestimated yet substantial influence on ecosystem dynamics. We underscore the crucial role of fungal diversity in mediating enzymatic activities vital for organic matter breakdown across the ocean's full vertical and horizontal dimensions.

Keywords: 3D Biogeography, Carbohydrate-Active Enzymes (CAZymes), Marine Biogeochemistry, Ocean Warming, Pelagic fungi.

Abstract

Marine fungi in China: diversity, distribution and novel taxa**Meng-meng Wang, Wei Li***Shantou University, Shantou, China**Email of the corresponding author mmwang@stu.edu.cn^{*}; liwei@stu.edu.cn[#]*

Background: China encompasses a vast area of diverse landscapes that support an exceptionally high level of fungal diversity. However, for a long time, questions such as how many marine fungal species exist in China, where they are distributed and what ecological functions they perform have remained unanswered. Over the past decade, our research group has systematically investigated the diversity, distribution patterns, and metabolic activities of marine fungi in Chinese seas through comprehensive sampling of intertidal, coastal, and deep-sea environments, employing both culture-dependent and culture-independent methodologies. This presentation will introduce our results related to fungal diversity, distribution and novel taxa based on cultural methods.

Methods: Methodology used in this study include sample collection, fungal strains isolation, DNA extraction, multi-locus sequences amplification and phylogenetic analyses, morphological observation, and statistical analysis.

Results: Our study systematically sampled diverse marine habitats along China's coastal gradient, spanning intertidal zones, marginal zones and deep-sea environments. From over 1000 samples of marine algae, sediments and seawater, we isolated approximately 10000 fungal strains, representing ~ 800 species. Among these, 150 were novel (with 37 formally described) and nearly 200 were new records for China or marine ecosystems. Taxonomic analyses revealed Ascomycota as the dominant phylum, with Dothideomycetes, Eurotiomycetes and Sordariomycetes being the most prevalent classes – consistent with global marine fungal distribution patterns. Habitat-specific diversity analysis showed marine sediment, particularly intertidal deposits, hosted the highest species richness (accounting for ~67% of total diversity). Geographically, southern China's intertidal zones exhibited peak species richness, followed by northern intertidal zones, marginal zones, and deep-sea environments – a latitudinal gradient aligning with climatic influences on fungal distribution. Additionally, we documented 20 unpublished novel species, supported by morphological photographs and multi-locus phylogenetic analyses to validate their taxonomic distinctiveness.

Conclusion: This comprehensive study demonstrates the remarkable fungal diversity along China's coastal ecosystems, with marine sediments--particularly intertidal zones--serving as biodiversity hotspots. The dominance of Ascomycota classes (Dothideomycetes, Eurotiomycetes, and Sordariomycetes) underscores their ecological adaptability in marine environments. Geographically,

the southern intertidal regions exhibit the highest species richness, revealing a latitudinal diversity gradient influenced by environmental factors. Notably, the discovery of 150 novel species (37 formally described) and 200 new records significantly expands our understanding of marine fungi. These findings, supported by morphological and phylogenetic evidence, highlight the importance of continued exploration and taxonomic documentation in understudied marine habitats. Future research should focus on elucidating the ecological roles of these fungi and their potential biotechnological applications.

Keywords: Algicolous fungi, Benthonic fungi, Mycoplankton, New species, Taxonomy

Abstract

The Contribution of Pelagic Fungi to Ocean Biomass

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Metagenomic analysis has recently unveiled the widespread presence of pelagic fungi in the global ocean, yet their quantitative contribution to carbon stocks remains elusive, hindering their incorporation into biogeochemical models. Here we revealed the biomass of pelagic fungi in the open ocean water column by combining ergosterol extraction, Calcofluor-White staining, CARD-FISH, and microfluidic mass sensor techniques. We compared fungal biomass to the biomass of other more studied microbial groups in the ocean such as archaea and bacteria. Globally, fungi contributed 0.32 Gt C (CI: 0.19-0.46), refining previous uncertainty estimates from two orders of magnitude to less than one. While fungal biomass was lower than that of bacteria, it exceeded that of archaea (Archaea:Fungi:Bacteria biomass ratio of 1:9:44). Collectively, our findings reveal the important contribution of fungi to open ocean biomass and, consequently, the marine carbon cycle, emphasizing the need for their inclusion in biogeochemical models.

Keywords: mycoplankton, marine carbon cycle, microbial loop

Abstract

TAXONOMIC NOVELTY IN BIONECTRIACEAE, INCLUDING A SYNOPSIS OF ACCEPTED GENERA

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Abstract

Bionectriaceous fungi are ecologically diverse, being corticolous, fungicolous, herbicolous, lichenicolous or coprophilous. They are mainly inhabiting terrestrial or freshwater ecosystems, with limited occurrences in marine environments. Our study of palm-associated fungal diversity across China and Thailand revealed *Pseudobionectria phoenicis* gen. et sp. nov., *Protocreopsis palmarum* sp. nov., and a new record of *Lasionectriella rubioi*. The new taxa were identified based on morphology and an updated multi-gene phylogeny. *Protocreopsis palmarum* was found on two different terrestrial palms and morphologically resembles *P. korfii* and *Nectriopsis hongkongensis*. *Pseudobionectria* is distinguished from other genera in *Bionectriaceae* by its superficial, globose, brownish-orange ascomata with a black crown that is flattened on the top and hyphal appendages that merge with the subiculum below. Besides, *Pseudobionectria* was collected on the mangrove floor and is likely to be an intertidal species, which enhances our understanding of bionectriaceous fungal diversity in marine habitats. In this study, a synopsis of the genera within *Bionectriaceae* is also provided, including information on the type species, morphological characteristics, lifestyles, molecular data and key publications.

Keywords: Bionectriaceous, Hypocreales, Palm fungi, Phylogeny, Taxonomy

Abstract

Fungal diversity and ecological patterns in seamounts of the Northwest Pacific Ocean

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Deep-sea represents a biodiversity hotspot of microbial life. Recently, fungi have been demonstrated to represent a key element of deep-sea microbial communities and trophic networks. Nonetheless, this component has received less attention in relation to prokaryotes, hampering the complete understanding of diversity patterns at all levels of ecosystem organization and ecosystem functioning. The Northwest Pacific has many deep-sea seamounts, large basins, and trenches, creating heterogeneous environments for various kinds of benthic organisms. It also gains global attention with rich mineral resources and high fishery yields. So, the aim of this project is to evaluate fungal diversity and ecological patterns in seamounts of the Northwest Pacific Ocean. As part of the 2024 Western Pacific International Cruise for the Digital Deep-Sea Typical Habitats, United Nations Ocean Decade Programme sediment samples were collected in August 2024 onboard the Research Vessel Shen Hai, Yi Hao. The eDNA was obtained from sediment cores, and baseline data on fungal diversity was generated using NGS-sequencing (MinION, Oxford Nanopore, Oxford, UK). Diversity patterns at the spatial scale and functional diversity were also analyzed. Our results evidenced a rich and diverse fungal community characterized by both, filamentous and yeast forms, and dominated by the Ascomycota. Remarkably, we detected a high proportion of widely distributed, uncultured oceanic phylotypes, suggesting the copious occurrence of novel fungal lineages with limited distribution to deep-sea sediments. Our findings revealed distinctive fungal assemblages at the local scale, which could be linked to site-specific processes that should be further evaluated. Interestingly, these fungal diversity patterns are consistent with former faunistic data, hinting chemical and physical heterogeneity as the main drivers of the community structure at microscopic and macroscopic levels. This work represents an important step towards understanding fungal diversity and distribution in deep-sea ecosystems.

reveal the diversity and connectivity of local species.

Keywords: Ascomycota, deep-sea fungi, marine microfungi, NGS

Abstract

A Marine-Specific Defense Strategy: ROS Signaling Activates Antifungal Biosynthesis in *Alternaria alternata* during Cocultivation

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Microorganisms form complex ecological networks of interactions, with secondary metabolites (SMs) playing a crucial role in microbial communication. While fungal coculture is an established strategy to activate silent biosynthetic pathways, the specific triggers and regulatory mechanisms, particularly in marine-derived fungi, remain poorly understood. This study explored the cocultivation of marine fungi *Alternaria alternata* and *Nigrospora sphaerica*, uncovering significant SM production changes. To comprehensively analyze the response, we integrated metabolic profiling with transcriptomic analysis. The biosynthetic gene clusters (BGCs) and transcription factor *aohR* in marine *A. alternata* were activated, leading to enhanced production of alternariol (AOH), a compound inhibiting fungal competitors. Whereas the activation was not present in the coculture of terrestrial strains of *A. alternata*. Transcriptomic and metabolic analyses further revealed that reactive oxygen species (ROS) play a crucial role as signaling molecules in these marine fungal interactions. Elevated ROS levels triggered the up-regulation of oxidative stress response genes in marine *A. alternata*, regulating the expression of *aohR*, which subsequently induced the biosynthesis of AOH. The essential role of PKS was confirmed through gene knockout, which regulated AOH production and established its pivotal function in the observed chemical defense. Our findings elucidate a ROS-mediated regulatory mechanism in marine fungal competition, providing novel insights into microbial ecology and a promising avenue for discovering new antibiotics..

Keywords: cocultivation, fungi, gene clusters, oxidative stress, secondary metabolite

Abstract

Biodegradation of PAHs by manglicolous fungi from a chronically contaminated site

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Polycyclic aromatic hydrocarbons (PAHs) contamination in mangroves occurs due to the introduction of wastewater, road runoffs, offshore activities, flaring processes, gasoline engine exhaust, and oil spills. Petroleum hydrocarbons are considered one of the greatest threats to coastal environments. Moreover, PAHs are of great medical and environmental relevance due to their carcinogenic, mutagenic, teratogenic, and ecotoxic properties. The bioprospecting of hydrocarbonoclastic microorganisms is relevant for the bioremediation of coastal sites. Thus, this work aimed to isolate fungi from a chronically polluted mangrove in Mexico and evaluate their ability to simultaneously degrade a mixture of 16 PAHs. Submerged root sections were collected from three mangrove species (*Rhizophora mangle*, *Laguncularia racemosa*, and *Avicennia germinans*) and incubated in humid chambers. From this, axenic cultures were obtained. Subsequently, a polyphasic taxonomic assignment was performed to identify the isolates. To determine hydrocarbon degradation, bioassays were run with 19 selected fungal strains using a mixture of 16 PAHs: acenaphthene, acenaphthylene, anthracene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, benzo[*a*]pyrene, chrysene, dibenzo[*a,h*]anthracene, fluoranthene, fluorene, indeno[1,2,3-*cd*]pyrene, naphthalene, phenanthrene, and pyrene). Gas chromatography was used to evaluate changes in the concentration of the PAHs mixture after the bioassays. Our results showed that all tested strains grew in the PAHs mixture as a sole carbon source. Likewise, some isolates demonstrated a greater ability to grow in the presence of PAHs in relation to a labile carbon source (controls). The evaluated fungal isolates showed a high potential for the degradation of PAHs. These findings highlighted the relevance of mangrove fungal bioresources as a viable alternative for the bioremediation of chronically contaminated coastal sites.

Keywords: Polycyclic aromatic hydrocarbons; manglicolous fungi; degradation

Abstract

TAXONOMIC NOVELTY IN BIONECTRIACEAE, INCLUDING A SYNOPSIS OF ACCEPTED GENERA

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Abstract

Bionectriaceous fungi are ecologically diverse, being corticolous, fungicolous, herbicolous, lichenicolous or coprophilous. They are mainly inhabiting terrestrial or freshwater ecosystems, with limited occurrences in marine environments. Our study of palm-associated fungal diversity across China and Thailand revealed *Pseudobionectria phoenicis* gen. et sp. nov., *Protocreopsis palmarum* sp. nov., and a new record of *Lasionectriella rubioi*. The new taxa were identified based on morphology and an updated multi-gene phylogeny. *Protocreopsis palmarum* was found on two different terrestrial palms and morphologically resembles *P. korfii* and *Nectriopsis hongkongensis*. *Pseudobionectria* is distinguished from other genera in *Bionectriaceae* by its superficial, globose, brownish-orange ascomata with a black crown that is flattened on the top and hyphal appendages that merge with the subiculum below. Besides, *Pseudobionectria* was collected on the mangrove floor and is likely to be an intertidal species, which enhances our understanding of bionectriaceous fungal diversity in marine habitats. In this study, a synopsis of the genera within *Bionectriaceae* is also provided, including information on the type species, morphological characteristics, lifestyles, molecular data and key publications.

Keywords: Bionectriaceous, Hypocreales, Palm fungi, Phylogeny, Taxonomy

Abstract

Diversity and Organic Acid Metabolites of *Aspergillus* Isolated from Freshwater Environments

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Freshwater ecosystems harbor diverse fungal communities that play crucial roles in nutrient cycling and organic matter degradation. In this study, we investigated the species composition and metabolite profiles of 52 *Aspergillus* isolates collected from freshwater environments in Korea. Phylogenetic identification revealed three dominant species: *A. japonicus*, *A. niger*, and *A. tubingensis*. Comparative metabolomic analysis using LC/MS demonstrated both common and species-specific organic acid production. Core metabolites such as citric acid, malic acid, succinic acid, gluconic acid, and phosphoric acid were consistently detected across isolates, indicating their fundamental role in central carbon metabolism. In contrast, distinct metabolic signatures were observed among species. *A. japonicus* was characterized by the production of catechin, lactic acid, pyruvic acid, and glucoheptonic acid, suggesting a potential for antioxidant and functional metabolite biosynthesis. *A. niger* uniquely produced high-molecular-weight organic compounds (e.g., C₂₇H₃₀O₁₅, C₂₈H₈O₁₁), reflecting its capacity for specialized secondary metabolism. *A. tubingensis* displayed diverse sugar acid derivatives, including 2-deoxy-5-keto-D-gluconic acid and acetylpyruvate, highlighting its role in acidification and carbon flux regulation. These findings underscore the metabolic diversity of freshwater-derived *Aspergillus* and their ecological significance in shaping biogeochemical processes, while also providing valuable insights for potential industrial applications in organic acid production and functional compound discovery.

Keywords: *Aspergillus*, freshwater fungi, organic acids, biodiversity, secondary metabolites

Abstract

Culturable mycobiota from the lower Yarlung Zangbo River BasinMin Tan, Fang Liu, Lei Cai[#]*State Key Laboratory of Microbial Diversity and Innovative Utilization, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China.*

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The Qinghai-Xizang Plateau, known as the "Third Pole", constitutes a globally significant biodiversity hotspot with heightened sensitivity to climatic change. It hosts extensive undocumented microbial diversity and a rich reservoir of novel biosynthetic gene clusters, offering exceptional opportunities for the exploration of microbial dark matter. In our study, using the dilution plate method, a total of 1,776 culturable fungal strains were isolated from water, soil, and sediment samples collected at 67 sampling sites across the lower reaches of the Yarlung Zangbo River Basin. Based on preliminary ITS sequence analysis, the isolates were classified into 466 species, belonging to 248 genera, 123 families, 57 orders, 18 classes, and 6 phyla. Among these, the phylum Ascomycota exhibited the highest species diversity (approximately 100 species), followed by Basidiomycota and Mucoromycota. Comparative analysis with the GenBank and UNITE databases revealed that ITS sequences of approximately 150 fungal strains showed < 98% similarity to the known species. We are currently conducting morphological descriptions and systematic studies on taxa that have been confirmed as new species through multi-gene phylogenetic analysis.

Keywords: culturable; fungal diversity; ITS DNA barcodes; morphology; systematics

Abstract

Species Diversity of Yeast from Freshwater and Soil Habitats in Korea

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Yeasts are an ecologically and biotechnologically significant group of fungi, yet their diversity across various habitats remains unexplored. During a survey of yeast diversity in soil and freshwater samples collected from different locations in Korea, 19 isolates were obtained using the dilution plating method. Based on morphology and molecular phylogenetic analyses using the ITS region and the D1/D2 domain of the large subunit (LSU) rRNA gene, a total of 13 species were identified. These species belonged to ten genera, including *Apiotrichum*, *Candida*, *Debaryomyces*, *Filobasidium*, *Galactomyces*, *Geotrichum*, *Pichia*, *Rhodotorula*, *Saccharomycopsis*, and *Wickerhamomyces*. Among them, three species in the genera *Debaryomyces*, *Saccharomycopsis*, and *Wickerhamomyces* are proposed as new species. Additionally, three species *Apiotrichum loubieri*, *A. sporotrichoides*, and *Geotrichum sinense* are new records for Korea. This poster provides comprehensive descriptions of the morphological, physiological, and cultural characteristics, as well as the phylogenetic status of representative strains of each species.

Keywords: Diversity, Taxonomy, Yeast

Abstract

Exploring the Diversity of *Rhizophydiales* (*Chytridiomycota*) in Soil and Freshwater Environments in Korea

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Chytridiomycota, commonly known as chytrids, is the most species-rich phylum among basal fungi. Members of the *Chytridiomycota* are widely distributed worldwide and have been identified in freshwater, terrestrial, and marine ecosystems. However, there have been little information on taxonomic studies of the phylum in Korea. During an investigation on the diversity of the order *Rhizophydiales* in soil and freshwater environments collected from different areas in Korea, 11 strains were isolated. Based on morphological characteristics and phylogenetic analyses of the internal transcribed spacer (ITS) and large subunit (LSU) regions of rDNA, six species were identified. These species belong to four genera, *Globomyces*, *Gorgonomyces*, *Paranamyces*, and *Rhizophydium*. Four species were represented as new records and two as novel species. Herein, detailed morphological and molecular characteristics of the newly described and monograph of species in *Rhizophydiales* in Korea are provided.

Keywords: Diversity, *Rhizophydiales*, Taxonomy, Basal fungi

Abstract

Aquatic Sordariomycetes of Panay Island, Philippines

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Sordariomycetes is the second largest class of Ascomycota, characterized by perithecial ascomata and inoperculate unitunicate or non-fissitunicate asci. Although predominantly composed of terrestrial taxa, several sordariomycetous species have also been reported in aquatic environments. This study documents 43 species of Sordariomycetes in aquatic habitats comprising 27 marine and 16 freshwater taxa across 39 genera, 16 families, and ten orders. Marine species were isolated from decayed submerged substrates, leachates, and coastal waters distributed across 18 genera, 12 families, and seven orders. Freshwater species were obtained from decayed wood substrates in freshwater streams and lakes, representing 12 genera, nine families, and six orders. Species identification was carried out using combined morphological and phylogenetic analysis using the ITS molecular data. Several taxa represent new records of aquatic Sordariomycetes in the Philippines. Following on earlier foundational studies of aquatic mycology in the country, this study provides critical baseline data on the underexplored fungal diversity in aquatic ecosystems and underscores the need for continued research in the region.

Keywords: aquatic Sordariomycetes, fungal biodiversity, Philippine fungal studies, tropical mycology

Abstract

Aquatic Dothideomycetes of Panay Island, Philippines

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Dothideomycetes is the largest and most ecologically diverse class of Ascomycota, characterized by bitunicate asci with fissitunicate dehiscence. In aquatic ecosystems, they play important roles as decomposers and colonize a broad range of substrates. This study reports 17 aquatic species of Dothideomycetes from Panay Island, comprising eight freshwater and nine marine taxa collectively representing seven genera, 12 families, and four orders. Freshwater species were isolated from submerged decayed substrates and freshwater ponds and spanned seven genera, seven families, and three orders (Cladosporiales, Pleosporales, Tubeufiales). Marine species recovered from coastal waters and leachates were distributed across nine genera, six families, and three orders (Cladosporiales, Mycosphaerellales, Pleosporales). Species identification was conducted through morphological characterization and phylogenetic analysis based on ITS sequences. Although investigations in Panay Island are limited, several taxa represent new records for the Philippines. These findings indicate the potential for further novel discoveries and emphasize the taxonomic and ecological importance of aquatic fungal species. Building upon earlier foundational surveys, this study highlights the need for continued exploration into fungal diversity of aquatic ecosystems.

Keywords: aquatic Dothideomycetes, fungal biodiversity, Philippine fungal studies, tropical mycology

Abstract

Unveiling the Compartmental Diversity and Hidden Ecological Roles of Seagrass-Associated Fungi

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Fungal associations remain understudied in many marine habitats, typically within seagrass meadows. Seagrasses are the only submerged marine flowering plants, yet previous studies of their associated fungi have often been limited by culture-based approaches and a narrow focus on specific seagrass compartments, leaving the ecological functions of these fungi largely unresolved. This presentation integrates the main findings from a series of our studies on fungi associated with the seagrass *Halophila ovalis* (Hydrocharitaceae) in Hong Kong. We identified culturable fungi inextricably associated with the seagrass, assessed the fungal diversity and fungal-prokaryotic networks across multiple compartments of *H. ovalis*, and employed HPLC-MS/MS analysis, transcriptomics, and *in vitro* plant growth assays for functional verification. From the mycobionts isolated from *H. ovalis* roots and rhizomes, we proposed *Halophilomyces hongkongensis*, a novel fungal species in a new genus with antibacterial potential that belongs to the Lulworthiaceae family, a group known for its obligate marine fungi. Compartmental fungal profiling using Illumina amplicon sequencing revealed that the below-ground tissues of *H. ovalis* exhibited a remarkably narrow fungal spectrum that was dominated by and enriched with *H. hongkongensis*, suggesting a possible seagrass-fungus symbiosis. The role of *H. hongkongensis* was further supported by its confirmed ability to produce the plant hormone indole-3-acetic acid (IAA), higher expression of IAA-biosynthesis genes in field-collected *H. ovalis* roots and rhizomes compared to rhizosphere sediments, and its verified plant growth-promoting activity on *Arabidopsis thaliana*. These complementary results indicate that *H. hongkongensis* functions in facilitating seagrass growth. Furthermore, fungal-prokaryotic interdomain network analysis highlighted the critical roles of fungi, such as acting as keystone taxa and maintaining network stability. Together, these findings advance our understanding of the compartmental distribution of seagrass fungi and provide the first evidence of their ecological roles through interactions with both the host seagrass and co-associated prokaryotic communities.

Keywords: Lulworthiaceae fungi, microbial interdomain network, novel taxa, plant-microbe interaction, seagrass

Abstract

Species Diversity of Yeast from Freshwater and Soil Habitats in Korea

Oh Beom Seok*, Ki Hyun Kang*, Su Jin Kim, Thuong T.T. Nguyen and Hyang Burm Lee[#]

Environmental Microbiology Lab, Dept. of Agricultural Biological Chemistry, College of Agriculture & Life Sciences, Chonnam National University, Gwangju 61186, Korea

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Yeasts are an ecologically and biotechnologically significant group of fungi, yet their diversity across various habitats remains unexplored. During a survey of yeast diversity in soil and freshwater samples collected from different locations in Korea, 19 isolates were obtained using the dilution plating method. Based on morphology and molecular phylogenetic analyses using the ITS region and the D1/D2 domain of the large subunit (LSU) rRNA gene, a total of 13 species were identified. These species belonged to ten genera, including *Apiotrichum*, *Candida*, *Debaryomyces*, *Filobasidium*, *Galactomyces*, *Geotrichum*, *Pichia*, *Rhodotorula*, *Saccharomycopsis*, and *Wickerhamomyces*. Among them, three species in the genera *Debaryomyces*, *Saccharomycopsis*, and *Wickerhamomyces* are proposed as new species. Additionally, three species *Apiotrichum loubieri*, *A. sporotrichoides*, and *Geotrichum sinense* are new records for Korea. This poster provides comprehensive descriptions of the morphological, physiological, and cultural characteristics, as well as the phylogenetic status of representative strains of each species.

Keywords:Diversity, Taxonomy, Yeast

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Abstract

The Forest Mycobiome in the Era of Global Change

Author: Claire Willing

Abstract: Underground fungal communities are fundamental to life on Earth. Quietly below the forest floor, these fungi act as ecosystem engineers, controlling nutrient availability and carbon storage in forests. What is more, plants are never found in nature without their mycobiomes and these communities can play critical roles in plant physiology and function. Just as explorations human mycobiomes have revolutionized our understanding of human health, forest mycobiomes represent a critical axis of forest health, especially in a changing climate.

Abstract

Comparative Genomic and Functional Analysis of Coastal Oil-Degrading Fungi

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Fungi can secrete large amounts of degradative enzymes and represent an effective tool for ecological remediation. While numerous fungal species have been isolated from polluted environments, their common traits—especially at the genomic level—have received limited attention. To address this gap, we conducted comparative analyses on samples collected from polluted and unpolluted beach sites in Singapore after the 2024 coastal oil spill incident. A total of 35 fungal isolates belonging to 13 genera were obtained, including *Trichoderma*, *Talaromyces*, and *Cladosporium*. Their hydrocarbon-degrading potential was systematically assessed through growth assays using diesel-amended media, hyphal attachment observations, cellulolytic activity tests, and comparative genomic analyses. Most isolates were unable to utilize gasoline as the sole carbon source; nevertheless, filamentous fungi exhibited the ability to colonize oil-contaminated media via hyphal attachment. Notably, strains S2 (*Trichoderma harzianum*), T2 (*Dothideomycetes sp.*), and A6 (*Fusarium solani*) grew robustly on media containing 2% diesel as the sole carbon source and displayed strong degradation capabilities toward long-chain alkanes. Comparative genomic analyses revealed that oil-degrading fungal genomes harbor more abundant lignin-degrading genes, whose abundance was positively correlated with biomass in oil-containing media. Furthermore, screening against a bacterial degradation enzyme database identified numerous homologous putative proteins, including LadA and AlmaA, which may function as fungal hydrocarbon-degrading enzymes. In addition, nitrogen addition experiments demonstrated that supplemental nitrogen can enhance both the biomass and degradation efficiency of oil-degrading fungi. These results improve current knowledge and offer a genomic foundation for developing fungal bioremediation strategies.

Keywords: Oil spill; Bioremediation; Hydrocarbon-degrading enzymes; Comparative genomics

Abstract

Transcriptomic trade-offs drive active fungal adaptation at the continental scale**Cong Wang***Institute of Microbiology, Chinese Academy of Sciences*

The microbial genome forms the basis for microorganisms to adapt to environments and exert ecosystem functions. While recent studies have investigated the geographical distribution patterns of bacterial genomic traits using metagenomics, little is known about the biogeography of fungal genomic traits. To address this, the present study explored the biogeography of soil fungal genomic traits across 36 typical Chinese forests using amplicon high-throughput sequencing and metatranscriptomic sequencing. The results showed that fungal taxonomic diversity did not significantly change along the latitude gradient and was weakly correlated with environmental factors, whereas fungal gene functional diversity exhibited a distinct latitude pattern and was tightly correlated with environmental factors. In contrast to fungi in cold, humid, nutrient-rich soils, fungi in warm, humid, nutrient-scarce soils had lower functional diversity, shorter transcript lengths, and a lower phosphorus cost for amino acid production, but exhibited stronger gene expression in cell structure repair and defense mechanisms. Furthermore, when the soil was extremely dry, soil moisture became the dominant factor influencing fungal functional expression. In drought-affected soils, fungi enhanced functions related to trehalose synthesis, glucan metabolism, and cell cycle control in response to dryness. These findings reveal, at the genomic level, how fungi adapt to different habitats, which is critical for understanding microbial adaptive strategies and the mechanisms by which they contribute to ecosystem processes.

Abstract

Dung Beetle iDNA Provides an Effective Way to Detect Diverse Mycological Communities

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Fungi play crucial ecological and economic roles, yet their diversity and distribution remain poorly known and challenging to assess. Using recent advances in invertebrate-derived DNA (iDNA) for biodiversity monitoring, we investigated the potential of dung beetle iDNA for fungal sampling and monitoring. We sampled two habitats (rainforest vs. rubber plantation) and seasons (dry vs. rainy) in tropical Xishuangbanna, southwest China. We extracted, amplified and identified 9259 unique fungi Amplicon Sequence Variants (ASVs) from the gut of three species of dung beetles (*Paragymnopleurus* sp., telecoprids; *Onthophagus diabolicus*, paracoprids; and *Onthophagus* cf. *gracilipes*, endocoprids). Fungal community composition was different across habitats and seasons, with the highest diversity found in the rainy season rainforest. Our results were consistent with previous eDNA-based studies based on soil samples in the detection of habitat differences (both approaches were able to detect low diversity of Basidiomycota in rubber plantations). However, our approach outperformed soil-based eDNA studies in being able to detect fungal occurrences associated with seasonal precipitation patterns. Our findings highlight the utility of dung beetle

iDNA to uncover spatiotemporal dynamics of fungal communities across different habitats. The use of iDNA broadens fungal biodiversity research, strengthens fungal monitoring to assess anthropogenic impacts and presents opportunities to conserve fungal diversity.

Keywords: biodiversity monitoring, dung beetles, fungi, iDNA, Xishuangbanna

Abstract

Electrical Information Flow Across the Sporocarps of Two Ectomycorrhizal Fungi in the Field

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Background

Ectomycorrhizal fungi, which spread their mycelial networks beneath the forest floor and establish symbiotic relationships with plant roots, may potentially mediate signal transmission between plants, including electrical signaling. In this study, we report on the patterns of electrical potentials recorded in the sporocarps of two ectomycorrhizal fungi species in the genus *Hebeloma* and their responses to artificial disturbance events.

Methods

In the 5m x 5m quadrat in a forest dominated by *Quercus serrata*, the addition of urea led to a bloom of sporocarps in October 2022. We selected 37 sporocarps for electrical potential recording by attaching electrodes. DNA analysis revealed that the sporocarps consisted of two species: *Hebeloma danicum* and *H. melleum*, including some clones. Causal analysis of the electrical potentials indicated causal relationships among the sporocarps, regardless of clone or species.

Results

Some “hub” sporocarps in the causality network were also identified. Artificial disturbance (such as water or urine addition) to certain sporocarp caused some changes in the electrical potential of the focal sporocarp and apparently influenced the electrical potentials of neighboring sporocarps. However, when tap water was added to the entire quadrat area, the magnitude of causal relationships among the sporocarps was significantly reduced.

Conclusion

These findings suggest that sporocarps on the forest floor may communicate via electrical signals, but that a sufficient water supply allows them to function more independently, temporally reducing their causal relationships.

Keywords: ectomycorrhizal fungi, electricity, information flow

Abstract

An Noncanonical S-R-C Adaptive Trajectory of Leaf mycobiome**Abstract:**

Phyllosphere plant-fungal interactions are crucial for crop health, yet how leaf metabolite dynamics direct fungal functional succession remains unclear. Using field-based holomics, we show that developmental shifts in sorghum leaf metabolomes drive a noncanonical temporal trajectory of fungal life-history strategies from stress-tolerator (S), through ruderal (R), to competitor (C). Young leaves deploy antifungal metabolites that select for stress-tolerators (S-strategists) with expanded genomes. A transient increase in maltose abundance during flowering promotes the proliferation of fast-growing ruderal (R-) strategists. As plants mature, competitive (C-) strategists rely on secondary metabolism to persist in crowded communities. We further detect asynchrony between taxonomic and functional β -diversities, and an integrated metric of the two is negatively correlated with expression of fungal genes encoding secreted carbohydrate-active enzymes and effectors. Collectively, these findings identify developmental stage-specific leaf metabolomes as key determinants of phyllosphere fungal adaptation and functioning.

Abstract

Topical Probiotic for Bats Against White-Nose Syndrome

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Without proper intervention, wildlife diseases can have drastic consequences for species. White-nose syndrome (WNS), which is caused by the fungus *Pseudogymnoascus destructans* (*Pd*), has devastated millions of several North American bat species. Here, we describe the development and testing of a probiotic cocktail for potential mitigation of WNS. Because of the absence of WNS in British Columbia (BC), Canada, we sourced probiotics from the skin microbiomes of healthy bats in this province. Four bacterial isolates with high anti-*Pd* activities showed complementary growth and combined effects in inhibiting *Pd in vitro*. We applied a mixture of these four *Pseudomonas* bacteria to: (i) bat roosts during captive trials of bats during the summer, and (ii) hibernating bats in a late fall captive trial. Using quantitative PCR analyses, we confirmed successful long-term adherence of the probiotic cocktail in bat boxes, successful transfer of these microbes to the wings of bats within the local population. Through weekly examinations of live bats in the captive trial by veterinarians, and comprehensive tissue examinations of euthanized animals after the completion of the trial, we found no adverse health effects that could be attributed to colonization of bacteria from the probiotic cocktail within bat wing tissues. Probiotic cells on roosts and wings showed negative correlation with *Pd* cells in Washington State field trial. Our probiotics mixture represents a promising biocontrol agent against WNS in western Canada and potentially the Pacific Northwest of North America.

Keywords: bats, disease management, microbiome manipulation, probiotics, white-nose syndrome (WNS)

Abstract

Bacterial-Mycelium Hybrids for Biomineralization-Based Self-Healing in Bioconcrete Structures

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Self-healing bioconcrete represents a sustainable innovation in construction materials, leveraging microbial processes to autonomously repair cracks and enhance durability. This literature review explores the emerging field of bacterial-mycelium hybrids for biomineralization-based self-healing in bioconcrete structures, synthesizing advancements in microbial-induced calcium carbonate precipitation (MICP). Traditional bacterial approaches, such as those utilizing *Bacillus* species (e.g., *B. sphaericus* and *Sporosarcina pasteurii*), have demonstrated effective crack sealing through ureolytic and non-ureolytic pathways, achieving up to 95% strength recovery and superior resistance to environmental stressors. Complementary fungal mechanisms, particularly involving filamentous mycelium from species like *Trichoderma reesei* and *Neurospora crassa*, offer advantages in forming extensive hyphal networks for larger crack healing and organomineralization via chitin nucleation sites. Although direct studies on bacterial-mycelium hybrids are limited, interactions between bacteria and fungi—such as fungal hyphae serving as vectors for bacterial dispersal—suggest synergistic potential for enhanced biomineralization efficiency and adaptability in harsh concrete environments. Key findings highlight improved mechanical properties, durability, and eco-friendliness, with challenges including microbial viability and scalability. Future research directions emphasize hybrid system optimization, interdisciplinary modeling, and field applications to advance sustainable infrastructure.

Keywords: Bacterial-mycelium hybrids, Biomineralization, Microbial-induced calcium carbonate precipitation, Self-healing bioconcrete

Abstract

Intraspecific Diversity of *Phytophthora cinnamomi* and Potential Geographic Distribution in China

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Background *Phytophthora cinnamomi* is widely recognized as a highly destructive plant pathogen, capable of infecting over 5,000 plant species, posing a significant threat to agricultural and forestry ecosystems. This species is globally distributed and considered as one of the most invasive and threatening plant pathogens. Although *P. cinnamomi* demonstrates strong adaptability and has a broad host range, research on its intraspecific diversity remains limited. Specifically, the phenotypic, genotypic, and virulence variations among strains from different habitats and host species are insufficiently investigated.

Methods In this study, 11 *P. cinnamomi* strains from various countries, regions, and host species were collected. Morphological characteristics observation, infectivity assessment and mitochondrial genomic comparative analysis were conducted. Additionally, the suitable habitats for *P. cinnamomi* in China were predicted using the MaxEnt model.

Results This study provides critical insights for controlling *P. cinnamomi* diseases and assessing the risk of foreign invasions. The key findings are as follows:

- 1) Significant differences in phenotypic traits—including morphological characteristics, optimal growth temperature, and growth rate—were observed among the 11 *P. cinnamomi* strains.
- 2) Infectivity levels varied significantly among the strains. Furthermore, infectivity differences between A1 mating-type strains were less pronounced than those between A2 mating-type strains.
- 3) Phylogenetic analysis of the mitochondrial genome and protein-coding genes revealed higher polymorphism in the non-coding regions of the *P. cinnamomi* mitochondrial genome.
- 4) The MaxEnt model predicted that suitable habitats for *P. cinnamomi* are primarily concentrated in the continental coastal areas south of the Yellow River in China, with a tendency to expand northeastward.

Conclusion Different strains of *P. cinnamomi* exhibit variations in phenotypic traits, infectivity, and genetic diversity, highlighting substantial intraspecific variability. Furthermore, the potential suitable habitats for *P. cinnamomi* in China are more extensive than previously reported. This study provides the first prediction of *P. cinnamomi*'s potential distribution in China, laying a foundation for more detailed disease modeling and offering valuable insights for disease control and invasion management.

Keywords:Infectivity assessment, Intraspecific diversity, MaxEnt model, Mitochondrial genome, *Phytophthora cinnamomi*

Abstract

Growth Assessment of Endolichenic Fungi under Acid Rain Conditions

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Sulphate when dissolve in rainwater become sulfuric acid and fall as acid rain. This has the potential to affect lichen growth, including those associated with their thallus. The current study investigated the growth responses of twelve endolichenic fungi (ELF) associated with the fruticose lichen *Ramalina* to acidic rain condition. The ELF isolates were then grown on potato dextrose agar (PDA) with Na₂SO₄ treatment at 2.55 mg/L concentration and pH 5.03, and their colony diameters were measured daily for seven days. Our results showed moderate growth for most of the ELF isolates compared to control (without Na₂SO₄ treatment, pH 5.6). *Ramalina*-ELF E7B.5, identified as *Nigrospora chinensis*, showed 76% colony diameter reduction. However, there are *Ramalina*-ELF isolates that are more tolerant to acid rain conditions. *Ramalina*-ELF E6D.4.1 and *Ramalina*-ELF E6B.3.1, both identified as *Xylaria* sp., showed moderate reduction of colonial growth, only at 13% and 17%, respectively. Two isolates, *Ramalina*-ELF E10A.1.2, identified as *Xylaria arbuscula*, and *Ramalina*-ELF E10C.1.2, identified as *Xylaria* sp., showed tolerance to sulfate and acid stress as indicated by their similar colony diameter with or without acid rain condition. Our study demonstrated strain-specific response of endolichenic fungi associated with *Ramalina* to simulated acidic rain condition.

Keywords: acidic rain, colony diameter, fungal growth, pH, sulphate

Abstract

Date palm (*Phoenix dactylifera*) root and bulk soil fungal communities are distinct and altered by salinity of irrigation water

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Abstract

Date palm (*Phoenix dactylifera*) roots and the surrounding bulk soil host compositionally and functionally distinct fungal communities, and irrigation water salinity is an important driver of that differentiation. We compared root-associated and bulk-soil fungal communities from date-palm farms irrigated with non-saline freshwater versus saline groundwater using high-throughput ITS metabarcoding and multivariate community analyses. Root fungal communities exhibited lower species richness than bulk soil and were dominated by a narrower set of taxa, indicating host selection of specialized symbionts. Saline groundwater irrigation significantly reduced root-associated fungal richness and altered overall community structure and guild composition (notably reductions in saprotrophic and changes in pathotrophic groups), while also shifting bulk-soil fungal assemblages and the pool of shared operational taxonomic units between compartments. Multivariate models and variance partitioning identified irrigation water electrical conductivity (EC) and associated soil salinity as strong correlates of community turnover, consistent with a salinity-filtering effect that modulates both deterministic (environmental selection) and stochastic assembly processes. These findings demonstrate that irrigation source — and the salinity it imposes — reshapes the fungal microbiome of date palms at both root and soil scales, with potential implications for plant health and resilience in arid agroecosystems managed with saline water.

Keywords: Arid Environment, Date palm, Fungal diversity, ITS sequencing, Irrigation water

Abstract

Interspecific Divergence in Transcriptional Regulation of an Aldo-Keto Reductase Drives Fungal Ecological Sorting in Saline Habitats

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Fungi exhibit remarkable ecological plasticity, yet the genetic mechanisms enabling niche expansion into osmotically challenging environments remain poorly understood. Through a 2,700 km coastal survey, we identified *Trichoderma asperelloides* as a dominant halotolerant species in saline soils, while congeners exhibited limited resilience. Functional screening of a salt-induced cDNA library in *Saccharomyces cerevisiae*, combined with gene deletion and phylogenetic analyses, revealed that interspecific divergence in halotolerance is primarily attributed to the transcriptional regulation of *gld1*, encoding an aldo-keto reductase (AKR) involved in accessory glycerol biosynthesis. *T. asperelloides* exhibited sustained, high-amplitude induction of *gld1* (up to 300-fold) under osmotic stress, whereas other species showed transient or weak responses. Deletion of *gld1* abolished glycerol accumulation and Na⁺ homeostasis, impairing saline growth. In synthetic communities, the Δ *gld1* mutant was outcompeted under salinity, while gene complementation restored dominance. These findings underscore the role of transcriptional evolution in *gld1* as a key driver of ecological sorting in saline habitats, highlighting how accessory gene recruitment facilitates fungal opportunism and niche expansion.

Keywords: Dominant species, Ecological sorting, Glycerol dehydrogenase, Osmoregulation, *Trichoderma asperelloides*

Abstract

Structural and Functional Characterization of Fungal Communities in Post-fire Pine Forests of Nyingchi, Tibet

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Wildfire, as a critical ecological disturbance, profoundly shapes the structural and functional characteristics of fungal communities in high-altitude forest ecosystems. The present study investigated the assembly mechanisms and functional traits of post-fire fungal communities in three months at a burnt pine forest in Nyingchi (Linzhi), Tibet, integrating PacBio full-length ITS amplicon sequencing, microbial screening and cultivation, and source tracking. The results revealed that fire significantly altered the fungal composition in surface litter: Ascomycota significantly (e.g., *Trichoderma* and *Penicillium*) replaced *Basidiomycota* as dominant taxa, accompanied by reduced α -diversity. The β NTI analysis indicated deterministic processes (environmental filtering) dominating the assembly of surface burnt litter communities, while stochastic processes governed those of deep litter and unburnt areas. The enrichment of key taxa such as *Trichoderma* spp. was found significantly correlated with the litter pH and the total carbon content, and co-occurrence network analysis highlighted its negative interactions with multiple fungal groups. The further source tracking analysis revealed 59.3% of surface burnt litter fungi originated from unknown sources (likely long-distance dispersal) and 21.5% from the neighbouring deep litter, whereas deep litter communities primarily relied more on local "seed banks" of microbes (44.8%). Notably, saprotrophs dominated burnt litter (>75%), increasing functional redundancy and reducing functional diversity, potentially heightening ecosystem vulnerability. These findings elucidate the early-time succession patterns and environmental drivers of fungal communities in high-altitude post-fire ecosystems, offering insights for managing fire-prone forests under climate change.

Keywords: Postfire mycobiome, Microbial dispersal, *Trichoderma*

Abstract

Investigation of Pathogenic Microsporidia in sewage sludge and environmental water.

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[Objective]

Microsporidia are obligate intracellular parasitic eukaryotes capable of infecting nearly all animal phyla. Over 1,200 species have been described, although fewer than 20 are known to infect humans. *Enterocytozoon bieneusi* is the predominant cause of human gastrointestinal microsporidiosis, with potential for waterborne and foodborne outbreaks. Water, vegetables, and animal reservoirs are regarded as the most likely routes of transmission; however, few studies have investigated environmental sources. This study aimed to detect microsporidian DNA in aquatic environments and estimate potential infection routes based on their distribution and diversity.

[Methods]

Samples were collected from multiple sites in the West Japan including sewage and fecal waste treatment facilities, rivers, lake, and irrigation canals. As Microsporidia are difficult to culture or observe directly in sludge, we employed DNA metabarcoding with universal microsporidian-specific primers. Solids from water and sludge were concentrated by filtration or centrifugation, followed by total DNA extraction. Approximately 500-bp amplicons of the small subunit ribosomal RNA gene were sequenced on an Illumina MiSeq platform (Macrogen Japan), and the resulting rawdata were analyzed using mothur and BLASTn to identify species pathogenic to humans.

[Results and Discussion]

In the sewage and fecal waste samples, *E. bieneusi* was sporadically detected, suggesting that gastrointestinal infections due to this species are likely to be infrequent in the studied area. In contrast, sequences with high similarity to *Endoreticulatus* sp., a systemic microsporidian pathogen previously reported from Thailand, were detected at higher frequencies than *E. bieneusi*. They were also found in the river and canal samples. *Vittaforma corneae*, a known cause of human keratitis that also infects arthropods, was detected in all samples from the rivers and canals. Most of the sequences obtained were more closely related to those from human corneas than to those from arthropods. Microsporidia in irrigation canals may contribute to the higher incidence of keratitis among agricultural workers.

Keywords: DNA Metabarcoding, Infection Routes, Microsporidia, Sewage Sludge, Environmental Water.

Abstract

Exploring the Utilization Value of Freshwater Bioresources: Statistical Data Analysis of Bacteria and Fungi

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Freshwater ecosystems in South Korea harbor a rich diversity of microorganisms, including bacteria and fungi with promising but underexplored biotechnological potential. However, their utilization has been limited due to insufficient functional and genetic information. To address this, the Nakdonggang National Institute of Biological Resources (NNIBR) launched the project “Sustainable Exploration of Biotechnological Potentials of Freshwater Bioresources”, producing diverse datasets on traits such as antibacterial activity, enzyme functions, and pigment biosynthesis. The dataset included physiological activities of bacteria (1,819 entries) and fungi (418 entries). Statistical profiling identified top-performing taxa, and correlation analysis revealed meaningful associations, such as a strong positive correlation between bacterial and antifungal activities. Genus-level patterns highlighted *Bacillus*, *Streptomyces*, and *Pseudomonas* as frequent among bacteria, and *Trichoderma* among fungi, with strong links to antimicrobial and enzyme-related functions. These results provide a scientific foundation for identifying promising taxa and demonstrate the potential of freshwater bioresources in agriculture and environmental sustainability.

Keywords: Freshwater bioresources, bacteria, fungi, Statistical analysis

Abstract

Expanding Truffle Cultivation: Co-occurrence of *Tuber melanosporum* and *T. borchii* on *Pinus pinea*

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Abstract

This study presents the first scientific confirmation of *Tuber melanosporum* ascocarp production on *Pinus pinea* in a commercial truffle orchard in Australia, where both *T. melanosporum* and *T. borchii* have been harvested since 2015. High-throughput metabarcoding was used to analyze fungal community composition and spatial patterns of colonization. Mycorrhizae of both *Tuber* species were detected on *P. pinea* roots. In soil pits measuring approximately 15 × 15 cm, a pattern of spatial dominance was observed in which either *T. melanosporum* or *T. borchii* tended to prevail, suggesting possible localized competition. *T. melanosporum* exhibited a stronger suppressive effect on other ectomycorrhizal fungi compared to *T. borchii*. Dominant non-*Tuber* taxa included *Rhizopogon*, *Suillus*, *Inocybe*, *Tomentella*, and *Amphenema* species. Both *Tuber* species were also detected in low abundance on roots of common weed species such as *Arctotheca calendula*, *Hypochoeris radicata*, *Sonchus oleraceus*, and members of Poaceae and *Trifolium*. These findings highlight *P. pinea* as a drought-tolerant, commercially viable host for truffle cultivation. Co-cultivation of *T. melanosporum* and *T. borchii* may extend the ascocarp harvest season. Further research is needed to assess long-term persistence and the influence of environmental factors on species interactions.

Keywords:(Truffle, Mycorrhizal interactions, High-throughput metabarcoding)

Abstract

Biodegradability Assessment of Compostable Plastics under Biodegradable Condition using Fungal Species isolated from Freshwater Related Environment

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Biodegradable plastics have been developed to replace conventional petroleum-based plastics to reduce plastic waste. Biodegradable plastics are divided into two types: “biodegradable” plastics and “compostable” plastics. Compostable plastics account for the largest market size in the bioplastic market due to their excellent physical properties. However, they require harder conditions (industrial composting condition, 58°C) to decompose. Therefore, compostable plastics have a significantly low decomposition rate under general landfill conditions, which also affects the reduction of waste. Therefore, it is important to improve the decomposition conditions of these biodegradable plastics.

In this study, we tried to find fungal strains that could decompose compostable plastics under biodegradable condition, 25°C. We selected polylactic acid (PLA), the most widely used compostable plastic, as the target material. First, we studied the decomposition mechanism of PLA and related enzymes in a previous study. We quantitatively analyzed the activity of the enzymes involved in the degradation of PLA among 130 fungal strains isolated from a freshwater-related environment. selected 4 fungal strains that had high enzyme activity. We selected fungal strains with high enzyme activity and treated the commercially available PLA product with liquid culture media. Finally, we analyzed the degradation rates over time under the biodegradable condition. As a result, 4 fungal strains, including the *Apiotrichum porosum* NNIBRFG1603 strain, showed a high PLA degradation rate under biodegradable conditions. Under 25°C condition, 90% of the PLA product was degraded within 6 months, which means that PLA can be decomposed under general landfill conditions.

Keywords: Biodegradation, Compostable plastics, Freshwater-related fungi

Abstract

Tolerance of *Trichoderma asperellum* to Copper and Nickel Stress: Implications for Bioremediation of Heavy Metal Contaminated Soils

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Heavy metals such as copper and nickel are widespread soil contaminants, often originating from mining, smelting, and industrial effluents, where they accumulate to levels that can inhibit microbial growth and disrupt ecological balance. Addressing heavy metal stress is critical not only for maintaining soil health but also for restoring biological functions in contaminated ecosystems. This study evaluated the tolerance of *Trichoderma asperellum* to increasing concentrations of copper and nickel (300, 600, 900, 1200 ppm) using colony diameter (cm) as a growth metric. Cultures were incubated under controlled conditions, and growth was measured over time. For copper, colony diameter declined from 7.2 ± 0.3 cm in the control to 2.8 ± 0.2 cm at 1200 ppm. The Kruskal–Wallis test revealed a significant overall difference ($H = 18.72$, $df = 4$, $p = 2.3 \times 10^{-8}$), with Dunn–Bonferroni post hoc analysis confirming strong inhibition at higher concentrations ($p < 0.001$). For nickel, growth decreased from 8.1 ± 0.2 cm in the control to 6.5 ± 0.3 cm at 1200 ppm, with significant differences detected ($p = 0.0035$), particularly at 900 ppm ($p < 0.01$) and 1200 ppm ($p < 0.001$). Time-resolved assays further showed progressive inhibition over five days under nickel stress. These results demonstrate a dose-dependent reduction in *T. asperellum* growth, with copper exerting a more pronounced toxic effect than nickel. The findings highlight the resilience of *T. asperellum* under moderate heavy metal stress, supporting its use as both a bioindicator of metal toxicity and a promising candidate for bioremediation strategies in mining-impacted and industrially contaminated soils.

Keywords: bioremediation, copper, fungal growth inhibition, heavy metals, *T. asperellum*, tolerance assay

Abstract

Inhibitory activities of six local endophytic fungi as potential biocontrol agents of cabbage Fusarium Wilt

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Six local EF were isolated and identified using morphological and molecular approaches, specifically targeting ribosomal DNA (rDNA) through ITS1 and ITS4 primers. Their antagonistic activity against FOC was assessed using the Dual Culture Assay (DCA), revealing varying degrees of inhibition. Among the isolates, *Daldinia eschscholtzii* ($56.14\% \pm 2.77$) exhibited the highest inhibition, followed by *Arthrinium phaeospermum* ($30.47\% \pm 3.51$), *Colletotrichum siamense* ($25.83\% \pm 4.64$), *Diaporthe* sp. ($25.13\% \pm 8.95$), and *Glomerella cingulata* ($13.68\% \pm 5.65$). In contrast, *Colletotrichum fructicola* ($-16.62\% \pm 3.41$) exhibited no inhibitory effect, indicating its limited biocontrol potential. With *D. eschscholtzii* exhibiting the highest inhibitory activity among the tested endophytes, a further study was conducted to compare its biocontrol potential alongside widely recognized fungal antagonists, *Trichoderma* species, in suppressing FOC. The DCA demonstrated that *T. harzianum* ($60.96\% \pm 3.10$), *T. asperellum* ($57.41\% \pm 4.12$), and *T. virens* ($53.94\% \pm 2.20$) exhibited significant inhibition of FOC, outperforming *D. eschscholtzii* ($19.34\% \pm 2.85$). Meanwhile, *T. atroviride* ($27.77\% \pm 4.92$) displayed moderate inhibition. The decline in the antagonistic activity of *D. eschscholtzii* may be attributed to repeated subculturing, which can lead to metabolic shifts and reduced biosynthesis of antifungal secondary metabolites, ultimately affecting its biocontrol efficacy. These findings highlight *D. eschscholtzii* as a promising biocontrol candidate while reinforcing the established antagonistic efficacy of *Trichoderma* species.

Keywords: biocontrol, cabbage Fusarium wilt, *Daldinia eschscholtzii*, dual culture assay, endophytic fungi, *Fusarium oxysporum* f. sp. *conglutinans*, ITS rDNA, *Trichoderma* spp.

Abstract

Investigation of Pathogenic Microsporidia in sewage sludge and environmental water.

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Keywords: DNA Metabarcoding, Infection Routes, Microsporidia, Sewage Sludge, Environmental Water.

Abstract

Two Novel Species, *Phytophthora umbellatus* sp. nov. and *Phytopythium niubeiliangense* sp. nov., from a Subtropical Island in Japan and a Warm-Temperate Forest in China

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Background The Oomycetes are renowned for harboring several important pathogenic species, such as *Phytophthora* and *Pythium*. Up to now, the 2025 edition of the "Catalogue of Life China" has recorded a total of 41 species of *Phytophthora* and 80 species of *Pythium*. Phylogenetically, *Pythium* has been reclassified multiple times: initially split into 11 clades, clade K was later moved to *Phytopythium*. The genus *Phytophthora* is one of the most important and well-researched groups, generating serious and widespread diseases that have an impact on the world's ecology, economy, society, and science. The majority species of *Pythium* are pathogens causing seedling damping-off, root rot, and fruit rot. Thus, Precise taxonomic identification serves as the foundational step for implementing effective control strategies against plant diseases.

Methods Strain isolation—Sampling was conducted on Amami Island in Japan, for the *Phytophthora* strain. Fallen leaves were collected from areas near rivers where the current was slow. The *Phytopythium* strain was isolated from the rhizosphere soil of in the Qinling Mountains of China, using the soil dilution plating method and Nystatin-Ampicillin-Rifampicin-Miconazole (NARM) medium to purify. DNA extraction and amplification—For the *Phytophthora* isolate, ITS-rDNA, *cox1*, *ypt1*, and *tigA* were amplified and sequenced. For the *Phytopythium* isolate, ITS-rDNA, LSU-rDNA, *cox1*, *cox2*, and β -*tub* were amplified and sequenced. Phylogenetic analyses—For the construction of phylogenetic trees, Bayesian Inference (BI) and Maximum Likelihood (ML) methods were employed. Sequencing, assembly, and annotation of mitogenome—The quality-assured DNA library was sequenced using an Illumina NovaSeq 6000 instrument. Morphological observation—Observed and recorded its characteristics during both the sexual and asexual reproductive stages.

Results Based on these distinct morphological features and taxonomic status, we formally describe the two strains as new species: *Phytophthora umbellatus* sp. nov. and *Phytopythium niubeiliangense* sp. nov. The key findings are as follows:

1) *Phytophthora umbellatus* sp. nov.:

Phylogenetic analysis—Our new isolate clustered with the *Phytophthora* subclade 9c species (*P. richardiae*, *P. macrochlamydospora*, and *P. quininea*), demonstrating distinct placement with high support values (100/1). Therefore, it can be concluded that the new isolate is genetically affiliated with subclade 9c. Mitogenomic analysis—The mitochondrial genome of *P. umbellatus* strain AM18FRL12 has a length of 39,675 bp. This mitochondrial genome contains 40 protein-coding genes (PCGs), two rRNA genes, and 25 tRNA genes and exhibits distinct gene

rearrangement compared to other published *Phytophthora* mitochondrial genomes. Taxonomy—A non-papillate, homothallic *Phytophthora* strain. It can be differentiated from *P. richardiae* (homothallic) by the presence of chlamydospores and its aplerotic oospores.

2) *Phytopythium niubeiliangense* sp. nov.:

Phylogenetic analysis—*Phy. niubeiliangense* was close to *Phy. aichiense*, with high bootstrap and posterior probability values (97/1). Taxonomy—A papillate, asexual *Phytopythium* strain. However, *Phy. niubeiliangense* differs morphologically from *Phy. aichiense* by its elongated hyphal swellings (which are irregular to globose), the absence of sexual structures, and a lower optimum growth temperature.

Conclusion In this study, two isolates were described and defined as novel species: a non-papillate, homothallic *Phytophthora* strain collected from a subtropical island in Japan, and a papillate, asexual *Phytopythium* strain collected from a warm-temperate forest in China. Genus such as *Phytophthora* and *Pythium* are of both pathological and ecological importance to agricultural and natural ecosystems worldwide. This study presents a new *Phytophthora* species and another new *Phytopythium* species from varied habitat and countries, will contribute valuable insights into the broader understanding of the two genera.

Keywords: *Phytophthora*; *Phytopythium*; Morphology; Phylogenetic; Mitochondrial genome

Abstract

Fungal Diversity of Spoil Heaps in the Czech Republic (Central Europe)Ondřej Martínek^{##}, Jiří Kout*Department of Biology, Geoscience & Environmental Education, University of West Bohemia, Pilsen, Czech Republic**Email of the corresponding author omartinek280@gmail.com*

Brown-coal mining in the Sokolov region (western part of the Czech Republic, Central Europe) has left behind extensive spoil heaps, which today represent unique anthropogenic habitats. These areas are subject to ongoing reclamation, yet they also provide valuable sites for studying biodiversity. Mycological surveys were carried out in 2024–2025 on two spoil heaps. There were chosen 34 micro-localities in total with 10 meters radius on the two spoil heaps. Research took approximately 10 minutes mycological inspection at each micro-location and focused on macromycetes. Until today there are revealed 84 fungal species respectively 92 fungal species on the second one. More precisely, 33 mycorrhizal species and 51 lignicolous species were found on the first one none of which are ascomycetes, while 48 mycorrhizal and 44 lignicolous species have been confirmed on the second one of which are five ascomycetes so far. According to Sorensen's similarity index ($S = 0.25$), the soil heaps have a moderate to weak similarity between the two communities. The findings highlight the ecological importance of spoil heaps as refuges for fungal biodiversity and their potential role in supporting rare taxa (there were found 18 species listed in the Czech's Red List) within disturbed landscapes. In conclusion, it could be said that even areas affected by coal mining in the past, which have subsequently undergone reclamation, can be interesting habitats with a high ecological value.

Keywords: Europe, fungi, recultivation

Abstract

Mycological Research of Waterlogged Sites in the Czech Republic

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Waterlogged sites in the Czech Republic (Central Europe) are poorly studied from a mycological perspective. The aim of this study was to conduct an inventory survey and evaluate the species diversity of fungi in waterlogged alder forests. Repeated collections were carried out at four sites covering an area of 1 ha, with a total of 27 collections. A total of 135 species of macromycetes from the *Ascomycota* and *Basidiomycota* phyla were found. Saprotrophic lignicolous fungi predominated (92 species), most of which caused white rot (67 species). Mycorrhizal species were less common (13). From a taxonomic perspective, the most species-rich order was *Agaricales*, comprising 18 families and 45 species. The second most diverse order was *Polyporales*, represented by 12 families and 35 species. Two species from the Red List of the Czech Republic were recorded – *Phleogena faginea* (Fr. & Palmquist) Link and *Steccherinum oreophilum* Lindsey & Gilb. (both NT, near threatened). Preliminary results show a high species diversity of saprotrophic lignicolous fungi and a lower representation of mycorrhizal species.

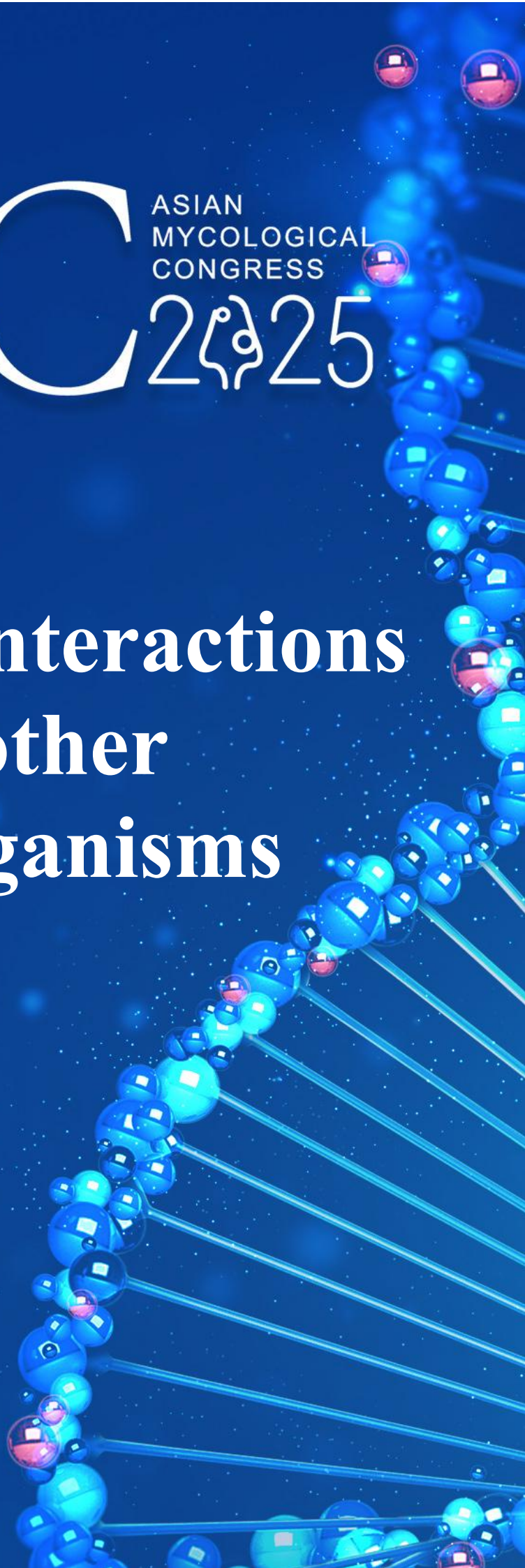
Keywords: ecology of fungi, macromycetes, mycology.

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Session 13 Interactions with other Microorganisms



Abstract

Antimicrobial Metabolites of *Bacillus velezensis* FZB42 Reshape the Rice Rhizosphere Microbial Community and Enhance Host Resistance to *Rhizoctonia solani*

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Abstract

Biocontrol agents (BCAs) and their metabolites offer a green and sustainable solution to emerging food production challenges, but knowledge of their role in shaping rhizosphere microbial communities is still lacking. To address this knowledge gap, the current study evaluated the effect of a biocontrol bacterium *Bacillus velezensis* FZB42, its mutants for fengycin AK2 (*ΔfenA*), surfactin CH01 (*ΔsrfA*) and bacillomycin D AK1 (*ΔbmyA*) and corresponding pure metabolites fengycin (Fin), surfactin (Sin), and bacillomycin D (Bin) on induced systemic resistance (ISR) in rice against pathogen *Rhizoctonia solani* and rhizosphere microbial communities' composition. The biocontrol agent FZB42, its mutants, as well as their metabolites, strongly inhibited *R. solani* mycelium growth in petri plate assay. The SEM and TEM analyses found deformed and destroyed cells as well as impairing the plasma cell membranes of mycelia growth. The bacterial strains and their crude extracts as well as metabolites showed strong antifungal potential due to the induction of severe oxidative stress in the mycelial cells. The 16S and ITS2-1 rRNA gene sequence data revealed that the application of specific treatments reshaped the rhizosphere microbial communities and significantly altered their diversity. Furthermore, after the application of mutants in the rhizosphere, different bacterial phyla (*Chlamydiae*, *Verrucomicrobia*, *Armatimonadetes*, and *Candidatus saccharibacteria*), and fungal phyla (*Chytridiomycota*, *Aphleidiomycota*, and *Blastocladiomycota*) which were not found in FZB42 and CKH. The application of pure metabolites in the rhizosphere also had various bacterial phyla (*Chlamydiae*, *Armatimonadetes*, and *Planctomycetes*) and fungal phyla (*Basidiobolomycota*, *Blastocladiomycota*, and *Mortierellomycota*) which were not present in control and disease. This means that the metabolites played a crucial role in preventing disease by directly inhibiting growth and reshaping microbial communities. The intra-kingdom network analysis revealed a decreased community number and modularity but an improved number of connections per node. Moreover, FZB42, its mutants, and pure metabolites protected plants by inhibiting the growth of *R. solani* and activating plant-induced systemic resistance by regulating defense genes. Overall, our findings suggest that the FZB42 and

their metabolites Fin, Sin, and Bin can be used as BCAs to achieve sustainable disease management due to their antimicrobial properties, capability to induce systemic resistance, and ability to promote a healthy and green state of the microbial community.

Keywords: *Rhizoctonia solani*, Biocontrol agents, Disease suppression, Induced system resistance, Rhizosphere microbiota.

Abstract

Ecological Genomic Research in *Trichoderma* Unveils Risks and Opportunities for Its Use in Sustainable AgricultureFeng Cai^{1,*,#}, Irina S. Druzhinina^{2,#}¹State Key Laboratory of Biocontrol, School of Ecology, Sun Yat-sen University, Shenzhen, China²Royal Botanic Gardens, Kew, Richmond, United KingdomCorresponding author: Feng Cai, caif8@mail.sysu.edu.cn; Irina S. Druzhinina, i.druzhinina@kew.org

Plant-beneficial mycoparasitic fungi from the genus *Trichoderma* enhance sustainable agriculture by controlling pathogens and improving crop immunity, stress tolerance, and yield. However, their occasional but increasing pathogenicity in plants, humans, and mushrooms underscores the need to understand the evolutionary mechanisms shaping their ecological versatility. This study used machine learning based on Support Vector Machines (SVMs) to correlate genomic data from 37 *Trichoderma* strains with over 140 phenotypic traits. Results depict *Trichoderma* as an ancient, genetically distinct, and ecophysiological diverse genus, adapted to arboreal phyllospheric microbial mats of tropical rainforests. Genomic analyses revealed that at least 10% of the core genome, encoding regulatory genes, enzymes, transporters, small secreted proteins, and genes without known function, is directly functionally linked to phenotypes (phenotype-associated genes, PAGs). Our data suggest patterns of character displacement, where closely related sympatric species exhibit distinct reproductive and ecological strategies, while distantly related species may converge on adaptive traits such as occasional terrestrialization or endophytism. These findings provide an evolutionary framework for understanding fitness trade-offs between environmental opportunism and ecological specialization. While some *Trichoderma* species exhibit traits of potential biosafety concerns, the genus's vast ecophysiological diversity allows for the targeted development of safe bioeffectors.

Keywords: Comparative genomics, Environmental opportunism, *Hypocreales*, Phenogenes, SVM

Abstract

Synthetic Phyllosphere Microbial Communities Promote Wax Gourd Growth and Suppress Leaf Blight by Reshaping Microbial Communities

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Abstract

The role of rhizosphere microbes in suppressing plant diseases is well-studied, but the response of phyllosphere microbes to disease invasion remains largely unexplored. In this study, we isolated biocontrol and plant growth-promoting bacterial strains from leaves of the wax gourd plant. *In vitro* tests on 63 bacterial strains revealed that 15, 11, and 12 strains produced amylases, cellulases, and proteases, respectively. Moreover, 14 strains solubilized organic phosphates, 13 inorganic phosphorus, and 3 showed high indole-3-acetic acid (IAA) production. Five strains exhibited strong antifungal activity against *Fusarium graminearum*, *F. equisetum*, *F. oxysporum*, *Colletotrichum capsici*, *Coccidioides phaseolus*, and *Phytophthora capsici*. These five wax gourd leaf strains, along with the previously isolated *Bacillus thuringiensis* GBAC46, were used to construct four synthetic bacterial communities (SynComs: A, B, C, and D). SynCom A showed reduced swarming motility and thinner biofilms, while SynComs B, C, and D displayed higher motility and well-structured, uniform biofilms. Additionally, the crude extract from SynComs significantly inhibited the growth of *P. capsici*. In pot experiments, SynCom B significantly enhanced plant growth, increasing root and shoot length, fresh and dry weights, and stem diameter. All four SynComs reduced the disease index compared to the control, with SynCom B showing the highest efficacy (40.48%) against *P. capsici* by modulating defense genes *ERF1* and *EIN3*. In the presence of *P. capsici*, SynComs reshaped bacterial communities, such as Shannon, Simpson, and Inv_simpson diversity, enriching beneficial genera like *Bacillus*, *Delftia*, and *Pseudomonas* in the wax gourd phyllosphere. These findings reveal novel mechanisms of SynCom-mediated leaf blight suppression, supporting its potential as a biopesticide.

Keywords: Phyllosphere, Synthetic microbial communities, Biofilm, Swarming, Biological control.

Abstract

Aspects of Plant Parasitism and Defense Against Mycoparasitism in the Broad-host-range Oomycete Plant Pathogen *Pythium myriotylum*

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The oomycete *Pythium myriotylum* is a broad-host-range plant pathogen whose genome encodes an expanded arsenal of putative virulence factors. *P. myriotylum* causes ginger (*Zingiber officinale*) soft rot disease, and this disease can be partly controlled by biological means using a mycoparasite (*Pythium oligandrum*). Our aims were to investigate proteins that *P. myriotylum* could potentially use in its parasitism of ginger, and potentially in defense against mycoparasitism. Key methods used included transcriptomics to identify candidate *P. myriotylum* proteins, and heterologous production of NEP1-like proteins (NLPs), and Kazal-type protease inhibitors (KSPIs), and (bio)chemical analysis of protein function and related plant and mycoparasite components. During infection of ginger leaves, a subset of NLPs, which were expanded in the *P. myriotylum* genome, were highly upregulated. Two of the *P. myriotylum* NLPs (Pm_NLP1 and Pm_NLP4) were cytotoxic to ginger leaves, and analysis of the sphingolipid content of ginger leaves showed high proportions of the sphingolipid type (Series A GIPCs) that NLPs are known to bind with and cause cytotoxicity. The cytotoxicity supports the potential of the NLPs to contribute to the *P. myriotylum* parasitism of ginger. The soft-rot disease can be partly controlled by a mycoparasite, and in the process of parasitizing *P. myriotylum*, a subset of *P. myriotylum* KSPIs were highly upregulated. One of the KSPIs (Pm_KSPI-11) inhibited mycoparasite protease activity, and was a strong inhibitor of trypsin with an inhibition constant (K_i) of ~ 5 nM. The inhibitory properties of Pm_KSPI-11 supports the potential of *P. myriotylum* to defend against the mycoparasite. Analysis of proteins in the expanded genome of *P. myriotylum* suggests ways to improve control of soft rot disease, such as by targeting NLPs or the sphingolipids they bind to, and also by engineering the mycoparasite to evade the protease inhibitors.

Keywords:oomycetes; parasitism; antagonism; NLPs; protease inhibitors;

Abstract

qPCR-Based Quantitative Assessment of Wood-Rotting Fungi during Early Stages of Environmental Wood Decay

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Quantifying microbial biomass in decaying wood is essential for understanding the decomposition process. In environmental wood decay, wood-rotting fungi, associated fungal groups, and bacteria interact in complex ways to drive decomposition. When analysing microbial community structures, DNA is usually extracted in bulk and separated into fungal and bacterial groups using barcoding sequences. The relative proportions of these groups are critical for reconstructing the community structure. We developed a quantitative PCR (qPCR)-based method to evaluate microbial biomass in decayed wood. Representative wood-rotting fungi (*Fomitopsis palustris*, *Trametes versicolor*, *Trichoderma reesei*) and common bacterial species (*Bacillus subtilis*, *Escherichia coli*) were used to establish standard curves, targeting the ITS region for fungi and partial 16S rDNA for bacteria. DNA was then extracted from wood blocks experimentally decayed for different periods by *F. palustris*, and fungal biomass was quantified using the calibration curves. In addition, fungal and bacterial DNA from other decayed wood samples were quantified using the same qPCR approach. Standard curves constructed with plasmid DNA containing genomes and barcode sequences of five test microorganisms confirmed the quantitative reliability of the assay. Using this approach, we quantified the directional growth of fungal mycelia in 6 × 6 cm wood blocks colonized by *F. palustris* and evaluated the fungal-to-bacterial DNA ratio in timber logs that had been buried underground as building foundation piles. qPCR-based quantification offers a practical approach to evaluate microbial biomass in decayed wood and contributes to understanding fungal roles in environmental wood decomposition.

Keywords: wood decay, qPCR, microbial biomass, fungi–bacteria interactions, environmental wood decomposition

Abstract

Antagonistic Activity of Ramalina- and Usnea-associated Endolichenic Fungi against *Fusarium oxysporum* Schltdl

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Endolichenic fungi (ELF), filamentous fungi associated with the thalli of the fruticose lichens *Ramalina* and *Usnea*, are tapped in this study to control the plant pathogen *Fusarium oxysporum* that infects banana plantations in the Philippines leading to low product yield and high economic losses. We hypothesized that ELF could antagonize plant pathogens and poise as a promising source of natural agrochemicals. In this study, we isolated ELF from the two lichen hosts collected in six sampling localities across Northern and Central Philippines. From the 824 ELF isolates, *Usnea*-ELF (n=578) had the highest number than *Ramalina*-ELF (n=246). Most of the ELF isolates were isolated from lichen thalli collected in Iloilo (*Ramalina*, 137 ELF) and Benguet (*Usnea*, 156 ELF). From our isolates, we selected 60 ELF, and these were tested against *F. oxysporum* using the agar-based dual-culture assay. Our results showed the following types of interactions: Type A (6 ELF, mutual intermingling of test fungi), Type B (34 ELF, mutual inhibition upon contact), Type C (1 ELF, mutual inhibition at a distance), Type D (13 ELF, inhibition of one organism upon contact), and Type E (6 ELF, inhibition of one organism at distance). Of these interaction types, Types D and E are promising for the bioprospecting of natural bioactive compounds. Our results further showed that the 14 ELF isolates, that is, 8 *Ramalina*-ELF and 6 *Usnea*-ELF, inhibited the growth of the plant pathogen, thereby winning over *F. oxysporum*. Computation of their percent inhibition indicates a range of 13% up to 56%. Two promising ELF isolates, identified as belonging to genus *Xylaria*, inhibited more than 50% of the growth of *F. oxysporum*. Antagonism was due to inhibitory activity of the ELF hyphae upon contact with the hyphae of the fungal pathogen, potentially through mycoparasitism and/or direct competition. Our study highlights the important roles of endolichenic fungi in lichen symbiosis and in the search for novel, eco-friendly fungicidal compounds.

Keywords: agrochemicals, biocontrol, bioprospecting, fungal natural products, fruticose lichens

Abstract

Aspects of Plant Parasitism and Defense Against Mycoparasitism in the Broad-host-range Oomycete Plant Pathogen *Pythium myriotylum*

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The oomycete *Pythium myriotylum* is a broad-host-range plant pathogen whose genome encodes an expanded arsenal of putative virulence factors. *P. myriotylum* causes ginger (*Zingiber officinale*) soft rot disease, and this disease can be partly controlled by biological means using a mycoparasite (*Pythium oligandrum*). Our aims were to investigate proteins that *P. myriotylum* could potentially use in its parasitism of ginger, and potentially in defense against mycoparasitism. Key methods used included transcriptomics to identify candidate *P. myriotylum* proteins, and heterologous production of NEP1-like proteins (NLPs), and Kazal-type protease inhibitors (KSPIs), and (bio)chemical analysis of protein function and related plant and mycoparasite components. During infection of ginger leaves, a subset of NLPs, which were expanded in the *P. myriotylum* genome, were highly upregulated. Two of the *P. myriotylum* NLPs (Pm_NLP1 and Pm_NLP4) were cytotoxic to ginger leaves, and analysis of the sphingolipid content of ginger leaves showed high proportions of the sphingolipid type (Series A GIPCs) that NLPs are known to bind with and cause cytotoxicity. The cytotoxicity supports the potential of the NLPs to contribute to the *P. myriotylum* parasitism of ginger. The soft-rot disease can be partly controlled by a mycoparasite, and in the process of parasitizing *P. myriotylum*, a subset of *P. myriotylum* KSPIs were highly upregulated. One of the KSPIs (Pm_KSPI-11) inhibited mycoparasite protease activity, and was a strong inhibitor of trypsin with an inhibition constant (K_i) of ~ 5 nM. The inhibitory properties of Pm_KSPI-11 supports the potential of *P. myriotylum* to defend against the mycoparasite. Analysis of proteins in the expanded genome of *P. myriotylum* suggests ways to improve control of soft rot disease, such as by targeting NLPs or the sphingolipids they bind to, and also by engineering the mycoparasite to evade the protease inhibitors.

Keywords:oomycetes; parasitism; antagonism; NLPs; protease inhibitors;

Abstract

Ecological Genomic Research in *Trichoderma* Unveils Risks and Opportunities for Its Use in Sustainable Agriculture

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Plant-beneficial mycoparasitic fungi from the genus *Trichoderma* enhance sustainable agriculture by controlling pathogens and improving crop immunity, stress tolerance, and yield. However, their occasional but increasing pathogenicity in plants, humans, and mushrooms underscores the need to understand the evolutionary mechanisms shaping their ecological versatility. This study used machine learning based on Support Vector Machines (SVMs) to correlate genomic data from 37 *Trichoderma* strains with over 140 phenotypic traits. Results depict *Trichoderma* as an ancient, genetically distinct, and ecophysiologically diverse genus, adapted to arboreal phyllospheric microbial mats of tropical rainforests. Genomic analyses revealed that at least 10% of the core genome, encoding regulatory genes, enzymes, transporters, small secreted proteins, and genes without known function, is directly functionally linked to phenotypes (phenotype-associated genes, PAGs). Our data suggest patterns of character displacement, where closely related sympatric species exhibit distinct reproductive and ecological strategies, while distantly related species may converge on adaptive traits such as occasional terrestrialization or endophytism. These findings provide an evolutionary framework for understanding fitness trade-offs between environmental opportunism and ecological specialization. While some *Trichoderma* species exhibit traits of potential biosafety concerns, the genus's vast ecophysiological diversity allows for the targeted development of safe bioeffectors.

Keywords: Comparative genomics, Environmental opportunism, *Hypocreales*, Phenogenes, SVM

Abstract

Multi-omics Analysis of Pathogenic Mechanisms and Disease-resistant Breeding of Yellow-root Disease in *Ganoderma lingzhi*

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Abstract:

Background: Yellow-root disease of *Ganoderma lingzhi* represents a critical constraint to the development of the *Ganoderma* industry, resulting in deterioration of fruiting body quality and yield losses of 30-50%. However, the causative pathogen and pathogenic mechanisms remain unclear, severely impeding effective disease control and breeding of resistant cultivars. This study aimed to elucidate the molecular mechanisms underlying this disease through multi-omics approaches and provide a foundation for disease-resistant breeding.

Methods: ITS amplicon sequencing and pathogen isolation were conducted on diseased fruiting bodies and cultivation substrates collected from major *G. lingzhi* production areas in China, followed by pathogenicity verification through Koch's postulates. The genome of *Xylogone ganodermophthora* was sequenced and assembled using next-generation sequencing technology, and virulence factor genes were predicted based on the PHI-base database with stringent criteria (alignment length ≥ 80 amino acids/240 nucleotides, E-value $< 1e-30$, identity $\geq 96\%$). Metabolomic profiles of healthy and diseased fruiting bodies were systematically analyzed using UPLC-MS/MS-based untargeted metabolomics. Additionally, disease-resistant mutants were screened through chemical mutagenesis combined with ARTP (atmospheric and room temperature plasma) mutagenesis. Growth performance of the mutant and parental strains was compared on media supplemented with pathogen metabolites, and the molecular mechanisms underlying stress resistance in the mutant strain were elucidated through integrated transcriptomic and metabolomic analyses.

Results: ITS amplicon sequencing revealed that the relative abundance of *Xylogone ganodermophthora* reached 60-70% in both diseased fruiting bodies and cultivation substrates. Combined with back-inoculation verification and re-isolation results, *X. ganodermophthora* was identified as the causative agent of yellow-root disease in *G. lingzhi* for the first time. Genome-wide analysis identified six high-confidence candidate virulence factor genes with phenotypes including reduced virulence, loss of pathogenicity, chemistry target: resistance to chemical, lethal,

and unaffected pathogenicity. Metabolomic analysis revealed 24 significantly differential metabolites in diseased fruiting bodies compared to healthy tissues, predominantly alkaloids, terpenoids, phenolic acids, and flavonoids, which were likely induced by pathogen infection or produced as stress responses in *G. lingzhi*.

One disease-resistant mutant strain was successfully obtained, exhibiting significantly faster mycelial growth than the wild-type strain under pathogen metabolite stress. Transcriptomic and metabolomic analyses revealed that the mutant and wild-type strains shared 473 core stress-responsive genes and 624 metabolites. The mutant strain specifically up-regulated 142 genes and 289 metabolites under stress conditions, constitutively expressed 13 genes and 151 metabolites that remained up-regulated under stress after mutagenesis, and possessed 117 unique stress-responsive genes and 113 unique metabolites. In-depth data mining identified significant associations between 69 mutant-specific genes and 72 metabolites, which were functionally enriched in carbohydrate metabolism and lipid metabolism pathways, indicating that the mutant strain enhanced stress resistance primarily through strengthened carbohydrate and fatty acid metabolism.

Conclusion: This study identified the causative pathogen of yellow-root disease in *G. lingzhi* and systematically elucidated the molecular mechanisms of pathogenesis and disease resistance at the genomic, transcriptomic, and metabolomic levels. The disease-resistant germplasm resources generated provide important theoretical foundations and superior genetic materials for green prevention and control of yellow-root disease and breeding of resistant *G. lingzhi* cultivars.

Keywords:(Provide 3 to 5 keywords alphabetically)

Ganoderma lingzhi; yellow-root disease; *Xylogone ganodermophthora*; multi-omics; pathogenic mechanism; disease-resistant breeding

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Session 14 Fungi Plant Interaction



Abstract

Progress in *Fusarium* systematics and *Fusarium* pathobiome**Shiling Han and Lei Cai *#**

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Fusarium species are among the most destructive crop pathogens, causing diseases such as ear rot, crown rot, and wilt diseases, while producing mycotoxins that threaten food safety. Although significant advances have been made in the taxonomy and pathogenic biology of this genus, critical questions persist concerning the delimitation of *Fusarium* and allied genera, the diversity of crop-associated *Fusarium* species, and the validity of the forma specialis concept. As a foundation, we reconstructed a genome-wide species tree using 1,001 homologous loci from 228 genomes, including 40 newly sequenced in this study. The resulting phylogeny strongly supports the “narrow” generic concept of *Fusarium* (= *Gibberella*). Building on this framework, we conducted a large-scale citizen science survey of diseased crops, including maize, rice, wheat, tomato, cucumber, and banana, across China’s major agricultural regions. A total of 2,679 *Fusarium* strains were isolated from 486 diseased crop samples. Using multi-locus phylogeny combined with morphological traits, these strains were identified to 64 species, including 15 newly described taxa. We also provide an updated global checklist of cereal-associated *Fusarium*, with 39 species newly recorded worldwide and 52 newly recorded in China. Pathogenicity assays following Koch’s postulates uncovered extensive cross-host infections, with up to 21 species associated with the same wilt disease. More than half of the diseased samples exhibited multiple species co-infections, revealing significant inconsistencies with the traditional forma specialis framework. These findings challenge the long-standing paradigm of host specificity and support a shift towards a “pathobiome” perspective, where disease dynamics are largely driven by community-level interactions rather than single pathogens. Our study offers one of the most comprehensive assessments of *Fusarium* diversity in agricultural systems to date. It not only clarifies the phylogenetic relationships and global distribution of crop-associated *Fusarium*, but also fundamentally transforms our understanding of host-pathogen interactions, thereby contributing to the development of more sustainable and ecologically informed disease management strategies.

Keywords: Diversity, Forma specialis, Pathobiome, Pathogenicity, Phylogeny

Abstract

Tracking plant pathogen emergence using thousand-genome panels**Daniel Croll***Institute of Biology, University of Neuchâtel, Switzerland**daniel.croll@unine.ch*

Adaptation of plant pathogens proceeds at speeds that easily overwhelm the rate of resistant cultivar deployment and fungicide development. Low cultivar diversity and the application of single fungicides can exacerbate these dynamics. Understanding the molecular basis of pathogen adaptation is critical to define more sustainable containment strategies. Emerging traits such as virulence on resistant cultivars or fungicide resistance often appear in a geographically structured manner. Such geographic mosaics can be determined by regional variation in selection pressures or as a consequence of the pathogen's population history. I will introduce the use of very large panels of pathogen genomes from the same species. Such genome panels can recapitulate historic colonization patterns and reveal constraints on pathogen genetic diversity. In combination with phenotyping assays, large genome panels enable the tracking of fungicide resistance mutations across continents and reveal how effector gene loci are undergoing adaptive sequence rearrangements. Recent research has pointed to selfish elements (i.e. transposable elements) as key factors in pathogen evolution, facilitating adaptation to biotic and abiotic factors in their environment. Selfish elements, by nature, can also impact the integrity of genomes and lead to deleterious dynamics for the pathogen. In conclusion, large-scale genomic investigations of individual pathogen species unravel essential mechanisms of pathogen adaptation.

Keywords: Population genomics, crop pathogens, wheat disease

Abstract

Melatonin-Induced Reprogramming Enhances *Brassica juncea* Plant Defense and Reduces Phytopathogen *Alternaria brassicae* Virulence

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Brassica juncea (Indian mustard, brown mustard, or leaf mustard) is a vital oilseed and vegetable crop belonging to the family Brassicaceae. It is widely cultivated in South Asia, including India, as well as in parts of China and Europe. In India, it is the second most important oilseed crop after groundnut, contributing significantly to edible oil production. Its productivity is severely constrained by *Alternaria brassicae*, a major fungal phytopathogen responsible for significant yield losses worldwide. In this study, we attempted to elucidate the protective role of exogenous melatonin (MT) in reprogramming defense responses of *Brassica juncea* plants, with a particular focus on mitigating the virulence of the fungal phytopathogen *Alternaria brassicae*. The exogenous application of melatonin (200 μ M) markedly reduced disease severity in *B. juncea* by up to 75%. MT treatment delayed infection onset, minimized necrotic lesion development, and strengthened plant defense responses through enhanced chlorophyll and proline accumulation, improved water retention, and reduced oxidative stress.

Biochemical analyses further revealed lower levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content, alongside elevated activities of antioxidant enzymes, confirming MT's role in alleviating oxidative damage. In addition, HRMS-based metabolite profiling of *A. brassicae* grown in the presence of MT demonstrated extensive reprogramming of fungal metabolism, including increased production of antifungal metabolites and reduced levels of antioxidant and indole derivatives, indicating weakened fungal defense system and reduced pathogenicity.

Collectively, these findings provide clear evidence that melatonin exerts contrasting effects on host and pathogen by simultaneously reprogramming plant defense mechanisms and disrupting fungal metabolism. This work establishes melatonin as a promising eco-friendly antifungal molecule, offering a sustainable alternative to chemical fungicides for protecting *B. juncea* and potentially other crops.

Keywords: *Alternaria brassicae*; *Brassica juncea*; Melatonin; Oxidative stress

Abstract

Role of circadian clock in regulating pathogenicity in *Fusarium oxysporum*Qiaojia Lu¹, Muqun Yu¹, Xianyun Sun¹, Xiao Liu^{1*#}¹ Institute of Microbiology, Chinese Academy of Sciences, Beijing, China

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Abstract :

Circadian clocks have evolved to enhance the fitness of organisms by improving their ability to adapt to daily environmental changes. Circadian clocks are known to modulate host immune responses to pathogen infections, yet their role in influencing pathogen pathogenesis remains unclear. To investigate the specific role of the circadian clock in pathogenic fungi, we examined the presence of a functional circadian clock in *Fusarium oxysporum*, which has multiple circadian clock genes *frqs* due to gene duplication events, with *Fofrq1* being the primary circadian clock gene. The pathogenesis of *F. oxysporum* in plants is controlled by its circadian clock, with infections causing severe disease symptoms at dawn. Notably, disruption of clock genes dramatically reduces fungal pathogenicity. Mechanically, circadian clocks regulate fungal-plant interactions by controlling the expression of pathogenic factors, including FoZafA, which enables the pathogen to adapt to zinc starvation within the plant, and FoCzf1, which governs the production of the toxin fusaric acid. In summary, we elucidated the important function of the circadian clock in modulating the pathogenicity of *F. oxysporum* by regulating zinc starvation response and secondary metabolite production.

Keywords: Circadian clock, fungal-host interaction, *Fusarium oxysporum*, secondary metabolism, transcription factor

Abstract

Rice phyllospheric *Pantoea* spp. suppress blast and bacterial blight diseases

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Abstract

Background: Rice is a major food crop in China as well as Asia, yet its production is threatened by microbial diseases including blast disease caused by fungal pathogen (*Magnaporthe oryzae*) and bacterial blight caused by several bacterial pathogens. To screen for bacterial microbiota associated with rice blast occurrence, and/or contributing to disease resistance, we performed microbiota analysis with rhizosphere soil, root, stem, and leaf samples of blast susceptible (CO39) and resistant (Y33R) rice grown in a blast disease nursery garden.

Methods: Full-length 16S rRNA sequencing (PacBio) was used to characterize the rhizosphere/plant microbiota of blast-susceptible (CO39) and resistant (Y33R) rice. Bioinformatics analyses included ASV generation, taxonomic annotation, and diversity analyses (α/β -diversity, LEfSe). *Pantoea* strains were isolated on TSB medium and identified via phylogenetic analysis of 16S rRNA/housekeeping genes and ANI. Blast (*Magnaporthe oryzae*) and bacterial blight (*Xanthomonas oryzae* pv. *Oryzae* and *Pantoea ananatis* SC7) were induced by conidial spraying or leaf clipping. The biocontrol efficacy of A25-11 (*P. ananatis* consortium) and B10-A1 (*P. dispersa*) was tested in greenhouse and field trials (Yangjiang Station). Immune priming was assessed by RT-qPCR (*OsPRI0*, *PBZ1*, *NPRI*), ROS detection (DAB/chemiluminescence), and SA pathway inhibition (AIP). Plant growth-promoting traits (IAA, phosphate solubilization, nitrogen fixation, and siderophores production) were quantified by LC-MS/biochemical assays. Statistics: ANOVA, Student's t-test, PERMANOVA ($p < 0.05$).

Results: Our result showed no significant difference in microbiota of rhizosphere soil, root, or leaf between these two rice cultivars, but stem microbiota were significantly different. *Pantoea* spp. were enriched in stem of blast susceptible rice, suggesting that it may play a role after fungal

infection. A total of 822 bacterial strains were isolated from the phyllospheric (including leaf and stem) samples of Y33R and CO39 rice, among which 3 strains were identified as *P. ananatis* and 1 strain was identified as *P. dispersa*. Average Nucleotide Identity (ANI) analysis confirmed that A25-H1 and B10-A1 strain was *P. ananatis* and *P. dispersa* respectively. The *P. ananatis* consortium (A25-F1, A25-G1, and A25-H1 combination) A25-11 and *P. dispersa* strain B10-A1 displayed suppressive effect on blast disease when they were applied to the susceptible rice CO39. Although a *P. ananatis* strain SC7 has been reported to cause bacterial blight in rice, A25-11 or B10-A1 was non-pathogenic to rice under experimental conditions. Furthermore, they could also suppress bacterial blight caused by SC7 or *Xanthomonas oryzae* pv. *oryzae* strain Pxo99A. A25-11 and B10-A1 did not affect the growth of *M. oryzae* mycelia in confrontation culture, but induced transcription of rice immunity genes and promoted ROS accumulation, suggesting that the biocontrol effect of A25-11 or B10-A1 may lie on immunity priming. We further showed that A25-11 and B10-A1 possessed growth promoting capacity including indole 3-acetic acid (IAA) production, phosphate solubilization, nitrogen fixation, and siderophore production. Under field condition, the consortium A25-11 and strain B10-A1 could effectively suppress leaf and panicle blast.

Conclusions: Overall, this study established a microbiome method for identifying the rice bacterial communities of agricultural significance, with capacity of rice disease management and/or growth promotion.

Keywords: *Pantoea* spp.; phyllospheric bacterial microbiota; plant growth promoting (PGP) ; rice bacterial blight; rice blast disease.

Abstract

Harnessing Mycobiomes and Biotic Interactions for Crop Improvement and Climate Mitigation

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Mutualistic interaction with soil-borne fungi is a hallmark of a broad range of land plants which rely on such rhizosphere symbioses to assimilate nutrients and/or acquire stress tolerance. Using metagenomics, we identified the rhizosphere mycobiota associated with natural habitats and model plants *Arabidopsis*, rice, barley, wheat and soybean. Coupled with function-guided assays we isolated 20 beneficial fungal strains that individually or as SynComs enhance seed germination, plant growth, drought tolerance, and/or disease resistance, in addition to significant biomass and yield increase in target crops. Cell biology of the fungus-root interactions revealed 3 novel interfaces and abilities in inducing such robust growth. Untargeted metabolomics revealed an upregulation of important primary and secondary metabolites, such as sugars, sugar alcohols, phenylpropanoids, glyoxylate, and glucosinolates in the fungus-inoculated host plants. Biochemical analyses provided molecular insights into cross-kingdom transport of fungus-derived beneficial metabolites, phytohormones (mimics) and assimilated phosphate as important contributing factors for such robust growth in the host. The most significant yield increase (~50%) was observed in plants inoculated with a beneficial fungal endophyte, *Tinctoporellus species* isolate AR8, in nutrient-limiting soils. Mechanistically, AR8 colonized the root cortex/endosphere and channeled the metabolic flux to phenylpropanoids and requisite secondary metabolites to promote plant growth. Mycobiont-induced biosynthesis of auxin improved root growth and provided an intrinsic source for long-distance signaling that enhanced shoot biomass. Chemical complementation with exogenous p-coumaric acid restored auxin signaling and enhanced growth in endophyte-inoculated *pall* mutant, thus implicating the phenylpropanoid-auxin nexus as a novel regulator of symbiotic plant growth. Comparative metabolomics further established hydroxycinnamic acid and p-coumaric acid as major plant-growth-promoting hubs that bridge the phenylpropanoid and auxin signaling in the cross-kingdom symbiotic interaction model. Lastly, we will discuss how a novel growth-defense axis anchored by phenylpropanoids and auxin enables such symbiotic interaction and outcomes.

Keywords: Fungal endophyte, Plant-fungus interaction, Phenylpropanoids, Symbiosis

Abstract

Bioprospecting of soil microbiota for the improvement of degraded agricultural lands

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Soil is a diverse ecosystem that harbors a large microbial biomass that plays a key role in biogeochemical cycles. Fungi, through their coenzymatic activities, decompose organic matter and produce byproducts that are useful for bacteria. This facilitates the biotransformation of elements such as phosphorus (P), nitrogen (N), carbon (C), and sulfur (S), making them available for plants. However, intensive agricultural practices such as monocultures and the use of agrochemicals alter the biogeochemical properties of soil, increasing erosion and salinization. This leads to desertification and infertility. Therefore, the accelerated loss of soil fertility due to land use change to intensive agricultural systems must be mitigated to ensure sustainable food security and resilient ecosystems. This study evaluated the effect of interactions between soil bacteria and fungi *in vitro* and *in situ* conditions, using samples collected from a managed site with agroecological practices in Mexico, aiming to improve the biogeochemical properties of the soil. Soil samples were collected from a chamomile (*Matricaria chamomilla*) plot during the flowering phenological stage. Eleven microorganisms (7 fungi and 4 bacteria) were isolated and variables such as pH, moisture, and total and dissolved forms of N, C, and P, as well as enzymatic activity, were characterized. Microorganisms were identified, and *in vitro* bioassays were conducted using Biolog plates. Interaction bioassays were conducted to design microbial consortia for *in situ* experiments. Finally, *in situ* bioassays were conducted in pots with chamomile plants to evaluate changes in soil biogeochemical properties and plant development in response to the microbial consortia. A broad metabolic range was observed among the microorganisms, particularly we detected isolates able to degrade substrata that are related to essential nutrient cycles, especially nitrogen. Moreover, the establishment of synergies between bacteria and fungi was mainly recorded. For instance, the interaction between *Mortierellaceae* sp. and *Bacillus* sp. 3. In addition, this consortium promoted the plant development and a prominent production of biomass. This work evidenced that the interactions between soil bacteria and fungi represent a promising tool for improvement the biogeochemical properties of agricultural soils and development of medicinal plants in Mexico.

Keywords: biogeochemical cycles; coenzymatic activity; interactions; agroecological practices

Abstract

From Soil to Roots: Molecular Insights into Fungal Diversity and Ecology of Endangered Chinese Cycads (Cycadales)

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Root-associated fungi, including fungal endophytes and arbuscular mycorrhiza, play key roles in plant ecology and function, yet remain poorly characterized in ancient gymnosperm like the Cycads. We investigated herein the fungal communities in three distinct niches: coralloid roots, rhizosphere soil, and bulk soil of Chinese cycads, including the endemic species *Cycas debaoensis* and *C. fairylakea*. Samples were collected across four sites: a botanical garden, a reintroduction site, and two natural habitats. Molecular metabarcoding based on *ITS* gene region was performed while soil physicochemical properties were measured and correlated with fungal community composition. A total of 17,234 fungal operational taxonomic units (OTUs) were detected with communities dominated by Ascomycota, followed by Basidiomycota and Mortierellomycota. Arbuscular mycorrhizal fungi, under the phylum Glomeromycota, were detected exclusively in roots at specific sites highlighting the presence of mycorrhizal component. The coralloid roots were enriched with several species of fungal endophytes such as *Gliocladiopsis*, *Metacordyceps*, and *Trechispora*. Soils, on the other hand, harboured mainly generalist and saprotrophic fungi, including *Mortierella*, *Archaeorhizomyces*, and *Penicillium*. Alpha diversity was higher in soils than in roots, and beta diversity analyses revealed clear niche- and site-specific clustering. Co-occurrence network analyses showed tightly modular, positive associations among root endophytes, intermediate complexity in rhizosphere networks, and highly connected bulk soil networks with more negative interactions indicating possible cooperation and competition across niches. These findings highlight that habitat filtering and niche differentiation may shape fungal communities in endangered cycads with roots supporting unique endophytic communities while soils favouring generalists and competitive taxa. Overall, our study reveals diverse fungal communities and ecological patterns, providing insights into their implications for the conservation of cycads.

Keywords: Cycadales, fungal diversity, habitat filtering, *ITS* genes, metagenomics

Abstract

A Novel Mitochondrial Regulon for Ferroptosis During Fungal Pathogenesis

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Ferroptosis remains an underexamined iron- and lipid peroxides-driven cell death modality despite its importance to several human and plant diseases and to immunity thereof. Here, we utilized chemical cell biology, molecular genetics and biochemical analyses to gain insights into how the fungal pathogen *Magnaporthe oryzae* undergoes ferroptosis strictly in the spore cells to successfully transit to infectious development. We reveal a complex functional interdependency and crosstalk between intrinsic ferroptosis and autophagy-mediated mitochondrial degradation. Mechanistically, the requirement of mitophagy for ferroptotic cell death was attributed to its ability to maintain a pool of metabolically active mitochondria. Pharmacological disruption of the electron transport chain or membrane potential led to complete inhibition of ferroptosis, thus simulating the loss of mitophagy phenotypes. Conversely, increased mitochondrial membrane potential in a mitophagy-defective mutant alleviated the ferroptosis defects therein. Graded inhibition of mitochondrial coenzyme Q biosynthesis with or without ferroptosis inhibitor liproxstatin-1 distinguished its antioxidant function in such regulated cell death. Membrane potential-dependent regulation of ATP synthesis and iron homeostasis, as well as dynamics of tricarboxylic acid cycle enzyme AcoA (aconitase A) in the presence or absence of mitophagy, mitochondrial poisoning or iron chelation further linked mitochondrial metabolism to ferroptosis. Last, we present an important bioenergetics- and redox-based mitochondrial regulon essential for intrinsic ferroptosis and its precise role in fungal pathogenesis leading up to the establishment of the devastating rice blast disease.

Keywords: Cell death; iron; mitochondrial metabolism; mitophagy; rice blast

Abstract

Inhibitory Effects and Mechanism of the Volatile Compound 2-Phenylethanol from Mango on *Colletotrichum siamense*

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Mango anthracnose is one of the major pre- and post-harvest diseases of mango, which occurs in all mango-producing areas in the world and causes substantial economic losses. At present, mango anthracnose is mainly controlled by chemicals, but the types of chemical pesticides used for mango postharvest anthracnose control are limited. 2-Phenylethanol is an aromatic alcohol compound with several biological activities and has antifungal activity against anthracnose. In the study, 2-PE was identified from the volatile compounds of diseased mango and was absent in healthy ones. *In vitro* assays demonstrated strong inhibitory effects of 2-PE fumigation on mycelial growth and conidial germination of *C. siamense*, with EC₅₀ values of 60.36 $\mu\text{L/L}$ and 60.82 $\mu\text{L/L}$, respectively. Morphological and ultrastructural changes, including hyphal deformation and organelle degradation, were observed under light and transmission electron microscopy. The mortality rate of conidia of *C. siamense* was 100% after six hours of 4000 $\mu\text{L/L}$ 2-PE immersion. *In vivo* fumigation and immersion treatments significantly reduced disease incidence in mango fruits, showing 60.11% and 75.99% efficacy, respectively. Fumigation with 2-PE had an inhibitory effect on conidial germination on mango fruits. RNA-seq analysis revealed 3135 differentially expressed genes in *C. siamense* under 2-PE stress, with significant enrichment in ribosome biosynthesis and amino acid metabolism pathways. Furthermore, the knockout of the ergosterol biosynthesis gene *CsErg6*, achieved via homologous recombination, reduced sensitivity to 2-PE, while complementation restored wild-type phenotypes, suggesting that *CsErg6* is a potential target. In this study, the relationship between 2-PE and the *CsErg6* gene was investigated at the gene level, which lays the foundation for exploring the target of 2-PE's action.

Keywords: Mango, Volatile substances, 2-phenylethanol, *Colletotrichum siamense*, Gene knockout

Abstract

The Interactions between *Peronophythora litchii* Cell Wall Degrading Enzymes and Host Immunity

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Abstract: Background: *Peronophythora litchii* (oomycete) causes litchi downy blight, relying on cell wall-degrading enzymes (CWDEs) for infection. However, how its CWDEs interact with litchi immunity remains unclear; this study aimed to address this. Methods: Yeast two-hybrid, Co-IP, GST pull-down, CRISPR/Cas9, VIGS, and RT-qPCR were used to identify CWDE-host protein interactions and analyze their roles in virulence and immunity. Results: 1) *P. litchii* PIPAE5 (pectin acetylesterase) interacts with litchi LcLTP1, destabilizing LcLTP1 to suppress SA signaling; 2) PIPeL1/PIPeL1-like (pectate lyases, PeLs) enhance virulence via enzymatic activity, while litchi LcPIP1 binds them to induce SERK3-dependent immunity; 3) PIPeL8 (PeL) triggers cell death via NbSOBIR1/NbMEK2, and litchi LcAP1 suppresses this to boost resistance. Conclusion: These findings reveal *P. litchii* CWDEs' dual roles (virulence/immunity induction) and host countermechanisms, providing targets for litchi disease-resistant breeding.

Keywords: Cell wall-degrading enzymes, *Peronophythora litchii*, Plant immunity, Salicylic acid signaling, Virulence factor

Abstract

The study of LtmilR2 in regulating pathogenicity of *Lasiodiplodia theobromae* to promote infection.

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Lasiodiplodia theobromae (*L. theobromae*) is the causative agent of grapevine canker disease which is serious threat to global grape production. Currently, no effective pesticides are available to manage this disease. In this study, LtmilR2 was identified as an Argonaute1 (AGO1)-dependent small RNA produced by *L. theobromae*. Functional analysis revealed that both LtAGO1 and LtmilR2 negatively regulate the pathogenicity of *L. theobromae*. Through degradome sequencing, we identified *LtRASGEF*—a guanine nucleotide exchange factor involved in RAS signaling—as the target gene of LtmilR2. LtmilR2 was confirmed to be able to suppress the expression of *LtRASGEF*. Pathogenicity assays demonstrated that LtRASGEF is essential for fungal virulence. Furthermore, following infection, the expression of LtmilR2 rapidly decreased, while *LtRASGEF* expression quickly increased and positively regulated infection. Finally, we observed that the growth of *L. theobromae* was inhibited when an RNA duplex targeting LtmilR2 was delivered via the star polycation (SPc) nanocarrier, suggesting that LtmilR2 may serve as an RNA-based pesticide target for grapevine canker disease management.

Keywords: Grapevine canker disease; *Lasiodiplodia theobromae*; plant immunity; miRNA; RNA pesticide

Abstract

Ferroptosis as a Conserved Pathogenicity Switch in Plant-Pathogenic *Colletotrichum* spp.

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Abstract:

Background: Anthracnose, caused by hemibiotrophic fungi of the genus *Colletotrichum*, is a globally prevalent and destructive plant disease. The appressorium, a specialized infection structure derived from the conidium, is essential for host penetration.

Methods: We investigated the role of ferroptosis in the pathogenicity of *Colletotrichum higginsianum*. Matrix-assisted laser desorption/ionization time-of-flight imaging mass spectrometry (MALDI-TOF IMS) identified pyochelin as the antifungal compound secreted by the biocontrol bacterium BX1. Scanning electron microscopy (SEM), live-cell staining, and live-cell fluorescence imaging were used to monitor conidial programmed cell death during appressorium maturation. Calcein-AM and C11-BODIPY^{581/591} fluorescent probes were employed to visualize intracellular ferric ions and lipid peroxides, while transmission electron microscopy (TEM) was used to assess mitochondrial ultrastructure, confirming ferroptosis. Functional analyses of iron chelation, chemical ferroptosis inhibition, and *ChNOX2* gene deletion were performed, and pathogenicity was evaluated using detached leaf and potted plant assays.

Results: Conidial cell death began in cells farthest from the appressorium and progressed sequentially. This ferroptotic process depended on ferric ions and lipid peroxidation. Iron chelation or ferroptosis inhibition preserved conidial viability, impaired appressorial invasion, and significantly reduced pathogenicity. *ChNOX2* deletion reduced lipid peroxidation, inhibited ferroptosis, lowered turgor pressure, and nearly abolished pathogenicity. Similar ferroptotic patterns were detected in other pathogenic *Colletotrichum* species, indicating genus-wide conservation.

Conclusion: Our findings first identify ferroptosis as a conserved pathogenicity switch in *Colletotrichum* spp., reveal the antifungal mechanism of a pyochelin-producing biocontrol bacterium, and highlight ferroptosis inhibitors as potential broad-spectrum fungicides.

Keywords: conidial cell death, ferroptosis, *Colletotrichum higginsianum*, NOX, pathogenicity

Abstract

The Interactions between *Peronophythora litchii* Cell Wall Degrading Enzymes and Host Immunity

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Abstract: Background: *Peronophythora litchii* (oomycete) causes litchi downy blight, relying on cell wall-degrading enzymes (CWDEs) for infection. However, how its CWDEs interact with litchi immunity remains unclear; this study aimed to address this. Methods: Yeast two-hybrid, Co-IP, GST pull-down, CRISPR/Cas9, VIGS, and RT-qPCR were used to identify CWDE-host protein interactions and analyze their roles in virulence and immunity. Results: 1) *P. litchii* PIPAE5 (pectin acetylesterase) interacts with litchi LcLTP1, destabilizing LcLTP1 to suppress SA signaling; 2) PIPeL1/PIPeL1-like (pectate lyases, PeLs) enhance virulence via enzymatic activity, while litchi LcPIP1 binds them to induce SERK3-dependent immunity; 3) PIPeL8 (PeL) triggers cell death via NbSOBIR1/NbMEK2, and litchi LcAP1 suppresses this to boost resistance. Conclusion: These findings reveal *P. litchii* CWDEs' dual roles (virulence/immunity induction) and host countermechanisms, providing targets for litchi disease-resistant breeding.

Keywords: Cell wall-degrading enzymes, *Peronophythora litchii*, Plant immunity, Salicylic acid signaling, Virulence factor

Abstract

Role of circadian clock in regulating pathogenicity in *Fusarium oxysporum*

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Abstract :

Circadian clocks have evolved to enhance the fitness of organisms by improving their ability to adapt to daily environmental changes. Circadian clocks are known to modulate host immune responses to pathogen infections, yet their role in influencing pathogen pathogenesis remains unclear. To investigate the specific role of the circadian clock in pathogenic fungi, we examined the presence of a functional circadian clock in *Fusarium oxysporum*, which has multiple circadian clock genes *frqs* due to gene duplication events, with *Fofrq1* being the primary circadian clock gene. The pathogenesis of *F. oxysporum* in plants is controlled by its circadian clock, with infections causing severe disease symptoms at dawn. Notably, disruption of clock genes dramatically reduces fungal pathogenicity. Mechanically, circadian clocks regulate fungal-plant interactions by controlling the expression of pathogenic factors, including FoZafA, which enables the pathogen to adapt to zinc starvation within the plant, and FoCzf1, which governs the production of the toxin fusaric acid. In summary, we elucidated the important function of the circadian clock in modulating the pathogenicity of *F. oxysporum* by regulating zinc starvation response and secondary metabolite production.

Keywords: Circadian clock, fungal-host interaction, *Fusarium oxysporum*, secondary metabolism, transcription factor

Abstract

Rice phyllospheric *Pantoea* spp. suppress blast and bacterial blight diseases

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Abstract

Background: Rice is a major food crop in China as well as Asia, yet its production is threatened by microbial diseases including blast disease caused by fungal pathogen (*Magnaporthe oryzae*) and bacterial blight caused by several bacterial pathogens. To screen for bacterial microbiota associated with rice blast occurrence, and/or contributing to disease resistance, we performed microbiota analysis with rhizosphere soil, root, stem, and leaf samples of blast susceptible (CO39) and resistant (Y33R) rice grown in a blast disease nursery garden.

Methods: Full-length 16S rRNA sequencing (PacBio) was used to characterize the rhizosphere/plant microbiota of blast-susceptible (CO39) and resistant (Y33R) rice. Bioinformatics analyses included ASV generation, taxonomic annotation, and diversity analyses (α/β -diversity, LEfSe). *Pantoea* strains were isolated on TSB medium and identified via phylogenetic analysis of 16S rRNA/housekeeping genes and ANI. Blast (*Magnaporthe oryzae*) and bacterial blight (*Xanthomonas oryzae* pv. *Oryzae* and *Pantoea ananatis* SC7) were induced by conidial spraying or leaf clipping. The biocontrol efficacy of A25-11 (*P. ananatis* consortium) and B10-A1 (*P. dispersa*) was tested in greenhouse and field trials (Yangjiang Station). Immune priming was assessed by RT-qPCR (*OsPRI0*, *PBZ1*, *NPRI*), ROS detection (DAB/chemiluminescence), and SA pathway inhibition (AIP). Plant growth-promoting traits (IAA, phosphate solubilization, nitrogen fixation, and siderophores production) were quantified by LC-MS/biochemical assays. Statistics: ANOVA, Student's t-test, PERMANOVA ($p < 0.05$).

Results: Our result showed no significant difference in microbiota of rhizosphere soil, root, or leaf between these two rice cultivars, but stem microbiota were significantly different. *Pantoea* spp. were enriched in stem of blast susceptible rice, suggesting that it may play a role after fungal

infection. A total of 822 bacterial strains were isolated from the phyllospheric (including leaf and stem) samples of Y33R and CO39 rice, among which 3 strains were identified as *P. ananatis* and 1 strain was identified as *P. dispersa*. Average Nucleotide Identity (ANI) analysis confirmed that A25-H1 and B10-A1 strain was *P. ananatis* and *P. dispersa* respectively. The *P. ananatis* consortium (A25-F1, A25-G1, and A25-H1 combination) A25-11 and *P. dispersa* strain B10-A1 displayed suppressive effect on blast disease when they were applied to the susceptible rice CO39. Although a *P. ananatis* strain SC7 has been reported to cause bacterial blight in rice, A25-11 or B10-A1 was non-pathogenic to rice under experimental conditions. Furthermore, they could also suppress bacterial blight caused by SC7 or *Xanthomonas oryzae* pv. *oryzae* strain Pxo99A. A25-11 and B10-A1 did not affect the growth of *M. oryzae* mycelia in confrontation culture, but induced transcription of rice immunity genes and promoted ROS accumulation, suggesting that the biocontrol effect of A25-11 or B10-A1 may lie on immunity priming. We further showed that A25-11 and B10-A1 possessed growth promoting capacity including indole 3-acetic acid (IAA) production, phosphate solubilization, nitrogen fixation, and siderophore production. Under field condition, the consortium A25-11 and strain B10-A1 could effectively suppress leaf and panicle blast.

Conclusions: Overall, this study established a microbiome method for identifying the rice bacterial communities of agricultural significance, with capacity of rice disease management and/or growth promotion.

Keywords: *Pantoea* spp.; phyllospheric bacterial microbiota; plant growth promoting (PGP) ; rice bacterial blight; rice blast disease.

Abstract

The Mechanism of Microbiome-Pathogen Fungus Interactions in Regulating Sugarcane Smut

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Sugarcane is a major economic crop in tropical and subtropical regions worldwide, whose healthy growth is closely associated with the microbiome. However, the interactions among sugarcane, pathogens, and the microbiome remain poorly understood, and the influence of microbial community structure on disease development is unclear. This study integrated metagenomic sequencing and culturomics to systematically analyze the rhizosphere soil, roots, and infected whips of healthy and diseased sugarcane plants. Alpha diversity analysis revealed that microbial diversity was highest in soil and decreased progressively from soil to roots and then to infected whips. Principal coordinate analysis (PCoA) further indicated significant differences in microbial community structure across these niches. Differential abundance analysis using STAMP showed significant enrichment of genera such as *Microbacterium* and *Pantoea* in infected whip tissues, which are functionally associated with plant growth regulation and pathogenesis, suggesting their potential role in the infection process of *Sporisorium scitamineum*. Through culturomics-based isolation and high-throughput 16S rRNA gene sequencing, multiple bacterial isolates, including *Pantoea*, were obtained. In vitro confrontation assays demonstrated that *Pantoea* significantly inhibits the mycelial growth of *S. scitamineum* and exhibits plant growth-promoting traits, results that were further validated in pot experiments. This study preliminarily reveals the shifts in the microbiome during disease development and their potential functional implications. Future work will employ molecular approaches to elucidate the biocontrol mechanisms of *Pantoea*, with the aim of explaining the pathogenic mechanisms of sugarcane smut from a pathogen–host–microbiome interaction perspective. These findings provide a theoretical basis and technical support for harnessing beneficial microbial resources and developing microbiome-based strategies for sustainable disease management.

Keywords: Sugarcane smut; Metagenomics; Microbiome; High-throughput isolation and cultivation

Abstract

Differential Impacts of Nitrogen Deposition and Litter Addition on Root-Associated Fungal Communities in a Subtropical *Pinus massoniana* Forest**Jiuchun Wu¹, Guiyun Yuan¹, Feng Jiang¹, Chao Shen¹, Xueguang Sun¹***¹Institute for Forest Resources & Environment of Guizhou, Guizhou Key Laboratory of Forest Cultivation in Plateau Mountain, College of Forestry, Guizhou University, Guiyang, 550025, China*

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Climate change is increasing forest litterfall, while anthropogenic nitrogen (N) deposition has risen sharply, especially in subtropical regions. Both nutrient inputs alter soil chemistry and microbial dynamics. However, their distinct effects on root-associated fungal communities—crucial for plant nutrient acquisition and ecosystem functioning—are poorly understood. In a subtropical *Pinus massoniana* forest, we applied short-term treatments of N additions (0, 5, and 10 g·m⁻²·a⁻¹) and litter addition, and measured soil nutrients, enzyme activities, and root fungal community structure. Soil pH and hydrolyzable N increased under high N treatment, while available phosphorus (AP) decreased across all N and litter additions. Enzyme activities (urease, β -glucosidase, and laccase) were enhanced by certain N and litter treatments. High-throughput ITS sequencing yielded 603 unique fungal ASVs, dominated by ectomycorrhizal (ECM) and saprotrophic taxa. After 6 months, moderate N (5 g·m⁻²·a⁻¹) and litter treatments boosted symbiotrophic fungi abundance, with no change under high N. By 12 months, both moderate N and litter treatments elevated symbiotrophs and suppressed saprotrophs. Overall fungal alpha diversity remained largely stable, though species richness (Chao1, ACE) declined under moderate N by 12 months. Community composition diverged significantly under N5 and litter treatments compared to controls. Linear discriminant analysis highlighted distinct biomarkers (e.g. *Russula murrillii* under moderate N, *Lactarius salmonicolor* under high N, and *R. cremicolor* under litter). Structural equation modeling revealed that soil phosphorus (total and available) and enzyme activities (urease, β -glucosidase) were primary drivers of community shifts. These results demonstrate that increased litterfall and N deposition have contrasting influences on root fungal assemblages by modifying soil resources and functional traits, highlighting complex soil–fungus interactions in subtropical forests.

Keywords: nitrogen deposition, litter addition, root fungal communities, ectomycorrhizal fungi

Abstract

Melatonin-Induced Reprogramming Enhances *Brassica juncea* Plant Defense and Reduces Phytopathogen *Alternaria brassicae* Virulence

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Brassica juncea (Indian mustard, brown mustard, or leaf mustard) is a vital oilseed and vegetable crop belonging to the family Brassicaceae. It is widely cultivated in South Asia, including India, as well as in parts of China and Europe. In India, it is the second most important oilseed crop after groundnut, contributing significantly to edible oil production. Its productivity is severely constrained by *Alternaria brassicae*, a major fungal phytopathogen responsible for significant yield losses worldwide. In this study, we attempted to elucidate the protective role of exogenous melatonin (MT) in reprogramming defense responses of *Brassica juncea* plants, with a particular focus on mitigating the virulence of the fungal phytopathogen *Alternaria brassicae*. The exogenous application of melatonin (200 μ M) markedly reduced disease severity in *B. juncea* by up to 75%. MT treatment delayed infection onset, minimized necrotic lesion development, and strengthened plant defense responses through enhanced chlorophyll and proline accumulation, improved water retention, and reduced oxidative stress.

Biochemical analyses further revealed lower levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content, alongside elevated activities of antioxidant enzymes, confirming MT's role in alleviating oxidative damage. In addition, HRMS-based metabolite profiling of *A. brassicae* grown in the presence of MT demonstrated extensive reprogramming of fungal metabolism, including increased production of antifungal metabolites and reduced levels of antioxidant and indole derivatives, indicating weakened fungal defense system and reduced pathogenicity.

Collectively, these findings provide clear evidence that melatonin exerts contrasting effects on host and pathogen by simultaneously reprogramming plant defense mechanisms and disrupting fungal metabolism. This work establishes melatonin as a promising eco-friendly antifungal molecule, offering a sustainable alternative to chemical fungicides for protecting *B. juncea* and potentially other crops.

Keywords: *Alternaria brassicae*; *Brassica juncea*; Melatonin; Oxidative stress

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Abstract

Effects on the Sporogenesis and Biocontrol functions of *Trichoderma* spp. by the mycoviruses

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Trichoderma spp. are known to impact host plant growth and defense activities. With the findings of the mycoviruses from *Trichoderma* spp., more and more effects on the sporogenesis and biocontrol functions of host induced by mycoviruses were known. In our research, *Trichoderma harzianum* partitivirus 2 (ThPV2) from *T. harzianum* strain (T673) showed higher mycelial density, conidiospore, and chlamydospore production than in the virus-infected strain T673-F, as well as moderately but statistically significantly improved biocontrol activity when compared with strain T673-F in the experiments with cucumber seeds inoculated with *Fusarium oxysporum* f. sp. *cucumerinum*. *Trichoderma harzianum* mycovirus 1 (ThMV1) from *T. harzianum* strain (T525) not only affected the phenotype of the host strain but also reduces its biocontrol function on *F. oxysporum* f. sp. *cucumerinum*. Using the yeast two hybrid nuclear system to screen, 57 proteins involving the interaction between T525 and ThMV1 have been obtained, among which 40 proteins interacted with ThMV1-RdRP, 29 proteins interacted with ThMV1-CP and 12 proteins interacted with both ThMV1-RdRP and ThMV1-CP. For the hexokinase1 (HXK) also was the interacted protein with ThMV1-CP, the hexokinase gene (*hvk*) knockout mutant (T525^{-hvk}) and reverse mutant (T525^{Rhvk}) of T525 were obtained. connecting the results of transcriptomic analysis, Real-time RT-PCR and the phenotype of cucumber, the interaction of ThMV1-CP/HXK in T525 was proved to negatively regulate the disease resistance pathway of T525 and positively regulate the growth promotion effect of T525 in the cucumber.

Keywords: *Trichoderma* spp.; mycovirus; effect; interaction

Abstract

Fusarium solani (HC01) enhances rapid agarwood resin production with a medically accepted level of agarotetrol

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Abstract

Agarwood, a valuable spice and traditional medicine, is appreciated not only for its unique aroma and pharmacological properties but also for its very high economic value. However, as wild agarwood resources become increasingly scarce, artificial induction methods have emerged as an alternative, with fungal induction proving relatively effective. This study selected *Fusarium solani* (HC01), isolated from naturally occurring agarwood resin in Guangdong Province, as a candidate fungus for promoting agarwood formation using the agar-wit method. From the results, resin formation was observed after 15 days of inoculation. By the sixth month, the agarotetrol content of the produced agarwood resin exceeded the pharmaceutical standard. At the same time, levels of several key secondary metabolites were significantly elevated compared to the control. This discovery is promising that *F. solani* (HC01) can reliably and quickly stimulate the production of agarwood resin. The product's quality meets medicinal standards, opening up promising opportunities for its use. In addition, this strain could be a key contributor to research and industrial use of artificial induction of agarwood, offering a new perspective on this field.

Keywords: biotechnology, fungus for promoting *Aquilaria* plants, green agriculture, sustainable method, Thymelaeaceae

Abstract

Identification of a *Coprinellus radians* Isolate and Its Role in Selenium Nanoparticle Biosynthesis

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Abstract: At present, numerous studies have focused on the enrichment of trace element selenium by edible and medicinal fungi, aiming to further enhance their nutritional value and biological efficacy. To explore fungal resources with selenium nanoparticle (SeNPs)-transforming capability, a fungal strain J2 was isolated from the wood of *Dalbergia odorifera* (Jiang zhen xiang). Morphological observations revealed that strain J2 had a yellow colony center, with colorless or pale yellow filamentous hyphae presenting radial long strip edges; the hyphae gradually turned yellow and eventually brown. The spores of J2 were smooth and dark brown. Based on ITS identification and whole-genome draft sequencing, strain J2 was identified as *Coprinellus radians*. Using sodium selenite as the selenium source, the SeNPs transformation and yield characteristics of strain J2 were investigated. The results showed that strain J2 could produce SeNPs in media containing 1, 3, 6, and 9 mmol/L sodium selenite. After 15 days of cultivation, the selenium transformation rates were 94.48%, 98.59%, 62.01%, and 46.67%, respectively, and the corresponding SeNPs yields were 22.16 g/kg, 98.40 g/kg, 138.88 g/kg, and 113.63 g/kg, respectively. Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS) analyses demonstrated that the intracellular SeNPs were spheroid-like or disc-shaped, with good dispersibility and a particle size range of 200–500 nm. The mechanism underlying SeNPs synthesis by this strain was determined via transcriptomic and proteomic approaches. This study provides a theoretical basis and strain resource for the application of *Coprinellus radians* in the biological synthesis of SeNPs. As a natural microbial resource, its metabolic product (SeNPs) is easily absorbed and utilized by crops, which is highly consistent with the development needs of green agriculture and provides a new path for the implementation of functional agriculture.

key words: *Coprinellus radians*; Selenium Nanoparticles; conversion rate

Abstract

Antifungal Nanoparticle and agents against Citrus rot of Sweet Orange

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Sweet orange (*Citrus sinensis*) is a key fruit crop in Nigeria and beyond, prized for its nutritional and for economic value. However, post-harvest fungal rot, caused by *Aspergillus flavus* and *Lasiodiplodia theobromae*, causes 20–50% losses, endangering production. Synthetic fungicides, while effective, risk environmental harm, health hazards, and resistance, prompting the need safer alternatives. This study assessed *Trichoderma asperellum*, *Ocimum gratissimum* essential oil (EO), and biosynthesized silver nanoparticles (AgNPs) as antifungal agents. Both fungi showed high virulence (74–75% rot incidence) with strongly inhibited *A. flavus* (27.89–65.81 mm) but not *L. theobromae*. Also EO outperformed mancozeb, inhibiting *L. theobromae* (45.74–61.68 mm at 0.25–1.00 µg/ml vs. 25.33 mm at 2 µg/ml) and *A. flavus* (14.01–38.66 mm). AgNPs were highly effective, suppressing *L. theobromae* (64.33 mm at 12.5 µg/ml) better than mancozeb (36.33 mm at 50 µg/ml). AgNPs were stable, crystalline (54.87 nm), and capped with bioactive compounds. EO's potency stemmed from terpenoids (e.g., linalool) and phenolics (e.g., eugenol). These results highlight *T. asperellum*, EO, and AgNPs as sustainable alternatives to synthetic fungicides for controlling citrus post-harvest rot, promoting eco-friendly agriculture.

Keywords: Antifungal activity, *Citrus sinensis*, Oils, Rot, AgNPs, *Trichoderma asperellum*

Abstract

ANTIBACTERIAL NANOPARTICLES FROM THE AQUEOUS EXTRACT OF *Ganoderma sessile*

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Ganoderma is a genus of mushrooms with a repository of rich medicinal properties. In this study, *Ganoderma sessile* was grown, and the water extract was used to synthesize silver nanoparticles (AgNPs). and the nanoparticles produced were screened for antibacterial activity. Gas chromatography- Mass spectroscopy analysis (GC-MS) of the extract was also determined to find out possible constituents of the mushroom responsible for the synthesis and activity. Nanoparticle synthesis was done under room temperature and at 60°C and nanoparticle was characterized using Ultraviolet -Visible spectroscopy (UV-Vis) and Fourier transform infra spectroscopy (FTIR). Results showed that nanoparticles precipitated at both temperatures. The synthesized AgNPs at room temperature exhibited a strong antibacterial activity against Gram-negative bacteria (*Escherichia coli* and *Pseudomonas* spp.) and Gram-positive bacterium (*Staphylococcus aureus*). It showed the strongest inhibition against *Staphylococcus aureus* at 11.00 ± 5.66 mm on the third day of the test. It also performed better than the standard (amoxicillin) against *Pseudomonas* species with 7.50 ± 0.71 mm zone of inhibition. UV-Vis spectra showed absorption peaks between 330 -390 nm and 330-395nm for nanoparticles at room temperature and 60°C respectively which fall within the standard range. The FTIR showed peaks at wavelength $1636.30131 \text{ cm}^{-1}$; known as conjugated stretching of the carbonyl and C=C double bond group due to diatomic unit vibrations. Excellent aromatic ring stretching and 2102.21854 for terminal alkyne C $\equiv$ C stretch and the peak $3317.33068 \text{ cm}^{-1}$ which is a broad band spectrum representing stretching of hydroxyl group. Major peaks produced by the mushroom in the GC-MS analysis include these terpenoids and other secondary metabolites Cyclopropanoethanal, 2-octyl-trans-2-Decen-1-ol, trifluoroacetate, 4-(6-Methyl-4-methylene-3, 4, 5, 6-tetrahydro-2H-pyran-2-yl)-1-butanol, Octadecanoic acid, Ethyl Oleate, n-Hexadecanoic acid which could be responsible for the synthesis and activity of the nanoparticles.

Keywords: Antibacterial, *Ganoderma*, Metabolites, Nanoparticle and Spectroscopy

Abstract

The Interactions between *Peronophythora litchii* Cell Wall Degrading Enzymes and Host Immunity

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Abstract: Background: *Peronophythora litchii* (oomycete) causes litchi downy blight, relying on cell wall-degrading enzymes (CWDEs) for infection. However, how its CWDEs interact with litchi immunity remains unclear; this study aimed to address this. Methods: Yeast two-hybrid, Co-IP, GST pull-down, CRISPR/Cas9, VIGS, and RT-qPCR were used to identify CWDE-host protein interactions and analyze their roles in virulence and immunity. Results: 1) *P. litchii* PIPAE5 (pectin acetyltransferase) interacts with litchi LcLTP1, destabilizing LcLTP1 to suppress SA signaling; 2) PIPeL1/PIPeL1-like (pectate lyases, PeLs) enhance virulence via enzymatic activity, while litchi LcPIP1 binds them to induce SERK3-dependent immunity; 3) PIPeL8 (PeL) triggers cell death via NbSOBIR1/NbMEK2, and litchi LcAP1 suppresses this to boost resistance. Conclusion: These findings reveal *P. litchii* CWDEs' dual roles (virulence/immunity induction) and host countermechanisms, providing targets for litchi disease-resistant breeding.

Keywords: Cell wall-degrading enzymes, *Peronophythora litchii*, Plant immunity, Salicylic acid signaling, Virulence factor

Abstract

Research and Application of *Clonostachys rosea* in Controlling against Diseases of Vegetables and Fruits

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Clonostachys rosea is a new kind of biocontrol resources which has shown great potential in disease management. In our research, *C. rosea* was widely collected and highly-efficient strains were developed to act against various plant diseases. By sclerotia-trapping method, a total of 1300 mycoparasites were isolated from 600 soil samples of vegetable gardens and farm lands in 22 provinces in China, and the species *C. rosea* was detected in almost 70% of the locations surveyed. Targeting at Fusarium root rot of vegetables, *C. rosea* isolates FJWY-12, HL-1-1 and SYP-1 presented strong antagonistic activity and parasitic ability to the pathogenic fungi, and these biocontrol strains significantly improved seed vitality index and promoted plant growth as well. In greenhouse trial, the control efficiencies of the *C. rosea* agents to root rot diseases reached to 65-70%. To reveal the mycoparasitism mechanisms of the biocontrol fungus, a transcriptome of *C. rosea* under the induction of sclerotia of *Sclerotinia sclerotiorum* was analyzed, from which a series of parasitism-related genes, including perilipin, monooxygenase, heat shock protein, and short-chain dehydrogenase were identified, and the regulation of MAPK signaling pathway involving mycoparasitic process was explored. Meanwhile, the functions of *C. rosea* controlling against plant nematodes were also investigated. *C. rosea* isolates associated with root-knot nematodes of vegetables and fruits were separated, and highly-efficient strains capable of colonizing eggs and killing juveniles were obtained through a high throughput screening in 24-pore tissue culture plates. It was found that the metabolites of the *C. rosea* strains greatly inhibited the hatch of nematode eggs and decreased the migration and aggregation of juveniles to cucumber root tips. Pot experiment was conducted in greenhouse and the results showed that the disease indices of nematodes on tomato were significantly reduced and high control efficiencies of 79.3-86.2% were achieved in the treatments of *C. rosea* strains HLF-14, NYC-16 and Per7. It was also found that the stem diameters of tomato seedlings doubled, the fresh weights of plants increased by two times, and chlorophyll contents increased by 50% after inoculated with *C. rosea* fermentation broth. Bioassay indicated that the activities of antioxidant enzymes POD, CAT, and SOD increased rapidly as well. In field, the control efficacies of the three *C. rosea* isolates were over 60%, and the densities of nematodes in soil were sharply suppressed. This study is of great significance for the development and utilization of *C. rosea* biocontrol agents and green management of plant diseases.

Keywords: *Clonostachys rosea*, mycoparasitism, biological control, plant fungal disease, root-knot nematode

Abstract

Studies of *Trichoderma* spp., Scent leave oil and Nanoparticle against Citrus rot

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Sweet orange (*Citrus sinensis*) suffers significant post-harvest losses (20–50%) due to *Aspergillus flavus* and *Lasiodiplodia theobromae*. Synthetic fungicides pose environmental and health risks, necessitating safer alternatives. This study evaluated *Trichoderma asperellum*, *Ocimum gratissimum* essential oil (EO), and biosynthesized silver nanoparticles (AgNPs) against these pathogens. Fungal identification and pathogenicity used rDNA-ITS sequencing and Koch's postulate. Paper disc assay was used for antifungal test. Result showed that *T. asperellum* strongly inhibited *A. flavus* (27.89–65.81 mm) but was less effective against *L. theobromae*. EO outperformed mancozeb, showing superior inhibition of *L. theobromae* (45.74–61.68 mm at 0.25–1.00 µg/ml vs. 25.33 mm at 2 µg/ml) and moderate activity against *A. flavus* (14.01–38.66 mm). AgNPs were highly effective, suppressing *L. theobromae* (64.33 mm at 12.5 µg/ml) better than mancozeb (36.33 mm at 50 µg/ml). Characterization confirmed stable, crystalline AgNPs (54.87 nm) with bioactive capping agents. GC-MS revealed EO's key antifungal components (linalool, eugenol). These findings demonstrate *T. asperellum*, EO, and AgNPs as sustainable, eco-friendly alternatives to synthetic fungicides for managing citrus post-harvest rot, supporting safer agricultural practices.

Keywords: Antifungal, AgNPs, *Citrus sinensis*, Oils, Rot, *Trichoderma*

Endophytic Fungi: Insights into Ecology, Diversity, and Biotechnological Potential

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Abstract

Endophytic fungi reside inside healthy plant tissues without causing any detectable symptoms may provide protection to host against biotic and abiotic stresses. The entry of endophytes to their host is very interesting as plant release the chitin deacetylase (CDA) to mask the activity of acetyl group of chitin. *Thevetia pruviana*, *Nerium indicum* were assessed to harbor fungal endophytes in different tissues through culture dependent and culture independent (NGS) methods to present the holistic view of fungal diversity. The isolation and characterization of bioactive substances from culture filtrates is done using bioassay guided fractionation and spectroscopic methods. Using the spectroscopic methods, some natural products such as terrin, javanicin, terrin and milbemycin have been reported from my lab. Using the epigenetic modulators (5-azacytidine and sodium butyrate), the production of metabolites in *Cladosporium gloeosporioides* was assessed against the control.

The fungal endophytes isolated from salt tolerant wheat varieties (KRL-210, KRL-213 and KRL-19) have generated tolerance in salt sensitive variety (PBW-343). *Aspergillus medius* (K19TR/200), *Cladosporium parahalotolerant* (K13TR/150) isolated from salt tolerant wheat were identified through molecular method. These isolates were involved in increasing the root-shoot length, proline and MDA contents.

A fungal isolate used to synthesize carbon nanodots (CNDs) through a simple and ecofriendly hydrothermal treatment. The optical and structural characteristics of the CND were investigated using XRD, TEM, UV-Vis and fluorescence spectroscopy methods. Elemental analyses and FTIR have been done to highlight the surface functionality. CNDs have been employed as a inducer of plant growth promotion.

Key words: Fungal diversity, Endophytes, Salt stress, Cryptic metabolites, Carbon nanodots

Abstract

Avocado root rot disease and biological control with an emphasis on the potential of microbial control to *Phytophthora*

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Avocado is a kind of economic plants in tropical and subtropical regions. To date, over 70 countries and regions possess substantial avocado cultivation bases, of which Top 5 producing countries of avocados are Mexico, Colombia, Dominican Republic, Peru and Indonesia. Plantation area and production of avocado developed well since 1961 and particularly sharply increased since around 2010. Even so, avocado plantations face a huge challenge. On a global scale, compared to other disease, root rot is the most destructive avocado disease because root rot diseases almost are caused by soil-borne pathogens and they easily disrupt the dynamic stability and interconnectedness within ecosystems of rhizosphere microbial communities, in addition, they spread fast and are difficult to contain their proliferation when the severity happened. Finally, they lead to a substantial economic loss and lower income for producers. Avocado root rot caused by *Phytophthora cinnamomi* was first reported from Puerto Rico by Tucker in 1929. Since then, or maybe also previously, all substantial avocado cultivation bases in our planet face the threat from avocado root rot caused by *P. cinnamomi*. Root rot disease caused by *P. cinnamomi* is regarded as the most destructive disease on avocado worldwide. Chemical and physical control strategies are used but not sustainable pathways for inhabitation of *P. cinnamomi*. Chemical method prolonged use has led to decreased sensitivity and the development of resistance and physical control has incomplete efficacy, high costs, risks of soil ecological damage, and operational complexity. Currently, although the identification of biological control agents for *P. cinnamomi* has been limited, biological control can be still a promising direction. The present study illustrates the potential of microbial control to *Phytophthora* by *Pseudomonas chlororaphis* subsp. *aurantiaca* using dual culture assay and filtrates extract antagonistic experiment. Furthermore, ultra performance liquid chromatography mass spectrometry and gas chromatography mass spectrometry demonstrate that Phenazine-1-carboxylic acid, 2-Hydroxyphenazine and ammonia are main compounds of anti-*Phytophthora cinnamomi*. In addition, the present study aims to provide five valuable cases based on the utility of plant growth-promoting microorganism (bacteria and fungi) to illustrate the potential of microbial control for *Phytophthora*. These support the use of microbial control measures as a safe and effective treatment for avocado root rot diseases in the future, promising a sustainable development for avocado production.

Keywords: Arbuscular mycorrhiza-forming fungi, Avocado, Biocontrol, *Phytophthora cinnamomi*, Plant growth-promoting microorganism

Abstract

Fighting Fungi, Feeding Roots: Biocontrol and Plant Growth Promoting Potential of Endolichenic Fungi Associated with the Fruticose Lichen *Usnea*

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Plant growth-promoting (PGP) traits due to plant-associated microorganisms are crucial to enhance crop productivity and ensure sustainable agriculture. Endolichenic fungi (ELF), a unique group of fungal symbionts that inhabit healthy lichen tissues, share ecological similarities with fungal endophytes in plants, and thus, can be tapped as bioinoculants. In this study, forty ELF isolated from the fruticose *Usnea* were evaluated for their PGP traits, particularly for the production of indole-3-acetic acid (IAA) and phosphate solubilization, and for antagonistic activity against the pathogen *Fusarium oxysporum*. IAA production was assessed in culture medium supplemented with L-tryptophan. Our results showed that 60% of the ELF isolates exhibited IAA activity, with IAA concentration reaching up to 45 µg/mL. Among these, ELF 5MB3 produced the highest IAA level with 45.9 ± 0.03 µg/mL of equivalent IAA. Phosphate solubilization was evaluated through agar-based assay with Pikovskaya's medium. Solubility index implies that 24 ELF isolates were able to solubilize insoluble phosphate, with indices of 2.02–2.22. Among these, ELF 1MA2 has the most notable phosphate solubilization. Assays for antagonistic activities showed that ELF inhibited *F. oxysporum* by up to 57%, with mutual inhibition upon mycelial contact being the most observed interaction between the test pathogen and the ELF. Our findings indicated that ELF possess multiple PGP traits, highlighting their potential as novel bioinoculants for sustainable crop production through IAA-mediated plant growth promotion, improved nutrient availability via phosphate solubilization, and the potential suppression of soil-borne pathogens through antagonistic activity.

Keywords: fungal antagonism, IAA, phosphate solubilization, plant growth promotion

Abstract

Trichoderma azadirachtae Ta3302: A Novel Biocontrol Agent for Banana Fusarium Wilt and a Promoter of Plant Growth

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Fusarium oxysporum f. sp. *cubense* tropical race 4 (FocTR4) is responsible for significant losses in banana production. In this study, we explore the potential of *Trichoderma azadirachtae* 3302 strain (Ta3302), isolated from *Azadirachta indica* rhizosphere soil, for controlling banana *Fusarium* wilt and promoting plant growth. Ta3302 significantly reduces disease incidence and severity, with an antifungal inhibition rate of $76.96\% \pm 1.48\%$ against the mycelial growth of FocTR4-14013 and a coverage rate of 100%. It can wrap or penetrate FocTR4-14013 hyphae and allow them to thrive. Ta3302 can grow normally under various stress conditions, including high salt (7% NaCl), low temperature (15°C), high temperature (35°C), drought (40% PEG), and strong acidity and alkalinity (pH 3/11), exhibiting excellent stress resistance. Ta3302 also promotes banana seedling growth, increasing fresh root weight, fresh crown weight, and plant height by 104.86%, 199.49%, and 49.75% respectively, with a 10% sterile fermentation broth treatment compared to the 10% Potato dextrose broth group. A 2% Ta3302 mycelial suspension treatment achieves 76.96% control efficiency, whereas a 10% Ta3302 fermentation broth treatment reduces disease index by 44.44% and increases fresh crown weight by 199.49%. Microbial analysis showed that Ta3302 enriches *Trichoderma* in roots and inhibits the *Fusarium* community. Ta3302 offers a promising biocontrol solution for sustainable banana production.

Keywords: Biocontrol, induced resistance, plant-growth promoting, soil-borne disease, *Trichoderma azadirachtae*

Abstract

Screening and Identification of Phosphate-Solubilizing Fungi from Agricultural Soils in Guangdong Province, China

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Phosphorus is a critical macro-element required for nearly all major metabolic activities in plants. However, 95–99% of the phosphorus in soil typically exists in insoluble mineral forms and therefore cannot be directly utilized by plants. Consequently, several soil fungi, particularly phosphate-solubilizing fungi, play a vital role in converting insoluble phosphorus into soluble forms that can be readily assimilated by plants. The present study aimed to isolate and identify phosphate-solubilizing fungi from agricultural soils in Guangdong Province, China. Fungi were isolated using the serial-dilution spread plate technique on Pikovskaya's agar. A total of nine positive fungal strains (MBSZU 25-065 to MBSZU 25-073) were obtained. These strains were initially identified using internal transcribed spacer (ITS) rDNA regions, and their ability to solubilize insoluble phosphate [$\text{Ca}_3(\text{PO}_4)_2$] was further evaluated using the agar plate assay. The strains were classified into two genera *Aspergillus* and *Penicillium*, and identified as three species including *Aspergillus neoniger* (MBSZU 25-065, MBSZU 25-066, and MBSZU 25-071), *A. trinidadensis* (MBSZU 25-067), and *Penicillium oxalicum* (MBSZU 25-068, MBSZU 25-069, MBSZU 25-070, MBSZU 25-072, and MBSZU 25-073). All fungal strains were able to solubilize insoluble phosphate, with phosphate solubilization index values ranging from 2.1 to 2.3. This study enhances Guangdong's phosphate-solubilizing fungi resources, and the identified high-efficiency strains show promise for localized use in biofertilizers to improve soil phosphorus availability, reduce chemical fertilizer use, and support sustainable subtropical agriculture.

Keywords: Fungal identification, Fungal isolation, Phosphate solubilization, Soil fungi

Abstract

Fusarium solani (HC01) promotes rapid agarwood production with a medicinally accepted agarotetrol levelTian-Ye Du ^{1*}, Feng Zhao ¹, Saowaluck Tibpromma ^{2#}, Samantha C. Karunarathna ^{2#}¹ Yunnan International Joint Laboratory for Digital Conservation and Germplasm Innovation and Application of China-Laos Tea Resources, School of Tea and Coffee, Pu'er University, Pu'er 665000, P.R. China² Center for Yunnan Plateau Biological Resources Protection and Utilization & Yunnan International Joint Laboratory of Fungal Sustainable Utilization in South and Southeast Asia, College of Biology and Food Engineering, Qujing Normal University, Qujing 655099, P.R. China

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Abstract

Agarwood, a precious spice and traditional medicine, is not only valued for its unique aroma and pharmacological activities but also possesses an extremely high economic value. However, with the increasing depletion of wild agarwood resources, artificial induction techniques have become an alternative, with fungal induction being considered a relatively effective method. However, the currently available induction strains are limited, and there is a lack of dominant strains capable of consistently producing high-quality agarwood. This study aimed to identify candidate fungi that can effectively promote agarwood formation, thereby contributing to the preservation of this valuable resource. To evaluate the effect of fungi on agarwood formation, *Fusarium solani* (HC01) isolated from naturally occurring agarwood resin in Guangdong Province was selected for this study. The Agar-Wit method was used to conduct this study by using the fermentation broth of our fungal strain, *F. solani* (HC01). The crude extract of our strain was inoculated into healthy agarwood (*Aquilaria sinensis*) trunks via an infusion device and sealed to prevent contaminating microbes from interfering and the control group only had wounds and no fungal fermentation liquid was added. The first observation was on the 15th day, the results and compound composition were subsequently monitored every three months for a total of one year. The results of this study found that the experimental group was able to observe the obvious formation of resin on the 15th day, and in the sixth month, the content of agarotetrol in the produced agarwood resin exceeded the pharmaceutical standard, and the levels of several key secondary metabolites were significantly elevated compared to the control. Our study has discovered a promising new way to induce agarwood formation in fungi. We found that *F. solani* (HC01) can reliably and quickly stimulate the production of agarwood resin. The quality of the product meets medicinal standards, offering exciting prospects for its application. This strain, therefore, could be a significant player in the research and industrial application of artificial induction of agarwood, providing a fresh perspective on this field.

Keywords: biotechnology, green agriculture, sustainable development, Thymelaeaceae, Wood of Gods

Abstract

The Interactions between *Peronophythora litchii* Cell Wall Degrading Enzymes and Host Immunity

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Abstract: Background: *Peronophythora litchii* (oomycete) causes litchi downy blight, relying on cell wall-degrading enzymes (CWDEs) for infection. However, how its CWDEs interact with litchi immunity remains unclear; this study aimed to address this. Methods: Yeast two-hybrid, Co-IP, GST pull-down, CRISPR/Cas9, VIGS, and RT-qPCR were used to identify CWDE-host protein interactions and analyze their roles in virulence and immunity. Results: 1) *P. litchii* PIPAE5 (pectin acetylesterase) interacts with litchi LcLTP1, destabilizing LcLTP1 to suppress SA signaling; 2) PIPeL1/PIPeL1-like (pectate lyases, PeLs) enhance virulence via enzymatic activity, while litchi LcPIP1 binds them to induce SERK3-dependent immunity; 3) PIPeL8 (PeL) triggers cell death via NbSOBIR1/NbMEK2, and litchi LcAP1 suppresses this to boost resistance. Conclusion: These findings reveal *P. litchii* CWDEs' dual roles (virulence/immunity induction) and host countermechanisms, providing targets for litchi disease-resistant breeding.

Keywords: Cell wall-degrading enzymes, *Peronophythora litchii*, Plant immunity, Salicylic acid signaling, Virulence factor

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Session 16 Mycorrhiza and Endophytic Fungi



Abstract

Melanization and diploidization of a dark septate endophyte: strategies for saline adaptation?

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A new species of dark septate endophyte (DSE), *Laburnicola rhizohalophila* was isolated and identified from the roots of the halophyte *Suaeda salsa*. This fungus significantly promoted plant root development and salt tolerance. Population genetic analysis revealed a complex population structure and genetic differentiation of *L. rhizohalophila*, as well as a ~20 kbp genomic island exhibiting high divergence and strong sign of positive selection. This region contains a melanin-biosynthetic gene cluster. Moreover, isolates from two subpopulations exhibited some degrees of heterozygosity, suggesting the possible formation of diploid strains. To further confirm evidence of diploidization and identify potential haploid parents, we generated chromosome-level assemblies for five representative isolates from structured subpopulations using Hi-C and PacBio HiFi technologies. Evidence from chromosome number, genome size, gene count, flow cytometry, and cytology confirmed that strains JP19 and JP8 are diploids, with genome size of approximately 130 Mb. Genomic features, phylogenomics, and macrosynteny have provided clear evidence for the origin of intraspecific diploid-like hybrids. JP19 originated from hybridization between two haploid parents, isolates JP11 and R22, while one parent of JP8 is JP44, with the other parent unknown but genetically close to JP11. Notably, the JP19 phenotype stood out as an outlier and exhibited a conditional fitness advantage when exposed to a range of abiotic stresses compared with its parents. By comparing the gene expression patterns in each hybrid parent triad under the four growth conditions, the mechanisms underlying growth vigor were corroborated through an analysis of transgressively upregulated genes enriched in membrane glycerolipid biosynthesis and transmembrane transporter activity. In vitro assays suggested increased membrane integrity and lipid accumulation, as well as decreased malondialdehyde production under optimal salt conditions (0.3 M NaCl) in the hybrid. These attributes have been implicated in salinity tolerance. This study establishes an ideal model for deepening the understanding of adaptive mechanisms in *L. rhizohalophila* and paves a way for selecting diploid DSEs as robust fungal inoculants for improving plant salt tolerance.

Keywords: Dark septate endophytes; diversity of chromosome number; parasexuality; heterosis

Abstract

Exploring the Response Mechanism of *Laccaria japonica* to Blue and Red Light

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Ectomycorrhiza (ECM) represents a key mutualistic interaction between fungi and plant roots, playing vital ecological and economic roles in forest ecosystems. While ECM formation is known to be influenced by environmental and molecular factors, the specific regulatory mechanisms underlying root colonization and sporocarp development remain insufficiently understood. Light is a critical environmental factor influencing the growth and development of saprotrophic fungi, yet its role in ECM fungi remains poorly understood. In this study, we investigated the effects of blue, red, white light, and darkness on *L. japonica*, an ECM fungus native to East Asia. Both free-living mycelia and *Pinus densiflora* seedlings inoculated with

L. japonica were subjected to these light conditions to assess impacts on mycelial growth, ECM root formation, and sporocarp primordium formation. Blue and white light significantly inhibited mycelial growth, whereas red light showed no inhibitory effect and resembled growth under darkness. Notably, all light treatments suppressed ECM formation compared to darkness, with blue light causing the most significant reduction. To explore the molecular basis of these responses, transcriptomic analysis and gene ontology (GO) enrichment were performed on mycelia exposed to blue, red, and dark conditions, followed by RT-qPCR validation of selected genes. The results revealed that gene expression profiles under red light closely resembled those under darkness, whereas blue light triggered a distinct transcriptional response. These findings shed light on the regulatory role of specific spectrum in ECM development and highlight light as a significant, previously underappreciated factor in the biology of ECM fungi.

Keywords: Ectomycorrhiza; *Laccaria japonica*; Light signaling; Transcriptome analysis

Abstract

Diversity of ectomycorrhizal fungi in pecan orchards in China and mycorrhizal inoculation of pecan seedlings with two indigenous fungi

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Pecan trees (*Carya illinoensis*) have been introduced and grown widely in China for commercial nuts. However, little is known on the diversity and community assembly of ectomycorrhizal fungi associated with it in China. Based on root tip samples from Anhui, Guizhou, Hubei, Hunan, Sichuan, Yunnan and Zhejiang provinces, ectomycorrhizal fungal (EMF) diversity and EMF communities associated with pecan in China were investigated using Illumina sequencing and Sanger sequencing. The results showed that there are at least 81 OTUs from 15 ECM lineages associated with pecan trees within China. Among these, 3 OTUs were shared among pecan, pine and oaks; 8 OTUs were detected on both pecan and oak trees; and 15 OTUs were shared by pecan and pine trees, while 55 OTUs were detected only on pecan samples. Based on current knowledge of ECM fungi associated with pecan trees, *Scleroderma yunnanense* and *Tuber lijiangense*, two indigenous ECM fungi with high commercial value, were selected and successfully synthesized with pecan seedlings.

Keywords: Edible fungi; Fungal Diversity; Fungal Resources; Macrofungi; Mycorrhizal synthesis

Abstract

Unlocking the hidden role of arbuscular mycorrhizal hyphae in regulating nitrate transformationJunling Zhang^{1*}, Ruotong Zhao^{1,2}, Xia Li^{1,3}, Guang He^{1,4}

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Arbuscular mycorrhizal fungi (AMF) contribute to nitrogen (N) uptake by plants. However, the role of AMF in N transformation is under explored. Here we demonstrated that AMF and hyphosphere microbes form the alliance to regulate the fate of nitrate (NO_3^-) by transforming nitrate into ammonium or N_2 to facilitate plant N uptake or reduce N emissions. Here we combined $^{15}\text{NO}_3^-$ tracing, shotgun metagenomics and bacterial isolation to test whether AMF simultaneously (i) enhance nitrate ammonification (DNRA) to retain N as NH_4^+ and (ii) strengthen the N_2O sink by recruiting/activating clade-I *nosZ* carriers. In hyphosphere versus bulk soil, AMF hyphae significantly increased NH_4^+ and $^{15}\text{NH}_4^+$ derived from $^{15}\text{NO}_3^-$ (1.42–5.01 $\times$), with metagenomes showing enrichment of *nirB/nirD* and prevalence of a *nirBD*-carrying *Paenibacillus* sp. (isolate DP01). Hyphal exudates boosted DNRA efficiency and biofilm formation by DP01, while microensors detected accelerated O_2 depletion at hyphal surfaces, consistent with formation of reduced niches favorable for ammonification. In residue patches, AMF reduced denitrification-derived N_2O emissions by up to 63%, coincident with selective enrichment and activation of N_2O -reducing *Pseudomonas* (including a clade-I *nosZ*-positive *P. fluorescens* JL1). Hyphal carboxylates acted as attractants and stimulants, triggering *nosZ* expression and N_2O consumption; re-inoculation tests and an 11-year field survey further linked hyphal length density with clade-I *nosZ* abundance. Together, these results reveal a cross-kingdom synergy whereby AMF hyphae co-opt DNRA bacteria to retain N and empower N_2O -reducers to mitigate emissions, thereby improving nitrogen use efficiency while lowering climate forcing. Given the ubiquity of AMF and nitrate-ammonifying/ N_2O -reducing microbes, leveraging the hyphosphere-microbiome interactions offers a tractable route for sustainable nitrogen management.

Keywords: Arbuscular mycorrhizal fungi; DNRA; *nirB*; *nosZ*; *Paenibacillus*; *Pseudomonas*

Abstract

Regional differences dominate the incidence, and severity in endophytic microbe community of *Amomum taso-ko* in Yunnan, China

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[Background] *Amomum tsao-ko* within the family of *Zingiberaceae*, is a perennial herb mainly distributed in southwest China including Yunnan, Guangxi, Guizhou and northern Vietnam. Its

dried fruits are popularly consumed as foods and medicines with digestion-promoting, anti-diabetic, anti-microbial, anti-obesitic, anti-inflammatory, and neuroprotective effects. However, most plantation was suffering the threaten of fruit rot mainly caused by *Fusarium* spp. Growing evidence suggests that disease occurrence in plants is often accompanied by changes in the associated microbiome. **[Method]** This study investigated the diversity and community structure of endophytic fungi and bacteria associated with diseased and healthy fruits of *Amomum tsao-ko* from Wenshan Prefecture, Honghe Prefecture, Nujiang Prefecture, Yunnan, China by high-throughput sequencing. **[Result]** From the analysis of CAP, the presence or absence of disease, disease severity, and regional differences all have a significant impact on the similarity of microbial community structure and species composition (beta diversity), but the measured variables of disease and disease severity on microbial community changes is less than 10%. Regional differences not only have a significant impact on fungal community composition, but could explain over 20%. From the PCoA results, it can be seen that samples from the same region tend to cluster together, and regional differences are the dominant factor affecting the composition of the microbiota. However, healthy and diseased samples from the same region are clearly separated, indicating that the low explanatory power of disease status and severity on microbiota changes is likely due to the greater influence of regional differences. For bacteria, disease and its severity are the biggest influencing factors, which can explain 60-80% of changes in the microbiota; However, although the impact on fungi is also significant, the explanatory power is small, mainly due to the significant regional differences in fungal community composition, which are the main factors affecting fungal community composition. **[Conclusion]** Disease incidence and severity are the biggest factors on endophytic bacterial diversity, whereas regional differences mainly affect fungal community composition in *Amomum taso-ko*.

Keywords: *Amomum tsao-ko*, fruit rot, disease severity, regional differences, microbial community structure

Abstract

Endophytic Fungi: Insights into Ecology, Diversity, and Biotechnological Potential

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Endophytic fungi reside inside healthy plant tissues without causing any detectable symptoms may provide protection to host against biotic and abiotic stresses. The entry of endophytes to their host is very interesting as plant release the chitin deacetylase (CDA) to mask the activity of acetyl group of chitin. *Thevetia pruviana*, *Nerium indicum* were assessed to harbor fungal endophytes in different tissues through culture dependent and culture independent (NGS) methods to present the holisitc view of fungal diversity. The isolation and characterization of bioactive substances from culture filtrates is done using bioassay guided fractionation and spectroscopic methods. Using the spectroscopic methods, some natural products such as terrin, javanicin, terrin and milbemycin have been reported from my lab. Using the epigenetic modulators (5-azacytidine and sodium butyrate), the production of metabolites in *Cladosporium gloeosporioides* was assessed against the control.

The fungal endophytes isolated from salt tolerant wheat varieties (KRL-210, KRL-213 and KRL-19) have generated tolerance in salt sensitive variety (PBW-343). *Aspergillus medius* (K19TR/200), *Cladosporium parahalotolerant* (K13TR/150) isolated from salt tolerant wheat were identified through molecular method. These isolates were involved in increasing the root-shoot length, proline and MDA contents.

A fungal isolate used to synthesize carbon nanodots (CNDs) through a simple and ecofriendly hydrothermal treatment. The optical and structural characteristics of the CND were investigated using XRD, TEM, UV-Vis and fluorescence spectroscopy methods. Elemental analyses and FTIR have been done to highlight the surface functionality. CNDs have been employed as a inducer of plant growth promotion.

Key words: Fungal diversity, Endophytes, Salt stress, Cryptic metabolites, Carbon nanodots

Abstract

Endophytic Dothideomycetes and Sordariomycetes from Philippine Endemic Annonaceae as Sources of Antibacterial, Antitubercular, and Antioxidant Extracts

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The global rise of antibacterial resistance, tuberculosis, and oxidative stress-related disorders underscore the need for novel sources of antibiotics and antioxidants. To address this, we investigated EF of the classes Dothideomycetes and Sordariomycetes isolated from Philippine endemic Annonaceae species. Twelve EF were cultivated in four media (YM 6.3, ZM ½, Q6 ½, and brown rice) and sequentially extracted with ethyl acetate and methanol, yielding 48 crude extracts. Antibacterial activity was evaluated against ESKAPE pathogens using REMA, antitubercular activity against *Mycobacterium tuberculosis* H₃₇R_v using MABA MIC₉₀, and antioxidant potential by DPPH assay. Among the extracts, 23 inhibited ESKAPE pathogens, 2 suppressed *M. tuberculosis* growth, and 18 exhibited free-radical scavenging activity. ITS-based phylogeny and morphological analysis identified 11 distinct EF as sources of these bioactive extracts. Notably, brown-rice extract of *Chromolaenicola* sp. displayed potent anti-ESKAPE activity (MIC = 7.81–250 µg/mL), while brown rice extract of *Diaporthe longicolla* and YM extract of *Endomelanconiopsis* sp. inhibited *M. tuberculosis* by 90% and 96% at 64 µg/mL, respectively. In addition, the ZM extract of *Chaetomium* sp. (IC₅₀ = 56.03 µg/mL) exhibited comparable RSA to antioxidant drugs BHT (IC₅₀ = 24.57 µg/mL) and ascorbic acid (IC₅₀ = 1.18 µg/mL). Among the four media, brown-rice media supported the highest secondary metabolite production. The mycochemical screening of active extracts revealed anthrones, terpenes, sterols, and indoles as major constituents. This study also provides the first records of *Chromolaenicola* sp. and *D. longicolla* from *O. cumingiana* and *Endomelanconiopsis* sp. from *A. cagayanensis*. These findings position endophytic Dothideomycetes and Sordariomycetes from Philippine Annonaceae as promising sources for novel antibacterial, antitubercular, and antioxidant compounds warranting targeted isolation and structure elucidation.

Keywords: antibiotic, antioxidant, ESKAPE, fungal secondary metabolites, *Mycobacterium tuberculosis* H₃₇R_v

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Session 17 Evolution and Diversification



Abstract

Ecological Diversification and Versatility of Plant-Associated Fungi Revealed by Studies on Endophytic Fungi

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Fungal endophytes are characterized as an ecological group of fungi that are intimately associated with living plants. They derive protection, nutrients, and enhanced tolerance to environmental stresses from host plants, while often providing the host with various physiological and/or ecological benefits. However, these fungi have not been unequivocally and unambiguously defined by unique biological properties; an endophytic lifestyle alone does not characterize all of them. They are not phylogenetically restricted and are ubiquitous in terms of both geographic distribution and host plant phylogeny. In this paper, I interpret the versatile lifestyles of diverse endophytic fungi based on three case studies. This interpretation highlights the role of high phenotypic plasticity—their capacity for physiological and ecological acclimation to variable and often unexpectedly changing environmental conditions—and their resilience to hostile host responses within dynamic plant-fungus communities. The first case study involves *Phyllosticta capitalensis*. Since its initial reports as an orchid leaf spot pathogen in 1908, this species was considered to be an orchid pathogen for over a century. However, in 2001, it was discovered to inhabit living leaf tissues of several ericaceous plants, and it has since been documented in many other plants worldwide. Given its cultivation characteristics and ecological traits, this fungus serves as a compelling example for studies on the biogeography and population genetics of plant-associated microfungi. The second case study focuses on xylariaceous fungi, which are well known as soil-borne decomposers and represent one of among the most important groups of saprotrophs in forest ecosystems. Since the 1980s, however, fungal endophyte studies have shown that several species within the Xylariaceae also exist as foliar endophytes. We have isolated endophytic Xylariaceae from leaf tissues of various plants, primarily across Japan and Thailand. Our studies have revealed a high phylogenetic diversity of xylariaceous fungi living endophytically. Consequently, we hypothesize that many xylariaceous fungi initially live as foliar endophytes, later transitioning to a role as plant decomposers. The third case study is *Hymenoschyphus fraxineus*. This fungus, which has been threatening *Fraxinus* plants (Oleaceae) in Europe since the early 1900s, is also found to live as an endophyte in *Fraxinus* plants within its native East Asia range. The study of its occurrence in native habitats is essential to fully unveiled the true biological nature of *H. fraxineus*. Thus, acclimation-based research has revealed many aspects of endophytic fungal biology, providing a foundation for understanding the diverse lifestyles of all plant-associated fungi. Fungal ecological roles were historically confined to rigid categorizations (e.g., mutualistic,

commensal, parasitic, and saprotrophic). However, the study of fungal endophytes now necessitates a fundamental reassessment of their ecological flexibility and versatility.

Keywords: lifestyles, *Hymenoschyphus fraxineus*, *Phyllosticta capitalensis*, Xylariaceae

Abstract

Macroevolution of Hymenochaetales, Basidiomycota

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Macroevolution bridges long-term evolutionary processes with ecological environments, revealing what determines species diversity, but such research was rarely performed in macrofungi. Hymenochaetales, a speciose order of mushroom-forming fungi within Agaricomycetes, Basidiomycota, possesses highly diverse morpho-ecological traits, which makes it exceptional for macroevolution research. To promote the conservation and utilization of fungal resources in Hymenochaetales that includes medicinal species and forest pathogens and thus is important economically, we first construct the latest taxonomic framework of this order from both morphological and phylogenomic/multilocus-based phylogenetic perspectives on the basis of a long-term taxonomic investigation. A total of 15 families (eight newly erected), and 84 genera with 65 in and 19 out of the 15 families are accepted in Hymenochaetales. Then, we analyze speciation and extinction patterns of this order using a novel, nearly comprehensive phylogeny. The results show that Hymenochaetales had a convoluted and eventful evolutionary history, shaped by an early adaptive period, followed by a general rate slowdown interrupted by local rapid radiations in the Cretaceous. Moreover, the ancestor of the order has undergone rapid phenotypic diversification into a range of fruiting body morphologies and lifestyles, of which crust-like morphologies and ‘pileate-sessile’ forms typical of bracket-fungi became dominant among extant taxa. Diversification rate showed significant correlations with paleotemperature, morphological and ecological traits; however, teasing apart the contributions of individual clades revealed that these were mostly driven by the Mid-late Cretaceous rapid radiation of the genus *Phylloporia*, a taxonomically diverse group of plant parasites. We further explore the spatiotemporal pattern of this genus in China. It is inferred that the highly suitable habitat of *Phylloporia* concentrated in southern, especially southeastern, China, where the ancestor of *Phylloporia* originated 77.74 million years ago and then dispersed to other parts of China. Benefitting from the available suitable habitats, *Phylloporia* rapidly diversified after its divergence in Southeast China. Then, the net diversification rate slowed down when the rapidly diversifying species filled available niches in Southeast China and the dispersed species in other parts of China inhabited the less suitable and unsuitable habitats. During adaptive evolution, precipitation, temperature and the host plant are the major environmental variables that shape the spatiotemporal pattern of *Phylloporia*. In conclusion, we reveal a complex evolutionary history of Hymenochaetales, and uncover novel patterns of phenotype evolution and diversification in mushroom-forming fungi.

Keywords: diversification, spatiotemporal pattern, species diversity, trait evolution

Abstract

Astromycology – Fungal Adaptation and Innovation in Space Environments

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Fungi represent a vast and largely understudied domain of microbial life, with only a small fraction of their estimated diversity being formally described. In the context of space exploration, filamentous fungi have emerged as both a challenge and an opportunity. Historically dismissed as contaminants in spacecraft and orbital habitats, certain fungal species have demonstrated resilience and adaptability under extreme conditions, raising concerns about biodeterioration and astronaut health. Simultaneously, their metabolic versatility offers high potential for biotechnological innovation in extraterrestrial environments, including spacecrafts, space stations, and even future bases on the moon or Mars.

Our research investigates fungal behaviour under space-relevant stressors, including simulated microgravity, lunar and Martian gravity (using a 3D-clinostat), and hypergravity conditions (using the European Space Agency (ESA)'s large diameter centrifuge, at European Space Research and Technology Center (ESTEC), Netherlands). We have been researching strain-specific morphological adaptations and consistent biosynthesis, focusing on functional nanoparticles, under altered gravity. Our findings suggest that selected fungal taxa may be harnessed for *in situ* resource utilization (ISRU), biomanufacturing, and life support systems in long-duration missions.

By integrating astromycology into broader space bioscience frameworks, we aim to promote international collaboration and advocate for fungi as key players in sustainable extraterrestrial ecosystems.

Keywords: Astromycology, Filamentous Fungi, Simulated altered gravity, Sustainable Space Missions, Planetary Protection

Abstract

Cenozoic Climate and Ectomycorrhizal Symbiosis Drove Rapid Diversification in BoletalesGang Wu^{1,2,*,#}, Zhu-Liang Yang^{1,2,#}*1 Key Laboratory of Phytochemistry and Natural Medicines, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, 650201, China**2 Yunnan Key Laboratory for Fungal Diversity and Green Development, Kunming 650201, Yunnan, China**Email of the corresponding author: wugang@mail.kib.ac.cn, fungi@mail.kib.ac.cn*

The evolutionary success of ectomycorrhizal (ECM) fungi remains poorly understood, particularly how climatic shifts and symbiotic partnerships together drove their diversification. Here, we reconstruct the evolutionary history of the hyper-diverse order Boletales (~3,000 species) using a comprehensive genus-level phylogeny based on 53 genes from 769 species. We show that Boletales originated in the Early Jurassic (189 Ma), but its major diversification pulses occurred during the Cenozoic, being associated with periods of global cooling and temporally linked to the intensification of East Asian monsoon. The first pulse commenced at the Cretaceous–Paleogene (K–Pg) boundary (~67 Ma), and the second began in the Early Miocene (~19 Ma). These accelerations were primarily driven by the acquisition and expansion of ECM symbiosis, which significantly outperformed the stipitate-pileate mushroom form in boosting diversification rates. Our results demonstrate that the interplay of climate fluctuation, and symbiotic partnership underpinned the rapid diversification of ECM fungi during the Cenozoic, offering a unified framework for understanding the evolution of plant-associated fungi.

Keywords: co-diversification, ectomycorrhizal symbiosis, fungal evolution, paleoclimate, phylogenomics

Abstract

Horizontal Gene Transfer as a Driver of Fungal Adaptation and Virulence Evolution

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Horizontal gene transfer (HGT) is increasingly recognized as a driver of fungal adaptation, yet its diversity and mechanisms remain incompletely understood. Through a systematic survey of 829 representative fungal genomes, we detected 20,093 prokaryote-derived genes arising from 8,815 distinct HGT events. Lifestyle was found to be a major determinant of HGT distribution: parasites harbored the greatest number of transferred genes, followed by saprotrophs, while symbionts contained the fewest. Most acquired genes were involved in metabolism and cellular functions and were under purifying selection. Notably, intron-containing HGT genes exhibited significantly higher expression levels than intronless ones, suggesting that intron gain may facilitate their functional integration. Building on this broad-scale analysis, we examined the transfer of the wheat pathogen virulence gene *ToxA*. Screening 2,137 fungal assemblies revealed its presence in *Alternaria ventricosa*, nearly identical to copies in *Bipolaris sorokiniana* and *Pyrenophora tritici-repentis*, embedded within a conserved ~14 kb *ToxhAT* element. Comparative analysis demonstrated that *ToxhAT* was independently mobilized by three distinct Starship transposons—*Frontier*, *Sanctuary*, and *Horizon*—highlighting the critical role of transposon activity in enabling adaptive gene transfer. Together, these findings provide complementary insights into the dynamics of fungal HGT: while lifestyle shapes the overall diversity and functional integration of transferred genes, transposons act as powerful vehicles for mobilizing key adaptive traits such as virulence factors. This dual perspective emphasizes the evolutionary significance of HGT in fungal diversification and host–pathogen interactions.

Keywords: Fungi; Horizontal gene transfer; Adaptation; Lifestyle; Transposons

Abstract

The Evolution of Ectomycorrhizal Symbiosis and Host Shifts Enhance Diversification of Amanitaceae (Agaricales, Basidiomycota)Qing Cai^{1,2*}, Yang-Yang Cui^{1,2}, Zhu L. Yang^{1,2#}¹State Key Laboratory of Phytochemistry and Natural Medicines, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China²Yunnan Key Laboratory for Fungal Diversity and Green Development, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China

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Amanitaceae is a cosmopolitan family comprising approximately 700 species. The family includes both ectomycorrhizal (ECM) and saprotrophic (SAP) fungi, and the ECM species is much more diverse than the SAP ones. Previous studies primarily focused on the phylogeny and species diversity of the family. However, our understanding of the evolution of ECM symbiosis in the family and the correlation between the acquisition of this type of nutritional mode and the diversification of *Amanitaceae* remains largely unknown. In this study, we reconstructed the phylogeny of *Amanitaceae* by using 500 single-copy orthologous genes from 130 representative species. Stable isotopic analyses of carbon and nitrogen were used to assess the nutritional modes of amanitas. Finally, we investigated whether the acquisition of ECM symbiosis facilitated the diversification of *Amanitaceae* by dating the divergence time, performing ancestral state reconstructions of nutritional modes and host plants, estimating the diversification rates, and developing a number of time-, trait- and paleoenvironment-dependent models based on a multi-locus (nrLSU, ITS, *tefl-α*, and *rpb2*) phylogeny with a broad sampling (ca. 560 species). The results indicate that the evolution of ECM symbiosis has a single evolutionary origin in *Amanitaceae*. The earliest explosive diversification detected in the family coincided with the acquisition of the ECM symbiosis with angiosperms during the middle Cretaceous. The recent rapid diversification was primarily triggered by the host-plant shifts from angiosperms to the mixed forests dominated by Fagaceae and Pinaceae, or specifically to Pinaceae. This study provides a good example of integrating phylogeny, the evolution of nutritional modes, and ecological analyses

to elucidate the mechanisms underlying the evolutionary diversification of fungi. Furthermore, it provides new insights into how the evolution of ECM symbiosis has facilitated the diversification of mushroom-forming fungi.

Keywords: Ecological opportunities, Rapid diversification, Stable isotopes, Temporal dynamics

Abstract

The Silk Road of Yeast

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The yeast *Saccharomyces cerevisiae* has been used by humans for baking and the production of alcoholic beverage for thousands of years. The earliest archeological evidence for a wine-like beverage production dates from about 7000 BC in China. Recent extensive field survey and population genetics and genomics studies of *S. cerevisiae* worldwide have shown that the global genetic diversity of the species is mainly contributed by the strains from Far East Asia, and the ancient basal lineages of the species have been found only in China, supporting an ‘out-of-China’ origin hypothesis. Interspecific hybridization between the ale beer yeast *S. cerevisiae* and the wild yeast *S. eubayanus* created the low-temperature tolerant lager beer yeast *S. pastorianus*. Our population genetics and genomics studies showed that *S. eubayanus* originated from the Tibetan Plateau and migrated to Europe probably through the Silk Road. Our recent phylogenomic analysis focusing on wine *S. cerevisiae* strains showed that the strains associated with the fermentation of Msalais, which is known as a living fossil of traditional wine production in Xijiang, formed distinct lineages basal to the Georgian and Wine/European lineages and possess much higher sequence diversity than the latter two lineages. The result implies that wine yeast probably originated in West China and was transferred to Europe also through the Silk Road.

Keywords: Beer yeast, Evolution, *Saccharomyces cerevisiae*, wine yeast

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Entomogenous fungi



Abstract

Entomogenous? Yes, but not only!

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Entomogenous (entomopathogenic) fungi of the order *Hypocreales* are widely studied for their diversity, infection mechanisms, and potential as mycoinsecticides. However, their ecological roles extend far beyond insect pathogenicity. Many strains can establish as endophytes or colonize the rhizosphere, exerting beneficial effects on plants. These fungi therefore represent an untapped reservoir of microbial resources with dual potential—as both mycoinsecticides and biofertilizers/biostimulants—for sustainable agriculture.

In this study, we screened a large collection of Hypocrealean entomogenous fungi for their capacity to promote plant growth and suppress invertebrate pests in rice cultivation. Our results revealed that not only *Metarhizium* species, known for their ecological versatility, exhibit strong dual functionality, but also taxa with more specialized entomopathogenic lifestyles, such as *Cordyceps*, can contribute to rice growth promotion. These findings highlight the ecological plasticity of Hypocrealean entomogenous fungi and warrant deeper investigation into the plant–microbe–soil interactions underlying their multifunctionality.

Abstract

Longifolene-mediated attraction of insects to *Metarhizium*-colonized cadavers enables construction of mosquito-attracting fungi**Dan Tang, Yubo Zhang, Weiguo Fang**

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Abstract

Metarhizium-based mycoinsecticides show promise in controlling insecticide-resistant mosquitoes, with efficacy dependent on infection rates. We showed that *Metarhizium*-colonized insect cadavers release the volatile longifolene to attract and infect healthy insects, facilitating spore dispersal. We identified the responsible odorant receptors in *Drosophila melanogaster* and *Aedes albopictus*. The virulent mosquito pathogen *M. pingshaense* was engineered to express pine longifolene synthase so that as well as cadavers it also produced a large amount of longifolene on a mass-production medium for fungi. The transgenic spores effectively lured and killed male and female *Ae. albopictus*, *Anopheles sinensis*, and *Culex pipiens*. This attraction to wild-caught mosquitoes extended over an area 12-fold larger than World Health Organization-recommended Western African experimental huts. Mosquito-attracting flowering plants, but not human presence, competed with transgenic *M. pingshaense* for attractiveness, but mortality remained over 90%. This study uncovered an active spore dispersal mechanism in broad-host-range entomopathogenic *Metarhizium*, enhancing mosquito control efficacy.

Abstract

Biosynthesis system establishment of high-value compounds in edible fungi: cordycepin in *Cordyceps militaris* as an example

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Abstract *Cordyceps militaris* is a kind of medicinal and edible mushroom with diverse bioactive compounds. With the development of synthetic biology, *C. militaris* shows the potential to be constructed as a cell factory for high-value compounds. Cordycepin is one of the most valuable bioactive ingredients naturally produced by *C. militaris*, with the advantages of an intrinsic high yield. Combining the genome-scale metabolic network model iPC1469 and rational analysis, 14 knockout candidate genes and 18 overexpression candidates were selected. All candidate genes were verified by applying the CRISPR-LITE system and the pZFE episomal system. 6 knockout strains were successfully obtained, in which 4 of them showed the ability to increase cordycepin yield, while the CM15 Δ *isn1* strain got the highest increase by 37.31%. 18 overexpression strains were constructed, of which 9 genes were able to increase cordycepin yield. Divided the biosynthetic pathway of cordycepin into PPP module, purine metabolic module, and IMP module, CM15/pZFE-*g6pd*, CM15/pZFE-*purDM*, and CM15/pZFE-*purA2* in those modules obtained the highest cordycepin yield with an increase by 387.05%, 657.43%, and 207.00%, respectively. This work successfully mined 13 genes that increase the yield of cordycepin, deepening the understanding of cordycepin biosynthesis and providing solid data for subsequent metabolic engineering.

Keywords *Cordyceps militaris*; cordycepin; gene editing; metabolic engineering

Abstract

Integrated studies of *Cordyceps blackwelliae*: Artificial Cultivation, Functional Genomics, and Pharmacological Properties

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Abstract: *Cordyceps blackwelliae* is a fungal species newly reported by Mongkolsamrit et al. in 2018 based on specimens collected from Thailand. In 2019, our research team successfully isolated and obtained pure cultures of this fungus from specimens collected at Danxia Mountain in Shaoguan, Guangdong Province, China. Subsequent studies revealed that this strain grows rapidly on artificial media and readily forms fruiting bodies under induced conditions. In recent years, our team has conducted systematic research on this fungus, including:

- (1) Fruiting body cultivation and bioactivity evaluation: We successfully induced the formation of fruiting bodies using substrates such as rice, wheat, and tussah pupae. Both liquid-fermented mycelia and artificially cultivated fruiting bodies exhibited significant antioxidant, fibrinolytic, and antimicrobial activities.
- (2) Molecular identification: We developed mitochondrial DNA-based molecular markers for precise differentiation of *C. blackwelliae* from closely related species.
- (3) Cultivation optimization: We performed systematic investigation of nutritional and environmental conditions for mycelial growth, leading to the identification of optimal media formulations and cultivation parameters.
- (4) Genome analysis: We completed the sequencing, assembly, and annotation of both the nuclear and mitochondrial genomes, uncovering an abundance of secondary metabolite gene clusters.
- (5) Developmental mechanisms: We integrated multi-omics data—including genomics, transcriptomics, and metabolomics—to elucidate the molecular regulatory networks governing mycelial growth and fruiting body development.
- (6) Pharmacological activity: Metabolomics and network pharmacology studies identified potential bioactivities, including anti-breast cancer, anti-prostate cancer, and anti-Alzheimer's effects. *In vitro* experiments confirmed its efficacy against triple-negative breast cancer and revealed a multi-component, multi-target, multi-pathway synergistic mechanism.

To date, this work has yielded six peer-reviewed publications and two Chinese patents (one granted), establishing a foundation for the further development and industrial applications of *C. blackwelliae*.

Keywords: bioactive compounds, *Cordyceps blackwelliae*, developmental mechanism, fruiting body cultivation, genome

Abstract

Unveiling a novel entry gate: Insect foregut as an alternative infection route for fungal entomopathogens

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Abstract

Insects and their natural microbial pathogens are intertwined in constant arms races, with pathogens continually seeking entry into susceptible hosts through distinct routes. Entomopathogenic fungi are primarily believed to infect host insects through external cuticle penetration. In this study, we have identified a new variety of *Beauveria bassiana*, designated as *Beauveria bassiana* var. *majus* (Bbm), capable of infecting lepidopteran larvae through both the integument and the foregut. The dual routes of infection significantly accelerate the speed of lethality, offering a promising approach for the effective management of lepidopteran pests. Notably, Bbm strains invade the alkaline foregut of lepidopteran larvae through a novel pH-responsive pathway involving the Aia-PalC-PacC signaling cascade. Overall, our findings uncover the insect foregut as an alternative and previously unrecognized route of infection by entomopathogenic fungi. We have also identified that the pH-responsive Aia-PalC-PacC signaling cascade facilitates fungal tolerance to alkaline foreguts, thereby enabling intestinal infection. The discovery of Bbm, with enhanced infectivity through both the integument and the foregut, opens a promising way for improving the efficacy of fungal insecticides, leading to efficient and environmentally friendly pest control.

Abstract

Advanced Genetic Toolkits for Precision Genome Engineering and Metabolic Reprogramming in *Cordyceps militaris*

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The entomopathogenic fungus *Cordyceps militaris* is a globally significant bio-resource with immense medicinal and economic value. Despite its promise as a versatile synthetic biology chassis, its development is notably hampered by fundamental knowledge gaps and technical challenges inherent to many under-researched macrofungi. Crucial aspects of its biology, including host-pathogen interactions, the molecular mechanisms of fruiting body emergence from the insect host, and the severe strain degeneration observed in cultivation, remain largely enigmatic. Furthermore, macrofungi often exhibit lower gene editing efficiencies than model filamentous fungi, and current genetic tools frequently suffer from issues such as off-target effects and the metabolic burden associated with constitutive gene expression. To overcome these critical hurdles, we report the development of two next-generation genetic toolkits. Building upon our prior advancements in efficient, marker-free large genomic fragment deletion with the CRISPR-Cas9-TRAMA system, we developed and optimized a high-fidelity CRISPR-Cpf1plus system. This new system incorporates refined Cas12a enzyme optimization for significantly improved editing efficiency. Crucially, the simpler guide RNA design inherent to Cas12a, a distinct advantage over Cas9, renders it exceptionally practical for multiplex gene editing. This system achieves efficient, marker-free deletion of large genomic loci and has been validated for its high specificity, showing no detectable off-target mutations, thus offering a superior platform for precise genetic interrogation. Concurrently, a tightly regulated tetracycline-inducible (Tet-On) expression system was established. This platform facilitates dose-dependent control of gene expression, which we demonstrated by engineering a strain capable of efficient lignin valorization. Importantly, this inducible approach circumvents the growth defects typically associated with constitutive overexpression, highlighting its utility for complex metabolic engineering. Together, these advanced CRISPR-Cpf1plus and Tet-On toolkits provide the specificity and transcriptional control essential for sophisticated functional genomics and metabolic pathway optimization. This work not only furnishes critical tools to dissect the unique biology of entomopathogenic fungi but also strategically positions *C. militaris* as a powerful and versatile chassis for the sustainable production of pharmaceuticals, nutraceuticals, and for green biorefinery applications.

Keywords: Chassis development, CRISPR-Cas12a, Edible fungi, Inducible expression, Synthetic biology

Abstract

Research Progress and Application of Novel Food Resource——*Cordyceps guangdongensis*

Chenghua Zhang^{1*}, Xiaolei Deng^{1,2}, Hao Huang¹, Qiuju Huang¹, Guorui Zhong¹, Gangzheng Wang¹, Ting Li¹, Wangqiu Deng^{1#}, Taihui Li^{1#}

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Abstract: *Cordyceps guangdongensis* is a rare and endemic edible and medicinal *cordyceps* species discovered in China. The fruiting bodies of *C. guangdongensis* is rich in nutritional components, exhibits significant bioactive effects, and was approved as a novel food source by the Chinese government in 2013. Currently, it is recognized by the state as one of the four major *cordyceps* species safe for edible or medicinal use. Our research team has long been dedicated to the industrial development and application of *C. guangdongensis*. We have conducted a series of studies encompassing germplasm resource evaluation, strain selection, establishment of cultivation technology, detection of nutritional and bioactive components, bioactivity research, and product development. Currently, a mature germplasm resource evaluation system for *C. guangdongensis* has been established, on the basis of which several high-quality strains have been screened out, and mature industrial-scale cultivation has also been realized. Meanwhile, based on the excellent bioactivities of fruiting bodies, including antioxidant effects, immune system enhancement, treatment of chronic renal failure, alleviation of chronic bronchitis, reduction of obesity, lowering of blood lipids, and regulation of blood glucose, a range of related products have been developed, such as powder, tablets, beverages, and liquor. Thus, with the joint efforts in *C. guangdongensis* research, a preliminary industry-academia-research integrated mode focusing on R&D and application has been realized.

Keywords: *Cordyceps guangdongensis*; biological breeding; biological activities; industrial application

Funding: This research was supported by the the National Natural Science Foundation of China (No. 32472812, 32573109), the Guangzhou Science and Technology Planning Project (2025A04J4147, 202206010050)

Abstract

Species diversity of entomogenous fungi and its application in agriculture and forestry systems of Fujian Province, ChinaYongsheng Lin*, Jiao Yang, Huili Pu, Longbin Lin, Tianyu Tan, Chenjie Xiong, Junzhi Oiu[#][College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou, China] Corresponding author: junzhiqiu@126.com**Abstract**

Entomogenous fungi play vital roles in maintaining ecological balance, pest biological control, and serving as food and medicinal resources. They act as crucial regulators of insect population dynamics in various natural ecosystems, including sustainable forests and agriculture. It was characterized by abundant vegetation, a mild and rainy climate, and a high diversity of insect species, offering an ideal environment for the growth of entomogenous fungi in Fujian Province. Based on morphological and phylogenetic analyses, 438 specimens were collected from Fujian, encompassing 50 species from 10 genera in Cordycipitaceae (including 7 new species and 2 new records), 26 species from 7 genera in Clavicipitaceae (consisting of 4 new species and 1 new record), and 3 species from 2 genera in Ophiocordycipitaceae. This study significantly enriches the fungal resource diversity of the Asian continent and enhances our understanding of the evolutionary relationships and divergence patterns within subtropical fungal communities.

Keywords: Ascomycota; entomogenous fungi; morphological; phylogenetic analyses; new taxa; application

Abstract

Cryptic Species Revealed Through Multi-Locus Molecular Phylogenetic Analysis of *Ophiocordyceps* (Ophiocordycipitaceae, Hypocreales) on Dipteran Flies in Thailand

[Suchada Mongkolsamrit 1], [Donnaya Thanakitpipattana 1], [Wasana Noisripoom 1], [Kanoksri Tasanathai 1], [Kanraya Liangsiri 1,2], [Somruetai Jaiyen 1], [Nattawut Rungjindamai 2], [Marc Stadler 3], [Jennifer Luangsa-ard 1*#]

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Field surveys in Thailand revealed a diverse collection of fungal specimens parasitizing dipteran flies. Morphologically, these fungi resembled *Ophiocordyceps dipterigena*, yet multi-locus molecular phylogenetic analyses using ITS, LSU, *TEF1*, and *RPB2* sequences uncovered a cryptic species complex. Our results demonstrate a well-supported monophyletic clade, which we designated the “*O. dipterigena* complex”. Within this complex, we describe four new species with distinct host associations: *Ophiocordyceps floriformis* on robber flies (Asilidae); *O. muscae* on houseflies (*Musca domestica*); *O. tabani* on horse flies (*Tabanus* sp.); and *O. thilosuensis* on fruit flies (Tephritidae) and soldier flies (Stratiomyidae). Furthermore, we report the first record of *O. philippinensis* in Thailand, collected from a robber fly (*Clephydrona* sp.). Our findings highlight significant host specificity and reveal a greater diversity within this fungal group than previously recognized.

Keywords: cryptic species, entomogenous fungi, *Hymenostilbe*, new taxa, phylogenetic analyses

Abstract

Advanced Genetic Toolkits for Precision Genome Engineering and Metabolic Reprogramming in *Cordyceps militaris*

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The entomopathogenic fungus *Cordyceps militaris* is a globally significant bio-resource with immense medicinal and economic value. Despite its promise as a versatile synthetic biology chassis, its development is notably hampered by fundamental knowledge gaps and technical challenges inherent to many under-researched macrofungi. Crucial aspects of its biology, including host-pathogen interactions, the molecular mechanisms of fruiting body emergence from the insect host, and the severe strain degeneration observed in cultivation, remain largely enigmatic. Furthermore, macrofungi often exhibit lower gene editing efficiencies than model filamentous fungi, and current genetic tools frequently suffer from issues such as off-target effects and the metabolic burden associated with constitutive gene expression. To overcome these critical hurdles, we report the development of two next-generation genetic toolkits. Building upon our prior advancements in efficient, marker-free large genomic fragment deletion with the CRISPR-Cas9-TRAMA system, we developed and optimized a high-fidelity CRISPR-Cpf1plus system. This new system incorporates refined Cas12a enzyme optimization for significantly improved editing efficiency. Crucially, the simpler guide RNA design inherent to Cas12a, a distinct advantage over Cas9, renders it exceptionally practical for multiplex gene editing. This system achieves efficient, marker-free deletion of large genomic loci and has been validated for its high specificity, showing no detectable off-target mutations, thus offering a superior platform for precise genetic interrogation. Concurrently, a tightly regulated tetracycline-inducible (Tet-On) expression system was established. This platform facilitates dose-dependent control of gene expression, which we demonstrated by engineering a strain capable of efficient lignin valorization. Importantly, this inducible approach circumvents the growth defects typically associated with constitutive overexpression, highlighting its utility for complex metabolic engineering. Together, these advanced CRISPR-Cpf1plus and Tet-On toolkits provide the specificity and transcriptional control essential for sophisticated functional genomics and metabolic pathway optimization. This work not only furnishes critical tools to dissect the unique biology of entomopathogenic fungi but also strategically positions *C. militaris* as a powerful and versatile chassis for the sustainable production of pharmaceuticals, nutraceuticals, and for green biorefinery applications.

Keywords: Chassis development, CRISPR-Cas12a, Edible fungi, Inducible expression, Synthetic biology

Abstract

FIRST REPORT OF INSECT-PATHOGENIC FUNGI IN SELECTED AREAS OF NORTHERN MINDANAO

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Insect-pathogenic fungi, primarily from the families *Clavicipitaceae*, *Cordycipitaceae*, and *Ophiocordycipitaceae* (order Hypocreales), parasitize insects and produce striking fungal growths. Despite their ecological importance, insect-pathogenic fungi remain undocumented in the Philippines, limiting understanding of their role in biodiversity and biocontrol. This study presents the first recorded survey of these fungi in Northern Mindanao, specifically in Rogongon (Iligan City, Lanao del Norte) and Imbayao (Malaybalay City, Bukidnon), conducted from August 2024 to February 2025. A mixed sampling approach was used—combining opportunistic observations and targeted collection of infected hosts across life stages (larvae, pupae, adults), including caterpillars, beetles, bugs, dragonflies, cicadas, ants, and spiders in leaf litter, soil, or attached in plant foliage. Field data included *In situ* photographs (Nikon D3300), and GPS coordinates and elevation (Garmin eTrex 10). Collected samples were then stored in sterile Falcon tubes at 4°C. Fungal taxa were identified using reference materials based on macroscopic morphology; hosts were classified at the order level. Based on our data, two families were confirmed: *Ophiocordycipitaceae*: *Ophiocordyceps* (7), *Purpureocillium* (1), *Hirsutella* (1). *Cordycipitaceae*: *Cordyceps* (2), *Akanthomyces* (1), *Gibellula* (2), *Torrubiella* (1). Insect hosts spanned Hemiptera, Diptera, Lepidoptera, Hymenoptera, and Araneae. The presence of six genera and 17 suspected species highlights a rich but underexplored insect-pathogenic fungal biodiversity in the region prompting more in-depth biodiversity studies for future applications.

Keywords: cordyceps, fungi, ophiocordyceps, insects, Philippines

Abstract

Screening of Beta-Glucan Production from Insect-Pathogenic Fungi for Potential Cosmeceutical Applications**[Donnaya Thanakitpipattana^{1*}], [Jennifer Luangsa-ard¹], [Wai Prathumpai^{2#}]**¹*[Plant-Microbe Interaction Research Team, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Pathum Thani, Thailand]*²*[Biocontrol Technology Research Team, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Pathum Thani, Thailand]**Presenting author: donnaya.tha@biotec.or.th*, Corresponding author: wai.pra@biotec.or.th[#]*

Beta-glucans are polymers of D-glucose that are essential components of the cell walls of bacteria, plants, and fungi. In fungi, beta-glucans have been reported to provide numerous benefits for human nutrition and health, including anticancer, antimicrobial, immune-modulating, antioxidant, and blood glucose/lipid-regulating activities. In this study, 73 strains of insect-pathogenic fungi (IPF) were screened for beta-glucan production in two liquid media (PDB, and M102) for potential cosmeceutical applications. Forty-three strains produced more than 1 g/L of beta-glucan; in PDB, 15 strains produced more than 1.5 g/L and 23 strains more than 2.1 g/L in M102. Cytotoxicity tests on human dermal fibroblasts (HDFn) and mouse lung fibroblasts (NCTC clone 929) revealed that 68 strains in PDB and 69 strains in M102 showed no effect on HDFn cells, and all 73 strains showed no effect on NCTC clone 929 cells. Among the screened isolates, *Polycephalomyces phaothaiensis* BCC 84546, *Akanthomyces pistillariiformis* BCC 79835, *Cordyceps lepidopterorum* BCC 79842, and *Polycephalomyces* sp. BCC 85057 produced over 1 g/L of beta-glucan and exhibited molecular weights within the specified criteria. These strains will be further evaluated for cosmeceutical activity and scaled-up fermentation.

Keywords: Beta-glucans, Insect-pathogenic fungi, Cosmeceutical Applications

Abstract

Research Progress and Application of Novel Food Resource——*Cordyceps guangdongensis*

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Abstract: *Cordyceps guangdongensis* is a rare and endemic edible and medicinal *cordyceps* species discovered in China. The fruiting bodies of *C. guangdongensis* is rich in nutritional components, exhibits significant bioactive effects, and was approved as a novel food source by the Chinese government in 2013. Currently, it is recognized by the state as one of the four major *cordyceps* species safe for edible or medicinal use. Our research team has long been dedicated to the industrial development and application of *C. guangdongensis*. We have conducted a series of studies encompassing germplasm resource evaluation, strain selection, establishment of cultivation technology, detection of nutritional and bioactive components, bioactivity research, and product development. Currently, a mature germplasm resource evaluation system for *C. guangdongensis* has been established, on the basis of which several high-quality strains have been screened out, and mature industrial-scale cultivation has also been realized. Meanwhile, based on the excellent bioactivities of fruiting bodies, including antioxidant effects, immune system enhancement, treatment of chronic renal failure, alleviation of chronic bronchitis, reduction of obesity, lowering of blood lipids, and regulation of blood glucose, a range of related products have been developed, such as powder, tablets, beverages, and liquor. Thus, with the joint efforts in *C. guangdongensis* research, a preliminary industry-academia-research integrated mode focusing on R&D and application has been realized.

Keywords: *Cordyceps guangdongensis*; biological breeding; biological activities; industrial application

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Abstract

Screening of high-quality *Armillaria* sp. and the relationship between *Polyporus umbellatus* and *Armillaria* sp.

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Abstract

Background: In view of the few studies on the influence of *Armillaria* spp. infection on the content of the chemical components in different parts of *Polyporus umbellatus* sclerotium, this study determined the biomass of *P. umbellatus* sclerotium and the contents of ergosterol, polyporusterone A, polyporusterone B and polysaccharide in the separated cavity wall of the sclerotia and the uninfected part of the sclerotia in different harvesting years under the conditions of *A. gallica* and *A. mellea* infection respectively. **Methods:** According to the difference of content and dynamic changes of the polysaccharide and the steroid substances, the superior *Armillaria* sp. were screened to obtain the best harvest years of *P. umbellatus*. Using HPLC and UV-VIS spectrophotometry methods, the contents of ergosterol, polyporusterone A, polyporusterone B and polysaccharide in *P. umbellatus* sclerotia infected by the two *Armillaria* spp. in different years were determined. In addition, the differentially expressed genes related to *P. umbellatus* polysaccharide synthesis were screened according to the transcriptomic data of different parts of *P. umbellatus* after *A. mellea* infection. **Results:** With the increase of years, the biomass of sclerotia infected by different *Armillaria* spp. had significant differences, and there were significant differences in the four components of sclerotia. The four components of the separated cavity wall of the sclerotia were significantly higher than those of the uninfected part. The best harvest time was the third year after cultivation. Transcriptomic analysis showed that the infection of *Armillaria* spp. could significantly promote polysaccharide synthesis, which provided a basis for polysaccharide content determination at the molecular level. **Conclusion:** The study clarified the influence of different *Armillaria* spp. infection on the accumulation of chemical components of *P. umbellatus* sclerotia, laying a foundation for exploring the symbiosis mechanism and provided a scientific clue for screening superior *Armillaria* sp. and guiding the artificial cultivation of *P. umbellatus* sclerotia.

Keywords: *Armillaria* sp.; *Polyporus umbellatus*; polysaccharide; steroid; transcriptome

Abstract

Mushrooms for the Heart: A Systematic Review of Clinical Studies on Cardiovascular Benefits

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Cardiovascular diseases (CVDs) are leading causes of death worldwide, often linked to chronic inflammation. Diet-based interventions are key to prevention, and mushrooms represent a nutritional source rich in antioxidants, β -glucans, terpenoids, polyphenols, L-ergothioneine, and glutathione. While animal studies highlight mushrooms' protective effects against CVD risk factors, a systematic review of human studies is needed to consolidate the evidence. A systematic review was conducted according to PRISMA guidelines. Records published between 2015 and 2024 were retrieved from PubMed, Scopus, and Web of Science. Duplicates were removed, followed by stepwise screening of titles and abstracts. Eligible studies were subjected to a bias risk assessment using Office of Health Assessment and Translation (OHAT) method. Articles rated as "high bias" were excluded, while "low" and "moderate" bias studies were retained for synthesis. From an initial pool of 130 records, 12 duplicates were excluded. Title screening eliminated 90 articles, followed by abstract screening which removed another 16. Bias assessment identified no "high" bias studies; 10 were classified as "low bias" and 2 as "moderate bias." Ultimately, 12 studies were included in this review. Evidence indicated that mushroom consumption is associated with reduced CVD risk factors, including improvements in physical performance, lowered serum glucose, reduced hypertension, and attenuated atherosclerosis. Notably, species such as *Ganoderma lucidum* and *Pleurotus ostreatus* demonstrated consistent benefits. Some studies also reported modulation of gut microbiota and reduction of low-grade intestinal inflammation, further linking mushrooms to cardiometabolic health. This review highlights the promising role of mushroom supplementation as a dietary approach to CVD prevention. While findings indicate significant potential, future research should clarify species-specific effects and explore underlying bioactive compounds. Integrating mushrooms into daily diets may represent an accessible and natural strategy to support heart health.

Keywords: anti-inflammation, antioxidant, cardiometabolic, cardiovascular disease, clinical trial, mushrooms.

Abstract

Comprehensive Germplasm Resource Integration and Genetic Characterization of Wild Edible and Medicinal Fungi: From Database Construction to Industrial Application

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Abstract:

Background: Wild edible and medicinal fungi represent critical strategic biological resources with significant ecological, economic, and health values. However, systematic conservation and industrial development of wild edible and medicinal fungal germplasm resources have been impeded by scattered resource distribution, insufficient fundamental research, and limited availability of elite breeding germplasm in both species' diversity and quantity. This study aims to establish a comprehensive platform for sustainable utilization of fungal resources through extensive resource collection, systematic germplasm preservation, multi-omics characterization, and strategies for artificial domestication and strain improvement.

Methods: The nationwide survey and collection campaign for edible and medicinal fungal germplasm resources across diverse ecological regions in China was conducted, establishing a multi-tiered germplasm repository. Utilizing self-developed software tools and omics sequencing platforms, we constructed an integrated database for edible and medicinal fungal germplasm resources, which incorporates specimen metadata collections, living culture repositories, and genomic databases. Multi-omics approaches including whole-genome sequencing, comparative transcriptomics, and population resequencing were employed to elucidate genetic mechanisms underlying key traits. The database platform integrates multidimensional data layers encompassing taxonomic information, bioactive compound profiles (polysaccharides, triterpenoids, nutritional components, volatile compounds), geographical distribution patterns, and cultivation parameters, supplemented with visualization tools, precision retrieval algorithms, and coordinate-based mapping functionalities.

Results: The established germplasm repository for edible and medicinal fungi represents one of the world's most comprehensive fungal bioresource collections, comprising 25,600 specimens, over 10,000 preserved strains, and 28,600 molecular sequences. We accomplished the first genomic characterization of the novel species *Ganoderma leucocontextum*, the newly recorded species

Flammulina fennae, and the ectomycorrhizal fungus *Russula griseocarnosa*. Transcriptomic analysis identified key regulatory genes controlling sporulation in *Ganoderma*. Population genomic analyses of *Ganoderma* spp and *Cordyceps militaris*, combining nationwide resequencing data with phenotypic traits, revealed significant correlations between genetic diversity patterns and geoclimatic variables, providing novel insights into adaptive evolution and domestication potential. 616 economically valuable *Ganoderma* strains were successfully domesticated and generated a series of elite strains through mutagenesis and screening, achieving significant enhancement in bioactive metabolite production with 35-48% increase in polysaccharide yield and 22-31% increase in triterpenoid content compared to wild-type strains. The elite cultivar "Guangzhi No 3" was officially recognized as Guangdong Province's first *Ganoderma* variety, while large-scale cultivation of *G. leucocontextum* achieved a breakthrough from zero to commercial production in Tibet Autonomous Region, generating substantial socioeconomic benefits.

Conclusion: The comprehensive database system for edible and medicinal fungal germplasm resources addresses critical gaps in fungal germplasm conservation and genetic resource utilization in China. This multi-tiered database system, integrating extensive biobanking with comprehensive molecular and bioactive compound databases, provides essential foundational data for biodiversity conservation, functional genomics research, and fungal bioprospecting initiatives. The successful transformation of wild germplasm from a mere resource to a material for large-scale application has been achieved, moving from the field to laboratory preservation and from wild strains to high-quality cultivated strains. In particular, the practical value of this database system has been demonstrated by the development of superior strains and the implementation of regional demonstration cultivation projects. These achievements have significantly enhanced the competitive advantage of China's mushroom industry, providing robust support for rural revitalization strategies and the development of the Comprehensive Health Industry.

Keywords:

Wild Edible and Medicinal Fungi; Big Database Construction; Germplasm Resource Bank; Domestication; Omics data mining.

Abstract

Research progresses of anti-obesity function and breeding in *Cordyceps guangdongensis*

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Cordyceps fungi are rich in various bio-active nutrients, with certain species exhibiting considerable food and pharmaceutical values. As a rising star among new resource foods, *Cordyceps guangdongensis* has shown significant medicinal function such as treating chronic renal failure, improving chronic bronchitis, and combating fatigue. The polysaccharides (CGP) of *C. guangdongensis* fruiting bodies were obtained by hot water extraction and ethanol precipitation, and an ethanol extract (CGE) was prepared using 55% ethanol extraction. The compositions of both CGP and CGE were analyzed. In animal experiments where the obese mice induced by high-fat diet were orally administrated using CGP and CGE dissolved in physiological saline buffer, we found that 400 mg/kg/d of CGP and 100 mg/kg/d of CGE significantly reduced body weight, epididymal and inguinal fat mass, increased brown adipose tissue mass, improved glucose tolerance, and attenuated hepatic steatosis in obese mice. Both of CGP and CGE markedly lowered hyperlipidemia indices and the activities of multiple enzymes in serum, decreased the relative abundance of Firmicutes, but increased the abundances of beneficial bacteria such as *Bacteroidetes* and *Bifidobacterium* in fecal samples. In addition, they can promote short-chain fatty acid production and bile acid excretion, and regulate the expression of genes related to lipid and fat metabolism in the liver samples. Furthermore, atmospheric and room temperature plasma (ARTP) mutagenesis and double-deactivation protoplast fusion techniques were applied, yielding six mutant strains with over 30% increase for mycelial growth rates and two fusion strains with over 16% improvement in growth. These findings provide a theoretical foundation and practical basis for high-activity strain breeding of *C. guangdongensis*, and for the application of its fruiting bodies in the food, biopharmaceutical, and health product industries..

Keywords: anti-obesity, *Cordyceps guangdongensis*, gut microbiota, high-quality strain breeding

Abstract

Study on Phamarcological Activities of Famous Mycomedicine-Sanghuang

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Background: The traditional Chinese mycomedicine-Sanghuang had been recorded in Chinese herbal books of successive dynasties since the Qin and Han dynasties. Sanghuang has much efficacy and can treat various diseases in clinical practice of traditional Chinese medicine for several thousands years.

Methods: The paper conducts research on the traditional efficacy and mechanism of Sanghuang using modern pharmacological methods. The methods include textual research of ancient and modern literature, establishing pharmacological animal models, immunohistochemistry, metabolomics, transcriptomics, proteomics, liquid quality analysis, etc.

Results and Conclusion: 1. The detailed characteristics including morphology, habitat, distribution and modern pharmacological activity proved that *Inonotus hispidus* should be the ancient traditional Chinese medicine "Sanghuang".

2. Active substances and mechanism of traditional Chinese medicine Sanghuang in treatment of "Zhengjia-Jiju" syndrome. The results showed that the tumor inhibition rate of different growth stages of Sanghuang fruiting bodies was in the order of aging stage > bud stage > mature stage, and the tumor inhibition rate of different polar extracts of Sanghuang fruiting bodies from high to low was petroleum ether extract > crude polysaccharide > water extraction alcohol precipitation supernatant > ethanol extract. Through the in vivo pharmacological activity tracking, it was determined that the effective anti-tumor part of Sanghuang was petroleum ether extract (IPE) from the aging fruiting bodies of Sanghuang. The results showed that steroids and fatty acids were the main components of the extract, in which the relative contents of polyporone B, ergosterone peroxide, and ergosterone peroxide were higher in steroids. The *Lilrb4a*, *Nrp1*, *Gzma*, *GSTT1* and *Pdk4* genes/proteins have been confirmed to be the key regulators of the anti-tumor effect of IPE, which were verified by qRT-PCR and western blotting studies. The anti-tumor mechanisms of Sanghuang petroleum ether extract and ergosterone were played significantly by activating endogenous mitochondrial pathway, endogenous endoplasmic reticulum pathway, exogenous death receptor pathway, activating the immune pathway, activating phagosome pathway, promoting amino acid metabolism pathway, and inhibiting energy metabolism.

3. Study on the active components and mechanism of traditional Chinese medicine Sanghuang in treatment of "Rupi". Study on the therapeutic effect and metabolomics of *I. hispidus* extract on mammary gland hyperplasia model rats. After the intervention of different polar extracts of *I. hispidus*, various indicators proved that the ethyl acetate extract of *I. hispidus* had the best effect in the treatment of mammary gland hyperplasia, and no obvious adverse reactions were observed.

Small molecule metabolites such as D-erythrose 4-phosphate, N-acetyl-L-glutamate and L-proline may be potential targets for ethyl acetate extract of *I. hispidus* to treat HMG, and play a role through multi-component and multi-target. The mechanism of action was studied, and it was determined to be the effective substance of *I. hispidus* in the treatment of "Rupi". By regulating the key metabolic pathways such as steroid hormone biosynthesis pathway, estradiol is metabolized into inactive estriol in the liver to achieve the effect of treating "Rupi".

Keywords: Sanghuang, *Inonotus hispidus*, Pharmacological function, Antitumor, Mammary gland hyperplasia

Abstract

Screening and Identification of *Ganoderma lucidum*-Derived Active miRNAs Targeting KRAS Gene in Pancreatic Cancer

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Pancreatic cancer is a highly malignant tumor with current chemotherapy drugs exhibiting significant side effects and susceptibility to drug resistance, necessitating the development of novel therapeutic strategies. In recent years, RNA-based drugs have become a research hotspot due to their unique physicochemical properties. KRAS gene mutations are present in up to 90% of pancreatic cancer cases, but developing small molecule drugs directly targeting KRAS has been challenging. This study proposes an innovative concept of cross-kingdom regulation of human KRAS gene by *Ganoderma lucidum*-derived microRNAs (miRNAs), providing new insights for pancreatic cancer treatment. Through high-throughput sequencing, 168 miRNAs were identified from *Ganoderma lucidum*, and cross-analysis using four prediction platforms (including MiRanda) screened 48 candidate miRNAs potentially targeting KRAS. By constructing 25 miRNA expression vectors and validating them in PANC-1 cells, miR-0001 and miR-0002 were found to significantly downregulate KRAS gene expression, while the other 23 miRNAs showed upregulating effects. Pharmacodynamic studies demonstrated that transfection with miR-0001 and miR-0002 mimics inhibited PANC-1 cell proliferation by 40.72% and 20.49%, respectively. In a nude mouse xenograft model, the tumor inhibition rates in the mimics0001 and mimics0002 groups reached 87.90% and 81.69%, significantly higher than the negative control group (29.94%) and comparable to the chemotherapy drug gemcitabine (80.99%), with statistical significance ($P < 0.01$). Mechanistic studies using AlphaFold3 predictions revealed specific binding between mimics0001/0002 and the KRAS 3'UTR region. qRT-PCR and ELISA assays confirmed significant downregulation of KRAS mRNA and protein expression in transfected groups. Dual-luciferase reporter gene experiments further validated that these two miRNAs regulate gene expression by acting on the KRAS 3'UTR region. This study demonstrated for the first time that *Ganoderma* miRNAs can target and inhibit KRAS through cross-kingdom regulation, not only providing novel candidate RNA drugs for pancreatic cancer treatment but also establishing a research paradigm for developing functional miRNAs from traditional Chinese medicine, which holds significant importance for promoting the application of natural medicines in cancer therapy.

Keywords: *Ganoderma* miRNA, KRAS gene, Pancreatic cancer, small RNA drugs

Abstract

Molecular and Protein Structural Insights into the Self-Sterility Characteristics of *Ophiocordyceps sinensis***Xiu-Zhang Li*, Yu-Ling Li, Jia-Shi Zhu#***State Key Laboratory of Plateau Ecology and Agriculture, Qinghai Academy of Animal and Veterinary Sciences, Qinghai University, Xining, China**zhujosh@163.com*

Background: The *Cordyceps sinensis* insect–fungi complex consists of the fruiting body of *Ophiocordyceps sinensis* and the remains of a *Hepialidae* moth larva, containing a thick larval body wall with numerous bristles, intact larval intestine and head tissues, and fragments of other larval tissues. Studies of natural *C. sinensis* have revealed multicellular heterokaryotic microstructures and genetic heterogeneity, including 17 genome-independent genotypes of *O. sinensis*, >90 other fungal species across 37 genera, and larval genes. The sexual reproduction of *O. sinensis* relies on the synergistic interaction of the MAT1-1-1 and MAT1-2-1 proteins. The MAT1-1-1 protein contains a MAT α _HMGbox domain, while the MAT1-2-1 protein possesses an HMG-box_ROX1-like domain. Both domains are formed by a hydrophobic core with 3 α -helices, which are critical for DNA binding and reproductive regulation. **Methods:** More than 170 wild-type *C. sinensis* isolates were examined for the occurrence and transcription of the *MAT1-1-1* and *MAT1-2-1* genes. The encoded sequences of the MAT1-1-1 and MAT1-2-1 proteins were subjected to Bayesian clustering, ExPASy ProtScale plotting and AI-based AlphaFold modeling and correlated with changes in the hydrophobicity and secondary and tertiary structures of the DNA-binding domains. **Results:** The alternative splicing, differential cooccurrences and transcription of the *MAT1-1-1* and *MAT1-2-1* genes were determined. Truncation mutations and various amino acid substitutions were detected, resulting in heteromorphic stereostructures of the MAT1-1-1 and MAT1-2-1 proteins. MAT1-1-1 proteins exhibited 24 AlphaFold-predicted stereostructure morphs and were clustered into 6 Bayesian clades, while MAT1-2-1 proteins showed 21 AlphaFold stereostructure morphs, forming 5 Bayesian clades. Truncations and 1–3 amino acid substitutions within the MAT α _HMGbox and HMG-box_ROX1-like domains of the mating proteins resulted in altered stereostructures of the domains. The diverse MAT1-1-1 and MAT1-2-1 proteins in the *C. sinensis* insect–fungi complex were derived from different fungal sources. **Conclusion:** The findings at the genetic, transcriptional and protein structural levels suggest the self-sterility of *O. sinensis* and the need for mating partners to accomplish heterothallic or hybrid reproduction during the lifecycle of the *C. sinensis* insect–fungi complex. Sexual reproductive behavior is vital for the persistence of the population size of the LEVEL–II endangered natural insect–fungi complex on the Qinghai–Tibet Plateau.

Keywords: Altered tertiary structures of the MAT1-1-1 and MAT1-2-1 proteins; alternative splicing and differential occurrences and transcription of the *MAT1-1-1* and *MAT1-2-1* genes; *Cordyceps sinensis* insect–fungi complex; heterothallic and hybrid reproduction; self-sterility of *Ophiocordyceps sinensis*

Abstract

Comprehensive Germplasm Resource Integration and Genetic Characterization of Wild Edible and Medicinal Fungi: From Database Construction to Industrial Application

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Abstract:

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Methods: The nationwide survey and collection campaign for edible and medicinal fungal germplasm resources across diverse ecological regions in China was conducted, establishing a multi-tiered germplasm repository. Utilizing self-developed software tools and omics sequencing platforms, we constructed an integrated database for edible and medicinal fungal germplasm resources, which incorporates specimen metadata collections, living culture repositories, and genomic databases. Multi-omics approaches including whole-genome sequencing, comparative transcriptomics, and population resequencing were employed to elucidate genetic mechanisms underlying key traits. The database platform integrates multidimensional data layers encompassing taxonomic information, bioactive compound profiles (polysaccharides, triterpenoids, nutritional components, volatile compounds), geographical distribution patterns, and cultivation parameters, supplemented with visualization tools, precision retrieval algorithms, and coordinate-based mapping functionalities.

Results: The established germplasm repository for edible and medicinal fungi represents one of the world's most comprehensive fungal bioresource collections, comprising 25,600 specimens, over 10,000 preserved strains, and 28,600 molecular sequences. We accomplished the first genomic characterization of the novel species *Ganoderma leucocontextum*, the newly recorded species

Flammulina fennae, and the ectomycorrhizal fungus *Russula griseocarnosa*. Transcriptomic analysis identified key regulatory genes controlling sporulation in *Ganoderma*. Population genomic analyses of *Ganoderma* spp and *Cordyceps militaris*, combining nationwide resequencing data with phenotypic traits, revealed significant correlations between genetic diversity patterns and geoclimatic variables, providing novel insights into adaptive evolution and domestication potential. 616 economically valuable *Ganoderma* strains were successfully domesticated and generated a series of elite strains through mutagenesis and screening, achieving significant enhancement in bioactive metabolite production with 35-48% increase in polysaccharide yield and 22-31% increase in triterpenoid content compared to wild-type strains. The elite cultivar "Guangzhi No 3" was officially recognized as Guangdong Province's first *Ganoderma* variety, while large-scale cultivation of *G. leucocontextum* achieved a breakthrough from zero to commercial production in Tibet Autonomous Region, generating substantial socioeconomic benefits.

Conclusion: The comprehensive database system for edible and medicinal fungal germplasm resources addresses critical gaps in fungal germplasm conservation and genetic resource utilization in China. This multi-tiered database system, integrating extensive biobanking with comprehensive molecular and bioactive compound databases, provides essential foundational data for biodiversity conservation, functional genomics research, and fungal bioprospecting initiatives. The successful transformation of wild germplasm from a mere resource to a material for large-scale application has been achieved, moving from the field to laboratory preservation and from wild strains to high-quality cultivated strains. In particular, the practical value of this database system has been demonstrated by the development of superior strains and the implementation of regional demonstration cultivation projects. These achievements have significantly enhanced the competitive advantage of China's mushroom industry, providing robust support for rural revitalization strategies and the development of the Comprehensive Health Industry.

Keywords: Wild Edible and Medicinal Fungi; Big Database Construction; Germplasm Resource Bank; Domestication; Omics data mining.

Abstract

Species Diversity and Utilization of Medicinal Mushrooms in South China**Xi Lei ^{1,2*}, Chenghua Zhang ^{1#}**

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Abstract: South China boasts abundant fungal resources, yet systematic investigations and research on its medicinal fungi are lacking. Systematic analyses of medicinal mushrooms from South China were conducted through field investigation and literature review. There are 220 species of medicinal mushrooms in South China, belonging to 2 phyla, 5 classes, 17 orders, 59 families, and 117 genera. Approximately 92% of these species (202) belong to the phylum Basidiomycota. The dominant families include Polyporaceae (22), Russulaceae (21), and Ganodermataceae (11), while the dominant genera are *Russula* (14), *Ganoderma* (8), and *Trametes* (7). The medicinal mushrooms in South China exhibit diverse biological activities such as anti-tumor, antioxidant, anti-pathogenic, and immune-regulatory effects. Among them, more than 140 species have varying degrees of tumor or cancer inhibitory functions, 100 species possess antioxidant properties, 75 species demonstrate antibacterial and antiviral activities, and 50 species can regulate human immunity. *Tolypocladium guangdongense*, an endemic new resource of edible and medicinal mushrooms in South China, shows excellent medicinal effects including anti-fatigue, antioxidant, anti-avian influenza virus, lifespan extension, and prevention and treatment of chronic renal failure, holding broad prospects for development and application.

Keywords: Medicinal mushroom; biological activities; *Tolypocladium guangdongense*

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Diversity of Pathogenic Fungi in Ancient *Platycladus orientalis* and Biological Control

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The ancient oriental arborvitae (*Platycladus orientalis*) holds unique historical, cultural, and ecological significance in landscape settings. In Beijing, there are over 20,000 ancient oriental arborvitae trees, accounting for more than 50% of all ancient trees. Diseases affecting these trees are highly prevalent, with over 80% suffering from needle blight, followed by branch blight and branch canker, which affect approximately 35%. To systematically investigate the species diversity of pathogenic fungi in ancient oriental arborvitae in Beijing, this study extensively collected disease samples from 15 regions where these trees are concentrated, including Zhongshan Park, Ritan Park, Beihai Park (Tuancheng), the Ming Tombs, the Summer Palace, Xiangshan Park, Tanzhe Temple, Jietai Temple, Taiziyu Ancient Tree Garden, Hongluo Temple, the Temple of Heaven Park, the Imperial College (Confucius Temple), Ditan Park, the Labour People's Cultural Palace, and Fahai Temple. Fungal identification was performed using morphological and molecular phylogenetic methods, and pathogenicity was verified using Koch's postulates, yielding the following results. In total, 394 disease specimens were collected, comprising 127 needle disease samples and 167 branch disease samples. A total of 675 fungal strains were isolated from the boundary between diseased and healthy tissues, including 247 strains from needles and 428 strains from branches. Initial identification, based on colony morphology on MEA medium and high-resolution gene fragments, revealed that *Alternaria* species accounted for the highest proportion, with 196 strains (29.04%). To date, 46 pathogenic fungal species have been identified through morphological characteristics and multi-gene phylogenetic analyses based on ITS, LSU, *TEF1*, *RPB1*, *RPB2*, *TUB2*, *GAPDH*, *ALT*, *ACT*, and *CAM* genes. Among these, 9 are suspected new species: *Alternaria platycladi* sp. nov., *Curvularia* sp. nov. (two species), *Fusarium* sp. nov. (four species), *Nothophoma platycladus* sp. nov., and *Toxicocladosporium* sp. nov. In addition, 38 biocontrol fungal strains from the genus *Trichoderma* were isolated from the trunks of ancient arborvitae. Morphological and molecular phylogenetic analyses identified four species: *Trichoderma*

citrinoviride, *T. nordicum*, *T. obovatum*, and a new species, *Trichoderma* sp. nov. Confrontation assays demonstrated significant antagonistic effects against pathogens in all four species. Furthermore, six biocontrol bacterial strains were tested in the laboratory, including *Bacillus velezensis* GY5.4, *Bacillus safensis* XL3.4, *Bacillus amyloliquefaciens* GY2.1, *Bacillus licheniformis* B1, *Bacillus subtilis* B3, and *Paenibacillus polymyxa* JL14.4. Resistance screening for these biocontrol bacteria is still ongoing. Future research will focus on completing the identification of unclassified species and compiling a catalogue of pathogenic fungi associated with ancient oriental arborvitae in Beijing. Concurrently, we aim to propose effective biological control strategies for the diseases affecting these trees through antagonism experiments and field-based efficacy evaluations.

Keywords: Ancient Oriental Arborvitae (*Platycladus orientalis*), Beijing, Biological control, Pathogenic fungi, Species diversity

Abstract

Genome-scale phylogenetics and integrative species delimitation to re-clarify taxonomic boundaries on the species level of pestalotiopsis-like fungi

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Abstract

Pestalotiopsis-like taxa encompass a diverse group of fungi that are often associated with plant diseases, unique ecological interactions such as endophytism, and the production of novel chemical metabolites. Traditionally, classifying pestalotiopsis-like taxa has been challenging due to their significant phenotypic plasticity and the overlapping morphological characteristics among species. Identifying certain isolates at the species level remains problematic, or even impossible, with current DNA sequencing methods, which highlights issues with defining species boundaries. To refine species boundaries, we integrated evidence from single-gene phylogenies (ITS, *tef1*, and *tub2*), multi-gene phylogenetic analyses combined with several species delimitation methods (GCPSR—genealogical concordance phylogenetic species recognition; PTP—Poisson tree processes; mPTP—multi-rate Poisson tree processes; ABGD—automatic barcode gap discovery; and ASAP—assemble species by automatic partitioning), and genomic metrics including average nucleotide identity (ANI) and the proportion of shared core gene clusters within the pangenome. Two novel species, *Neopestalotiopsis camelliae* and *Pseudopestalotiopsis dasymaschalonis*, were introduced based on integrative analyses. Evidence of over-splitting was detected across all three genera. Consequently, five species complexes—*Pe. adusta* species complex, *Pe. brassicae* species complex, *Pe. clavata* species complex, *Pe. rosea* species complex, and *Ps. cocos* species complex—were established to accommodate lineages that form monophyletic clades in both multi-gene and genome-scale phylogenies but exhibit short internal branches, low statistical support, and indistinct species boundaries.

Keywords: Pestalotioid, Species boundaries, Species/species-complex, Sporocadaceae, Taxonomy

Abstract

World's deadliest mushrooms**Samantha Chandranath Karunarathna**

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Only a small percentage (approximately 1%) of the more than 14,000 known species of macrofungi produce fruitbodies (mushrooms) that are deadly or potentially deadly to humans. Despite this low number of deadly species, hundreds of people worldwide die each year because of 'mushroom' poisoning. Insufficient knowledge, unclear taxonomy, rising consumption of fungi, and misleading folklore, such as the 'silver spoon test' or 'peeling the cap,' are all contributing factors to these deaths. This work is a crucial discussion on the 37 macrofungal species known to be fatal. It has been meticulously compiled to raise awareness of food safety issues related to poisonous mushrooms and to expand the general public's and the scientific community's knowledge base on this topic. This work provides detailed information on the taxonomy, ecology, and distribution of these species, serving as a significant contribution to the field. In addition, this serves as a platform to present the latest information and catalyze further research into the taxonomy, toxicology, ecology, and associated medical treatments for poisonings caused by these macrofungi. Furthermore, we discuss methods for preventing future instances of poisoning and the necessity of developing regional-level educational and awareness programs where they would be most beneficial, offering hope for a safer future.

Key words: amatoxins, ascocarps, basidiocarps, gyromitrin, muscarine, mushroom poisoning, orellanine, virotoxins

Abstract

Exploring Fungal Diversity of the South China Sea Coast**Nimali Indeewari de Silva^{1*}, Sinang Hongsan^{1,2#}**

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Coastal regions encompass some of the most biodiverse and unique ecosystems on Earth. China is one of the countries possess numerous sea resources with vast maritime region. The South China Sea is the largest marginal sea in the western Pacific, covering ~3.5 million km² along the continental margin of East Asia and averaging over 2,000 m in depth. The South China coastline harbours different ecosystem types, i.e., coral reefs, seagrasses, mangroves, wetlands, bays, and coastal vegetation. The largest mainland coastline in South China sea can be seen in Guangdong Province which spans along in 14 coastal cities. The persistence of many fungal species relies upon diverse macro and micro habitats in coastal areas. Despite the availability of abundant ecological niches, research on the fungi inhabiting coastal South China region remains limited. Therefore, as a research team at the College of Life Science and Oceanography, Shenzhen University, we investigate fungal diversity from various coastal substrates, including dead plants, soil, and rocks. Currently we have observed numerous plant specimens and revealed 35 new fungal species, 40 new host records, and new collections from Dothideomycetes and Sordariomycetes (Ascomycota). We identified new plant pathogens records and confirmed their pathogenicity via Koch's postulates. Exploration of soil fungal diversity in natural and agricultural areas is another research goal in our team. Our findings show that majority of new fungal taxa and new records of soil inhabitant fungi belongs to Ascomycota. We studied these beneficial soil fungi to promote plant growth and soil health. The coastal environment is one of the extreme environments for rock inhabiting fungi since they are exposed to stresses, such as solar radiation, desiccation and rehydration, and temperature fluctuations. The peculiar morphological features based on melanisation and slow growth of rock inhabiting fungi are important in ecology, and potential in biotechnology. Thus, we conduct research to achieve a better understanding of the hidden biodiversity and their salt tolerant abilities and gene regulation.

Keywords: Plant pathogens, rock inhabiting fungi, saprobes, soil fungi, taxonomy

Abstract

Species Diversity and Taxonomy of Xylariales in Southwest China**Qi-Rui Li^{1,2*}, Ji-Chuan Kang²**

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Southwestern China, including Guizhou Province, Yunnan Province, Sichuan Province, and other provinces, harbors one of the world's largest areas of typical karst landforms. Its unique geographical environment supports a high diversity of fungal species, among which the Xylariales is an important taxonomic group. Since 2018 to the present, a total of more than 6,200 specimens have been collected in this region. By integrating morphology and multi-gene phylogenetic analyses (including ITS, LSU, *rpb2*, *TUB2*, and *TEF-1 α*), 12 families, 42 genera, and 453 species of Xylariales have been identified, including 193 new species. The collected specimens are mainly concentrated in the following families: Cainiaceae, Diatrypaceae, Hypoxylaceae, Pallidoperidiaceae, Vamsapriyaceae, and Xylariaceae. Targeted investigations were conducted on the genera *Xylaria*, *Hypoxylon*, and *Annulohypoxylon*, resulting in the identification of 73 species of *Xylaria*, as well as 85 species of *Annulohypoxylon* and *Hypoxylon*. In the systematic taxonomic study of Xylariales, several significant systematic taxonomy issues were identified: 1. A large number of genera remain classified under incertae sedis; 2. Xylariales still contains numerous polyphyletic and paraphyletic genera, such as *Xylaria*, *Hypoxylon*, and *Anthostomella*. We propose that the causes of these phenomena may include: 1. Lack of DNA sequences derived from type specimens; 2. A large number of evolutionary intermediate species remain undiscovered; 3. Convergent evolution; 4. Divergent evolution. Potential solutions to address these issues may include: 1. Collecting a large number of fresh specimens to fully discover evolutionary intermediate species and ensure the integrity of the evolutionary process; meanwhile, collecting specimens of type species for DNA sequences of type species; 2. Applying novel methodologies (e.g., chemical characteristics and genomic sequence information) to compensate for the limitation that existing traits are insufficient to fully reflect the evolutionary relationships among species. Southwestern China is rich in Xylariales resources, with a large number of undiscovered taxa. Comprehensive surveys in this

region will significantly contribute to the advancement of systematic and taxonomic research on Xylariales.

Keywords: Ascomycetes, China, Microfungi, Phylogenetic

Abstract

Amazing helicosporous hyphomycetes**Yong-Zhong Lu^{1,2,3*}, Jian Ma¹, Ji-Chuan Kang²**

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Helicosporous hyphomycetes represent a unique group of microfungi with significant applied value, capable of producing structurally novel bioactive natural products that hold potential for new drug development. However, these fungi present several challenges, including ambiguous interspecific boundaries, difficulties in identification, limited strain resources, and insufficient molecular data. Moreover, discrepancies exist between traditional morphological classification and molecular phylogenetic analyses: some morphologically conserved species exhibit significant genomic divergence, while morphologically distinct species may belong to the same molecular clade. In this study, we collected nearly 9,000 samples from various regions across China, obtaining over 1,100 helicosporous hyphomycete specimens. Through single-spore isolation and cultivation, 1,060 strains were acquired, significantly enriching the strain resources for this group. By integrating classical morphology with multi-gene (ITS, LSU, RPB2, TEF1 α) phylogenetic analyses, we identified 232 species belonging to 3 classes, 8 orders, 9 families, and 38 genera, including 18 novel genera, 136 new species, and 71 new combinations. The study further clarified the generic morphological characteristics of morphologically similar groups such as *Helicoma*, *Helicosporium*, *Helicomycetes*, and *Tubeufia*, resolving ambiguities in intergeneric and interspecific classification boundaries and revising the taxonomic systems of related genera. Additionally, 112 secondary metabolites were isolated and identified from 7 helicosporous hyphomycete strains, including 24 novel natural products. Notably, the new dimeric compound Neogrisphenol A, produced by *Neohelicosporium griseum*, exhibited significantly higher activity against human ovarian cancer cell line A2780 than the positive control drug, demonstrating potential for anti-ovarian cancer drug development. The novel compounds Rubracin D and Dirubracin A, derived from *Tubeufia rubra*, effectively reversed tumor cell multidrug resistance, suggesting potential as

tumor resistance reversal agents or chemosensitizers. This study provides a scientific foundation for microbial drug discovery.

Keywords: Microfungi, Ascomycetes, Phylogenetic analysis, Classical morphology

Abstract

Cordyceps* biodiversity and industrialization of *Ophiocordyceps sinensis

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Cordyceps sensu lato create tremendous economic value through their medicinal, biological, and nutritional properties. Extensive research has been conducted on these properties, which has translated into practical applications. Cordycepin is a crucial bioactive compound produced by various *Cordyceps sensu lato* species, but it is challenging to synthesize it in large quantities through industrial production. Therefore, it is imperative to emphasize the accurate classification and identification of *Cordyceps sensu lato* and to optimize the large-scale production conditions for *C. militaris* and cordycepin, as well as to explore the industrialization of *C. militaris*.

Our study has achieved several significant progress in tackling the challenges in these research fields. Firstly, utilizing morphological characteristics and multi-gene phylogenetic methods, we discovered two new hypocrealean families (Polycephalomycetaceae and Chlorocilliumaceae) and 200 new species from China, Thailand, and Russia. Secondly, we have conducted a study on cordycepin production via solid-state fermentation. We optimized the levels of variables using a Central Composite Design (CCD) experiment and predicted the maximum cordycepin yield of 18.92 mg/g using the optimized medium components. Thirdly, we improved the cordycepin separation and purification process, and our production line currently has a capacity of approximately 200 kg per year. Thirdly, Due to the scarcity of resources, wild *Ophiocordyceps sinensis* is extremely expensive, with prices ranging from several hundred to several thousand yuan per gram, depending on the specific specifications. In contrast, artificially cultivated *Ophiocordyceps sinensis* is significantly less expensive, with costs having decreased from over 30 yuan per piece for the first generation to less than 0.1 yuan per piece for the fourth generation. The end-product prices are more accessible, and the market potential is substantial.

In summary, this research has enriched the fungal biodiversity of *Cordyceps sensu lato* and provided an effective approach for enhancing the production of fruit bodies and cordycepin on a large scale. The strategies employed in this study could be widely applicable to other fermentation processes.

Keywords: *Ophiocordyceps sinensis*, new families, fourth generation, Species diversity

Acknowledgements: This work was supported by the National Natural Science Foundation of China (No. 32570025).

Abstract

Taxonomy and diversity of freshwater fungi in Thailand and southwestern China**Dan-Feng Bao¹, Yong-Zhong Lu^{2,3}, Zong-Long Luo^{4*}, Ji-Chuan Kang^{1*}**

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Freshwater fungi are ecologically critical fungi that drive nutrient cycling, organic matter decomposition, and ecosystem stability. Despite their vital role in maintaining aquatic ecosystem health, these fungi represent a vast, underexplored reservoir of chemical diversity with significant potential for bioactive compound discovery. However, global biodiversity assessments remain severely limited, with only ~3,870 formally described species (2–3% of known fungal diversity). Taxonomic uncertainties persist due to morphological plasticity and historical reliance on single-gene analyses, while evolutionary origins of these fungi, particularly within subclasses like *Diaporthomycetidae* and *Hypocreomycetidae*, remain poorly studied. In this study, approximately 2000 samples were collected from Thailand and Southwestern China with 850 fungal strains were obtained, based on phylogenetic analysis and morphological analysis, 266 species were identified distributed across 88 families, 35 orders, six classes, and three phyla, including 64 new species and three new combinations. Additionally, A multi-gene phylogenetic framework and molecular clock was constructed for the subclasses *Diaporthomycetidae* and *Hypocreomycetidae*. By integrating morphological characteristics, multi-locus phylogenetic analyses and divergence time estimates, this study resolved long-standing taxonomic uncertainties and misplacements of families, genera, and species, thereby refining the taxonomic framework for the two subclasses by introducing six orders, nine new families. Furthermore, Molecular clock and ancestral state reconstruction revealed that ancestor of *Hypocreomycetidae* originated in terrestrial habitats, the early-diverging lineages

within this class were predominantly terrestrial, while freshwater and marine fungi formed distinct branches, exhibiting a pattern of secondary evolutionary derivation. This study resolves long-standing taxonomic uncertainties and elucidates the evolutionary origins of freshwater fungi, providing critical insights for biodiversity conservation in freshwater ecosystems.

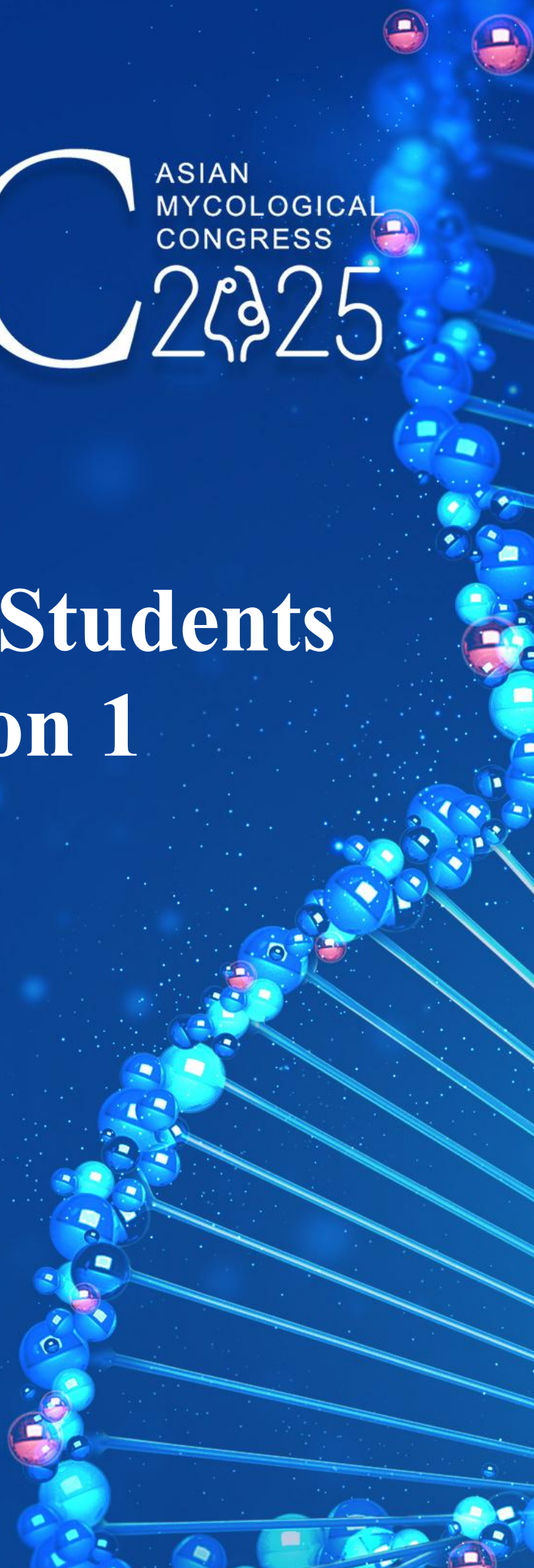
Keywords: Ancestral character analysis, *Diaporthomycetidae*, *Hypocreomycetidae*, Molecular clock analysis, Phylogeny

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Abstract

Phylogenetics of the Pluteaceae and Diversity of Species in Southwest China**[Xuan Chen*], [Zhu L. Yang#]***[State Key Laboratory of Phytochemistry and Natural Medicines, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China]*Email of the corresponding author: fungi@mail.kib.ac.cn

The family Pluteaceae (Basidiomycota, Agaricomycetes, Agaricales), saprophytic fungi comprises two genera *Pluteus*, and *Volvopluteus*. Accurate species delimitation is fundamental to phylogenetic and evolutionary studies; however, many described species within this group exhibit minimal morphological differentiation and high molecular similarity, suggesting possible over-splitting in current classifications.

In this study, we employed an integrative taxonomic approach that combines detailed morphological assessment with two molecular species delimitation methods (bPTP and BPP) to clarify species boundaries in Pluteaceae. We also reconstructed multi-locus phylogenies and phylogenetic networks to explore both divergent and reticulate evolutionary patterns.

Based on consensus results of species delimitation methods and morphology evidence, we recognized 57 species in *Pluteaceae*. Our results demonstrate persistent ambiguity in species boundaries across several clades. Phylogenetic network analyses revealed substantial reticulation, especially within the morphologically similar *Pluteus* section *Pluteus*, which has experienced at least four hybridization events and extensive introgression. These processes of reticulate evolution likely underlie both the observed morphological stasis and the challenges associated with species discrimination based on limited genetic markers.

To avoid excessive species splitting, we recommend adopting a BPP support threshold of 100% as a criterion for describing new species in *Pluteaceae*. This study not only provides a robust phylogenetic framework but also underscores the importance of reticulate evolution—driven by introgressive hybridization and incomplete lineage sorting—as a major factor complicating taxonomy and species delimitation in this family.

Keywords: Pluteaceae, Phylogenomics, Species delimitation

Abstract

Fungal Taxonomy in the Genomic Era: New Criterion and Applications

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The primary goal of taxonomy is to construct a natural classification system, or a “Tree of Life”. In modern fungal taxonomy, classification systems are primarily built on three principles: monophyly, diagnostic characteristics, and divergence times. In recent years, genomic data has greatly improved our understanding of the fungal Tree of Life, but also brings new challenges. Different genomic datasets or analytical methods sometimes yield conflicting, but highly supported, topologies, meaning well-supported “clades” in phylogenetic trees may not always be monophyletic. The three aforementioned principles are also insufficient to address this challenge. Using QuartetScores software to further analyse the phylogenomic results of the Subkingdom *Dikarya* within the Kingdom *Fungi*, we introduce the extended quadripartition internode certainty (EQP-IC) value as a novel criterion for constructing fungal classifications, with a recommended threshold of 0.1 for each taxonomic category. Suprageneric taxa with an EQP-IC value exceeding 0.1 exhibit reduced topological variation, suggesting a stronger correspondence with natural taxonomic category. This new criterion was also applied to investigate the derived suborders of mushroom-forming *Agaricales*, including three suborders, *Agaricineae*, *Pluteineae*, and *Tricholomatineae* (APT), that had been long-standing problems in phylogenetic analyses. In total, 142 genomes, including 64 newly generated ones, were utilized to reconstruct the phylogenetic relationships based on concatenated and coalescent methods. The phylogenetic incongruencies and evolutionary histories of APT were also investigated. Phytotop analyses revealed that widespread and high-level incomplete lineage sorting (ILS) and introgression/hybridization (IH) present among suborders within the APT, suggesting their relationships may be reticulate rather than strictly dichotomous. Three new suborders, *Amanitineae*, *Macrocytidiineae*, and *Omphalinineae* are added to the APT clade. The new combination *Baisuzhenia humphreyi*, new genus *Baisuzhenia*, new family *Baisuzheniaceae*, and new suborder *Baisuzheniineae* are proposed to accommodate *Stereopsis humphreyi*, which shows an independent, but close relationship with the clade formed by the six above-mentioned derived suborders of *Agaricales*.

Keywords: Bai Suzhen; Phylogenetic conflicts; New taxa; Systematics

Abstract

Species Diversity and their Applications of Macrofungi in Fujian Province, China

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Abstract: Macrofungi represent a highly taxonomically diverse biota within Earth biosphere, encompassing numerous Basidiomycota and Ascomycota taxa with morphologically, ecologically, and phylogenetically diverse fruiting bodies. Mountainous topography, subtropical monsoon climate, hydrological abundance, and exceptional forest coverage in Fujian of China create optimal edaphic-microclimatic conditions facilitating macrofungal biodiversity. In this study, we conducted comprehensive mycological surveys across diverse ecological habitats throughout Fujian Province, resulting in the procurement of 4,135 macrofungal voucher specimens. Based on morphological examination and phylogenetic analyses, we delineated 667 distinct macrofungal taxa, including the discovery and circumscription of 51 novel species representing significant contributions to mycological systematics. Furthermore, we seek to comprehensively document the geographical distribution patterns of Macrofungi across Fujian Province while simultaneously generating fundamental taxonomic reference data through integrated morphological observations and phylogenetic analyses, and enriching the taxonomic research on Asian Macrofungi. Meanwhile, some species including their most partial genes, proteins, metabolites and cultivars, can facilitate the development of agriculture and forestry and human progress.

Keywords: Basidiomycota, Ascomycota, Macrofungi, new species, innovation applications

Abstract

Revisiting Coelomycetes: An Integrative Review of Taxonomy and Phylogeny**Chao Chen ^{1,2*}, Kevin D. Hyde ^{1,2,3}, Ishara S. Manawasinghe ^{2#}, Ratchadawan Cheewangkoon ^{1#}**¹*Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand*²*Beijing Key Laboratory of Environment-Friendly Management on Fruit Diseases and Pests in North China, Institute of Plant Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, People's Republic of China*³*Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, Thailand; School of Science, Mae Fah Luang University, Chiang Rai, Thailand*Email of the corresponding author ishara9017@gmail.com; ratchadawan.c@cmu.ac.th

Coelomycete is the general term for asexual fungi that produce conidia inside a cavity, which are characterized as pycnidial, acervular, cupulate, pycnothyrial, or stromatic conidiomata. Coelomycetes comprise about 1400 genera, and include endophytic, saprobic and important plant pathogenic species. Historically, the taxonomy and classification of coelomycetes relied heavily on morphological characters such as conidiomatal type and conidial features, often leading to uncertain or polyphyletic groupings. Recent advances in molecular phylogenetics have significantly enhanced our understanding of their evolutionary relationships and enabled the integration of asexual and sexual morphs. However, morphological characters remain indispensable for taxonomy, as many genera still lack sequence data, especially when introducing new taxa. Previous monographs have provided systematic overviews of both hyaline and dematiaceous coelomycetes. However, these monographs remain incomplete, and the ongoing discovery of novel species, genera, and higher-level taxa shows the evolution of coelomycete taxonomy. This study synthesizes current knowledge on coelomycete diversity, highlights significant progress in multi-locus phylogenetic analyses and detailed morphological descriptions, and introduces several important Coelomycetous fungi, viz. *Bartalinia*, *Botryosphaeria*, *Colletotrichum* and *Phoma*. By providing an updated taxonomic framework and discussing persistent challenges, this study aims to serve as a foundational resource for future research into the taxonomy of these diverse fungi.

Keywords: Asexual fungi; Morphology; Multi-gene analysis

Abstract

Taxonomy and phylogeny of *Septoria* and *Septoria*-like fungi (Mycosphaerellaceae) from Xinjiang Autonomous Region, China

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Septoria (Mycosphaerellaceae, Ascomycota) is a species-rich phytopathogenic genus which comprise over 3,000 taxa that cause foliar necroses on diverse plant hosts. Extensive research has recognized numerous related genera collectively termed "Septoria-like fungi," including *Ramularia*, *Sphaerulina*, and *Nothoseptoria*. However, comprehensive molecular phylogenetic studies remain limited, particularly in Xinjiang, China, where diverse climatic gradients suggest substantial undiscovered diversity. This study presents comprehensive taxonomic and phylogenetic analyses of *Septoria* and allied genera from Xinjiang Autonomous Region and adjacent areas of northwestern China. A total of 95 fungal isolates were collected from 81 host plant species representing 51 genera and 32 families across diverse ecological niches. Multilocus phylogenetic analyses using ITS, LSU, rpb2, tef-1 α , tub2, act, and cal, combined with morphological characterization were used in species delineation. Based on integrated analyses, 28 *Septoria* species were identified, including 12 novel species and 10 new Chinese records. *Septoria anchusicola*, *S. artemisiae-macrocephalae*, *S. brassicicola*, *S. caraganicola*, *S. convolvulicola*, *S. epilobii-minutiflori*, *S. hejingensis*, *S. kareliniae*, *S. klaseae*, *S. polemonii-caerulei*, *S. rubiae-cordifoliae*, and *S. wusuensis* are introduced as new species. New Chinese records comprise *S. argyraea*, *S. astragali*, *S. cerastii*, *S. firouraghina*, *S. plantaginis-psylli*, *S. sedicola*, *S. senecionis*, *S. semicircularis*, *S. sii*, and *S. tosevi*. In addition, eight new *Septoria*-like taxa are described; *Apseudocercospora ligulariae*, *Neocaryophylloseptoria potentillae*, *Neopseudocercospora brassicicola*, *Neoseptoria tienshanensis*, *Nothopseudocercospora dictamni-dasy carp*, *Ramularia lonicericola*, *R. xinyuanensis*, and *Sphaerulina populi-euphraticae*. With the updated taxonomy, of *Septoria*-like fungi four new genera are proposed as *Interseptoria*, *Septoriaella*, *Septoriopsis*, and *Xeroseptoria*, together with one new combination *Caryophylloseptoria saponariae*. Furthermore, sequence data for 17 species are provided for the first time. These results reveal remarkable species richness of

Septoria and related genera in northwestern China. Our study significantly expands the understanding of this *Septoria*-like fungi and contributes substantially to global Mycosphaerellaceae taxonomy. This phylogenetic framework provides a foundation for future studies and emphasizes the need for continued biodiversity surveys in understudied Chinese regions.

Keywords: New species; Taxonomy; Phylogeny; Northwestern China

Abstract

Symphony of Fungi: untapping the Diversity of Fungi growing on Mangrove associates

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Mangrove-associated plants in the Gulf of Thailand support diverse vegetation communities and contribute to overall ecosystem diversity. These glycophytic species, tolerant of moderate salinity (3–5 Practical Salinity Unit), play a key role in the ecological balance of mangrove ecosystems through litterfall. Despite this, the diversity of fungi associated with these plants remains underexplored. Between 2023 and 2025, we conducted five sampling surveys along the Pranburi River and adjacent areas in Prachuap Khiri Khan, Thailand, to investigate micro-fungi associated with mangrove-associated plants, including *Acrostichum aureum*, *Hibiscus tiliaceus*, *Leucaena leucocephala*, and *Volkameria inermis*, along the Pranburi River in Prachuap Khiri Khan, Thailand. Our findings reveal *Sordariomycetes* (51%) was the dominant class, followed closely by *Dothideomycetes* (47%), with *Eurotiomycetes* comprising a smaller fraction (2%). Three novel species: *Neoarthrinium aquaticum*, *Oncopodiella lignicola*, and *Vaginatispora acrostichii* were reported from *Acrostichum aureum* and *Hibiscus tiliaceus*. Other prominent saprobic genera identified include *Verruculina enalia*, species of *Diaporthe*, *Lasioidiplodia*, *Lignicola*, and *Rhytidhysterium*. Additional genera, such as *Acrocalymma*, *Brevicollum*, *Bruenneofusispora*, *Chloridium*, *Corollospora*, *Cytospora*, *Diplocladiella*, *Heteroacremonium*, *Lundqvistomyces*, *Melomastia*, *Memnoniella*, *Montagnula*, *Neoarthrinium*, *Nigrograna*, *Okeanomyces*, *Oncopodiella*, *Paradietiarthrinium*, *Patellaria*, *Peroneutypa*, *Preussia*, *Pseudomelanconiella*, *Pseudoastrospheriellopsis*, *Saagaromyces*, *Sporidesmium*, *Vaginatispora*, and *Veronaea*, were observed. Fungal assemblages were most abundant on *Hibiscus tiliaceus* (53.1%), followed by *Volkameria inermis* (26.5%), *Leucaena leucocephala* (8.8%), *Acrostichum aureum* (7.1%), and *Acanthus ilicifolius* (4.4%). This can be attributed to the woody nature of the host, *Hibiscus*

tiliaceus, and its widespread availability across all sampling sites. Morphology and multi-gene phylogeny of nuclear large subunit ribosomal DNA (LSU), nuclear small subunit ribosomal DNA (SSU), internal transcribed spacer (ITS), beta-tubulin (*TUB2*), translation elongation factor 1-alpha (*TEF1*), and RNA polymerase II second largest subunit (*RPB2*), based on Maximum-likelihood, Maximum-parsimony, and Bayesian inference, confirm the placement of genera within the respective families. Given the vital role of micro-fungi in ecosystems as decomposers and the increasing anthropogenic threats, conserving fungi and raising awareness are crucial steps in protecting both the mangrove ecosystems and fungi.

Keywords: *Acrostichum aureum*, *Hibiscus tiliaceus*, *Leucaena leucocephala*, *Volkameria inermis*, Pranburi

Abstract

Saprobic Fungi on Seashore Plants: Taxonomic Insights from Coastal Thailand

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Coastal vegetation includes submerged or partially submerged plants such as seagrasses, macroalgae, mangroves, and salt marshes. These coastal ecosystems play crucial roles in mitigating coastal erosion, managing flooding, and buffering the impact of storms. Fungi, which are closely linked to these fertile habitats, contribute significantly to water and soil quality improvement. In this study, we investigate saprobic fungi on seashore plants, with a focus on salt marsh hosts *Suaeda maritima* and *Sesuvium portulacastrum*. The attached dead plant material was collected from the seashore of the central region of Thailand during high- and low-tide seasons from 2022 to 2024. Morphology and multigene phylogeny were used for fungal identification. More than 60% of 60 fungal samples belonged to *Dothideomycetes*, with *Pleosporales* as the dominant order. *Neocamarosporium* (*Pleosporales*, *Dothideomycetes*) and *Halocryptovalsa* (*Xylariales*, *Sordariomycetes*) were the most frequently observed genera. Although *Halosphaeriaceae* (*Microascales*, *Sordariomycetes*) is a common aquatic marine and freshwater fungal family, few species were collected and limited to submerged samples collected during high tide, for examples: *Saagaromyces* species. This study contributes to knowledge of fungal communities in marine-based habitats.

Keywords: aquatic fungi, fungal taxonomy, marine fungi, phylogeny, salt marsh fungi

Abstract

Systematics of Tropical Ant Nests-Associated Chaetothyriales

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Interactions between ants and fungi have been documented since last century. Chaetothyriales is an overriding fungal order that creates a symbiosis with ants residing in the ant nests. The oligotrophic lifestyle, melanized cell wall, and vast diversity of enzyme production fit chaetothyrialean species to survive in this unique niche. These enzymes are utilized in various industries, such as detergent production, food processing, bioremediation, and the breakdown of polymers. They also produce a range of bioactive compounds with potential applications in developing new drugs. Melanin pigments in the cell wall are used for cosmetics, food coloring, and as a protective coating for sensitive materials. Therefore, chaetothyrialean species are proposed for use not only in ecological aspects but also in the exploitation of novel biotechnological processes and biomolecules. The literature reports biodegradation is a significant character of most chaetothyrialean fungi, and ant nests are rich in hydrocarbons and volatile compounds released by ants and plants. Scientists paid less attention to the diversity of chaetothyrialean taxa and their utilization in this under-studied niche. Poor sample collection, unstable phylogeny in different scenarios, undetermined isolates in GenBank, and unavailability of protein-coding gene sequences for many species create incorrect classification and identification. A broad sample collection and multigene phylogenies with protein-coding genes are required for accurate delimitation of species and generic boundaries. In this ongoing study, we collected and isolated ant nests-associated fungal isolates from southwest China; and identified them by morphology and multigene phylogeny. This study will be found more species and novel species will be introduced, and the systematics of some incertae sedis taxa will be determined. The physiological performance of chaetothyrialean species will be determined.

Keywords: Bioremediation, Extremophiles, Fungal diversity, Symbiosis, Taxonomy

Abstract

Taxonomy Based on a Culture of a Species of the Genus *Pyxidiophora* (Laboulbeniomycetes) Isolated from Animal Cadaver

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The class Laboulbeniomycetes, including the orders Laboulbeniales, Herpomyces and Pyxidiophorales, are biotrophic parasites associated with arthropods and fungi. The Laboulbeniomycetous fungi except Pyxidiophorales are typically characterized by the loss of hyphae, entire life cycle on a host animal, and lacking asexual reproduction. On the other hand, the Pyxidiophorales is known to produce several different shapes of anamorphs: dispersal anamorphs and hyphal anamorphs. Its teleomorph has been discovered from various habitats like dung, submerged wood, rotting mushroom, and bark beetle galleries etc. It is believed that when a small animal like mite walks around perithecium, ascospores released from perithecium might attach to its surface. Then, ascospores directly divide into a few-celled thalli of dispersal anamorph which produce conidia. Pyxidiophorales which has intermediate life cycle between Laboulbeniales and other typical hyphal forming ascomycetes, has been considered as a missing link between them, but its morphological and ecological information has been poorly known. Therefore, establishing culture is indispensable for advanced studies for clarifying characters of the missing link such as mechanisms of attachment to their host animals. In the aid of bathing methods using animal cadavers (e.g., bones of pig and fish), perithecioid fungus was collected, which was identified as a fungus of the genus *Pyxidiophora* due to a flask-shaped perithecium with oval to irregularly angular cells and long necks constructed of parallel, narrow, and elongated cells. Phylogenetic analyses using a concatenated SSU + ITS + LSU rDNA region show that this fungus is involved in Pyxidiophorales, though we could not identify the species because of the lack of reference molecular data of the species of the order. In addition, no morphologically matching described species exists, and this is the first case of *Pyxidiophora* fungus detected from animal cadavers. Based on these facts, we concluded the present fungus is an undescribed species. When a single ascospore and a single hyphal anamorph conidium were isolated on CMA and Sh3A (0.3% shrimp powder agar), both of the spores germinated to be short hphyaes (or small conidia like *Thaxteriola*) on both of the media, but these hyphae stopped their growth. In the end, we succeeded in establishing dual culture by inoculating these short hyphae (and small codinida) with *Fusarium solani* which was isolated from the same habitat of the present fungus, which indicates the present fungus is a mycoparasite. Under the dual culture, the present fungus vigorously produced “*Gabarnardia*” type hyphal arthroconidia within 3-4 days, and also perithecia with mature ascospores within 5-7days, which proved the species to be homothallic. This is the first report of homothallic *Pyxidiophora* species whose

teleomorph can be easily induced under culture, which can be an ideal material to analyze the evolutionary history of the Laboulbeniomyces.

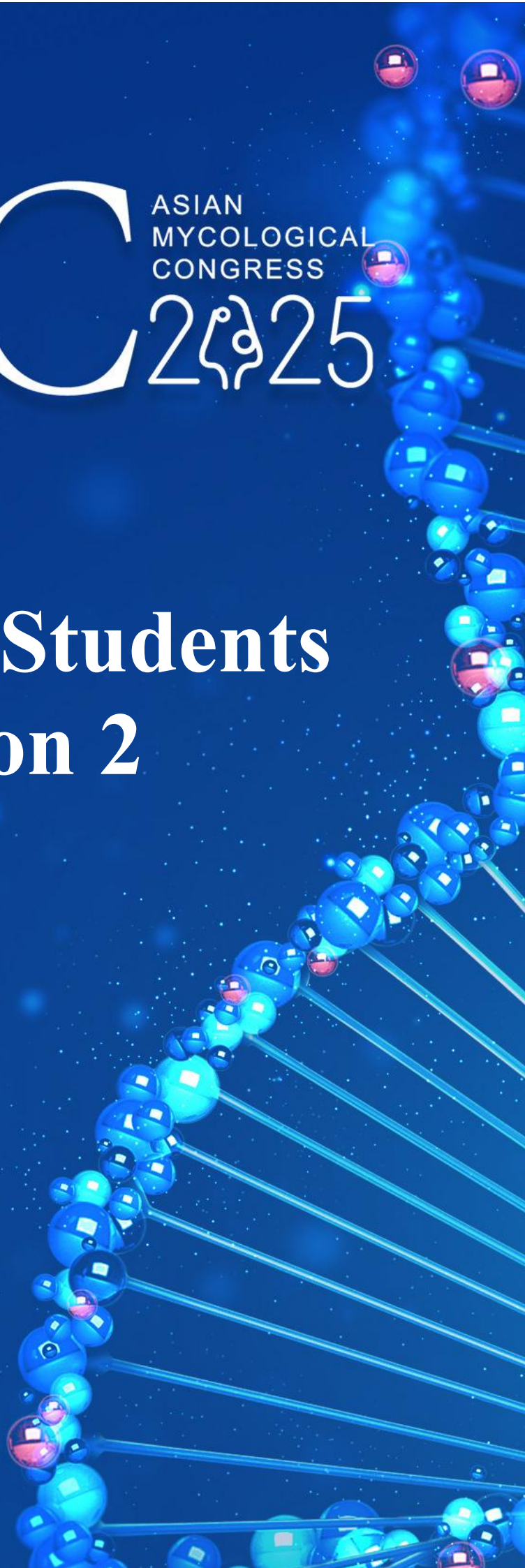
Keywords: Dual culture; *Fusarium solani*; mycoparasite; one new species; *Pyxidiophora*.

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Abstract

Endolichenic Fungi from Two Fruticose Lichens Exhibit Different Antagonistic Activities

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Endolichenic fungi (ELF) represent an understudied yet highly promising source of natural antifungal agents with potential application in sustainable agriculture. These filamentous microorganisms inhabit lichen thalli asymptotically and could be found in different lichen hosts. In this study, ELF were isolated from two fruticose lichens collected from ecologically distinct Philippine habitats: *Ramalina* from lowland Laguna (average annual temperature of 26–28 °C, 237 masl) and *Usnea* from highland Benguet (15–22 °C, 1,102 masl). Morphological characterization, coupled with ITS-based molecular analyses, identified two similar ELF isolates as *Xylaria arbuscula* (*Ramalina*-ELF-LS1A.4 and *Usnea*-ELF-BG1D.1) and *Nemania feicuiensis* (*Ramalina*-ELF-LS2B.1 and *Usnea*-ELF-BG4B.5.1). We then evaluated their antifungal activities against three agriculturally important phytopathogens: *Colletotrichum gloeosporioides*, *Cladosporium cladosporioides*, and *Fusarium oxysporum* using dual-culture and mycelial growth inhibition assays. ELF isolates from *Ramalina* exhibited markedly stronger antagonistic activity (*Ramalina*-ELF-LS1A.4: 35.19–50.94%; *Ramalina*-ELF-LS2B.1: 21.46–61.44%), and primarily with Type D interaction which involved direct hyphal contact and progressive overgrowth over the pathogens, compared with *Usnea*-derived isolates. Several ELF isolates grew over and coiled around the hyphae of *F. oxysporum*, suggesting direct contact inhibition via potential mycoparasitism. However, only *Ramalina*-ELF-LS2B.1 showed a clear zone of inhibition against *F. oxysporum*, indicating production of antifungal compounds. Our results suggest that the lichen host may significantly influence the ELF bioactivity and highlight the ecological importance of host-fungal interactions.

Keywords: bioactivity, *Nemania*, phytopathogenic fungi, *Xylaria*, Xylariaceae

Abstract

Culturable Root and Rhizosphere Mycobiota Associated with Grapevine Trunk Diseases in China

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Grapevine trunk diseases (GTDs) comprise a complex of diseases that significantly impact viticulture worldwide. Most GTD research targets above-ground pathogens, while below-ground fungal communities and their roles remain poorly understood. Additionally, culture-independent studies dominate the field, but they offer limited application for GTD management. Therefore, we investigated the fungal diversity associated with the roots and rhizosphere soil of both GTD-symptomatic and asymptomatic Red-globe grapevines from six major grape-growing regions in China (Guangxi, Hubei, Hunan, Shandong, Yunnan, and Zhejiang) using a culture-dependent approach. A total of 519 fungal isolates, representing 30 genera, were identified using ITS sequencing. The majority of isolates belonged to the phylum Ascomycota (98%), with Eurotiomycetes (50.2%) and Sordariomycetes (43.7%) being the dominant classes, followed by Dothideomycetes (5.3%), Agaricomycetes (0.6%), Mucoromycetes (0.8%), and Saccharomycetes (0.2%). Among the sampled regions, Shandong vineyards exhibited the highest fungal richness and diversity, followed by Yunnan and Zhejiang. Across all sites and compartments, the most abundant genera were *Penicillium* (31%), *Fusarium* (23%), and *Talaromyces* (14%). Well-known biocontrol genera such as *Clonostachys* and *Trichoderma* were consistently found across most samples. Additionally, Grapevine Black-Foot disease-causing genera, such as *Dactylonectria* and *Ilyonectria*, and grapevine dieback disease-causing *Lasiodiplodia* were detected in several regions. Despite their presence, no clear correlation was observed between the relative abundance of these genera and the manifestation of GTD symptoms, indicating that below-ground fungal communities may contribute more to disease resilience than direct symptom development. Further, other major GTD pathogens were not detected in any of the samples, suggesting their limited occurrence in below-ground compartments or a minor role in the surveyed vineyards. Overall, this study emphasizes the diversity and ecological significance of below-ground fungal communities and their potential contributions to GTD management.

Keywords: Culture-dependent, Fungal diversity, Grapevine diseases, *Vitis vinifera*

Abstract

A Comprehensive Study of Mycelium-Based Biomaterials from Potential *Panus* Species (Panaceae, Polyporales) in Thailand

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Mushroom mycelium-based biomaterials (MMB), considered an innovative alternative to plastics and foams, are eco-friendly, biodegradable, clean, cheap, and sustainable. In this study, fresh specimens of *Panus ciliatus* and *P. subfasciatus* were collected from mixed deciduous forests at Chiang Rai Province, Thailand, and identified through a combination of morphological characteristics and phylogenetic analyses based on the ITS region. *Panus ciliatus* is reported as a new geographical record from Thailand. MMB samples were developed using mycelia of these species and locally sourced sawdust. The materials were comprehensively characterised for their physical, mechanical, hydrodynamic, and chemical properties. Additional analyses included FTIR spectroscopy, thermogravimetric analysis (TGA), flammability testing, and soil burial degradability. Mycelial viability maintenance technique has been reported. Notably, this study presents the first comprehensive report on the application of *Panus* species in MMB development, characterisation, and product fabrication. The findings highlight the potential of MMB as a sustainable, eco-friendly alternative to conventional synthetic materials.

Keywords: biodegradable material, circular economy, mycelium-based application, new geographical record, sustainable product

Abstract

Expanding Truffle Cultivation: Co-occurrence of *Tuber melanosporum* and *T. borchii* on *Pinus pinea*

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Abstract

This study presents the first scientific confirmation of *Tuber melanosporum* ascocarp production on *Pinus pinea* in a commercial truffle orchard in Australia, where both *T. melanosporum* and *T. borchii* have been harvested since 2015. High-throughput metabarcoding was used to analyze fungal community composition and spatial patterns of colonization. Mycorrhizae of both *Tuber* species were detected on *P. pinea* roots. In soil pits measuring approximately 15 × 15 cm, a pattern of spatial dominance was observed in which either *T. melanosporum* or *T. borchii* tended to prevail, suggesting possible localized competition. *T. melanosporum* exhibited a stronger suppressive effect on other ectomycorrhizal fungi compared to *T. borchii*. Dominant non-*Tuber* taxa included *Rhizopogon*, *Suillus*, *Inocybe*, *Tomentella*, and *Amphenema* species. Both *Tuber* species were also detected in low abundance on roots of common weed species such as *Arctotheca calendula*, *Hypochoeris radicata*, *Sonchus oleraceus*, and members of Poaceae and *Trifolium*. These findings highlight *P. pinea* as a drought-tolerant, commercially viable host for truffle cultivation. Co-cultivation of *T. melanosporum* and *T. borchii* may extend the ascocarp harvest season. Further research is needed to assess long-term persistence and the influence of environmental factors on species interactions.

Keywords:(Truffle, Mycorrhizal interactions, High-throughput metabarcoding)

Abstract

Orchids inhabiting fungi and their ecological roles

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Orchids are one of the prominent products in Yunnan export industry. Although the diversity of wild orchid species is high in Yunnan, their presence in the market is limited. This could be attributed to insufficient knowledge of wild orchid domestication and challenges in seed germination. Orchid seeds depend on fungi for seed germination. Exploring and identifying orchid seed germination-promoting fungi (SGPF) and to conduct pilot research on their effect on seed germination is necessary. A systematic investigation into the taxonomy of SGPF will facilitate the accurate identification of these fungi. The endophytic fungi from mature orchid roots, and saprobic mushroom species around the orchid roots will be collected. The surface sterilized root samples will be used to isolate root endophytes and mushroom species will be isolated from fruiting bodies or spores. Morphological characteristics will be examined from sporulating cultures and fresh mushrooms. Fungal DNA will be extracted from fresh mycelia and multigene phylogeny of ITS, LSU, rpb2, tef1- α , beta tubulin will be carried. The seed germination-promoting abilities of these fungal isolates will be tested on several commercial and medicinal orchid seeds in greenhouse conditions. The best-performing strains will be selected for preparing fungal inocula.

Keywords: Ecological functions, Endangered plant, Endophytes, Fungal diversity, Symbiotic fungi

Abstract

Study of the Characteristics and Roles of Volatile Compounds in *Termitomyces* Fungi for Termite Recognition and Symbiosis**Qianqian Tao ^{1*}, Itthayakorn Prompttha ^{1#}, Chun-I Chiu ^{2#}, Yuwei Hu ^{3#}**¹*Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand;*²*Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand;*³*Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan, 650201, China.*Email of the corresponding author: itthayakorn.p@cmu.ac.th; chuni.chiu@cmu.ac.th; huyuwei@mail.kib.ac.cn

Termitomyces R. Heim is a genus of mushroom-forming fungi renowned for its culinary flavor and high market value, attributed to their unique texture, nutrition content, and ethnomedicinal importance. These fungi grow in a mutualistic relationship with termites of the subfamily *Macrotermitinae* and are distributed across tropical and subtropical regions of Sub-Saharan Africa, South Asia, and Southeast Asia. Volatile organic compounds (VOCs) emitted by *Termitomyces* serve as critical exogenous chemical signals that influence termite foraging behavior. However, the specific role of VOCs in mediating the symbiotic relationship between fungus-growing termites and *Termitomyces* remains unclear. This study aims to advance our understanding of the role of volatile substances in the termite-*Termitomyces* symbiosis, offering new insights into the mechanisms by which termites recognize their fungal crop. This study analysed the similarities and differences in the VOC profiles of various *Termitomyces* species from China and South Africa using gas chromatography-mass spectrometry (GC-MS) and headspace-gas chromatography-ion mobility spectrometry (HS-GC-IMS). The results indicate that termites potentially distinguish between different *Termitomyces* species based on volatile compound signals as biomarkers. Furthermore, we employed gas chromatography-electroantennographic detection (GC-EAD) technology and Y-tube olfactometer assays to validate termite behavioral responses and to screen for VOCs that attract termites and elicit foraging behavior. This research highlights the critical role of characteristic VOCs in *Termitomyces* recognition, advances our understanding of symbiotic mechanism and facilitates toward the domestication of *Termitomyces* in future research.

Keywords: GC-EAD, GC-MS, HS-GC-IMS, *Termitomyces* mushrooms, Volatile compounds

Abstract

Biodegradation of PAHs by manglicolous fungi from a chronically contaminated site

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Polycyclic aromatic hydrocarbons (PAHs) contamination in mangroves occurs due to the introduction of wastewater, road runoffs, offshore activities, flaring processes, gasoline engine exhaust, and oil spills. Petroleum hydrocarbons are considered one of the greatest threats to coastal environments. Moreover, PAHs are of great medical and environmental relevance due to their carcinogenic, mutagenic, teratogenic, and ecotoxic properties. The bioprospecting of hydrocarbonoclastic microorganisms is relevant for the bioremediation of coastal sites. Thus, this work aimed to isolate fungi from a chronically polluted mangrove in Mexico and evaluate their ability to simultaneously degrade a mixture of 16 PAHs. Submerged root sections were collected from three mangrove species (*Rhizophora mangle*, *Laguncularia racemosa*, and *Avicennia germinans*) and incubated in humid chambers. From this, axenic cultures were obtained. Subsequently, a polyphasic taxonomic assignment was performed to identify the isolates. To determine hydrocarbon degradation, bioassays were run with 19 selected fungal strains using a mixture of 16 PAHs: acenaphthene, acenaphthylene, anthracene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, benzo[*a*]pyrene, chrysene, dibenzo[*a,h*]anthracene, fluoranthene, fluorene, indeno[1,2,3-*cd*]pyrene, naphthalene, phenanthrene, and pyrene). Gas chromatography was used to evaluate changes in the concentration of the PAHs mixture after the bioassays. Our results showed that all tested strains grew in the PAHs mixture as a sole carbon source. Likewise, some isolates demonstrated a greater ability to grow in the presence of PAHs in relation to a labile carbon source (controls). The evaluated fungal isolates showed a high potential for the degradation of PAHs. These findings highlighted the relevance of mangrove fungal bioresources as a viable alternative for the bioremediation of chronically contaminated coastal sites.

Keywords: Polycyclic aromatic hydrocarbons; manglicolous fungi; degradation

Abstract

Bioprospecting of soil microbiota for the improvement of degraded agricultural lands

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Soil is a diverse ecosystem that harbors a large microbial biomass that plays a key role in biogeochemical cycles. Fungi, through their coenzymatic activities, decompose organic matter and produce byproducts that are useful for bacteria. This facilitates the biotransformation of elements such as phosphorus (P), nitrogen (N), carbon (C), and sulfur (S), making them available for plants. However, intensive agricultural practices such as monocultures and the use of agrochemicals alter the biogeochemical properties of soil, increasing erosion and salinization. This leads to desertification and infertility. Therefore, the accelerated loss of soil fertility due to land use change to intensive agricultural systems must be mitigated to ensure sustainable food security and resilient ecosystems. This study evaluated the effect of interactions between soil bacteria and fungi *in vitro* and *in situ* conditions, using samples collected from a managed site with agroecological practices in Mexico, aiming to improve the biogeochemical properties of the soil. Soil samples were collected from a chamomile (*Matricaria chamomilla*) plot during the flowering phenological stage. Eleven microorganisms (7 fungi and 4 bacteria) were isolated and variables such as pH, moisture, and total and dissolved forms of N, C, and P, as well as enzymatic activity, were characterized. Microorganisms were identified, and *in vitro* bioassays were conducted using Biolog plates. Interaction bioassays were conducted to design microbial consortia for *in situ* experiments. Finally, *in situ* bioassays were conducted in pots with chamomile plants to evaluate changes in soil biogeochemical properties and plant development in response to the microbial consortia. A broad metabolic range was observed among the microorganisms, particularly we detected isolates able to degrade substrata that are related to essential nutrient cycles, especially nitrogen. Moreover, the establishment of synergies between bacteria and fungi was mainly recorded. For instance, the interaction between *Mortierellaceae* sp. and *Bacillus* sp. 3. In addition, this consortium promoted the plant development and a prominent production of biomass. This work evidenced that the

interactions between soil bacteria and fungi represent a promising tool for improvement the biogeochemical properties of agricultural soils and development of medicinal plants in Mexico.

Keywords: biogeochemical cycles; coenzymatic activity; interactions; agroecological practices

Abstract

Spider Webs as Reservoirs of Fungal Diversity: Hidden *Cladosporium* Species in the Webs of *Cyclosa mulmeinensis*

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The web of *Cyclosa mulmeinensis* has been characterized as a durable silk structure, distinctly ornamented with organic debris, by which a favorable environment is provided for the colonization of saprobic microorganisms, particularly fungi associated with the decomposition of organic matter. In a preliminary investigation, fungal isolates were predominantly identified as belonging to the genus *Cladosporium*, a genus frequently found across a broad range of environments. Samples were obtained from three locations in Thailand—Phetchaburi, Pathum Thani, and Nakhon Nayok—and overlapping *Cladosporium* species were observed across all sites. Phylogenetic relationships were inferred using combined datasets of the ITS, actin, and Tef1- α gene regions. Through this analysis, 4 species were identified among 17 isolates within the *C. cladosporioides* complex, and an additional 4 species were determined among 5 isolates within the *C. sphaerospermum* complex. Notably, isolates BP1-002, BP1-054, BP2-001, BP26-037, BP26-047, and BP29-013 were placed in well-supported clades closely related to *C. tenuissimum*, *C. oxysporum*, and *C. colocasiae*, suggesting that potentially novel *Cladosporium* species may be associated with spider webs. These findings indicate that, rather than functioning solely as passive collectors of airborne spores, the webs of *C. mulmeinensis* may actively support fungal species exhibiting ecological associations with the web itself. Consequently, spider webs are emphasized as underexplored microhabitats that may serve as reservoirs of fungal diversity and promising sources for the discovery of novel taxa.

Keywords: *Cyclosa mulmeinensis*; Spider web; *Cladosporium*; Phylogenetic tree

Abstract

Integrated Assessment of Growth and Protein Content in Basidiomycetous Fungi for Mycoprotein Production

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The rising demand for sustainable and health-conscious protein sources has driven interest in fungal-derived mycoprotein as an alternative to conventional meat products. While commercial mycoprotein production predominantly utilizes *Fusarium venenatum*, concerns over mycotoxin potential and limited strain diversity indicate the need to explore safer and edible basidiomycetes. In this study, 28 species across four taxonomic orders within Agaricomycetes were screened for their potential as mycoprotein sources. Hyphal growth dynamics were measured on potato dextrose agar, and crude protein content was quantified from submerged mycelial cultures using the Kjeldahl method. Results revealed significant inter-order variation: *Polyporales* exhibited the fastest radial growth, while *Agaricales* grew the slowest. Highest crude protein levels were observed in *Inonotus obliquus* (41.98%), *Neolentinus lepideus* (40.27%), and *Bjerkandera adusta* (39.15%). The dual assessment of growth kinetics and nutritional value identified strains from *Gloeophyllales*, *Hymenochaetales*, and *Polyporales* as promising candidates for scalable mycoprotein development. These findings show the potential of basidiomycetous fungi as safe and effective sources of mycoprotein and provide a framework for future fermentation optimization and functional food innovation.

Keywords: Biotechnology, crude protein, submerged fermentation

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Abstract

Reexamination of *Pyrenophora* Species Associated with Barley and Wheat in Japan**Perry Lorraine Duran Canareab*#, Hana Tonamia, Koharu Sekiguchia, Miyu Fujitaa, and Chiharu Nakashimaab**^a*Graduate School of Bioresources, Mie University, Japan*^b*Department of Biological Sciences – College of Science, Central Luzon State University, Philippines*

Fungal pathogens pose significant threats to barley and wheat production, with species of the genus *Pyrenophora*. In Japan, only *P. tritici-repentis* had been known as a causal pathogen of the yellow spot of wheat. The preliminary phylogenetic study of isolates from symptomatic plants using the internal transcribed spacer (ITS) region indicated that several species of the genus *Pyrenophora* were associated with the leaf symptoms of the yellow spots. To enhance phylogenetic resolution, a concatenated sequence matrix, composed of ITS, RPB2, and GAPDH has been analysed to generate a robust phylogeny for accurate species cognition, as the examined *Pyrenophora* species, for Japanese isolates. The resultant phylogenetic tree generated by RAxML reveals considerable species diversity within the genus *Pyrenophora* in Japan. Notably, the examined Japanese isolates labeled as *P. tritici-repentis* formed a strongly supported clade with *P. trichostoma* in the phylogenetic tree with a combined data matrix. Although the latter species have been synonymized with the former species, nowadays, these are treated as independent species based on phylogeny, respectively. Then, 20 Japanese species of the genus *Pyrenophora* on barley and wheat, including *P. tritici-repentis* and *P. trichostoma*, were examined for the phylogenetic relationship using a concatenated sequence matrix. The results show the diversity of species in those plants and the necessity of reexamining species associated with barley and wheat in Japan.

Keywords: barley, phylogeny, *Pyrenophora*, wheat

Abstract

***Diaporthe* Diversity in Mangroves: Taxonomic novelties, pathogenic associations, and phylogenetic insights**

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Diaporthe species are frequently reported as endophytes on mangroves. However, *Diaporthe* species are also known pathogens affecting several important hosts worldwide and have yet to be explored in mangrove environments. In this study, 45 *Diaporthe* strains from symptomatic leaves and stems of several mangrove species were isolated from mangrove forests and estuaries in Thailand. A polyphasic approach, comprising morpho-cultural and multi-locus phylogenetic analyses (ITS, *TEF1-α*, *TUB2*, *CAL*, and *HIS3*), supported with pairwise homoplasy index tests, identified 10 *Diaporthe* species belonging to three sections (Eres, Foeniculina, Sojae). The species were associated with leaf spots and stem canker on various mangrove species. This included three novel species: *Diaporthe mangroviorum*, *D. narathiwatensis*, and *D. rhizophoracearum*. This study identified nineteen new host-pathogen associations for *D. arecae*, *D. azadirachtae*, *D. biconispora*, *D. charlesworthii*, *D. siamensis*, *D. ueckeri*, and *D. vitimegaspora*; as well as two geographical records for *D. azadirachtae* and *D. charlesworthii*. Pathogenicity was confirmed on mangrove hosts using detached leaf assays, showing varied levels of pathogenicity. We also present evidence supporting the synonymization of *D. eleutharrhenae*, *D. pseudobauhiniae*, *D. xishuangbannaensis*, and *D. yunnanensis* with *D. vitimegaspora*. Additionally, the taxonomic placements of 43 previously reported endophytic *Diaporthe* strains from mangroves were updated, highlighting the importance of using both morphological characteristics and multi-gene sequence analysis for accurate identification of endophytes. The findings of this study enhance our understanding and provide a more holistic view of the *Diaporthe* species within the mangrove ecosystem, including their lifestyles, etiology, and host range.

Keywords— host records, multi-locus phylogeny, new species, *Phomopsis*, synonyms

Abstract

The zinc metalloprotease CsMep maintains Zn homeostasis and regulates pathogenicity in *Colletotrichum sublineola* by modulating the aromatic amino acid biosynthesis pathway

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Abstract. Sorghum anthracnose, caused by *Colletotrichum sublineola*, severely affects leaves, stems, and panicles. This study characterizes CsMep, a zinc-dependent metalloprotease with a conserved HEXXH motif and an N-terminal signal peptide. CsMep localizes to the cell membrane, cytoplasm, and nucleus, and suppresses plant programmed cell death in an HEXXH-dependent manner. This suppression was accompanied by reduced ROS accumulation and cell necrosis, along with decreased electrolyte leakage in plant tissues. Deletion of *CsMep* impaired fungal pathogenicity, hindering hyphal growth, sporulation, and appressorium formation, while increasing sensitivity to ZnSO₄ stress. Under zinc treatment, the mutant showed upregulation of aromatic amino acid biosynthesis genes and accumulation of metabolites such as L-tryptophan and protocatechuic acid. These findings demonstrate that CsMep promotes virulence by regulating zinc stress adaptation and metabolic reprogramming, highlighting its potential as a disease control target.

KEYWORDS. *Colletotrichum sublineola*; zinc metalloprotease; pathogenicity; Zinc stress; aromatic amino acid metabolism

Abstract

Pathogenic and beneficial microbial communities determine the shelf life of stored 'Fengtangli' plums

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ABSTRACT

Fungal decay significantly contributes to postharvest fruit losses, influenced by the interaction between fruit and its microbiota. Despite their susceptibility to spoilage, plums have been underexplored in terms of microbial communities during storage. This study shows that while the diversity of endophytic microbes remains stable, epiphytic diversity decreases over time. Major pathogens identified in plums stored at room temperature include *Lasiodiplodia theobromae*, *Diaporthe* spp., *Botryosphaeria dothidea*, *Pseudomonas oryzae*, and *Pantoea* spp. After refrigeration, human pathogens such as *Streptococcus varani* and *Bacteroides* spp. were detected. Beneficial microbes from genera like *Aureobasidium*, *Marinomonas*, *Pantoea*, *Pseudomonas*, *Novosphingobium* and *Sphingomonas* may extend plum shelf life by suppressing pathogen growth. Metagenomic analysis revealed antibiotic resistance genes (ARGs) in both endophytic and epiphytic communities, predominantly within the *Pseudomonadota* phylum. These findings provide insights for microbiome-based strategies to mitigate postharvest diseases in plums and other stone fruit.

Keywords: Antibiotic Resistance Genes, Endophytic Microbiome, Epiphytic Microbiome, Fruit Rot, Stone Fruit

Abstract

Systematic Studies of Seed-Associated Fungi and Their Functional Characterization**Jin Zhang^{1,2*}, Ratchadawan Cheewangkoon^{1#}, Fuqiang Yu^{2#}, Indunil C Senanayake^{2#}**

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Seeds are the cornerstone of agricultural production, and their health directly affects crop yield and quality. The microorganisms frequently colonize seeds and these microorganisms affect on seed germination and seedling establishment, seed decaying and spoilage, disease control and pathogen shielding, nutrient liberation and delivery, and improve plant fitness. Studies on the systematics and functional traits of seed-associated microorganisms remain limited, hindering a comprehensive understanding of their ecological significance. Revealing the functional microbiome in seeds is important to determine their role. To address this knowledge gap, we isolate fungi and bacteria inhabiting stored-seeds, identify employing morphological characterization combined with phylogenetic analysis, identify functionally beneficial fungi and bacteria that promote seed germination and plant growth, and elucidate the metabolic interactions of different fungal genera and their resulting effects on host plants. In addition, a culture collection of seed-associated fungi will be established and maintained, providing a foundation for systematically characterizing their taxonomy and functional traits, identifying potential pathogenic and beneficial fungi, and utilizing microbial resources to enhance seed health and early seedling growth in agricultural production.

Keywords: Beneficial fungi, Functional traits, Metabolic interactions, Seed-associated fungi, systematics

Abstract

Taxonomy, enzymatic activity, and pathogenicity of entomopathogenic fungi isolated from various insect cadavers in Northern Thailand

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Owing to their ecological significance, commercial application for controlling insect pests, and unique infection mechanisms, studies on entomopathogenic fungi (EPF) show growing research interest. In the present study, we conducted a survey at a mountain forest in Chiang Mai Province, Northern Thailand, and collected mycosed insect cadavers and isolated. Morphological characteristics and multi-locus phylogenetic analysis using ITS, LSU, *rpb1*, *rpb2*, TEF, and TUB gene sequences were conducted for proper identification of the species. Fungal isolates were further screened for proteolytic, chitinolytic, and lipolytic activity (in triplicate for each) using an agar plate assay. The larval mortality bioassay was conducted by placing ten larvae of *Tenebrio molitor* in a PDA plate that had been grown with each fungal strain. Three replicates for each fungal strain were arranged here as well. In the field, seven insect cadavers were collected with infections of five different species of EPF. The collected isolations were identified as *Beauveria asiatica*, *B. bassiana*, *Clonostachys rogersoniana*, *Cordyceps blackwelliae*, *Isaria tenuipes* (two strains), and *Purpleocillium takamizusanense*. According to the enzymatic assay, *I. tenuipes* strain 82 showed the overall highest total enzymatic activity index, followed by *P. takamizusanense*, *C. rogersoniana*, *I. tenuipes* strain 81, *B. asiatica*, *B. bassiana*, and *C. blackwelliae*. Based on the larval cumulative mortality percentage (at the 10th day), *B. bassiana* (100%) was the most virulent species, followed by *B. asiatica* plus *C. blackwelliae* (86.66% each), *I. tenuipes* strain 82 (63.33%), *P. takamizusanense* (33.33%), *C. rogersoniana* (30%), and *Isaria tenuipes* strain 81 (23.33%). In

conclusion, our study has demonstrated the potential for a higher diversity of EPF in the study area and shown that the isolated fungi release key enzymes, such as protease, chitinase, and lipase, which are crucial in breaking the insect integument and advancing the infection. Despite the *B. bassiana*, no isolated strains indicated effective larvicidal activity. Thus, *B. bassiana* could be a potential candidate for inclusion in biocontrol formulations in future studies.

Keywords: *Beauveria bassiana*, Diversity, *Isaria tenuipes*, Protease, Virulence

Organizers

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Mycological Society of China

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