Identification and fungicides sensitivity evaluation of the causal agent of cobweb disease on Lyophyllum decastes in China

Keqin Peng
Guizhou University
Meiling Lin
Guizhou University
Xiaoxiao Yuan
Guizhou University
Changtian Li
Jilin Agricultural University
Xiangyu Zeng
Guizhou University
Yu Li
Jilin Agricultural University
Fenghua Tian
fhtian@gzu.edu.cn
Guizhou University

Research Article

Keywords: Koch's postulates, Antibacterial effect, Hypomyces odoratus, Multi-gene phylogenetic tree

Posted Date: October 14th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2128611/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at BMC Microbiology on May 24th, 2024. See the published version at https://doi.org/10.1186/s12866-024-03326-0.

Abstract

Background: Cobweb disease is a fungal disease that often occurs in the cultivation and production of edible fungi, which can harm a variety of cultivated edible fungi and cause serious losses. Cobweb disease is considered to be one of the four most serious fungal diseases in edible fungi. Symptoms suspected of cobweb disease were found during the cultivation of *Lyophyllum decastes* mushrooms. The objective of our study was to identify the cobweb pathogen and screen out the effective fungicides, so as to provide a reference for the comprehensive prevention and control of velvet mushroom cobweb disease.

Results: The causal agent for this cobweb disease was isolated from symptomatic samples and was found to be *Cladobotryum mycophilum* based on morphological characteristics, phylogeny (ITS, *RPB1*, *RPB2* and *TEF1-α*) and the cultural characteristics of two isolates on PDA and MEA medium. Results of pathogenicity tests also supported the conclusion that *C. mycophilum* is the pathogen responsible for this condition. The antibacterial effect of Prochloraz-manganese chloride complex, Trifloxystrobin and tebuconazole, and Difenoconazole among the tested fungicides is remarkable, with EC_{50} being 0.076 μg/mL, 0.173 μg/mL and 0.364 μg/mL respectively. These fungicides have good control effect, low toxicity, and have good application potential on *L. decastes*.

Conclusion: First report of *cladobotryum mycophilum* causing cobweb disease of *Lyophyllum decastes* in China.

Background

Lyophyllum decastes (Fr.) Singer, also known as antler mushroom, is a precious edible and medicinal mushroom (Fig. 1A). It is belongs to Basidiomycetes, Agaricomycetes, Agaricales, Lyophyllaceae. In Japan, there is a saying of “Smell then *Tricholoma matsutake*, eat then *Lyophyllum decastes*”, and in Europe it has the reputation of “Fried chicken mushroom” [1]. In China, *L. decastes* is mainly distributed in Liaoning, Jilin, Heilongjiang, Jiangsu, Qinghai, Sichuan, Guizhou, Yunnan and Xinjiang province [2]. Its fruiting body is delicate and refresh with high nutritional and medical value. *L. decastes* is rich in protein as well as an essential amino acid, polysaccharide (LDS), which has a series of important effects such as anti-tumor, hypoglycemic, hypolipidemic and antioxidant [3, 4]. According to statistics of China Edible Mushroom Association, the yeild of *L. decastes* in China has increased rapidly since 2015, and has reached 21,600 tons in 2019.

At present, cobweb disease is a common disease of edible mushroom. Its typical symptom is having cobweb-like mycelium on the surface of fruit bodies at the initial stage [5, 6]. In the middle and late stage, the mycelium cover the whole fruiting body and wrapped it, resulting in the decay of fruiting bodies and massive conidia spread rapidly casuing extensive harm. Cobweb disease, brown spot disease (caused by *Verticillium* spp.), green mold disease (*Trichoderma* spp.) and brown rot disease (caused by *Hypomyces perniciosus*) are considered to be the four most serious fungal diseases on many mushroom, such as *Flammulina velutipes*, *Pleurotus eryngii* var. *tuoliensis*, *Lentinula edodes*, *P. eryngii*, *Agaricus bisporus* and so on [5, 7]. Cobweb disease has seriously hindered the development of mushroom industry in China. It has occurred on mushroom such as *A. bisporus*, *P. eryngii*, *Coprinus comatus* and *Ganoderma lingzhi*, which has a great impact on agricultural economy and mushroom farmers' income [8–11]. Cobweb disease on mushrooms also occurs in different regions and countries. Cobweb disease caused by *C. mycophilum* and *C. varium* was reported on *A. bisporus*, *P. eryngii* and *F. velutipes* in Korea [12]. In the same period, *C. mycophilum* also caused cobweb disease of *A. bisporus* in Castilla La Mancha, Spain [13, 14] reported a cobweb disease on *A. bisporus* caused by *C. mycophilum*, which is becoming more and more serious in the cultivation of edible mushroom in South Africa. So it is urgent to study the prevention and control of

cobweb disease, as there are so many kinds of pathogens and a wide range of harm on mushroom cultivation. To prevent disease outbreaks, attention should be paid to improving the disease resistance of varieties, ensuring the vitality and purity of spawn, cleanliness of culture rooms, soil disinfection and standard operation in the process of cultivation. However, cobweb disease on mushroom is common [15], and there is no simple and effective means to control cobweb disease, resulting in a high risk of this disease. To tackle disease occurrence, fungicides are often applied as preventive treatments for extensive outbreaks [16].

In 2021, the author conducted disease investigation in the cultivation area of *L. decastes* in Baiyun District, Guiyang, Guizhou, China, and found a widespread disease suspected of cobweb disease. The disease is highly contagious and destructive that led to almost no harvest in two mushroom sheds, which poses a serious threat to the cultivation and production of *L. decastes*. The isolated pathogen was identified by combining the morphological characteristics and phylogenetic analysis, and its pathogenicity was verified according to Koch's rule. Furthermore, antibacterial effect of several fungicides were analyzed by mycelial growth rate method on the pathogen. Thus, the results of this study will provide a reference for the comprehensive prevention and control the disease on *L. decastes*.

Results

Disease symptoms identification

The symptoms of this disease were obvious in the middle and late growth stage of cultivation, and tended to be aggravated with the increase of the number of fruiting tides. The pathogen was first appeared on the base of the stalk of the covering soil or fruit body. Initially, the roots of the fruiting bodies were covered with white, coarse and cobweb-like mycelia, and then spread along the stalk to the cap. After that, the white flocculent mycelia of the pathogen would quickly cover the surrounding soil and fruiting bodies. Finally, the fruiting bodies were rotten, shrink, while making it dark brown and rancid, covered with massive of conidia, which can spread rapidly by air flow to adjacent ones (Fig. 1B-D). The disease spread rapidly in the whole shed, resulting in the abnormal growth of the mushroom and failure of harvest.

Pathogenicity results

A total number of 12 isolates were obtained from the diseased fruiting bodies, among which the strain 2021062102-1 and 2021062102-3 were pathogenic. Pathogenicity results showed that cobweb disease symptoms were visible 2 days after inoculation (Fig. 1F). Filaments similar to white hairs were produced at the inoculation point, and then gradually spread around, with clear symptoms developing subsequently at 2 days post inoculation. These symptoms resulting from artificial inoculation were similar to those observed in the field. The control was asymptomatic (Fig. 1E). The pathogens were consistently re-isolated from the infected fruiting bodies of *L. decastes* fulfilling Koch’s postulate, and were confirmed to be consistent with the inoculated strain by morphological characteristics.

Morphological description

Colonies grow rapidly on a 90 mm PDA plate and covering the petri dish after 3 days at 25℃; reverse initially yellowish ochraceous turning roseous or brownish red in 10 d (Fig. 2A-D). The aerial mycelium of the colony were lush and cotton-like, with massive conidia. Colonies grow slowly on a 90 mm MEA medium and grew all over the culture dish at 25℃ for 10 days, produced a large number of dense conidia (Fig. 2E-H). Conidiophores straight,

hyaline, branching profuse, irregular, tips simple, 24.5-37.6 × 4.0-6.7 μm (n=30) (Fig. 2I-J). Conidia hyaline, mostly ellipsoidal, 0-3 septate, bases rounded, slightly constricted at the septum, 17.3-27.2 × 7.9-10.4 μm (n=50) (Fig. 2K-N). According to the morphological characteristics, the isolate (2021062102-1) was identified as *Cladobotryum mycophilum*.

Phylogenetic analyses

The ITS-rDNA, *RPB1*, *RPB2* and *TEF1-α* genes of two isolates were amplified and sequenced with primers ITS4/ITS5, *cRPB1Af/RPB1Cr*,*RPB2-5f/RPB2-7cR* and *EF1-983f/EF1-2218r*, respectively. Sequences of the two isolates (GUCC202106: 2021062102-1, GUCC202107: 2021062102-3) were identical, and DNA sequences were deposited in GenBank (ITS, OK285275 OK285276; *RPB2*, OK458561 OK458562; *TEF1-α*, OK448484 OK448458;*RPB1*, OK513067 OK513068). A multigene phylogenetic tree, inferred by the ML (Maximum-Likelihood) method based on the concatenated ITS-*RPB1-RPB2-TEF1-α* sequences, confirmed the multiple isolates as *C. mycophilum*. According to comprehensive identification of the phylogenetic analysis, morphological characteristics and cultural characteristics, the isolates were identified as *C. mycophilum* (Fig. 3). The results were similar to morphological identification.

Effect of different fungicides on the cobweb disease pathogen of *Lyophyllum decastes*

The effect of nine fungicides on radial growth of the pathogen was studied to screen out which fungicides are highly effective against the pathogen. The average radial growth of fungus was significantly affected by different fungicides. The results of the nine fungicides screening showed that in PDA medium, the inhibition effect of all the fungicide was good. Among them, Prochloraz-manganese chloride complex (50% WP) were the most effective in controlling the pathogen, with EC_{50} being 0.076 μg/mL. Trifloxystrobin and tebuconazole (75% WDG) has the second effective in controlling the pathogen, with EC_{50} being 0.173 μg/mL. Furthermore, the effective in controlling the pathogen of Difenoconazole (10% WDG) was better, with EC_{50} being 0.364 μg/mL. Among them, the inhibitory effect of carvacrol (5% SL) was slightly worse than that of other fungicides (Table 2).

Discussion

The cobweb disease has been reported in all mushroom-growing countries around the world, which causes heavy economic losses, especially in the mid-1990s [21, 22]. In the late 1980s, the disease rarely occurred in cultivated edible fungi, and even if it occurred, it was easily controlled by fungicides. However, the use of fungicides gradually developed resistance to pathogens and eventually caused the arachnid epidemic in Ireland and the United Kingdom in the 1990s, where the annual production of *Agaricus bisporus* decreased by nearly 40% [8–11]. *C. mycophilum* has a wide host range that has been reported on *A. bisporus*, *Albatrellus* sp., *Lactarius mitissimus*, *L. mitissimus* cf. *vellereus*, *Russula* sp., *Coniophora* sp., *Megacollybia platyphylla*, *Inocybe* sp., *Armillaria mellea*, *Lycoperdon pyriforme*, *P. eryngii*, *P. ostreatus*, *G. lingzhi* [11–12, 19–20, 23]. The disease is prevalent especially in *A. bisporus*. Gea et al. [24] and Kim et al [25]. The prevention and control of the disease is mainly based on the principle of "Prevention first, comprehensive prevention and control", strictly control the production of each link, prevent the invasion of pathogens, if the disease after the application of chemical pesticides for prevention and control. In terms of prevention and control, we can create conditions suitable for edible fungi but not conducive to the growth and development of pathogens by controlling nutritional and environmental conditions, and we can also control the breeding and harm of hybrid bacterium by comprehensively using various prevention and control methods, so as to ensure the yield and quality of *Lyophyllum decastes*. However, it is a strategy to screen and use

effective fungicides in a targeted way.Attempted to study the prevention and control agents for *C. mycophilum*. But it has not been reported on *L. decastes*. This is the first report of *C. mycophilum* causing cobweb disease on cultivated *L. decastes* in the world.

Conclusions

L. decastes is an important edible and medicinal mushroom mainly cultivated in Shandong, Jiangsu, Fujian, Guangdong, Hubei and Guizhou Provinces, China. About 1 million sticks per year is being cultivated in Guizhou province. In June 2021, cobweb disease appeared on fruiting bodies of *L. decastes* in Guiyang, Guizhou Province, with 3-5% incidence rate, bringing great reduction yield. Initially, the roots of the fruiting bodies were covered with white, coarse and cobweb-like mycelia, and the cap and stipe were rapidly affected. Finally, the fruiting bodies were rotten, dark brown, rancid and covered with massive of conidia, which can spread rapidly by air flow to adjacent ones. Unlike the disease on *A. bisporus*, no symptoms of cap spotting were seen on the fruit bodies of *L. decastes* [17-20]. Two samples with typical symptoms which were collected from the location. Two strain 2021062102-1 and 2021062102-3 were pathogenic by Koch's postulate. On the basis of the phylogenetic, morphological and cultural characteristics analysis, the causal agent was introduced herein as *C. mycophilum*.

As species of *Cladobotryum* grow much faster than mushrooms, they spreads very fast in the fruiting body stage, which will cause serious economic losses. Therefore, the control of the cobweb disease should be applied at early as possible during the cultivation, whereas nine fungicides with recommend concentration were selected for the primary screening experiment. Afterwards, according to the preliminary screening results, different gradient treatments with appropriate concentrations carried out for the fungicides were applied in the culture media with three replicates to perform linear regression analysis and determine the half maximal effective concentration (EC_{50}) values. Prochloraz-manganese chloride complex (50% WP), Trifloxystrobin and tebuconazole (75% WDG), and Difenoconazole (10% WDG) were indicated to be effective fungicides among the nine candidates to control the pathogen, with EC_{50} being 0.076 μg/mL, 0.173 μg/mL and 0.364 μg/mL respectively. These fungicides have good control effect, low toxicity, and have good application potential on *L. decastes*. Management of the disease requires an integrated approach, among which cultural practices, physical and biological prevention and control must be emphasized for delaying the development of resistance and maintaining efficacy which directly impacts yield.

Methods

Pathogen isolation

Three diseased fruiting bodies of *L. decastes* were collected from a mushroom cultivation base, Guiyang (106°43'25" N, 26°43'41" E), Guizhou Province, China, on June 22nd 2021. Each diseased fruiting body was cleaned with flowing water and disinfect the surface firstly. Secondly, sections with about 0.3 cm square from the diseased fruiting body was cut off and surface sterilized with the following steps: immersed in 95% ethanol for 1 min, washed with ddH_2O 2 times, immersed in 75% ethanol for 30 s, and suspensions were spread on a potato dextrose agar (PDA) plate with three duplications and incubated at 25℃ in darkness. The pathogen of each duplicate was re-isolated and purified while the single colonies formed [26]. All cultures were deposited to Culture Collection of the Department of Plant Pathology, College of Agriculture, Guizhou University, China (GUCC).

Pathogenicity tests

All isolates were tested for pathogenicity using 2-3 cm high of the fruiting bodies following a modified protocol of Tian et al. [27], 10 healthy fruiting bodies were inoculated, with sterilized distilled water as control. All treated fruiting bodies were maintained in the same mushroom-growing space, under the conditions (16-18℃, 90-95% relative humidity). The pathogenicity test was assessed over 4 days. Re-isolated were performed from the infected fruiting bodies, and morphological and phylogenetic analysis were done as below. All experiments were conducted triplicate.

Morphological and molecular characterization

For the morphological observations of the colonies, the strains were grown on PDA and 1.5 % malt extract agar (MEA) medium, at 25°C in darkness [15]. The colony characteristics and microscopic morphological characteristics of mycelia, conidiophore and conidia were observed at 3, 5, 10 and 14 days. Conidia were measured from each isolate. The isolates were then identified based on the morphological characteristics of the conidia and conidiophores according to the descriptions from Gams and Hoozemans [28], Rogerson and Samuels [29]. Additionally, the molecular characteristics of the isolates, total genomic DNA was extracted from the colony of the isolates using a CWBIOTECH Plant Genomic DNA Kit (Changping, Beijing, China) following the manufacturer's protocol. PCR was set up using the following primers for amplification of the different gene regions: the internal transcribed spacer (ITS) region of the rDNA gene cluster were amplified by PCR with primers ITS4/ITS5 [30]. And three protein-coding genes were amplified using the following primers: the partial translation elongation factor 1-α (*TEF1-α*: *EF1-983f/EF1-2218r*) [31, 32]; RNA polymerase I second largest subunit (*RPB1*: *cRPB1Af/RPB1Cr*) [33]; RNA polymerase II second largest subunit (*RPB2*: *fRPB2-5f/fRPB2-7cR*)[34, 15], respectively.

The PCR was conducted in a Applied Biosystems, ProFlex™ PCR (, Waltham, Massachusetts, USA). The PCR reaction was performed with a 50 μL mixture consisting of 3.2 μL of dNTP mix (2.5 mM□μL-1), 0.2 μL of Taq polymerase (5 U□μL-1), 2 μL of genomic DNA (50 ng□μL-1), 4 μL of polymerase buffers (10× μL-1, Takara, Japan), and 2 μL of each primer (25 mM μL-1). Amplification of the ITS region was performed as follows: initial denaturation at 94°C for 5 min , 30 cycles of 30 s at 94°C, 30 s at 50°C, 30 s at 72°C, and with a final extension of 10 min at 72°C. For amplifying the *TEF1-α* protein-coding genes programming for an initial denaturation at 94℃ for 3 min followed by 35 cycles of 15 s at 94℃, 15 s at 55℃ and extension at 72℃ for 15 s; and *RPB1* region: initial denaturation 5 min at 94°C, 30 cycles of 30 s at 94°C, 30 s at 55°C, 30 s at 72°C; for *RPB2* region: initial denaturation at 95℃ for 3 min followed by 35 cycles of 15 s at 94℃, 15 s at 52℃ and extension at 72℃ for 30 s; and with the same final extension at 72℃ for 10 min. Electrophoresis was performed on 0.8% agarose gels stained with Gel Green. PCR products were sequenced by the same primers used for amplification by Qingke Biotech (Chengdu) Co., Ltd.

The sequences of ITS, *RPB1*, *RPB2* and *TEF1-α* genes from representative ex-type strains were selected for phylogenetic analyses and extracted from GenBank using BLAST. The obtained sequences were visualized and aligned using BioEdit [35] and compared against the non-redundant nucleotide collection (nr/nt) sequences present in the NCBI GenBank database using the Basic Local Alignment Search Tool (BLASTn) tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). As for building the phylogenetic trees, maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) were performed at the CIPRES web portal [36]. 24 phylogenetically related species of *Cladobotryum*, *as C. asterophorum*, *C. paravirescens*, *C. protrusum*, *C. prurpureum*, *Hypomyces subiculosus*, *H. samuelsii*, *C. tchimbelense*, *C. heterosporumne*, *C. indoafricum*, *C.*

multiseptatum, *H. dactylarioides*, *H. rosellus*, *C. rubrobrunnescens*, *C. tenue*, *C. mycophilum*, *C. semicirculare*, *H. australasiaticus*,et al. were used for phylogenetic analyses [15] (Table 1)

Screening of fungicides for prevention and control of cobweb disease causal agent on *Lyophyllum decastes*

Various fungicide, including Carvacrol (5% SL), Osthol (1% EW), Eugenol (0.3% SL), Propiconazole (25% EC), Triadimefon (20% EC), Trifloxystrobin and tebuconazole (75% WDG), Prochloraz-manganese chloride complex (50% WP), Pyraclostrobin (10% WDG) and Difenoconazole (10% WDG), were selected. Preliminary indoor screening of fungicides for prevention and control of cobweb disease agent on *L. decastes*: the methodology was modified as appropriate according to Chen et al.[37]. According to the active ingredients, nine kinds of low toxic fungicides were diluted with sterile water to make mother liquor of certain concentration. In order to determine the concentration range of each fungicide, a pre-test was carried out with a concentration gradient of 5 times for each fungicide. According to the volume ratio, the PDA medium containing fungicide was prepared with the amount of mother liquid : PDA =1:9 in a Petri dish with diameter of 9.0 cm. The pathogen filaments which were cultured grown on PDA medium at 25°C in darkness for 4 days were made into cake with a 5 mm hole punch. PDA medium with equal amount of sterile water without fungicide was used as control. The fungus cakes were transferred into the prepared medium, and incubated at 24°C in darkness. In this process, the growth of pathogen was observed to determine the initial concentration of each fungicide. Selecting the fungicide that could inhibit the pathogen and conduct further concentration screening test. According to the pre-test results, each fungicide was diluted into 6 concentration gradients according to the effective components. The method of inoculation and culture for each treatment was the same as above. The diameter of colonies was measured with crisscross method, when colonies in control almost covered the Petri dish. Inhibitory percentage on mycelia growth was calculated after treatment with different concentrations and fungicides. Inhibition of mycelial growth (%) = [(dimeter of mycelium in control -diameter of mycelium in treatment)/dimeter of mycelium in control]x100. Each treatment was repeated three times. The EC50 value of each fungicide was evaluated by using ANOVA and GraphPad Prism 7.0 program (GraphPad Software, La Jolla, CA, USA) in three replicates. The ANOVA was performed as per Duncan's multiple range test to determine the significant difference (* $p < 0.05$) [38].

Declarations

Data Analysis

All statistical analyses were conducted in MS Excel and SPSS statistics (version 19.0) software. The ANOVA was performed as per Duncan's multiple range test to determine the significant difference (* $p < 0.05$). Figures were generated using GraphPad Prism 7.0 program.

Author details

[1] Department of Plant Pathology, College of Agriculture, Guizhou University, Huaxi 550025, Guiyang, Guizhou, China. [2] Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, Nanguan 130118,Changchun, Jilin, China.[3] Institute of Edible Mushroom, Guizhou University, Huaxi 550025, Guiyang, Guizhou, China.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

Funding

This work was supported by the Science and Technology Projects of Guizhou Province Cultivation [grant number Support of QKH [2021] General 199]; Natural Science Research Projects of Guizhou Education Department [grant number QKH-KY[2021]054]; Major Special Characters of QianKeHe [grant number QKH[2019]3005-1]; and Science and Technology Innovation Team of Guizhou Province [grant number QKH-PTRC [2020]5001].

Authors' contributions

Keqin Peng: Data curation, Investigation,Writing - original draft. Meiling Lin: Data curation, Software. Xiaoxiao Yuan: Investigation, Methodology. Changtian Li: Supervision, Writing - review and editing. Xiangyu Zeng: Methodology, Software. Fenghua Tian: Conceptualization, Funding acquisition, Supervision, Visualization, Writing - review and editing. Yu Li: Conceptualization, Resources, Validation.

Acknowledgments

The authors would like to thank Guizhou Kaidong Technology Co., LTD for providing collection and research materials.

References

1. Qin CQ. Extraction, isolation, structure identification and antioxidant study of polysaccharides from fruiting body of *Lyophyllum decastes*. Zhejiang University of Technology, Hangzhou, China.2019.
2. Li Y, Li TH, Yang ZL, Tu LGE, Dai YC. Atlas of Chinese macrofungal fesources. Zhengzhou: Central Plains Farmers Press; 2015.
3. Jiang CR, Qu QR, Song KL, Zheng XY, Chen P, Wang Q. Advances in the study of chemical constituents and biological activities of *Lyophyllum*. J Fungal Res. 2022;20(01):72–8. https://doi.org/10.13341/j.jfr.2020.1326.
4. Zhang FP, Xu H, Qiu SF, Zhang JL, Wu XP, Fu JS. Study on antioxidant and liver protection of Polysaccharides from *Lyophyllum decastes*. Biotechnol Bull. 2021;37(11):92–100. https://doi.org/10.13560/j.cnki.biotech.bull.1985.2021-0891.
5. Carrasco J, Navarro MJ, Gea FJ. Cobweb, a serious pathology in mushroom crops: a review. Span J Agric Res2017;15(2), e10R01. https://doi.org/10.5424/sjar/2017152-10143.

6. Zhang QH, Wang W, Li CH, Wen ZQ. Biological characteristics of *Hypomyces aurantius* parasitic on *Hypsizygus marmoreus*. Mycosystema. 2015;34(3):350–6. https://doi.org/10.13346/j.mycosystema.140248.
7. Cao MT, Li B, Li H, Fang CC, He PX. Research progress of edible mushroom cobweb disease. J Edible Fungi. 2020;27(3):127–36.
8. Lan YF, An XR, Wang QW, Tang LA. Biological characteristics of *Cladobotryum mycophilum* causing cobweb disease on *Agaricus bisporus*. Edible Fungi of China. 2017;36(4):62–5. https://doi.org/10.13629/j.cnki.53-1054.
9. Tian FH, Li CT, Li Y. First report of *Cladobotryum varium* causing cobweb disease of *Pleurotus eryngii* var. tuoliensis in China. Plant Dis. 2018;102(4):826. https://doi.org/10.1094/PDIS-05-17-0741-PDN.
10. Wang GZ, Luo Y, Li JL. Characteristics of cob-web disease in fruiting bodies of *Auricularia cornea* and physiological feature and control strategy of the pathogenic fungus *Cladobotryum cubitense*. Chin J Microbiol. 2019;38(3):341–8. https://doi.org/10.13346/j.mycosystema.180270.
11. Xu R, Liu ZH, Fu YP, Li Y. Identification and biological characteristics of *Cladobotryum mycophilum* causing cobweb disease on *Ganoderma* lingzhi. Mycosystema. 2019;38(5):669–78. https://doi.org/10.13346/j.mycosystema.180328.
12. Back CG, Lee CY, Seo GS, Jung HY. Characterization of species of *Cladobotryum* which cause cobweb disease in edible mushrooms grown in Korea. Mycobiology. 2012;40(3):189–94. https://doi.org/10.5941/MYCO.2012.40.3.189.
13. Gea FJ, Navarro MJ, Carrasco J, González AJ, Suz LM. First report of cobweb disease on white button mushroom (*Agaricus bisporus*) in Spain caused by *Cladobotryum mycophilum*. Plant Dis. 2012;96(7):1067. https://doi.org/10.1094/PDIS-02-12-0120-PDN.
14. Chakwiya A, Linde EJ, Chidamba L, Korsten L. Diversity of *Cladobotryum mycophilum* isolates associated with cobweb disease of *Agaricus bisporus* in the south African mushroom industry. Eur J Plant Pathol. 2019;154(3):767–76. https://doi.org/10.1007/s10658-019-01700-7.
15. Põldmaa K. Tropical species of *Cladobotryum* and *Hypomyces* producing red pigments. Stud Mycol. 2011;68(68):1–34. https://doi.org/10.3114/sim.
16. Gea FJ, Navarro MJ, Santos M, Diánez F, Carrasco J. Control of fungal diseases in mushroom crops while dealing with fungicide resistance: a review. Microorganisms. 2021;9:581. https://doi.org/10.3390/microorganisms9030585.
17. Back CG, Kim YH, Jo WS, Chung H, Jung HY. Cobweb disease on *Agaricus bisporus* caused by *Cladobotryum mycophilum* in Korea. J Gen Plant Pathol. 2010;76:232–5. https://doi.org/10.1007/s10327-010-0236-3.
18. Carrasco J, Navarro MJ, Santos M, Diánez F, Gea FJ. Incidence, identification and pathogenicity of *Cladobotryum mycophilum*, causal agent of cobweb disease on *Agaricus bisporus* mushroom crops in Spain. Ann Appl Biol. 2016;168:214–24. https://doi.org/10.1111/aab.12257.
19. Gea FJ, Carrasco J, Suz LM, Navarro MJ. Characterization and pathogenicity of *Cladobotryum mycophilum* in Spanish *Pleurotus eryngii* mushroom crops and its sensitivity to fungicides. Eur J Plant Pathol. 2017;147(1):129–39. https://doi.org/10.1007/s10658-016-0986-7.
20. Gea FJ, Navarro MJ, Suz LM. Cobweb disease on oyster culinary-medicinal mushroom (*Pleurotus ostreatus*) caused by the mycoparasite *Cladobotryum mycophilum*. J Plant Pathol. 2019;101:349–54. https://doi.org/10.1007/s42161-018-0174-z.
21. Hoog GS de.. Notes on some fungicolous hyphomycetes and their relatives. Persoonia. 1978;10:33–81.

22. McKay GJ, Egan D, Morris E, Scott C, Brown AE. Genetic and morphological characterization of *Cladobotryum* species causing cobweb disease of mushrooms. Appl Environ Microbiol. 1999;65(2):606–10. https://doi.org/10.1128/AEM.65.2.606-610.1999.
23. Tamm H, Poldmaa K. Diversity, host associations, and phylogeography of temperate aurofusarin-producing *Hypomyces/Cladobotryum* including causal agents of cobweb disease of cultivated mushrooms. Fungal Biol. 2013;117(5):348–67. https://doi.org/10.1016/j.funbio.2013.03.005.
24. Gea FJ, Navarro MJ, Santos M, Diánez F, Herraiz-Peñalver D. Screening and evaluation of essential oils from Mediterranean aromatic plants against the mushroom cobweb disease, *Cladobotryum mycophilum*. Agronomy. 2019a;9(10):656. https://doi.org/10.3390/agronomy9100656.
25. Kim MK, Seuk SW, Lee YH, Kim HR. Cho KM.Fungicide sensitivity and characterization of cobweb disease on a *Pleurotus eryngii* mushroom crop caused by *Cladobotryum mycophilum*. Plant Pathol J. 2014;30(1):82–9. https://doi.org/10.5423/PPJ.OA.09.2013.0098.
26. Yuan XX, Peng KQ, Li CT, Zhao ZB, Zeng XY, Tian FH, Li Y. Complete genomic characterization and identification of *Saccharomycopsis phalluae* sp. nov., a novel pathogen causes yellow rot disease on *Phallus rubrovolvatus*. J Fungi. 2021;7(9):707. https://doi.org/10.3390/jof7090707.
27. Tian FH, Li CT, Li Y. First report of *Penicillium brevicompactum* causing blue mold disease of Grifola frondosa in China. Plant Dis. 2017;101(8):1549. https://doi.org/10.1094/PDIS-09-16-1301-PDN.
28. Gams W, Hoozemans AC. *Cladobotryum*-konidienformen von *hypomyces*-Arten. Persoonia. 1970;6(1):95–110.
29. Rogerson CT, Samuels GJ. Agaricicolous species of *Hypomyces*. Mycologia. 1994;86:839–66. https://doi.org/10.1080/00275514.1994.12026489.
30. White TJ, Bruns TD, Lee SB, Taylor JW. "Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. " PCR protocols: A guide to methods and applications. New York: Academic Press Inc.; 1990. https://doi.org/10.1016/B978-0-12-372180-8.50042-1.
31. Carbone I, Kohn LM. A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia. 1999;91:553–6. https://doi.org/10.1080/00275514.1999.12061051.
32. Rehner SA. Primers for elongation factor 1-Alpha (EF1-Alpha). 2001. http://www.aftol.org/pdfs/EF1primer.pdf.
33. Castlebury LA, Rossman AY, Sung GH, Hyten AS, Spatafora JW. Multigene phylogeny reveals new lineage for Stachybotrys chartarum, the indoor air fungus. Mycol Res. 2004;108(8):864–72. https://doi.org/10.1017/S0953756204000607.
34. Liu YJ, Whelen S, Hall BD. Phylogenetic relationships among ascomycetes, as inferred from RNA polymerase II phylogeny. Mol Biol Evol. 1999;16:1799–808. https://doi.org/10.1093/oxfordjournals.molbev.a026092.
35. Hall TA. BioEdit: a user-friendly biological sequence alignment editor. and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series. 1999;41:95–98. https://doi.org/10.14601/Phytopathol_Mediterr-14998u1.29.
36. Miller MA, Pfeiffer W, Schwartz T. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Gateway Computing Environments Workshop (GCE), New Orleans, 14 November IEEE. 2010;pp 1–8. https://doi.org/10.1109/GCE.2010.5676129.

37. Chen HZ, Yang HF, Yao KB, Shu ZL, Zhou HF, Zhuang YQ. Identification of pathogenic bacteria of rice seedling disease and determinatio:174–184.
38. Li WZ, Long YH, Mo FX, Shu R, Yin XH, Wu XM, Zhang RG, Zhang ZZ, He LN, Chen TT, Chen J. Antifungal activity and biocontrol mechanism of *Fusicolla violacea* J-1 against soft rot in kiwifruit caused by *Alternaria alternata*. J Fungi. 2021;7:937. https://doi.org/10.3390/jof7110937.

Tables

Table 1. Materials of *Cladobotryum* species used in phylogenetic analyses

Species	Strain	GenBank accession number			
		ITS	*RPB2*	*TEF-1a*	*RPB1*
C. asterophorum	CBS 676.77	FN859395	FN868649	FN868712	FN868776
C. paravirescens	TFC 97-23	FN859406	FN868660	FN868724	FN868787
C. protrusum	CBS 118999	FN859408	FN868662	FN868726	FN868789
C. protrusum	FSU 5877	FN859411	FN868665	FN868729	FN868792
C. prurpureum	CBS 154.78	FN859415	FN868669	FN868733	FN868796
Hypomyces samuelsii	G.J.S. 96-41	FN859448	FN868702	FN868766	-
C. tchimbelense	TFC 201146	FN859419	FN868673	FN868737	FN868800
C. heterosporumne	CBS 719.88	FN859398	FN868653	FN868716	FN868780
C. indoafricum	FSU 5807	FN859399	FN868654	FN868717	FN868781
C. multiseptatum	CBS 472.71	FN859405	FN868659	FN868723	FN868786
Hypomyces dactylarioides	CBS 141.78	FN859429	FN868683	FN868748	FN868809
Hypomyces rosellus	TFC 99-229	FN859441	FN868695	FN868759	FN868820
C. rubrobrunnescens	CBS 176.92	FN859416	FN868670	FN868734	FN868797
C. tenue	CBS 152.92	FN859420	FN868674	FN868738	FN868801
C. mycophilum	TFC 200102	FN859433	FN868687	FN868752	FN868813
C. mycophilum	TFC 98-25	FN85943	FN868688	FN868753	FN868814
C. mycophilum	TFC 05-93	FN859436	FN868690	FN868755	FN868816
C. semicirculare	CBS 705.88	FN859417	FN868671	FN868735	FN868798
Hypomyces australasiaticus	TFC 03-8	FN859428	FN868681	FN868746	FN868807
Hypomyces khaoyaiensis	G.J.S. 01-304	FN859431	FN868685	FN868750	-
Hypomyces armeniacus	TFC 02-86/2	FN859424	FN868678	FN868742	FN868804
C. cubitense	TFC 2007-13	AM779857	FN868652	FN868715	FN868779
Hypomyces gabonensis	TFC 201156	FN859430	FN868684	FN868749	FN868810
Hypomyces aurantius	TFC 95-171	FN859425	FN868679	FN868743	FN868805
Hypomyces lactifluorum	TAAM 170476	FN859432	EU710773	FN868751	FN868812
Hypomyces subiculosus	TFC 97-166	FN859452	FN868706	FN868770	FN868829
C. penicillatum	CBS 407.80	FN859407	FN868661	FN868725	FN868788

Table 2. The virulence effect of nine kinds of fungicides on the pathogen

Fungicides	Treatment concentration ug mL^{-1}					Toxicity regression equation	EC50/(ug mL-1)	Correlation coefficient
	T1	T2	T3	T4	T5			
Carvacrol (5% SL)	500.00	100.00	20.00	4.00	0.80	y=3.0897x+2.4323	6.777	0.9711
Osthol (1% EW)	50.00	10.00	2.00	0.40	0.08	y=1.0215x+4.3536	4.294	0.9619
Eugenol (0.3% SL)	30.00	6.00	1.20	0.24	0.05	y=2.0136x+5.0131	0.985	0.9725
Propiconazole (25% EC)	5.00	2.50	1.25	0.63	0.31	y=1.6649x+5.4195	0.560	0.9163
Triadimefon (20% EC)	5.00	2.50	1.25	0.63	0.31	y=1.0460x+4.8275	1.462	0.9979
Trifloxystrobin and tebuconazole (75% WDG)	1.39	0.35	0.09	0.02	0.01	y=1.3942x+6.0633	0.173	0.9572
Prochloraz-manganese chloride complex (50% WP)	1.00	0.33	0.11	0.04	0.01	y=0.7724x+5.8659	0.076	0.9759
Pyraclostrobin (10% WDG)	2.40	1.20	0.60	0.30	0.15	y=1.4208x+4.7917	1.402	0.9805
Difenoconazole (10% WDG)	10.00	1.00	0.10	0.01	0.00	y=0.8038x+5.3524	0.364	0.9688

Figures

Figure 1

Wild symptoms of causing cobweb disease on *Lyophyllum decastes* and pathogenicity tests of *Cladobotryum mycophilum* (2021062102-1). (A), Healthy fruiting bodies of *L. decastes.* (B-C), Rotten fruiting bodies at late stage of the disease. (D), White anamorph spread over *L. decastes*. (E), Pathogenicity tests, day 2 after inoculation, control, asymptomatic. (F), Pathogenicity tests, day 2 after inoculation of *C. mycophilum* 2021062102-1, diseased.

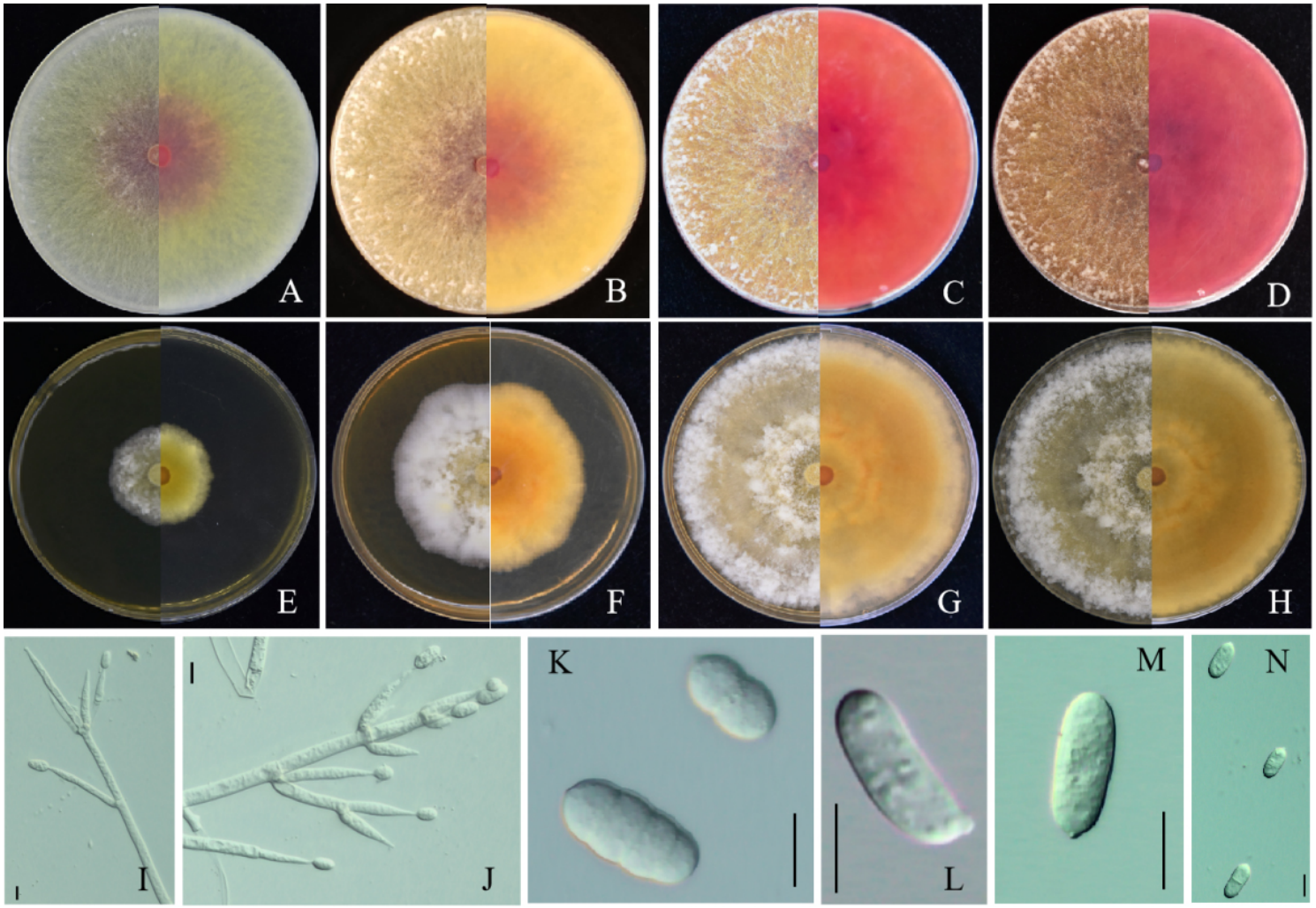

Figure 2

Morphology characterization of *Cladobotryum mycophilum* (2021062102-1). (A-D), Colony morphology on PDA medium at 25℃. A: after 3 days; B: after 5 days; C: after 10 days; D: after 14 days. (E-H), Colony morphology on MEA medium at 25℃. E: after 3 days; F: after 5 days; G: after 10 days; H: after 14 days. (I-J), Conidiophores cells straight, hyaline, branching profuse, tips simple, Bar=10 μm. (K-N), Conidia, with 0-3 septa, Bar=10 μm.

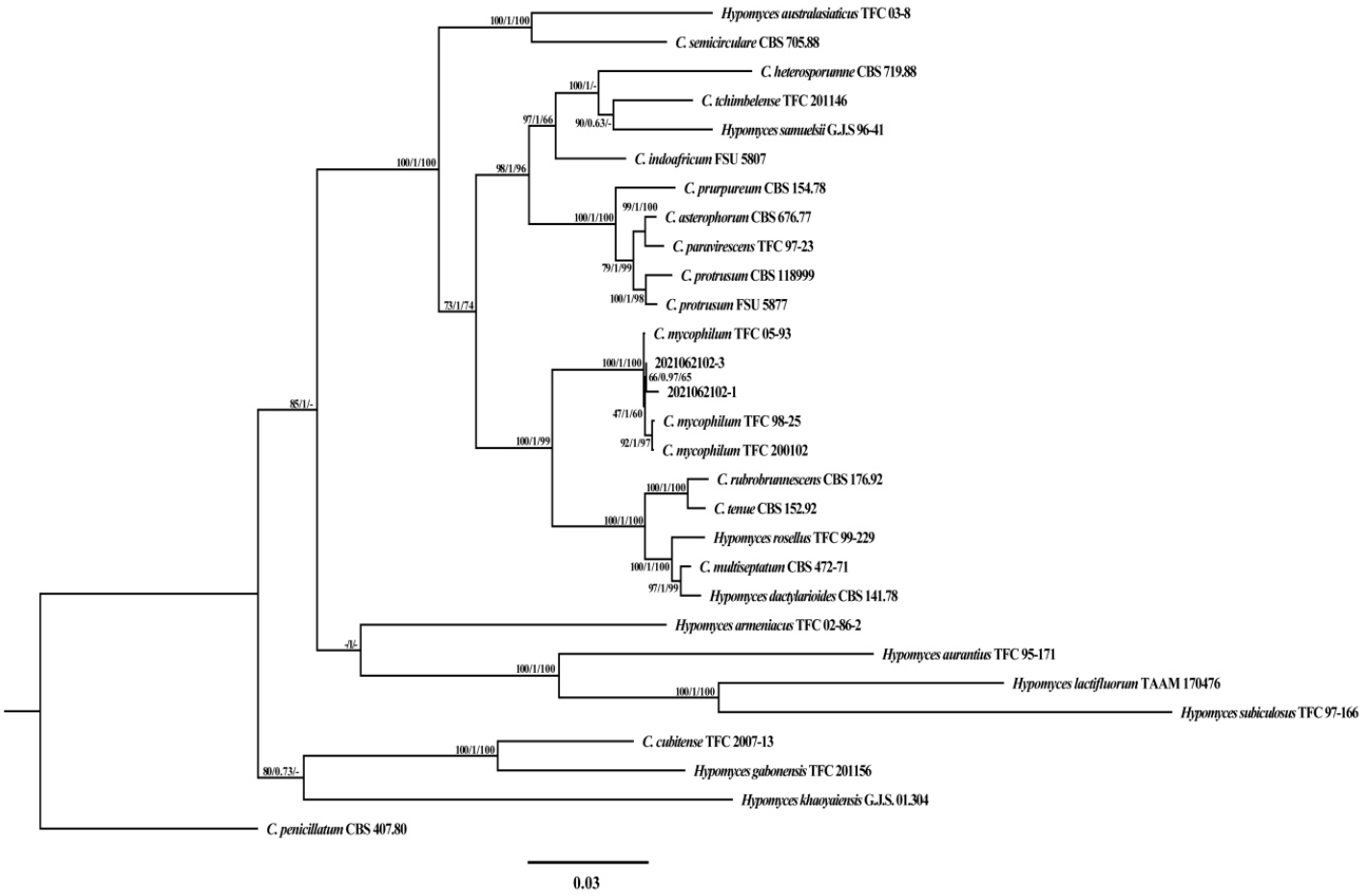

Figure 3

Multi-gene phylogenetic tree based on combined ITS*, RPB1*, *RPB2*, *TEF1-α*sequences. ML (maximum likelihood) and MP (maximum parsimony) bootstrap values greater than 50% are reported above the branches, BI (Bayesian inference) values > 0.90 are shown next to topological nodes and separated by "/". Bootstrap values < 50% and BI values < 0.90 are labeled with "-". The tree was rooted to *Cladobotryumpenicillatum* CBS 407.80.