First report of Fusarium solani causing root rot in Mushroom (Lyophyllum decastes) in China

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Short Report

Keywords: Mushroom (Lyophyllum decastes), Fusarium solani, Fungal disease, Root rot disease

Posted Date: August 15th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3244522/v1

Version of Record: A version of this preprint was published at Journal of Plant Diseases and Protection on October 1st, 2023. See the published version at https://doi.org/10.1007/s41348-023-00808-7.

Abstract

Lyophyllum decastes is a delicious mushroom with health care value, and has been successfully cultivated in many areas in China. In 2021 and 2022, root rot was found on the mushroom in a production base of Guiyang City, Guizhou province. Based on morphological and molecular identifications, the pathogenic fungus was identified as *Fusarium solani*. The pathogenicity was verified with Koch's postulates. This is the first report confirming the presence of this *Fusarium* causing root rot on *L. decastes* in China.

Full Text

Lyophyllum decastes (Fr.) Sing belongs to the family Tricolomataceae, a gray basidiomycete, is a popular culinary-medicinal mushroom due to its good flavor and excellent texture (Pokhrel et al. 2007). It has tremendous economic importance and was cultivated widely across China. Its fruit is a delicious mushroom and contains a number of health-promoting compounds (SOOD et al. 2016).

In Autumn 2021and 2022, root rot was found in Guiyang City of Guizhou Province, with 22% incidence in approximately 8.1 ha plantation of the mushroom (*L. decastes*). The early symptom appeared as irregular lesions on the root. The epidermis of susceptible sample was easy to be rot and become softening. White mycelium formed on spots at a later stage (**Fig. 1 a b**). Odorless decay and root rot symptoms were observed at a later stage, and the immature mushroom eventually wither and die.

Two methods (tissue separation and mycelium separation) were used to isolate pathogenic fungus. For the tissue separation method, symptomatic samples were surface sterilized in 75% ethanol for 10 s and 1% NaClO for 1 min, rinsed three times with sterile water. Small pieces were aseptically cut and incubated on PDA for 3 days at 25 °C, in the dark. For the mycelium separation method, the hyphal tip on the symptomatic samples was picked and incubated on potato dextrose agar (PDA) medium. The resulting cultures were incubated for 3 days at 25 °C, in the dark. (Leslie and Summerell 2006).

To study the pigmentation and growth rates, three isolates (LRGF001, LRGF002, LRGF003) were transferred onto fresh potato dextrose agar (PDA) plates and incubated under 12 h alternating light (black/white) at 25°C for 8 days. For microscopic observations, strains were transferred to carnation leaf-piece agar (CLA, Fisher et al. 1982) plates and incubated under 12 h alternating light at 25°C for 8 days. Thirty randomly selected conidia of each septation class (macro- and microconidia) were measured.

On PDA aerial mycelium uniformly cottony. Cultures grew fast, the growth rate (mm⁄day) on PDA at 25 °C in intermittent light ranged from 7.2 to 8.8 mm/day. The hyphae initially hyaline and mycelium became yellowish white, and purple in reverse after 8 days. At 25 °C, aerial conidiophores formed abundantly, unbranched or branched, up to 50 μm long, 3.5-7.0 μm at base. Phialides were more or less erect, subcylindrical, or cylindrical arising from conidiophores. The macroconidia are typically falcate, widest in the middle of their length, with 3-4-septate. The microconidia are oval, reniform, elongated oval to sometimes obovoid, with 0-2-septate. The size of conidia measures as follows: 0-1-septate = (5.5-18.5) μm × (2.5-3.5) μm, 2-4-septate = (22-40) μm × (2.5-4.5) μm (**Fig. 1 d e**). Chlamydospores are smooth walled. Morphological and cultural characteristics matched *Fusarium solani* (Perez et al. 2007, Perez et al. 2011, Yan et al. 2023).

To further confirm the identity of the fungal strain, all strains were grown on PDA with sterile dialysis membranes for 5 days. The mycelium grown over the membranes were harvested to a genomic DNA extraction and gene amplification of ITS and TEF-1α. Amplification of the TEF-1α gene and ITS regions was conducted using primer pair ef1 (5'-ATGGGTAAGGA(A/G)GACAAGAC-3')/ef2 (5'-GGA(G/A)GTACCAGT(G/C)ATCATGTT-3') for the translation elongation factor 1-α (TEF-1α) region and ITS1 (5'-TCCGTAGGTGAACCTGCGG-3')/ITS4 (5'-TCCTCCGCTTATTGATATGC-3') for the internal transcribed spacer (ITS) region (VEERARAGHAVAN et al. 2004; White et al. 1990). The ITS and TEF-α sequences were compared with other available *Fusarium* species sequences in the GenBank. Base on the NCBI-BLAST analysis of DNA sequences, ITS sequence showed 99-100% identity with *Fusarium* spp., including *Fusarium solani* (MG711902.1, KY745778.1, FJ874633.1, et al.), *F. metavorans* (OW988422.1, OW987888.1), *F. eumartii* (MH855784.1), *F. sporotrichioides* (EU520119.1), *F. quercinum* (OW986761.1). TEF-1α sequence showed 99-100% identity only with *F. solani* (MN650117.1, MN650105.1, OP778751.1, et al.) and revealed low similarity (<96%) to other species. The phylogenetic tree was generated using neighbor-joining (NJ) method in MEGA5.0. Bootstrap values for the maximum parsimony tree (MPT) were calculated for 1000 replicates. The tree generated from the combined dataset of ITS regions or TEF-α supported previously inferred *F. solani* (**Fig. 2 and Fig. 3**).

The ITS and TEF-α sequences of the 3 representative isolates obtained in this study were deposited in GenBank (ITS: OR349485, OR349621, OR349623; TEF-α: OR356109, OR356110, OR356111, respectively). All isolates (LRGF001, LRGF002, LRGF003) obtained in this study were deposited in Pathology Laboratory of Guizhou Institute of Biology.

In May to July 2022, Koch's postulates were checked by using asymptomatic mushrooms in two plantations of Baiyun county, Guiyang City. (26° 44' 40.9" N, 106° 43' 52.9" E). Mushrooms were inoculated by spraying with a spore suspension (10^6 conidia/mL). There were 10 replicates to three isolates (LRGF001, LRGF002, LRGF003), respectively. Spore suspension was prepared by suspending spores from *F. solani* cultures that were incubated on PDA at 25 °C with a 12-h photoperiod for 7 days. Inoculated mushrooms were wrapped with moist cotton and polyethylene bags for 24 h. Ten asymptomatic mushrooms were sprayed with sterile water served as controls. White mycelium (4 days after inoculation) and root rot symptom (8 days after inoculation) similar to those observed on naturally infected mushroom (**Fig. 1 c**). *F. solani* were reisolated from all inoculated plants, but not the controls.

Recent studies show that *F. solani* can infect a variety of hosts, including *Citrus reticulate* and *Chamaedorea cataractarum*, in Pakistan (Moosa et al. 2023; Haq et al. 2020), tomatoes (Debbarma et al. 2021), chickpea (Dell'Olmo et al. 2023), and so on. To the best of our knowledge, *F. solani* has hardly been reported as a pathogen causes mushroom diseases. This is the first report of *F. solani* causing root rot disease in mushroom (*Lyophyllum decastes*) in the world.

Declarations

Acknowledgements This research was supported by “Based on metagenomics technology to study the effects of morel pathogens, soil microorganisms, and soil properties on continuous cropping obstacles (Guizhou Academy of Sciences R 2021 No. 4)” and “Study on the applicability of pine and China fir aging to prepare for Fungus material of precious mushroom (Guizhou Province Cooperation Support, [2022]-114)”.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Figures

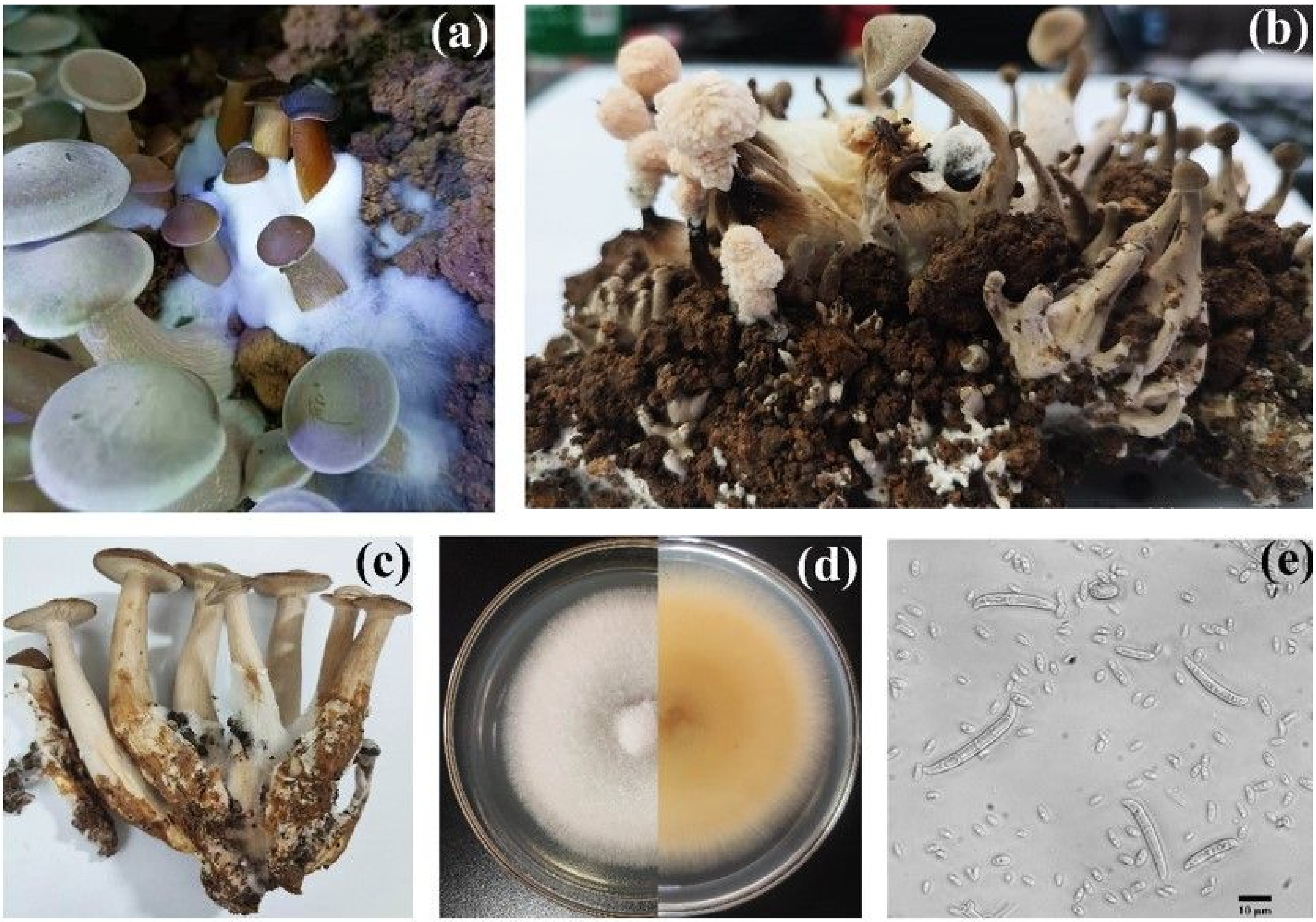

Figure 1

Root rot disease on mushroom (*Lyophyllum decastes*). (a, b) Root rot at disease early and late stage of the mushroom in the field, respectively. (c) 4 days after inoculation, similar symptoms were observed in the treatment (LRGF001). (d) *Fusarium solani* on PDA medium 3 days (front and back). (e) Conidia with 0-5-septate, bar=10 μm.

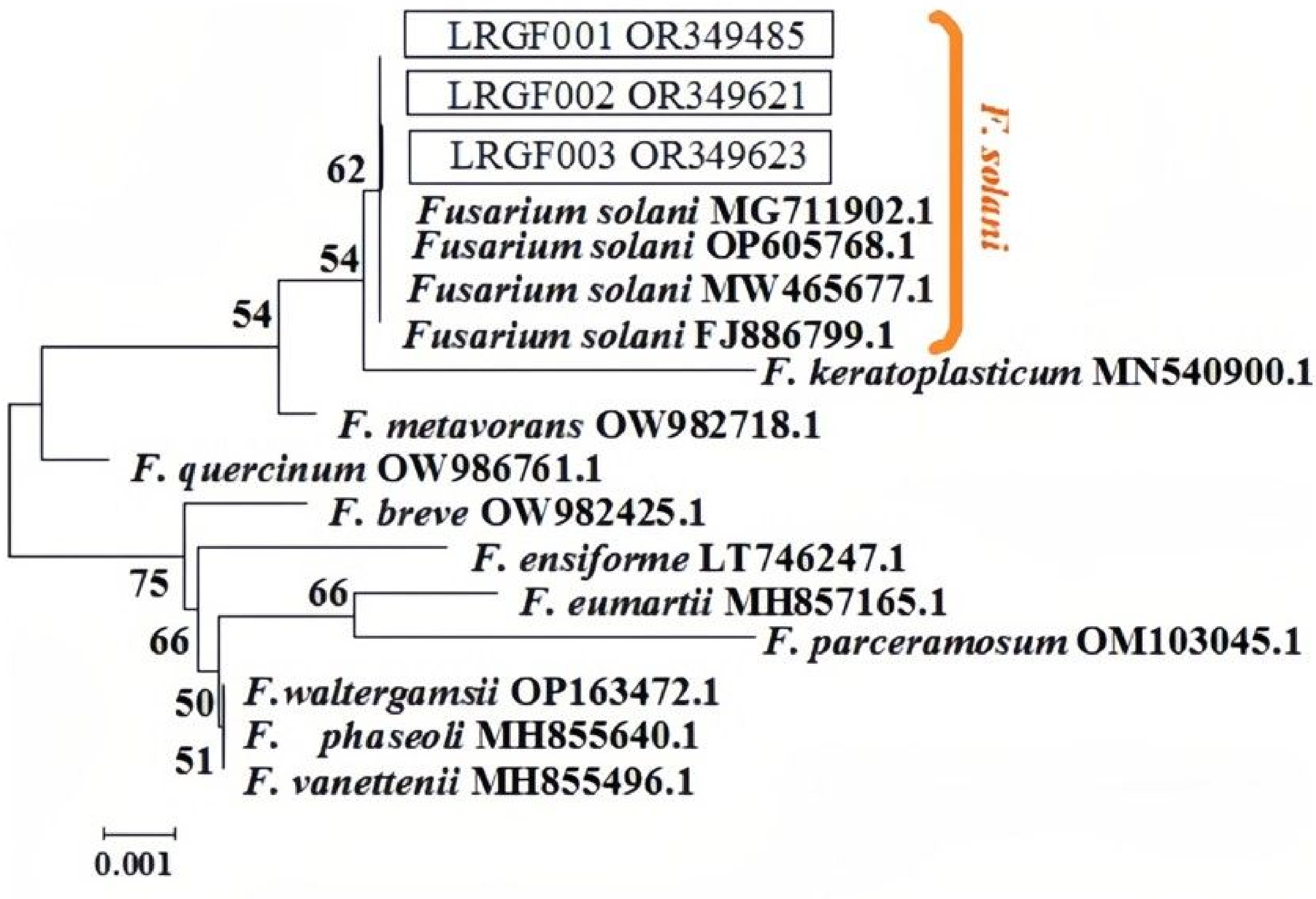

Figure 2

Phylogenetic tree produced from the internal transcribed spacer (ITS) region showing the phylogenetic relationships among *Fusarium*spp., using the neighbor-joining method.

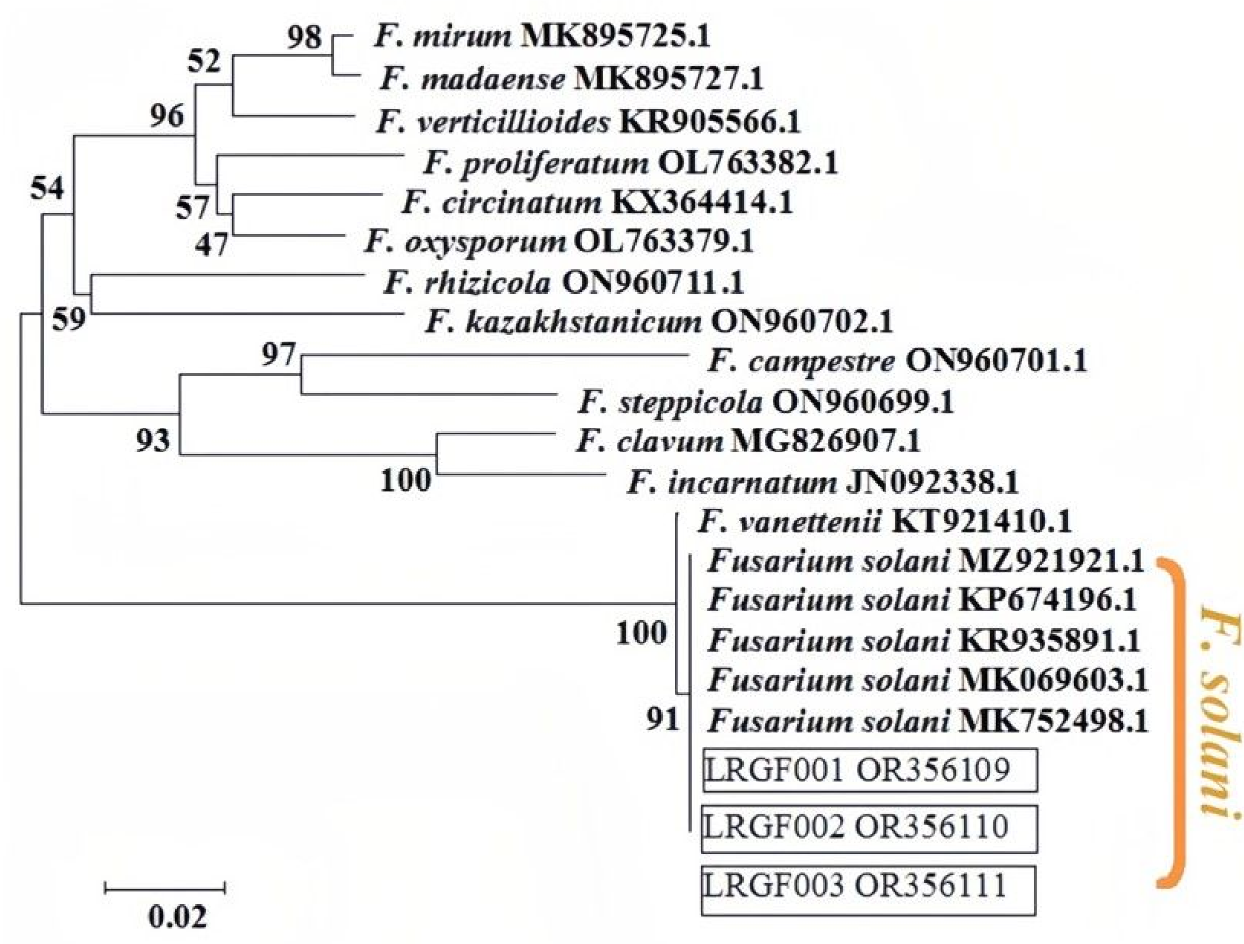

Figure 3

Phylogenetic tree produced from the translation elongation factor 1-α (TEF-1α) region showing the phylogenetic relationships among *Fusarium* spp., using the neighbor-joining method.