

Fusarium equiseti Causes Leaf Rot in Hylotelephium erythrostictum in China

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Article

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Abstract

Colonies with similar molecular characterisation were isolated from rotten leaves of *Hylotelephium erythrostictum* collected from the Chinese Medicine Herbal Cultural EXPO Garden of Three Gorges, Chongqing, China. In this study, the pathogen that caused these symptoms was identified as *Fusarium equiseti* using morphological observation, molecular characterisation (ITS, *RPB2* and *CAMD*) and a pathogenicity test. To the best of our knowledge, this is the first report of *F. equiseti* as a pathogen of *H. erythrostictum*. The findings of the present study are expected to contribute to the knowledge of *H. erythrostictum*-associated diseases in China and provide an essential foundation for the development of effective pre-harvest disease management strategies to curb disease spread.

1. Introduction

Hylotelephium erythrostictum (Miq.) H. Ohba, which is native to China, is a common, perennial fleshy herb of the *Crassulaceae* family¹ and was clearly classified as a traditional Chinese herbal medicine in Shennong's Classic of Materia Medica. Compounds that were found to significantly inhibit α -glucosidase activity, thereby control blood sugar levels, were isolated from *H. erythrostictum*². Moreover, the extract of its above-ground part has strong anti-oxidant and anti-inflammatory properties and can be used as an anti-inflammatory agent³. Over the last several years, because of its commercial value, the endive planted area has expanded on a yearly basis. However, successive planting has led to occurrence of disease in this plant. Only two diseases have been reported in *H. erythrostictum*, i.e., leaf spot caused by *Alternaria alternata* and powdery mildew caused by *Erysiphe sed*^{4–5}.

Fusarium spp. display a rich within-species variation⁶ and are highly regarded plant pathogens that have had a significant impact on production⁷. These pathogens severely restrict the yield of traditional Chinese medicinal plants and prevent them from undergoing normal growth and development. Several *Fusarium* spp., i.e., *Fusarium culmorum*, *Fusarium solani*, *Fusarium oxysporum* and *Fusarium redolens*, have been reported to cause diseases in valuable traditional Chinese herbs in China, such as *Dendrobium officinale*, *Lycium barbarum*, *Panax ginseng*, *Panax notoginseng* and *Gastrodia elata*^{8–12}. However, there have been no reports of *Fusarium* spp. infection in *H. erythrostictum*.

During 2023–2024, a severe leaf rot disease was observed on *H. erythrostictum* at the Three Gorges Chinese herbal medicine planting expo park, Kaizhou District (31°34'N; 108°25'E), Chongqing, China. The disease incidence was greater than 40% and caused economic loss of *H. erythrostictum*. Thus, this study aimed to use morphological and molecular characterisations to identify the pathogen that causes leaf rot in *H. erythrostictum*. The findings of this study will expand the knowledge of diseases in *H. erythrostictum* and provide scientific references for the identification and prevention of the occurrence of leaf rot.

2. Materials and methods

2.1. Fungus isolation and morphological observation

Tissue blocks (5 × 5 mm) cut from the infected–uninfected junction of the samples were surface sterilised in a 75% (vol/vol) ethanol solution for 1 min and a 3% (vol/vol) sodium hypochlorite solution for 3 min. Then, the blocks were washed three times with distilled water and placed on potato dextrose agar (PDA). Subsequently, they were cultured in a growth chamber at 25°C for 7 days¹³ and examined for morphological characteristics. The prepared fungal samples were placed on glass slides for subsequent microscopic examination. Morphological observations and size measurements of conidiophores and conidia were performed using an OLYMEUS, BX51 microscope (n = 50).

2.2 Pathogenicity test

To deduce whether the disease symptoms were caused by these isolates, healthy leaves of *H. erythrostictum* were rinsed with sterile water and leaves were stabbed at two sites symmetrically using a sterilised needle. The isolates were inoculated on one site¹⁴, whereas cultures of sterile PDA agar were used as negative controls on the other site. All leaves were covered with a transparent and sterile plastic bag, to maintain the humidity and prevent contamination. The experiment was performed in triplicate. Re-isolation and identification of the pathogenic species were performed, to fulfil Koch’s postulates¹⁵.

2.3. Molecular characterisation

The fungal isolates were incubated on a PDA plate at 25°C for 1 week. The mycelium was gently scraped with a sterile scoop, and DNA was extracted using the Plant Genomic DNA Kit (cwbio, Jiangsu). The internal transcribed spacer (ITS), the partial RNA polymerase second largest subunit (*RPB2*) and calmodulin (*CAMD*) were amplified using the following primer pairs: ITS1 (5'–TCCGTAGGTGAACCTGCGG–3')/ITS4 (5'–TCCTCCGCTTATTGATATGC–3'), *RPB2*-5F2 (5'–GGGGWGAYCAGAAGAAGGC–3')/*RPB2*-7cR (5'–CCCATRGCTTGTYRCCCAT–3') and *CL1* (5'–GARTWCAAGGAGGCCTTCTC–3')/ *CL2A*(5'–TTTTTGCATCATGAGTTGGAC–3')^{16–18}. The PCR mixtures included 7.4 µL of ddH₂O, 0.3 µL of each primer, 2 µL of DNA template and 10 µL of dNTP mix, for a total volume of 20 µL. The amplification conditions used for the ITS1/ITS4, *RPB2*-5F2/*RPB2*-7cR and *CL1*/*CL2A* primer pairs were as follows (Table 1). The PCR products were checked by electrophoresis on a 1.0% agarose gel in TAE buffer (0.5×) and observed under UV illumination.

Table 1
Gene/regions, primers and PCR protocols used in the study.

Gene/regions	Primer pairs	Optimised PCR protocols
ITS	ITS1/ITS4	94°C: 5 min; (94°C: 45 s, 55°C: 45 s, and 72°C: 1 min) × 35 cycles; 72°C: 10 min
<i>RPB2</i>	<i>RPB2</i> -5F2/ <i>RPB2</i> -7cR	94°C: 5 min; (94°C: 1 min, 56°C: 1 min, and 72°C: 80 s) × 36 cycles; 72°C: 10 min
<i>CAMD</i>	<i>CL1</i> / <i>CL2A</i>	94°C: 90 s; (94°C: 45s, 55°C: 45 s, and 72°C: 1 min) ×36 cycles; 72°C: 10 min

2.4. Phylogenetic analyses

Phylogenetic analysis was performed using the sequences of representative isolates obtained in this study via PCR amplification of genetic regions (ITS, *RPB2* and *CMDA*) that were validated with different species available on a database, and a strain from a different genus and species was selected as the outgroup (*Dactylonectria estremocensis* CBS 129085). The obtained sequences belonging to all gene regions were deposited in the GenBank database (<https://www.ncbi.nlm.nih.gov/genbank/>) and subjected to BLAST analysis using the NCBI and FUSARIOID (Polyphasic identification) database tool. The sequences of each gene/region were aligned using MEGA11¹⁹, and different single-gene sequences were assembled using the PhyloSuite software to generate concatenated sequences²⁰. A phylogenetic tree of the concatenated sequences (ITS, *RPB2* and *CAMD*) was constructed using the maximum-likelihood algorithm²¹. Branch stability was assessed through 1000 bootstrap replications.

3. Results

3.1. Symptom characteristics

A disease exhibiting rot symptoms was observed in the leaves of *H. erythrostictum* in Chongqing, China. This disease mainly damaged the middle and lower leaves of *H. erythrostictum* (Fig. 1a), with an incidence rate of more than 40% under high temperature and high humidity conditions. Early lesions appeared as brown irregular spots that increased in size and extended to dark-brown lesions with yellow halos around them from leaf tip (Fig. 1b); moreover, several small black dots were observed (Fig. 1c). In the later stage, the leaf tissues were rotted, causing abscission of the leaves.

3.2 Morphological characteristics of the pathogen

In this study, a total of 46 fungi were isolated from 15 diseased leaves of *H. erythrostictum*, 33 of which were a morphologically similar fungus, with a separation rate of 71.7%. The colonies grown on PDA formed abundant floccose aerial mycelia with irregular margins. As the culture time increases, the colour of the aerial mycelium changed from white to brownish yellow (Fig. 2a–b). The colour of the back of the leaves also changed from beige to brownish yellow. Aerial mycelia possessing conidia were observed using a microscope. The conidia were scythe-shaped, had a size of $21.5\text{--}31 \times 2.3\text{--}6.5 \mu\text{m}$ ($n = 50$) and exhibited 2–6 diaphragms (Fig. 2c). Old mycelia produced spherical, smooth and rough-walled chlamydospores, which formed intercalary, solitary chains and clumps (Fig. 2d).

3.3 Pathogenicity test

The representative JT29 and JT32 isolates were inoculated on wounded sites to evaluate pathogenicity. Moreover, cultures of sterile PDA agar were used as negative controls. After 11 days of culture, lesions similar to those observed in the field were observed on inoculated leaves, whereas the control plants remained healthy (Fig. 3). The strains that were re-isolated from the treated leaves exhibited identical

morphological characteristics to those of the aforementioned inoculated isolates; therefore, Koch's postulates were fulfilled.

3.4 Molecular characterisation and phylogenetic analysis

For molecular characterisation, DNA was extracted from representative isolates (JT29 and JT32). The sequences of ITS, *RPB2* and *CAMD* from the isolates were deposited in GenBank (JT29: ITS, OR888751; *RPB2*, OR892585; *CAMD*, PP543256 and JT32: ITS, OR888754; *RPB2*, OR892586; *CAMD*, PP543257). The phylogenetic tree revealed that the isolates JT29 and JT32 had a similarity of 100% and 99% with *Fusarium equiseti* CBS 307.94 (ITS, MH862468; *RPB2*, KU604327) and *F. equiseti* CBS 185.34 (ITS, MH855481; *RPB2*, MN170399; *CAMD*, MN170332) (Table 2, Fig. 4), respectively. Based on morphology and the results of the multi-locus phylogenetic analysis, the representative strains were identified as *F. equiseti*.

Table 2
GenBank accession number used in the phylogenetic tree.

Species	Isolate	GenBank accession number		
		ITS	<i>RPB2</i>	<i>CAMD</i>
<i>Fusarium equiseti</i>	CBS 307.94*	MH862468	KU604327	–
<i>Fusarium equiseti</i>	CBS 185.34*	MH855481	MN170399	MN170332
<i>Fusarium acuminatum</i>	DG1-1	OP897712	OR701935	–
<i>Fusarium acuminatum</i>	NJC24	OL655401	OL741723	–
<i>Fusarium alpinum</i>	LC6043	MW016688	MW474674	–
<i>Fusarium alpinum</i>	LC6045	MW016689	MW474675	–
<i>Fusarium andiyazi</i>	CBS 256.93*	KR071653.	KU604231	KU603955
<i>Fusarium andiyazi</i>	CBS 119857*	KR071651.	KT154004	MN534175
<i>Fusarium fujikuroi</i>	CBS 257.52*	MH857023	MW402812	MW402454
<i>Fusarium fujikuroi</i>	CBS 449.95*	KR071667	KU604259	KU603998
<i>Fusarium napiforme</i>	CBS 673.94*	KR071649	KU604237	KU603965
<i>Fusarium napiforme</i>	CBS 535.95*	KU604029	KU604236	KU603964
<i>Fusarium nygamai</i>	CBS 140.95*	KR071695	KU604264	KU603949
<i>Fusarium nygamai</i>	CBS 749.97*	MH862671	KU604262	MT010892
<i>Fusarium oxysporum</i>	CBS 132475*	MH866023	KU604313	–
<i>Fusarium oxysporum</i>	CBS 130302*	MH865881	MT010985	–
<i>Fusarium proliferatum</i>	CBS 189.38*	MH855944	KM232384	KM231395
<i>Fusarium proliferatum</i>	CBS 138981*	KT716199	KT716196	–
<i>Fusarium thapsinum</i>	CBS 113963*	KR071691	KU604296	KU603969
<i>Fusarium thapsinum</i>	CBS 109077*	KR071689	KU604295	KU603970
<i>Fusarium verticillioides</i>	CBS 122159*	KR071637	KU604224	KU603943
<i>Fusarium verticillioides</i>	CBS 576.78*	MH861171	KU604216	KU603936
<i>Dactylonectria estremocensis</i>	CBS 129085*	MH865182	KM232345	KM231328
Ex-Type (ex-epitype) strains marked with an *				

4. Discussion and conclusion

Based on morphological observation and molecular identification, the isolates obtained here were conclusively identified as *F. equiseti*. To the best of our knowledge, this is the first report of *F. equiseti* causing leaf rot in *H. erythrostictum* in China.

The fungal genus *Fusarium* comprises more than 400 species of plant-pathogenic fungi that harm a wide range of plants worldwide²². *F. equiseti* was first discovered and named by (Corda) Sacc.²³, and 25 strains of it have been included as pathogens in MycoBank (<https://www.mycobank.org/>).

Fusarium species are able to produce type A trichothecenes after infecting plants²⁴, which have an immunosuppressive effect in animals and humans because of their multiple inhibitory actions in eukaryotic cells²⁵. Traditional Chinese medicinal materials are gradually being included in the list of health products²⁶, type A trichothecenes will pose safety hazards to the production and development of it. Overall, it is necessary to prevent *F. equiseti* in the production of *H. erythrostictum*.

Declarations

IUCN policy statement

Hylotelephium erythrostictum is a medicinal herb and not a protected plant, all specimens were collected with permission from Three Gorges Chinese herbal medicine planting expo park, Chongqing, China. And the current research will not cause any kind of damage to the population of *Hylotelephium erythrostictum*. We confirm compliance with the IUCN policy for plant.

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Author contributions

F.Y. and J.J.: contributed to the fungus isolation, analysis of all results and wrote a paper. Z.S. and Q.X.: contributed to the analysis of morphological characteristics of the pathogen and pathogenicity test results. W.M.: contributed to the analysis of molecular characterisation and phylogenetic analysis results. M.L. and J.Q.: contributed to the overall experimental idea and data analysis.

Data availability statement

The data that support the findings of this study are contained within the manuscript. All the sequences used in this study have been successfully uploaded to the NCBI repository. The nucleotide sequences obtained with ITS, *RPB2* and *CAMD* have been uploaded successfully under the accession IDs (JT29: ITS, OR888751; *RPB2*, OR892585; *CAMD*, PP543256 and JT32: ITS, OR888754; *RPB2*, OR892586; *CAMD*, PP543257); respectively.

Competing interests statement

The authors declare no competing interests.

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Figures

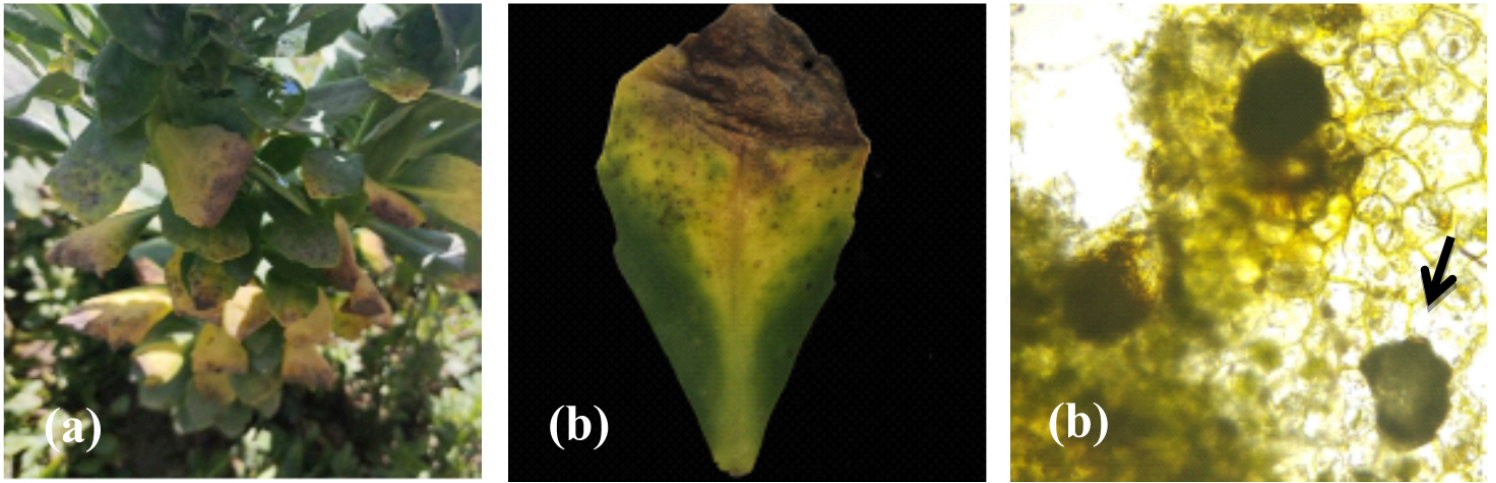


Figure 1

Symptoms of leaf rot in *Hylotelephium erythrostictum* in the field.

(a) The disease mainly occurred at the leaf margin; (b) diseased leaves after drying. (c) black dots detected under microscopic observation.

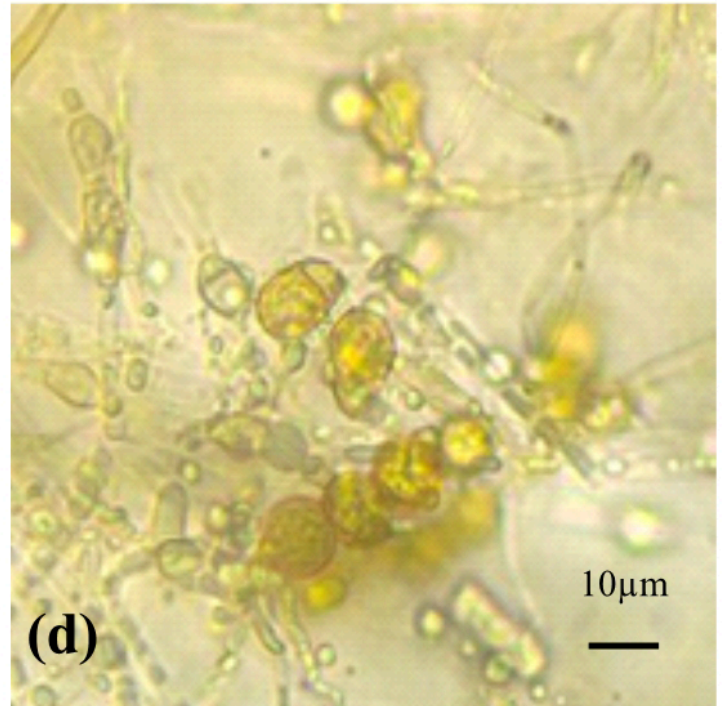
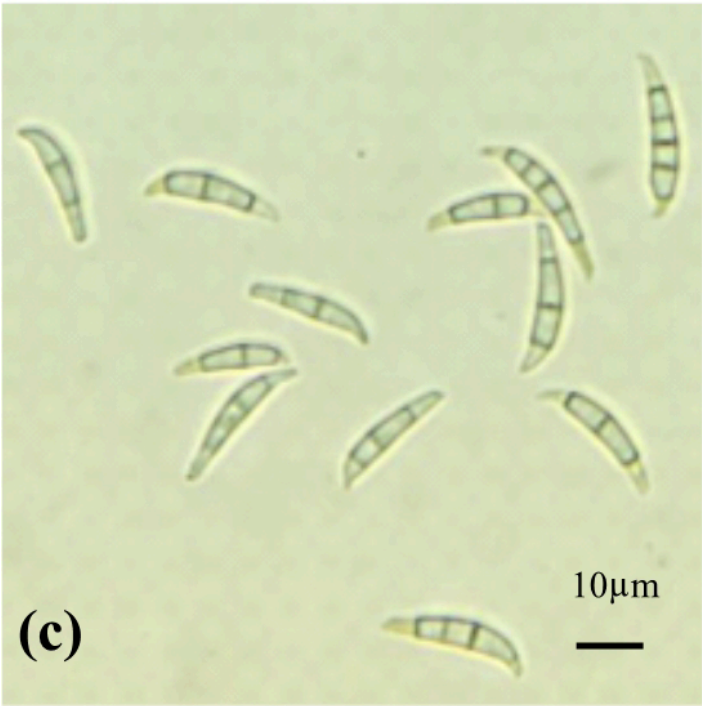
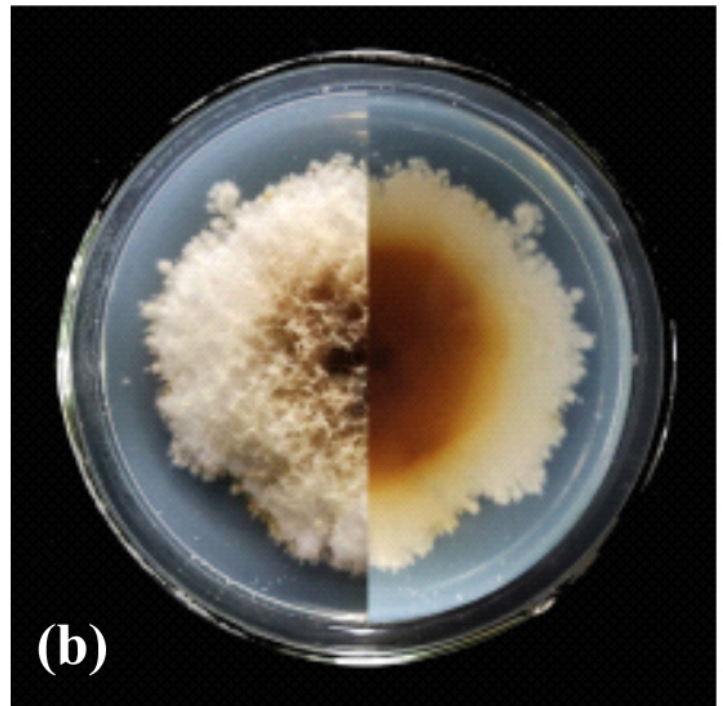
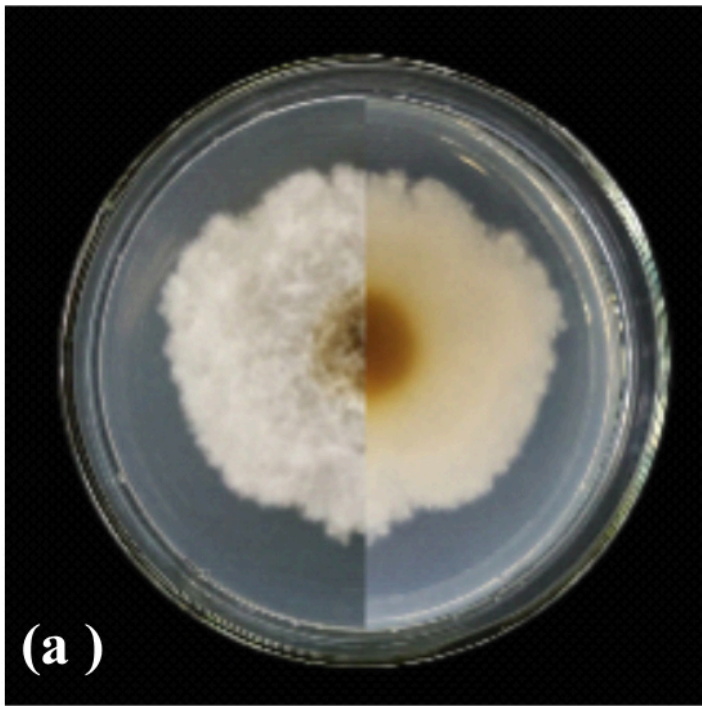


Figure 2

Morphological observation.

(a) A colony of *Fusarium equiseti* on PDA medium at 7 days after incubation; (b) a colony of *F. equiseti* on PDA medium at 12 days after incubation; (c) conidia; (d) chlamydospores.

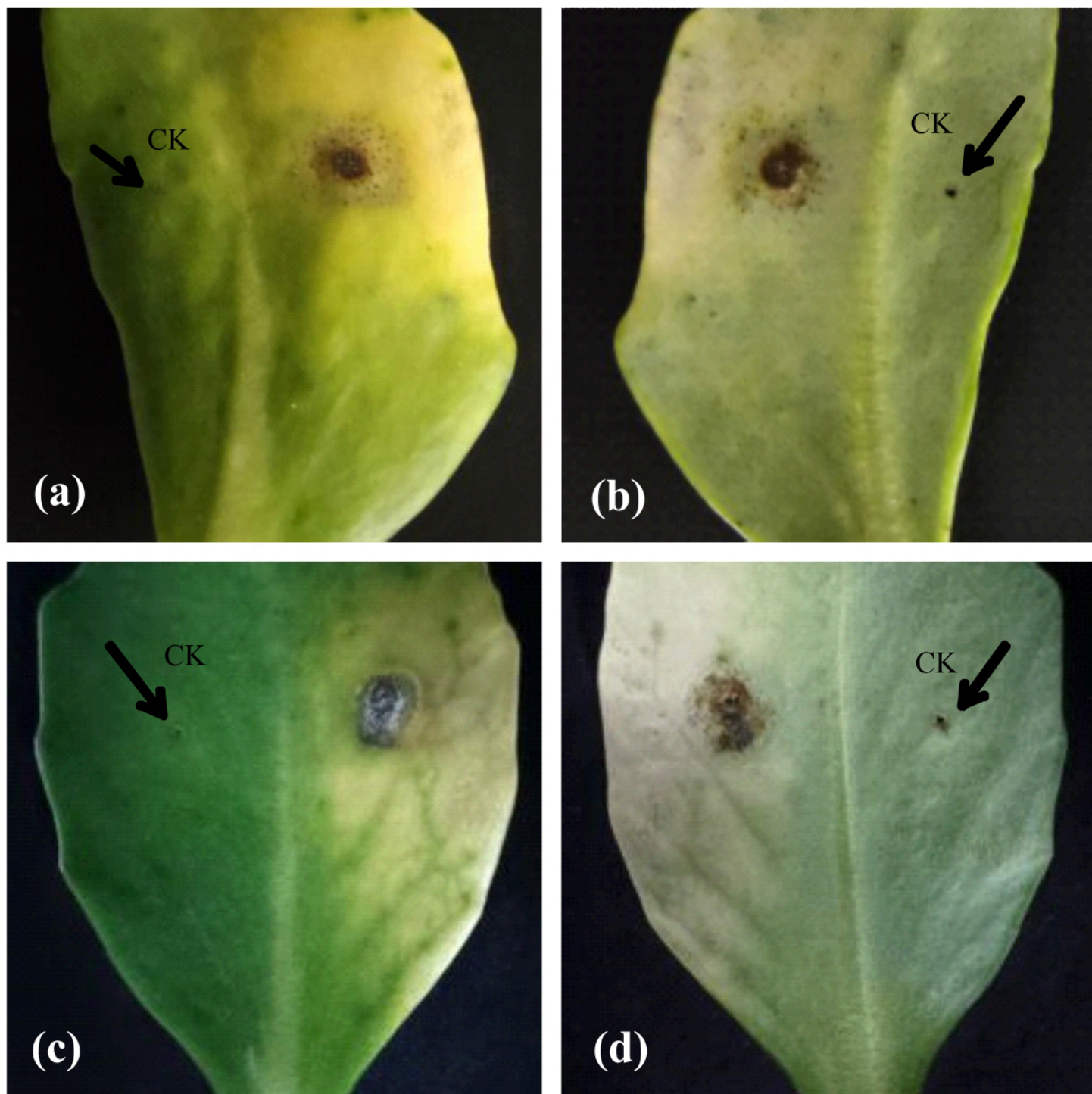


Figure 3

Pathogenicity test.

(a–b) Symptoms on healthy leaves inoculated with the JT29 isolate for 11 days; (c–d) Symptoms on healthy leaves inoculated with the JT32 isolate for 11 days.

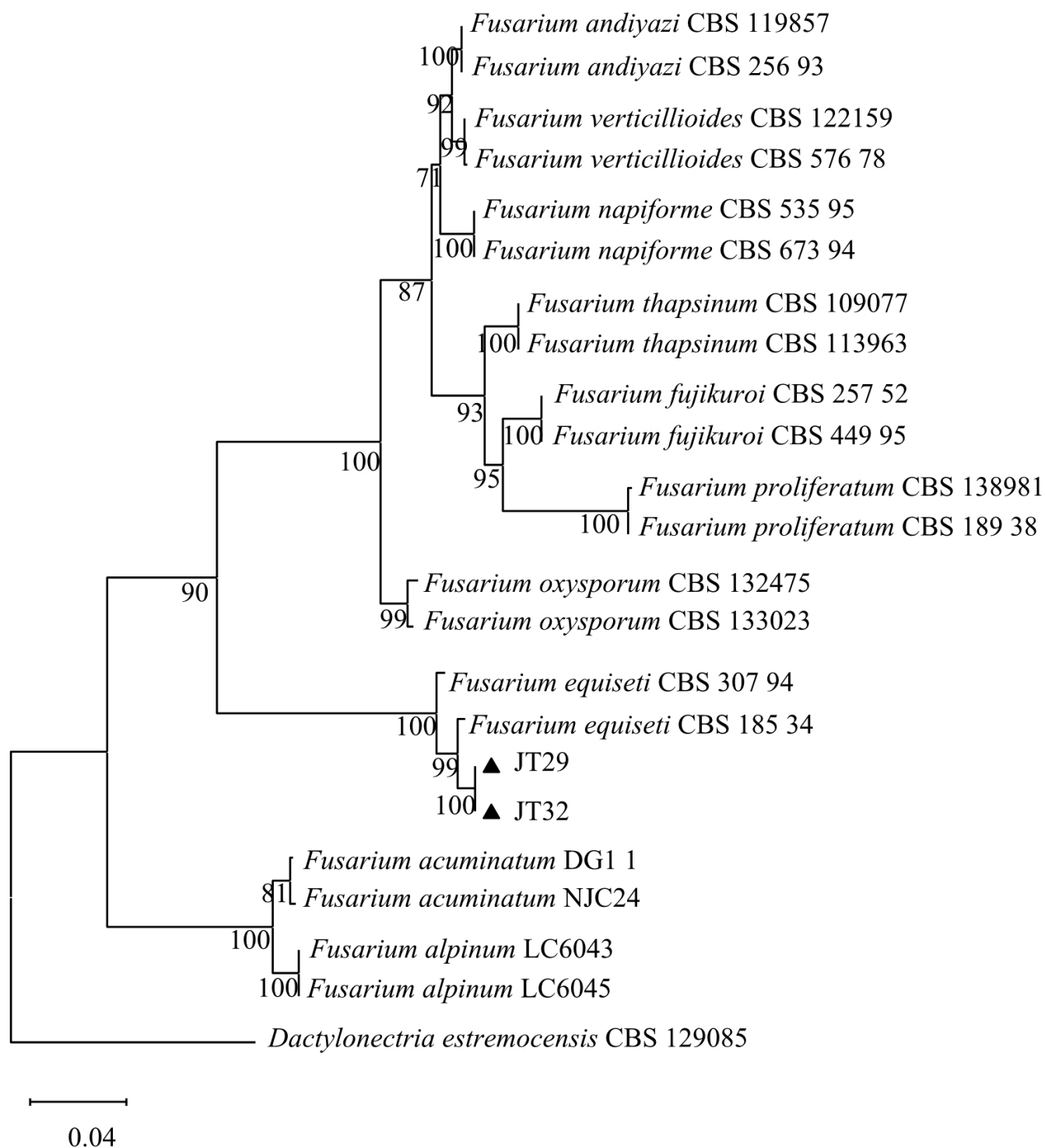


Figure 4

Phylogenetic tree obtained based on the combined ITS, *RPB2* and *CAMD* sequences of *Fusarium* spp. via a Kimura 2-parameter model using MEGA11.0. A bootstrap analysis using the maximum-likelihood method with 1,000 replicates was conducted. The representative isolates were JT29 and JT32. *Dactylonectria estremocensis* was used as the outgroup. The scale bar indicates the evolutionary distance (number of nucleotide substitutions per site).