

Postprint of Isolation and Identification of Pathogenic Fungi Causing Soft Rot in *Amorphophallus konjac*

Authors: Li Zhumei, Dong Kun, Zhang Yan'an, Gao Yong, Chen Hong, Fang Pingping, Lei Hongxian, Lu Xiaoqian, Chu Honglong

Date: 2023-07-13T00:00:00+00:00

Abstract

Konjac soft rot disease is a significant disease in konjac production and a primary constraint on the development of the konjac industry. Currently, it has been reported that konjac soft rot disease is mainly caused by bacteria, with rare reports of fungi causing soft rot in konjac corms. To determine the pathogen species and infection characteristics of soft rot disease in flower konjac (*Amorphophallus konjac*) from Qujing City, Yunnan Province, this study isolated fungi from diseased samples of flower konjac collected from Qujing City, Yunnan, via tissue isolation. The isolated fungi were identified via morphological observation combined with molecular methods based on ITS and LSU sequence analysis, and pathogenicity was assessed according to Koch's postulates. A dual inoculation experiment was performed with the identified pathogenic fungi and the bacterial pathogens of konjac soft rot. The results demonstrated that: (1) Three *Fusarium* species, namely *Fusarium concentricum*, *F. oxysporum*, and *F. ambrosium*, as well as one *Mucor* sp., one *Rhizopus* sp., one *Penicillium* sp., and one *Clonostachys* sp. were identified based on morphological and molecular evidence. (2) Statistical analysis revealed that *F. concentricum* exhibited the highest relative abundance (45.45%). (3) Pathogenicity tests following Koch's postulates demonstrated that *F. concentricum* was pathogenic. (4) Dual inoculation of konjac corms with *F. concentricum* and the pathogenic bacterium *Pectobacterium aroidearum* led to accelerated disease development, with significantly greater lesion tissue weight compared to single inoculation treatments with either *F. concentricum* or *P. aroidearum*. These findings suggest that konjac soft rot disease may be caused by fungal-bacterial co-infection. This study provides a theoretical foundation for the prevention and control of konjac soft rot disease.

Full Text

Preamble

Pathogenic Fungi Isolation and Identification from Rot Tissue of *Amorphophallus konjac* Corm

Li Zhumei¹, Dong Kun², Zhang Yan'an¹, Gao Yong¹, Chen Hong¹, Fang Ping-ping¹, Lei Hongxian¹, Lu Xiaoqian¹, Chu Honglong^{1,3*}

(1. College of Biological Resources and Food Engineering, Qujing Normal University, Qujing 655011, Yunnan, China; 2. Fuyuan Konjac Research Institute, Yunnan Academy of Agricultural Sciences, Fuyuan 655500, Yunnan, China; 3. Yunnan Engineering Research Center of Fruit Wine Technology Innovation and Application, Qujing Normal University, Qujing 655011, Yunnan, China)

Abstract: Konjac soft rot is a critical disease in konjac production and a primary factor limiting the development of the konjac industry. Current reports indicate that konjac soft rot is mainly caused by bacteria, with few documented cases of fungal-induced soft rot in konjac corms. To clarify the pathogen species and infection characteristics of soft rot in *Amorphophallus konjac* in Qujing City, Yunnan Province, this study isolated fungi from diseased konjac samples using tissue isolation methods. Isolated fungi were identified through morphological characterization combined with molecular analysis of ITS and LSU sequences, and pathogenicity was determined according to Koch's postulates. Dual inoculation experiments were conducted between the identified pathogenic fungi and konjac soft rot bacteria. The results showed: (1) Three *Fusarium* species (*Fusarium concentricum*, *F. oxysporum*, and *F. ambrosium*), one *Mucor* sp., one *Rhizopus* sp., one *Penicillium* sp., and one *Clonostachys* sp. were identified through morphological and molecular approaches. (2) Statistical analysis revealed that *F. concentricum* had the highest relative abundance at 45.45%. (3) Koch's postulates tests demonstrated that *F. concentricum* was pathogenic. (4) Dual inoculation of konjac corms with *F. concentricum* and the bacterial pathogen *Pectobacterium aroidearum* resulted in faster disease development, with significantly greater rotten tissue weight compared to single inoculation with either *F. concentricum* or *P. aroidearum* alone. These findings suggest that konjac soft rot may be caused by combined fungal and bacterial infection. This study provides a theoretical basis for the prevention and control of konjac soft rot.

Keywords: *Amorphophallus konjac*, soft rot, pathogenic fungi, phylogenetic analysis, pathogenicity

Document Code: A

Classification Number: Q949.32

Amorphophallus is a genus of perennial herbs in the family Araceae that primarily grows in high-altitude mountainous regions (Qiu & Chou, 1995). Ap-

proximately 170 species exist worldwide, mainly distributed in Vietnam, Myanmar, China, and Japan, with about 17 species found in China, concentrated in Guangdong, Sichuan, and Yunnan provinces. Konjac corms contain higher protein content than potatoes and sweet potatoes and are rich in dietary fiber. Konjac products are low in calories yet high in nutritional and medicinal value, offering benefits such as weight loss, blood pressure and glucose reduction, improved gut microbiota structure, and cancer prevention (Zhang et al., 2005; Chua et al., 2010; Srzednicki et al., 2020).

Konjac glucomannan (KGM), the main component of konjac flour (Li et al., 2010), is a water-soluble polysaccharide with diverse applications in food science, nutrition, biotechnology, pharmacology, and fine chemical engineering (Zhang et al., 2005; Chua et al., 2010; Behera & Ray, 2016; Zhu, 2018; Srzednicki et al., 2020). *Amorphophallus konjac*, one of the konjac species with the highest KGM content, is a major cultivated variety in China (Gao et al., 2022). As an important economic crop, konjac has become one of the most promising and competitively advantaged characteristic resource industries in agricultural economic development across Yunnan, Guizhou, and Sichuan provinces, and is a key crop promoted for rural revitalization.

Soft rot is the most severe disease affecting konjac. Due to the lack of sustainable and effective control measures, it is considered the most devastating threat to the konjac industry, occurring during both the growth and storage periods. During the growth period, soft rot symptoms include softening of stems and corms, leaf wilting, followed by blackening and rotting of corms with foul odor emission and plant collapse (Wei et al., 2020; Wang et al., 2021). During storage and planting periods, infected seed corms initially develop water-soaked brown striations on the epidermis that expand inward, with white tissue gradually turning gray to yellow-brown and exuding large amounts of thick bacterial fluid, leading to corm rot (Wang et al., 2021). Currently, soft rot is widespread in konjac cultivation areas, seriously hindering the development of China's konjac industry.

Reports indicate that konjac soft rot is primarily caused by bacterial pathogens including *Pectobacterium aroidearum*, *P. carotovorum* subsp. *carotovorum* (Pcc), *P. chrysanthemi*, and *Enterobacter* sp. (Wu et al., 2011; Xu, 2011; Huang et al., 2014; Wu et al., 2015; Sun et al., 2019; Wei et al., 2020; Zhang et al., 2022). Few studies have reported on pathogenic fungi causing konjac soft rot. He et al. (2016) identified *Fusarium solani* and *F. oxysporum* as pathogenic fungi causing root zone and root surface soil rot in konjac plants. Li et al. (2017) demonstrated through inoculation with mycelial blocks that *F. oxysporum* exhibited varying pathogenicity to different konjac varieties. Zhao et al. (2022) isolated and identified pathogens from konjac stem rot, also obtaining *F. oxysporum* and *F. solani*, but pathogenicity tests showed that the *F. oxysporum* strain (xymy-8) was non-pathogenic while *F. solani* strains (xymy-7, xymy-9) were pathogenic with varying virulence. Currently, the mainstream view holds that konjac soft rot is caused by bacteria (Xu, 2011; Wu et al., 2015; Wei et al., 2020; Zhang et al., 2022). However, observations

in Qujing City, Yunnan Province, revealed extensive fungal mycelium growth on diseased storage corms, on field-collected samples shortly after placement at room temperature, and even on soft rot tissues in the field. Studies on bacterial infection characteristics have shown that soft rot pathogens cannot directly infect konjac corms through natural openings but only through bud sheaths and wounds (Huang et al., 2014; Wu et al., 2021). The rapid outbreak of soft rot in the field may result from both pathogen accumulation and rain-mediated spread (Zhang et al., 2012), and potentially from fungal infection providing entry points for bacterial pathogens.

This study focused on konjac cultivation areas in Qujing City, Yunnan Province, targeting soft-rotted corms of *A. konjac*. Using fungal tissue isolation, morphological and molecular identification, Koch's postulates testing, and dual inoculation experiments with isolated pathogenic fungi and konjac soft rot bacteria, we aimed to address: (1) whether konjac soft rot is caused by fungi, bacteria, or a composite disease; and (2) the species, taxonomic status, and disease characteristics of pathogenic fungi associated with konjac soft rot. The findings will provide a theoretical basis for precise prevention and control of konjac soft rot in Yunnan.

1.1 Experimental Materials

Diseased *A. konjac* corms were collected from konjac cultivation bases in Fuyuan County, Zhanyi District, and Luliang County, Yunnan Province, with details provided in Table 1. Disease symptoms are shown in Figure 1 [Figure 1: see original paper]: leaves yellowed and wilted with plant collapse; stems and/or corms exhibited soft rot symptoms, with excavated corms appearing black, rotten, and emitting foul odor (Figure 1: A, B, D). Collected samples showed abundant white and/or yellow fungal mycelium at disease sites, with some samples developing extensive mycelium shortly after collection when stored at room temperature (Figure 1: B, C).

Table 1 General situation of sample sites

Sample sites	Sample time	Altitude (m)	Latitude	Longitude
Fuyuan County	-	-	25°43 0 N	104°12 13 E
Zhanyi District, Qujing	-	-	25°54 36 N	103°48 28 E
Luliang County	-	-	25°07 12 N	103°47 54 E

A. Whole plant soft rot symptoms; B. Stem soft rot; C. Early corm soft rot; D. Late-stage soft rot of stem and corm base.

Figure 1 Rot character of *Amorphophallus konjac*

1.2.1 Strain Isolation and Purification

(1) Fungal isolation

Diseased *A. konjac* corms with soft rot symptoms were washed under running water to remove surface soil. Tissue from the junction between diseased and healthy areas was cut into ~0.3 cm pieces, surface-sterilized in 75% ethanol for 30 s, and rinsed three times with sterile water. Three to five sterilized tissue pieces were transferred to PDA plates (containing 3‰ lactic acid), arranged evenly, numbered, sealed with parafilm, and incubated in darkness at 25 °C for 2–5 days.

(2) Fungal purification

After 2–5 days of incubation, different colored and shaped fungal colonies grew around tissue pieces. Mycelia from colony edges were transferred to new PDA plates using inoculation needles, numbered, and incubated at 25 °C in darkness. Growth was observed and recorded. The purification process was repeated twice.

1.2.2 Morphological Identification

After one week of culture, temporary slides of sporulating strains were prepared. Mycelial, sporulation, and spore structural characteristics were observed and photographed using a compound microscope (Olympus BX53). Spore sizes were measured using Image FrameWork software (20 spores per strain). Morphological identification followed the *Fungal Identification Manual* (Wei, 1979).

1.2.3 Molecular Identification

Mycelia were scraped from purified fungal cultures into 1.5 mL centrifuge tubes, ground in liquid nitrogen, and genomic DNA was extracted using the CTAB method. The LSU and ITS conserved regions were amplified using primer pairs LR0R (5'-GTACCCGCTGAACTTAAGC-3') and LR5 (5'-ATCCTGAGGGAACTTC-3') (Vilgalys & Hester, 1990), and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAGTCGTAACAAGG-3') (White et al., 1990), respectively. Amplified products were sequenced. Sequences were edited using BioEdit and subjected to Blastn analysis in the NCBI database. High-homology sequences were downloaded, with *Russula vesca* as the outgroup. A phylogenetic tree was constructed using MEGA-X (Clustal W, Neighbor-Joining, bootstrap=1,000).

1.2.4 Pathogenicity Testing

(1) Koch's postulates test

Mycelial plugs (0.5 cm diameter) from identified fungal cultures were placed on healthy konjac corm slices with the mycelial side contacting the tissue. Sterile agar plugs served as controls. Samples were incubated at 28 °C with high humidity, and soft rot symptoms were observed. Pathogenic fungi were re-isolated from diseased tissues.

(2) Bacterial and fungal co-inoculation test

Four treatments were established: Treatment 1 (F+P) received both *Fusarium concentricum* mycelial plug (0.5 cm diameter) and 20 L of *Pectobacterium aroidearum* suspension (OD₆₀₀=0.1); Treatment 2 (F) received only *F. concentricum* mycelial plug and 20 L sterile water; Treatment 3 (P) received only *P. aroidearum* suspension (20 L, OD₆₀₀=0.1) and sterile agar plug; Treatment 4 (CK) received sterile water and sterile agar plug. All treatments were incubated at 28 °C with high humidity for 3 days. Rotten tissue was excavated and weighed using a silicone spoon. Data were analyzed using one-way ANOVA in SPSS (IBM SPSS Statistics 19) with Tukey's test, and graphs were generated in Excel.

2.1 Isolation and Purification of Soft Rot Fungi from *A. konjac* Corms

Twenty-two fungal strains were isolated and purified from diseased *A. konjac* corm tissues, designated M1-M25 (M2, M13, and M16 failed purification). After 5 days of culture on PDA medium at 25 °C, colony morphology and microscopic structures were observed. Strains M1, M3, M4, M5, M6, M8, M9, M11, M14, and M25 grew rapidly, forming brick-red to purple or light purple colonies on both sides with neat edges and a villous texture, showing white cottony surfaces without obvious rings. Conidia measured approximately 7.51 μm × 3.51 μm, predominantly small and kidney-shaped; macroconidia were slightly curved, septate; hyphae were septate, produced red pigment, and sporulated centrally.

Strain M7 grew rapidly, forming red to light purple colonies in the center on both sides with white, irregular edges. Conidia measured ~7.60 μm × 4.03 μm; small conidia were oval, macroconidia were sickle-shaped and septate, producing red pigment. Hyphae were septate and sporulated intercalary.

Strains M10 and M15 grew rapidly, forming white colonies on both sides with neat edges and villous texture without obvious rings. Conidia measured ~10.77 μm × 5.92 μm, predominantly small and oval; macroconidia were slightly curved with one or no septa. Hyphae were transparent, septate, and sporulated intercalary.

Strains M12, M19, and M22 formed white to light yellow colonies with centers higher than edges, with or without rings. Conidia measured ~4.68 μm × 3.16 μm, elliptical, smooth, and transparent without septa. Hyphae were white, floccose, transparent, septate, with apical penicillate branching.

Strains M17 and M21 grew rapidly. Conidia measured ~5.54 μm × 4.01 μm; small conidia were elliptical, large conidia spherical. Hyphae had few branches, were transparent, with spherical sporangia at apices.

Strains M18, M20, and M23 grew rapidly, forming colonies with dark gray centers, white edges, and white reverse sides. Texture was loose with irregular edges, no rings, and black surfaces. Conidia were round or elliptical, measuring ~4.35 μm × 3.61 μm with thick walls. Vegetative hyphae were mostly aseptate,

produced yellow pigment, and formed sporangia at swollen apices.

Strain M24 grew rapidly, forming grayish-white to bluish colonies on the front and grayish-white on the reverse with irregular edges, villous texture, and obvious rings. Conidia measured $\sim 3.15 \mu\text{m} \times 2.67 \mu\text{m}$, spherical or oval with thick walls. Hyphae were septate and sporulated centrally or from apical sporulation structures (Figure 2 [Figure 2: see original paper], Figure 3 [Figure 3: see original paper]).

M1, M3-M12, and M14-M25 are isolate numbers. The same below.

Figure 2 Isolated strains cultured on PDA medium

Figure 3 Microscopic observation of isolated strains (Bar = 20 μm)

2.2 Molecular Identification of Soft Rot Fungi from *A. konjac* Corms

Genomic DNA was extracted from the 22 purified fungal strains. After ITS and LSU amplification and sequencing, Blastn analysis in the NCBI database was performed. Similar sequences for ITS and LSU genes of 22 strains were downloaded for phylogenetic tree construction. Results showed that strain M5 had 96.89% ITS sequence similarity with the type strain of *Fusarium concentricum*, while M1, M3, M4, M6, M8, M9, M11, M14, and M25 had >97.77% ITS and LSU sequence similarity with the *F. concentricum* type strain (Table 1). Phylogenetic analysis indicated these nine strains clustered with *F. concentricum* with 72% bootstrap support, confirming conspecificity.

Strains M10 and M15 clustered with *F. ambrosium* with 80% support and showed >97.82% ITS and LSU sequence similarity, confirming conspecificity. Strain M7 clustered with *F. oxysporum* with 99% support and 98.95% sequence similarity, confirming conspecificity.

Strains M12, M19, and M22 showed >99% ITS and LSU sequence similarity with *Clonostachys rosea* f. *catenulata* and clustered together with 84% support, confirming conspecificity.

Strain M17 showed 97.22% and 99.23% ITS and LSU sequence similarity with *Mucor bainieri* and clustered with 100% support, confirming conspecificity. Strain M21 showed 91.39% and 99.20% similarity with *M. bainieri* but clustered with 100% support, indicating it belongs to *Mucor* sp.

Strains M18 and M23 showed >98% ITS and LSU sequence similarity with *Rhizopus azygosporus* and clustered with 97% support, confirming conspecificity. Strain M20 showed 98.89% and 85.85% similarity with *R. azygosporus* and clustered with 100% support, indicating *Rhizopus* sp.

Strain M24 showed 91.80% ITS and LSU sequence similarity with *Penicillium solitum* and clustered with 96% support, indicating *Penicillium* sp. (Table 2, Figure 4 [Figure 4: see original paper]).

Table 2 Comparison results of isolation fungal ITS and LSU sequences from NCBI database

Strain No.	Nearest fungi	GenBank accession No. of nearest fungi	Identity (%)
M3, M4, M6, M9, M11, M14	<i>Fusarium concentricum</i>	NR111886.1, NG069847.1	ITS&LSU
M19, M22	<i>Clonostachys rosea f. catenulata</i>	NR173405.1, NG076685.1	ITS&LSU
M10, M15	<i>F. ambrosium</i>	NR103628.1, NG067371.1	ITS&LSU
M18, M20, M23	<i>Rhizopus azygosporus</i>	NR103653.1, NG066155.1	ITS&LSU
M17	<i>Mucor bainieri</i>	NR165993.1, NG063969.1	ITS&LSU
M24	<i>Penicillium solitum</i>	JX290030.1	ITS&LSU

Note: M1, M3-M12 and M14-M25 are isolate numbers. The same below.

In summary, morphological and molecular identification revealed seven fungal species: three *Fusarium* species (*F. concentricum*, *F. oxysporum*, and *F. ambrosium*) accounting for 43.86% of total isolates; and one species each of *Mucor bainieri*, *Rhizopus azygosporus*, *Penicillium* sp., and *Clonostachys rosea f. catenulata*, each representing 14.29% of total isolates.

Figure 4 Phylogenetic tree of strains

Based on identification results, isolation frequencies of different species were calculated. *Fusarium concentricum* showed the highest frequency with a relative abundance of 45.45%. *Clonostachys rosea f. catenulata* and *Rhizopus azygosporus* had higher frequencies at 13.64% each. *Fusarium ambrosium* and *Mucor bainieri* had lower frequencies at 9.09% each. *Fusarium oxysporum* and *Penicillium* sp. had the lowest frequencies at 4.55% each (Table 3), suggesting *F. concentricum* as the most probable pathogen causing konjac soft rot.

Table 3 Relative abundance of isolated strains

Nearest strains	Strain counts	No. of isolates	Relative
<i>Fusarium concentricum</i>	10	M1, M3, M4, M5, M6, M8, M9, M11, M14, M25	45.45
<i>F. ambrosium</i>	2	M10, M15	9.09
<i>F. oxysporum</i>	1	M7	4.55
<i>Rhizopus azygosporus</i>	3	M18, M20, M23	13.64
<i>Clonostachys rosea f. catenulata</i>	3	M12, M19, M22	13.64

Nearest strains	Strain counts	No. of isolates	Relative
<i>Mucor bainieri</i>	2	M17, M21	9.09
<i>Penicillium</i> sp.	1	M24	4.55
Total	22		100

2.3 Koch's Postulates Testing

Mycelial plugs (0.5 cm diameter) from the seven identified strains were inoculated onto healthy konjac corm slices. After 3 days of incubation at 28 °C with high humidity, slices inoculated with *F. concentricum* developed obvious foul odor and black soft rot symptoms near the inoculation sites, with expanding lesions matching konjac soft rot symptoms, while controls showed no symptoms. The inoculated strain was successfully re-isolated from diseased tissues, confirming *F. concentricum* as a potential pathogenic fungus (Figure 5 [Figure 5: see original paper]).

Co-inoculation experiments showed that the combined treatment with *F. concentricum* and *Pectobacterium aroidearum* (F+P) produced significantly greater rotten tissue weight than single inoculations (F or P), indicating that combined fungal and bacterial infection may accelerate disease development in field conditions. The rotten tissue weight also revealed that the bacterial pathogen *P. aroidearum* was more pathogenic than *F. concentricum* under the same conditions (Figure 6 [Figure 6: see original paper]).

Red frames indicate mock inoculation; A: *F. concentricum*; B: *F. oxysporum*; C: *F. ambrosium*; D: *Clonostachys rosea** f. *catenulata*; E: *Rhizopus azygosporus*; F: *Mucor bainieri*; G: *Penicillium* sp.*

Figure 5 Koch postulates test of isolated fungi

F+P: *F. concentricum** + *P. aroidearum*; F: *F. concentricum* alone; P: *P. aroidearum* alone; CK: control. Different letters indicate significant differences.*

Figure 6 Pathogenicity test of mixed pathogen inoculation

3 Discussion and Conclusion

Determining pathogen species is crucial for plant disease control. This study isolated and purified 22 fungal strains from diseased *A. konjac* corms, identifying seven species through morphological and molecular methods: three *Fusarium* species (*F. concentricum*, *F. oxysporum*, and *F. ambrosium*), and one species each of *Mucor bainieri*, *Rhizopus azygosporus*, *Penicillium* sp., and *Clonostachys rosea* f. *catenulata*. Koch's postulates testing revealed pathogenicity in *F. concentricum*, suggesting it as the likely fungal pathogen causing konjac soft rot in Qujing, Yunnan. *Fusarium concentricum* has been reported as a pathogen causing soft rot in pepper (*Capsicum annuum*) fruit (Wang et al., 2013), maize (*Zea mays*) ear rot (Du et al., 2020), *Podocarpus macrophyllus* wilt (Qin et al., 2021),

and roselle (*Hibiscus sabdariffa*) fruit blotch (Rahim et al., 2020). Large-scale konjac cultivation in Yunnan often involves intercropping with maize, which may increase the risk of both konjac fungal soft rot and maize ear rot. He et al. (2016) isolated *F. oxysporum* and *F. solani* from konjac rhizosphere and demonstrated that crude toxin extracts could cause corm rot. Li et al. (2017) also isolated *F. oxysporum* and *F. solani* from soft-rotted corms, finding both could infect *A. konjac* but *F. oxysporum* could not infect *A. bulbifer*. Zhao et al. (2022) similarly isolated *F. oxysporum* and *F. solani* from stem rot samples, but pathogenicity tests showed *F. oxysporum* (strain xymy-8) was non-pathogenic while *F. solani* strains (xymy-7, xymy-9) were pathogenic with varying virulence. This study also isolated *F. oxysporum* but found it less pathogenic than *F. concentricum*. These differences in *Fusarium* pathogenicity to konjac across studies may be related to strain virulence variation and regional cultivation differences.

Furthermore, dual inoculation of konjac corms with the bacterial pathogen *P. aroidearum* and the fungal pathogen *F. concentricum* produced significantly greater rotten tissue weight than single inoculations, indicating that combined bacterial and fungal infection accelerates konjac tissue soft rot. We therefore hypothesize that konjac soft rot may be a composite disease involving both fungi and bacteria. Unlike fungal pathogens that can form appressoria for active host penetration, bacterial pathogens require infection ports and must reach certain population densities at infection sites to cause disease (Yang et al., 2019; Chadha et al., 2022). Wu et al. (2021) investigated infection pathways of konjac soft rot pathogens and found that bacterial pathogens cannot directly infect intact corms or penetrate through natural openings, but only through wounds or growing tissues such as buds and scales. Huang et al. (2014) used GFP-labeled bacterial pathogens to study infection characteristics in konjac tissue culture seedlings through different inoculation methods (needle pricking, smearing, and root drenching), also finding that pathogens could not infect through natural openings or roots, but mainly through wounds. The rapid outbreak of soft rot in the field may result from both pathogen accumulation and rain-mediated spread (Zhang et al., 2012), and potentially from fungal infection of wounds providing entry channels for bacterial pathogens, leading to rapid disease onset.

This study also isolated saprophytic fungi including *Mucor*, *Rhizopus*, and *Penicillium* from the junction between diseased and healthy soft rot tissues. These fungi could not cause disease in healthy konjac corms, and their specific roles in soft rot development require further investigation. However, these saprophytic fungi can accelerate degradation of rotted tissue (Kavkler & Demšar, 2019) and secrete various polysaccharide-degrading enzymes during rapid proliferation (Lange et al., 2012), potentially affecting healthy corm tissues and accelerating disease development. Additionally, the biocontrol fungus *Clonostachys* sp., which preys on fungi and nematodes (Seenivasagan & Babalola, 2021), was isolated from soft rot tissues, possibly because secondary invading fungi and nematodes at diseased sites provide food sources.

Although numerous studies have reported on konjac soft rot control (Cui et

al., 2021; Dai et al., 2021; Zhao et al., 2021), few highly effective methods have been developed. This study clarified the fungal community composition in konjac soft rot tissues and, for the first time, identified *F. concentricum* as a pathogenic fungus causing konjac corm soft rot. Dual inoculation experiments with *P. aroidearum* and *F. concentricum* demonstrated that combined bacterial and fungal infection accelerates konjac tissue soft rot, indicating that konjac soft rot may be a composite disease involving both fungi and bacteria. This research identified pathogenic fungal species and disease characteristics of soft rot in *A. konjac* cultivation areas of Qujing, Yunnan, providing crucial practical guidance for disease prevention and control.

References

- BEHERA SS, RAY RC, 2016. Konjac glucomannan, a promising polysaccharide of *Amorphophallus konjac* K. Koch in health care[J]. Int J Biol Macromol, 92: 942-956.
- CHEN EF, LIU H, DING HB, et al., 2021. Cloning and Expression Analysis of *pelD* and *pelE* Genes from Konjac Soft Rot Pathogen[J]. SW China J Agric Sci, 34(3): 495-500. [CHEN Enfa, LIU Hui, DING Haibing, et al., 2021. Cloning and Expression Analysis of *pelD* and *pelE* Genes from Konjac Soft Rot Pathogen[J]. Southwest China Journal of Agricultural Sciences, 34(3): 495-500.]
- CHUA M, BALDWIN TC, HOCKING TJ, et al., 2010. Traditional uses and potential health benefits of *Amorphophallus konjac* K. Koch ex N.E.Br[J]. J Ethnopharmacol, 128(2): 26-78.
- CUI S, CHEN CL, FENG JH, et al., 2021. Characterization of *Pectobacterium aroidearum* Causing Konjac Soft Rot and Biocontrol Effect of *Bacillus velezensis*[J]. Chin Veget, (3): 83-93. [CUI Shuang, CHEN Changlong, FENG Jiahao, et al., 2021. Characterization of *Pectobacterium aroidearum* Causing Konjac Soft Rot and Biocontrol Effect of *Bacillus velezensis*[J]. China Vegetables, (3): 83-93.]
- DAI XF, ZHU L, ZHANG SL, et al., 2021. Screening of Antagonistic Actinomycetes Against *Amorphophallus* Soft Rot[J]. J SW Univ (Nat Sci Ed), 43(11): 9-17. [DAI Xuefeng, ZHU Li, ZHANG Shenglin, et al., 2021. Screening of Antagonistic Actinomycetes Against *Amorphophallus* Soft Rot[J]. Journal of Southwestern University (Natural Science Edition), 43(11): 9-17.]
- DU Q, DUAN C, LI S, et al., 2020. First report of maize ear rot caused by *Fusarium concentricum* in china[J]. Plant Dis, 104: 5.
- GAO Y, ZHANG Y, CHEN F, et al., 2022. A chromosome-level genome assembly of *Amorphophallus konjac* provides insights into konjac glucomannan biosynthesis[J]. Comput Struct Biotechnol, 20:
- HE F, ZHANG ZL, CUI M, et al., 2016. Identification and allelopathic effect of dominant fungi in rootzone of *Amorphophallus konjac* and screening of the bio-

control actinomycetes[J]. J NW A & F Univ (Nat Sci Ed), 44(4):157-167. [HE Fei, ZHANG Zhongliang, CUI Ming, et al., 2016. Identification and Allelopathic Effect of Dominant Fungi in Rootzone of *Amorphophallus konjac* and Screening of the Bio-control Actinomycetes[J]. Journal of Northwest A & F University (Natural Science Edition), 44(4): 157-167.]

HUANG L, LIU YX, REN XX, et al., 2014. Isolation, Identification and GFP Marker of Soft Rot Bacteria Strains in *Amorphophallus rivieri*[J]. GuiZhou Agric Sci, 42(12): 118-121. [HUANG Lu, LIU Yongxiang, REN Xiuxiu, et al., 2014. Isolation, Identification and GFP Marker of Soft Rot Bacteria Strains in *Amorphophallus rivieri*[J]. Guizhou Agricultural Sciences, 42(12): 118-121.]

KAVKLER K, DEMŠAR A, 2012. Impact of fungi on contemporary and accelerated aged wool fibres[J]. Polym Degrad Stabil, 97(5): 786-792.

LANGE L, PILGAARD B, HERBST FA, et al., 2019. Origin of fungal biomass degrading enzymes: Evolution, diversity and function of enzymes of early lineage fungi[J]. Fungal Biol Rev, 33(1): 82-97.

LI H, ZHU G, BOYCE PC, et al. Flora of China[M]. Beijing: Science Press, 2010: 23-33.

LI YB, BAO XK, WAN Q, et al., 2017. Isolation and pathogenicity of konjac root rot pathogenic fungi[C]//Proceedings of the Annual Meeting of Chinese Society for Plant Pathology. Shandong: Taian. [LI Yingbin, BAO Xiaokai, WAN Qi, et al., 2017. Isolation and Pathogenicity of Konjac Root Rot Pathogenic Fungi[C]//Proceedings of the Annual Meeting of Chinese Society for Plant Pathology. Shandong: Taian.]

CHADHA J, HARJAI K, CHHIBBER S, 2022. Revisiting the virulence hallmarks of *Pseudomonas aeruginosa*: a chronicle through the perspective of quorum sensing[J]. Environ Microbiol, 24(6):

QIN CD, JIANG Y, ZHANG R, et al., 2021. First report of *Fusarium concentricum* causing shoot blight on *Podocarpus macrophyllus* in china[J]. Plant Dis, 160(2).

QIU L, CHOU NX, 1995. Konjac Resource and Its Development and Utilization Value[J]. Territ & Nat Resour Study,(2): 73-74. [QIU Ling, CHOU Nongxue, 1995. Konjac Resource and Its Development and Utilization Value[J]. Territory & Natural Resources Study, (2): 73-74.]

RAHIM H, KAMARUDIN NS, MOHD MH, 2020. First report of *Fusarium concentricum* causing fruit blotch on roselle (*Hibiscus sabdariffa*)[J]. Australas Plant Dis, 15(1).

SEENIVASAGAN R, BABALOLA OO, 2021. Utilization of microbial consortia as biofertilizers and biopesticides for the production of feasible agricultural product[J]. Biology, 10(11): 1111.

- SRZEDNICKI G, BOROMPICHAICHARTKUL C, 2020. Konjac glucomannan-production, processing, and functional applications. Boca Ration: CRC Press, 1-300.
- SUN MM, 2019, Pathogen identification and rapid detection method development for soft rot of *Amorphophallus konjac*[D]. Wuhan Hubei: Huazhong Agricultural University. [SUN Miaomiao, 2019. Pathogen Identification and Rapid Detection Method Development for Soft Rot of *Amorphophallus konjac*[D]. Wuhan, Hubei: Huazhong Agricultural University.]
- VILGALYS R, HESTER M, 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species[J]. J Bacteriol, 172(8): 4238-4246
- WANG JH, FENG ZH, HAN Z, et al., 2013. First report of pepper fruit rot caused by *fusarium concentricum* in china[J]. Plant Dis, 97(12): 1657-1658.
- WANG ZM, LIU RN, DANG DZ, et al., 2021. Symptoms, influencing factors and control measures of konjac soft rot[J]. NW Horticult, (2): 47-48. [WANG Minzhen, LIU Runni, DANG Danzhou, et al., 2021. Symptoms, Influencing Factors and Control Measures of Konjac Soft Rot[J]. Northwest Horticulture (Comprehensive), (2): 47-48.]
- WEI H, YANG M, PEI W, et al., 2020. First Report of *Pectobacterium aroidearum* Causing Soft Rot of *Amorphophallus konjac* in China[J]. Plant Dis, 104(3): 969.
- WEI JC, 1979. Fungal Identification Manual[M]. Beijing: Science Press. [WEI Jingchao, 1979. Fungal Identification Manual[M]. Beijing: Science Press.]
- WHITE TJ, BRUNS T, LEE S, et al., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics[J]. ScienceDirect. PCR Protocols: 315-322.
- WU J, DIAO Y, GU Y, et al., 2011. Molecular detection of *Pectobacterium* species causing soft rot of *Amorphophallus konjac*[J]. World J Microb Biot, (27): 613-618.
- WU J, YANG C, JIAO Z, et al., 2015. Genetic relationships of soft rot bacteria isolated from konjac in China by Amplified Fragment Length Polymorphism (AFLP) and 16S rDNA gene sequences[J]. Agric Sci, 6: 717-723.
- WU JP, DIAO Y, GU YC, et al., 2021. Infection pathways of soft rot pathogens on *Amorphophallus konjac*[J]. Afr J Microbiol R, 4(14): 1495-1499.
- XU W, 2011. Isolation and Identification of the soft rot and *Sclerotium rolfsii* of Konjac and study of biological control in Langao county[D]. Yangling: Northwest A & F University. [XU Wei, 2011. Isolation and Identification of the Soft Rot and *Sclerotium rolfsii* of Konjac and Study of Biological Control in Langao County[D]. Yangling: Northwest A & F University.]

ZHANG HJ, SHAO M, DU P, et al., 2012. Effects of diversity cultivation of konjac and maize in controlling konjac's soft rot disease in Yunnan Province, Southwest China[J]. Chin J Ecol, 31(2): 332-336. [ZHANG Hongji, SHAO Mei, DU Peng, et al., 2012. Effects of Diversity Cultivation of Konjac and Maize in Controlling Konjac's Soft Rot Disease in Yunnan Province, Southwest China[J]. Chinese Journal of Ecology, 31(2): 332-336.]

ZHANG YA, CHU HL, YU LQ, et al., 2022. Analysis of the taxonomy, synteny, and virulence factors for rot pathogen *Pectobacterium aroidearum* in *Amorphophallus konjac* using comparative genomics[J]. Front Microbiol, 12: 679102.

ZHANG YQ, XIE BJ, GAN X, 2005. Advance in the applications of konjac glucomannan and its derivatives[J]. Carbohydr Polym, 60: 27-31.

ZHAO XL, HE SL, LIU SR, et al., 2022. Isolation and identification of three strains of pathogen causing konjac stem rot and studies on pathogenicity[J]. Chin Veget, (6): 56-63. [ZHAO Xingli, HE Shengling, LIU Sirui, et al., 2022. Isolation and Identification of Three Strains of Pathogen Causing Konjac Stem Rot and Studies on Pathogenicity[J]. China Vegetables, (6): 56-63.]

ZHAO XM, LI ZY, CUI M, et al., 2021. Preliminary Study on Soft Rot Control Technology of Konjac in Ankang[J]. Acta Agric Boreal-Occident Sin, 30(8): 1263-1270. [ZHAO Xiaoming, LI Zengyi, CUI Ming, et al., 2021. Preliminary Study on Soft Rot Control Technology of Konjac in Ankang[J]. Acta Agriculturae Boreali-occidentalis Sinica, 30(8): 1263-1270.]

YANG Z, DAI CC, WANG XX, et al., 2019. Advance in research on rhizosphere microbial mechanisms of crop soil-borne fungal diseases[J]. Acta Pedol Sin, 56(1): 12-22. [YANG Zhen, DAI Chuanchao, WANG Xingxiang, et al., 2019. Advance in Research on Rhizosphere Microbial Mechanisms of Crop Soil-borne Fungal Diseases[J]. Acta Pedologica Sinica, 56(1): 12-22.]

ZHU F, 2018. Modifications of konjac glucomannan for diverse applications[J]. Food Chem, 256: 419-426.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv — Machine translation. Verify with original.