Slippery Scar: A New Mushroom Disease in *Auricularia polytricha*

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A new disease, the slippery scar, was investigated in cultivated bags of *Auricularia polytricha*. This fungus was isolated from the infected mycelia of cultivated bags. Based on morphological observation, rDNA-internal transcribed spacer and 18S sequence analysis, this pathogen was identified as the Ascomycete *Scytalidium lignicola*. According to Koch's Postulation, the pathogenicity of *S. lignicola* to the mycelia of *A. polytricha* was confirmed. The parasitism of this fungus on mushroom mycelia in China has not been reported before.

**KEYWORDS**: *Auricularia polytricha*, Koch's Postulation, *Scytalidium lignicola*, Slippery scar disease

**Introduction**

*Auricularia polytricha* (Mont.) Sacc., which is rich in various nutrients such as polysaccharides and edible fibers, is called “jellyfish on the trees.” This fungus is wildly cultivated in China by the technology of sawdust substitute cultivation. It has special flavors, improves sexual function, clears away lung heat, activates the blood, alleviates pain, and so on. Recent medical studies show that the fruitbody of *A. polytricha* is an ideal healthy food because of its anti-tumor activity [1].

In May 2009, a new disease called the slippery scar by the local inhabitants of Shifang, Pengzhou, and Guanghan cities in Sichuan Province was found. The slippery scar disease occurs seriously; the infection rate in the early hyphae stage is about 2~5%, but it increases to 40~50% when the fruitbody forms in late April to mid-May. The rate reaches above 80% in June. Finally, the yield of *A. polytricha* declines to about 20~30% because of the slippery scar disease.

**Materials and Methods**

**Isolates collection.** The pathogen was isolated from cultivated bags collected in Shifang City, purified, cultured, and preserved. The tested cultivated variety of *A. polytricha* was Huanger No. 10, which was the main variety in Sichuan province.

**Pathogen isolation.** From 2009 to 2010, the symptom, status, and occurrence of the slippery scar disease was surveyed, observed, and recorded in Shifang, Pengzhou, and Guanghan cities, Sichuan Province. Conventional tissue isolation was carried out. The infected mycelia of *A. polytricha* which were infected by the pathogen 1~2 cm away from healthy mycelia, were then collected and placed in a potato dextrose agar (PDA) Petri dish and cultured at 25°C. Finally, the colony was purified and inoculated for further research.

**Pathogenicity test.** The cultivation materials were as follows: 78% sawdust, 20% bran, 1% lime, and 1% gypsum. Bagging, sterilization, and inoculation were performed according to the report of Zhang [2]. Two programs were used in the pathogenicity test. One program involved the simultaneous inoculation of the mycelia of *A. polytricha* and the isolated colony into both ends of cultivated bags. The other involved the inoculation of *A. polytricha* mycelia into cultivated bags first. The mycelia of the isolated colony were then inoculated onto the surface of mycelia when *A. polytricha* mycelia had grown over all the cultivated bags. The symptom of the two programs was observed and compared with the slippery scar disease to see if they appeared to be the same, if they were so, re-isolated the infected mycelia, and cultured in a PDA Petri dish at 25°C. Culture characteristics of the re-isolation were observed to see if

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they were the same as the inoculation. At last the re-isolation and the inoculation were co-cultured to see if there were antagonistic lines. If the two programs both appeared the same symptom of the slippery scar disease, meanwhile the characteristics of the re-isolation were exactly the same as the inoculation, and also there were no antagonistic lines between them, they had the same DNA sequence simultaneity, according to the Koch’s Postulation, the isolation was the pathogenic of the slippery scar disease definitely.

**Culture characteristics.** The screening test for the optimal culture conditions, such as temperature, pH, as well as C and N sources, was performed according to Li et al. [3] and Gao et al. [4]. Statistical analysis of the data for the macelia growth in different C and N sources were subjected to double-factors experiments of variances (ANOVA) with SAS ver. 9.2 (SAS Inc., Cary, NC, USA).

**DNA extraction and PCR amplification.** Genomic DNA was extracted according to Wu et al. [5]. The PCR amplification for internal transcribed spacer (ITS) was performed as follows: 1 cycle at 94°C for 5 min; 35 cycles at 94°C for 50 sec, 53°C for 50 sec, and 72°C for 60 sec; and a final cycle at 72°C for 10 min. The primers used were ITS1 (5’-TCCGTAGGTGAACCTGCGG-3’) and ITS4 (5’-TCCTCCGCTTATTGATATGC-3’) [6].

The PCR amplification for 18S was performed as follows: 1 cycle at 94°C for 5 min; 35 cycles at 94°C for 60 sec, 60°C for 60 sec, and 72°C for 60 sec; and a final cycle at 72°C for 10 min. The primers used were NS1 (5’-GTAGTCATATGCTTGTCTC-3’) and NS8 (5’-TCCGCA-GTITCACCTACGG-3’) [7]. The products were purified and sequenced by Genscript Company (Nanjing, China).

**Results**

**Symptoms and development.** The pathogen only infected the mycelia of *A. polytricha* either when the mycelia were growing or when they grew over the full bags, and not the fruit body. This feature is the outstanding characteristic of the slippery scar disease. Slippery, glossy, and dark-brown scars formed on the surface of infected mycelia of *A. polytricha* bags. There was a red-brown antagonistic line between the infected and healthy mycelia, and the margin of this line was irregular. The infected scar extended rapidly until it ran across the full bag. When the mycelia of *A. polytricha* were growing, the pathogen prevented their growth. Pathogen infection thoroughly corroded the mycelia. Sometimes, the pathogen infected and swallowed all mycelia of full cultivation bags of *A. polytricha* (Fig. 1).

The compost, which was far away from the mycelia of *A. polytricha*, also exhibited the slippery scar symptom. This finding indicated that the pathogen can also be saprophytic. The most important reason of its occurrence can be attributed to the incomplete sterilization of the compost or the breakage of cultivated bags in the process of cultivation.

Only a few bags were infected during winter and early spring; however, the infection rate increased with increased temperature. Pathogen reproduction accelerated, and the number of scars increased rapidly above 25°C. When the humidity of the shed was too high as a result of frequent spraying or pouring, the pathogen rapidly spread. The opening of the cultivated bags was the main infection pathway after the fruit bodies began to be harvested. The infection rate increased with the harvesting time from May to June.

**Isolation and culture characteristics.** Grey white and thin mycelia grew on the PDA plates within 2~3 days. After 3~4 days, the colony became dark brown and flat, and the aerial mycelia started to flourish. The colony surface was glossy and honeycomb shaped, and produced catenulate chlamydospores (Fig. 2).

The mycelia grew at 15~30°C and pH 3~9. They could...
fulfilled the PDA Petri dish, which was 9 cm wide, in 4~5 days at 25°C and pH 7, the growth rate was about 1 cm per day. There were significant effects of the temperature and pH on pathogen growth rate. And the optimal condition for mycelia growth was 25°C and pH 7 (Tables 1 and 2).

There were no differences in the colony morphology of the pathogen among various C and N sources, but the growth rates were different (Table 3). There were significant interactive effects of the carbon concentration and C:N ratios on pathogen growth. Mycelia growth varied among different carbon concentrations at the C:N ratios tested and among different C:N ratios of the same carbon concentration. The optimal interaction of carbon concentration and C:N ratio for mycelia growth was 6 g/mL and 40:1 for the pathogen.

Pathogenicity test. The symptom of the slippery scar appeared in both programs of the pathogenicity test. In the first case, the growth rate of the pathogen was about twice that of A. polytricha, and there was an obvious antagonistic line between them. In the other case, the mycelia of the pathogen covered and swallowed the healthy part of the host fungus quickly, and spread all around the inoculated spot. Eventually, the entire bag became full of the mycelia of the pathogen. The symptom and the culture characteristics of the re-isolation were the same as the slippery scar disease and the inoculation. Besides, there were no antagonistic lines in the co-culturing of the re-isolation and the inoculation. Meanwhile, PCR amplified the ITS sequence of the re-isolation by the way described in the method, it was 99% matching with the inoculation. All the results indicated that the re-isolation was the same as the inoculated fungus, so the pathogenicity of the isolation was confirmed (Fig. 3).

**Table 1. The growth of the pathogen mycelia at different temperature**

| Temperature (°C) | 15 | 20 | 25 | 30 | 35 |
|------------------|----|----|----|----|----|
| L (cm)           | 0.63 | 1.74 | 7.22 | 3.86 | 2.74 |

The L (cm) represent the diameter of the pathogen colony in three days at different temperature.

**Table 2. The growth of the pathogen mycelia at different pH**

| pH  | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
|-----|-----|-----|-----|-----|-----|-----|-----|
| L (cm) | 1.2 | 4.43 | 5.28 | 5.44 | 9   | 3.8 | 1.18 |

The L (cm) represent the diameter of the pathogen colony in four days at different pH.

**Fig. 3.** Infectious symptom from the inoculation test with the isolated fungus. A, The simultaneous inoculation of the mycelia of *Auricularia polytricha* and the isolated colony into both ends of cultivated bags; B, The inoculation of *A. polytricha* mycelia into cultivated bags first. The mycelia of the isolated colony were then inoculated onto the surface of mycelia when *A. polytricha* mycelia had grown over all the cultivated bags.

Morphological characteristics. The mycelia of the pathogen were 2~5 µm wide, septate, and branched. The chlamydospores were elongated, catenulated, 7~11 µm long, and 3~5 µm wide. The top of the chlamydospore was flat (Fig. 4).

**Table 3. Effects of carbon concentration and carbon to nitrogen (C:N) ratio on pathogen growth**

| Carbon (g/L) | 10:1 | 20:1 | 40:1 | 80:1 | 160:1 |
|--------------|------|------|------|------|-------|
| 6            | 1.19 | 1.795| 1.995| 1.5125| 1.795 |
| 8            | 0.955| 1.42 | 1.67 | 1.4475| 1.1625|
| 12           | 0.3825| 0.6875| 1.455| 1.3475| 1.2975|

(P > F <α) < 0.0001 < 0.05

Molecular identification. The sequences of ITS (551 bp) and 18S (1,671 bp) were submitted to GenBank (accession Nos. JQ691625 and JQ691626). The phylogenetic tree of 18S gene was constructed (Fig. 5). The results indicated that the ITS sequence matched with Ascomycota species, WP-2010 (GU591745.1, unpublished) by 99%, and 18S sequence matched with *Scytalidium lignicola* (GQ280419.1) by 99%. Therefore, this pathogen was identified as *S. lignicola*.

**Discussion**

*Scytalidium* was discovered by Pesante (1957, non-original description) [8], and *S. lignicola* is its representative species. To date, there are 18 species accepted. *Scytalidium*
Fig. 4. Pathogenic morphology of the slippery scar disease in *Auricularia polytricha*. A, Mycelia of the pathogen with septum, and branched (×40); B, Chlamydospores, elongated and catenulated (×40); C, Mature chlamydospores under a scanning electron microscope; D, Old chlamydospores are breaking under a scanning electron microscope.

Fig. 5. Phylogenetic relationship of the 18S gene. The tree was constructed based on 1,000-fold bootstrap analyses by the neighbor-joining method using the MEGA 5.0 program, and the evolutionary distances were computed using the maximum composite likelihood method.
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are widespread and they can cause ulceration, rot, and wilt by parasitizing on plants, such as apple, banana, citrus, fucus, grape, potato, walnut, yam and so on. *Scytalidium* has also been isolated from human feet in tropical regions, a henhouse, a mushroom house, greenhouse soil, mow, and roots of some plants [9].

Li and Sun [8] have isolated one species of *Scytalidium* from *Cordyceps sinensis* in Hualong District, QingHai Province, China, and designated it as a new species. However, this species is later found out to be a synonym of another species. Xu [10] and Zhang [11] have isolated one species of *Scytalidium*, but cannot confirm its exact species. Geng *et al.* [12] isolated some species of *Scytalidium* from soils in Tibet, QingHai, and Huanggang, Hubei province, China, and identified one (HSAUPII06 3138) of them as *S. lignicola*. However, the identification of its rDNA-ITS sequence and morphology is inconsistent to a remarkable extent; thus, further study is necessary.

The description of *S. lignicola* in MycoBank is as follows. The non-original description is colonies are light to grayish orange darkening to gray or black, with dark brown, thick-walled, catenulate chlamydospores. Unbranched fertile lateral branches fragment to form hyaline, narrow, cylindrical arthroconidium 4–9 µm × 1.5–2.5 µm, termed “conidia vera” by Pensate [8]. Sigler and Carmichael [13] have described in detail the catenulate chlamydospores as a second type of arthroconidium, but these are indehiscent structures. It can hydrolyze cellulose. The colonies on the PDA plates are effuse, flat with raised folds, cottony to woolly, initially whitish, and finally becomes dark grey to black. Hyphae are initially hyaline, and later become brown. Arthroconidia are hyaline, thin-walled, rectangular, about 5–8 µm × 2 µm. Conidia are chlamydospore-like (single or in chains), dark brown, thick-walled, swollen, and up to 7 µm wide. The ITS restriction map is based on CBS 387.59. Strain IP 2311-95 causes human mycetoma (J. W. Waller, personal communication, from MycoBank).

By comparing the data of the isolated pathogen and records of *Scytalidium*, this pathogen was identified as *S. lignicola*, considering its morphology, culture characteristics, and ITS, 18S DNA sequence.

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