Identification and Characterization of Trichoderma Species Damaging Shiitake Mushroom Bed-Logs Infested by Camptomyia Pest

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The shiitake mushroom industry has suffered from Camptomyia (gall midges) pest, which feeds on the mycelium of shiitake mushroom during its cultivation. It has been postulated that fungal damage of shiitake bed-logs is associated with infestation by the insect pest, but this is not well understood. To understand the fungal damage associated with Camptomyia pest, various Trichoderma species were isolated, identified, and characterized. In addition to two previously known Trichoderma species, T. citrinoviride and T. deliquescentes, two other Trichoderma species, T. harzianum and T. atroviride, were newly identified from the pest-infested bed-log samples obtained at three mushroom farms in Cheonan, Korea. Among these four species, T. harzianum was the most evident. The results of a chromogenic media-based assay for extracellular enzymes showed that these four species have the ability to produce amylase, carboxyl-methyl cellulase, avicelase, pectinase, and β-glucosidase, thus indicating that they can degrade wood components. A dual culture assay on PDA indicated that T. harzianum, T. atroviride, and T. citrinoviride were antagonistic against the mycelial growth of a shiitake strain (Lentinula edodes). Inoculation tests on shiitake bed-logs revealed that all four species were able to damage the wood of bed-logs. Our results provide evidence that the four green mold species are the causal agents involved in fungal damage of shiitake bed-logs infested by Camptomyia pest.

Keywords: Camptomyia, shiitake mushroom bed-logs, Trichoderma damage

Introduction

Shiitake (Lentinula edodes, Berk. Pegler) is an economically important edible and medicinal mushroom that is widely cultivated in Asian countries, including Korea, Japan, and China. Its cultivation has been increased around the world owing to its unique flavor, tastes, and nutritional values [16]. In general, shiitake is cultivated either by sawdust media-based methods or by bed-log-based methods. Bedlogs using oak timber have been used for the cultivation of high-quality shiitake. Recently, huge economic loss has occurred nationwide in Korea in shiitake farms where mushroom production was performed using bed-logs [19]. Farmers in the shiitake farms have blamed mushroom flies that newly emerged in the cultivation houses as the cause of bed-log damage. The flies’ influence has been investigated on several shiitake farms where disturbance of shiitake’s mycelial growth and fruit body formation occurred in mushroom fly-infested bed-logs. Large colonies of unknown dipteran insects were found under the bark of the damaged bed-logs and were identified as gall midges that taxonomically belong to the genus Camptomyia Kieffer (Diptera: Cecidomyiidae). Camptomyia corticalis and C. heterobia have been identified in damaged shiitake bed-logs [19]. The larvae of some Camptomyia species in this genus are known to feed on the fungi colonized in dead trees [14], and the larvae of the two Camptomyia flies were found to live on shiitake mushroom that colonized the oak bed-logs. Thus, these Camptomyia pests were associated with the shiitake damage in bed-logs. Because these mushroom fly species had not previously been recorded as mushroom pests, they were reported as such [19].

Green molds are the fungi that cause green mold diseases on cultivated mushrooms such as button mushroom (Agaricus
Hypocrea, Trichoderma, and Gliocladium are well-known taxonomic names for green molds. Among these, ascomycete Hypocrea is the teleomorph name of some of the anamorphic species of Trichoderma and Gliocladium. These anamorphic species produce huge numbers of green-colored spores that can easily disperse in mushroom cultivation houses. Since Camptomyia pests are mycophagous and signs of green mold are frequently observed in shiitake bed-logs, it has been postulated that the damage of shiitake mushroom bed-logs might be caused not only by the mushroom flies but also by green mold. In a previous work on green mold, Trichoderma citrinoviride and Gliocladium viride (an anamorph of Hypocrea lutea) were identified in shiitake bed-logs infested by mushroom flies [7, 8]. G. viride was later renamed to Trichoderma deliquescent [5]. Recently, T. harzianum and T. citrinoviride were isolated from Camptomyia larvae and adults that were caught from damaged shiitake bed-logs [12]. This isolation data displayed that there is a relationship between Trichoderma species and Camptomyia. Because Trichoderma spp. are known for their ability to degrade wood and their antagonistic properties on mushroom fungi [17, 20], they have been presumed possible agents of fungal damage on shiitake bed-logs. However, the damage to Camptomyia-infested shiitake bed-logs associated with Trichoderma species has not been fully elucidated yet.

The purpose of this study was to understand the fungal damage of shiitake bed-logs associated with the Camptomyia pest that occurred in Korea. Four Trichoderma species, which were possibly responsible for wood damage of Camptomyia-infested shiitake bed-logs, were isolated, identified, and evaluated for shiitake damage. We analyzed the competitiveness of the isolated Trichoderma species over shiitake mycelia growing on culture media plates and bed-logs, as well as their ability to produce extracellular enzymes that are responsible for wood degradation. Thus, we obtained evidence that they are the causal agents of fungal damage.

Materials and Methods

Sampling of Shiitake Bed-Logs Infested by Camptomyia Pests and Fungal Isolation

Three shiitake bed-logs infested by C. corticalis and C. heterobia were sampled in the summer season of 2009 from each cultivation house of three shiitake mushroom farms located in Cheonan, Korea. The damaged parts of the oak bed-log were easily distinguished from the normal parts by debarking the oak logs. The insect pest-infested parts of the oak bed-logs were cut, placed in plastic bags, and transferred to the laboratory. The transferred samples were chopped up into small pieces (1 cm × 1 cm × 0.5 cm) using a sterile chisel and hammer. To isolate the green mold species, the chopped small wood pieces were surface sterilized by soaking in 1% sodium hypochlorite solution for 2 min. After washing with sterile water three times, the wood pieces were placed on potato dextrose agar (PDA) plates supplemented with streptomycin (100 µg/ml) and the PDA plates were incubated at 25°C for 3–5 days. Mycelia that grew out from the small wood pieces and started to grow on PDA were separated with a sharp sterile needle and transferred to new PDA plates. After growing for 3–5 days, single spore isolates were obtained from the PDA-grown fungi. Single colony isolation was undertaken at least three times from the single spore-grown fungi. The fungal colonies thus obtained were initially grouped based on colony morphology and then randomly selected colonies from each group were subjected to species identification. The selected fungal colonies were kept on PDA plates for the experiment and stored at −70°C for preservation.

Morphology Observation

For the observation of fungal colony morphology, each single spore isolate was pre-cultured on a PDA plate at 25°C for 3 days. Agar plugs (5 mm diameter) were taken from the cultured fungal isolate and inoculated at an edge position on PDA plates and Czapek yeast extract agar plates, respectively, and incubated at 25°C for 3 days. Five replicates were prepared for each single spore isolate. Colony color and shape were examined by the naked eye. The fungal microstructures such as conidiophores and conidia were observed using the phase contrast microscope Axioskop 40 (Karl Zieiss, Germany) and scanning electron microscope (SEM) S4300 (Hitachi, Japan). For SEM observation, the specimens were prepared using 1% osmium tetroxide as described by Kim et al. [8]. For all isolates, morphological characteristics were compared with those of reference isolates [4]. For the observation of damaged wood pieces, a SZ61 stereo microscope (Olympus Opitical Co., Japan) was used.

Molecular Identification of Fungal Isolates

For fungal DNA preparation, the subject isolates were grown on PDA at 25°C for 3–5 days and their genomic DNAs were extracted by the drilling method described by Kim et al. [9]. The translation elongation factor 1-alpha gene (tef1) was PCR amplified using the TEF1 and TEF728 primer pair [3, 17], in a 50 µl reaction mixture using EmeraldAmp PCR Master Mix (Takara, Japan). The PCR was performed with the following directions: one cycle of pre-denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 56°C for 30 sec, and extension at 72°C for 1 min, and one cycle of final extension at 72°C for 5 min. The PCR products were purified using a PCR purification kit (NaviBiotech, Korea), cloned into pGEM T-easy vector (Promega Corp., USA), and sequenced by Macrogen Inc. (Korea). The determined nucleotide sequences were compared with known sequences of Trichoderma species through BLASTN at the GenBank database of the National Center for Biotechnology Information.
Information (http://www.ncbi.nlm.nih.gov/genbank) and TrichoBLAST at the website of the International Subcommission on Trichoderma and Hypocrea Taxonomy (http://www.isth.info/). The determined nucleotide sequences of tef1 were manually edited and aligned using the Biological Sequence Alignment Editor ver. 7.0.5. Molecular phylogenetic analysis was performed using the MEGA program [20], and a neighbor-joining tree was constructed using Kimura’s two-parameter model [10]. Bootstrap values were generated with 1,000 replicates. The reference tef1 sequences of related taxa were obtained from the GenBank database.

**Extracellular Enzyme Test**

To test the ability to degrade wood cell components, the fungi were grown on media containing 0.5% avicel (Sigma-Aldrich, USA), CM-cellulose (Sigma-Aldrich), β-cellobiose (Sigma-Aldrich), starch (Sigma-Aldrich), skim milk (Sigma-Aldrich), polygalacturonic acid (MP Biomedicals, USA), xylan (Sigma-Aldrich) as a carbon source, 0.1% yeast nitrogen base as their fundamental nitrogen source, and 1.5% agar powder. Congo red dye (0.5%) was used for chromogenic reaction due to its better performance in extracellular enzyme activity detection [24]. After 7 days of culturing at 25°C, we could observe the clear zone formed by reaction between the secreted extracellular enzymes and chromogenic substrates. The diameter of clear zone was measured and used for the basis of relative activity comparison of the tested extracellular enzymes.

**Antagonistic Ability and Inoculation Test on Oak Bed-Logs**

*Leninula edodes* (shiitake strain Sanzo 701Ho) was grown at 25°C on one side of PDA for 7 days to investigate the antagonistic behavior of the isolated *Trichoderma* species in vitro. Then, each test species was inoculated on the other side of the *L. edodes*-grown PDA and incubated for 14 days at 25°C. The dual cultured samples were examined for the existence or absence of antagonistic growth inhibition between *L. edodes* and each *Trichoderma* species. The experiment was repeated with three replicates.

To investigate the invasive behavior of the isolated green mold species on shiitake bed-logs in vivo, each *Trichoderma* species was grown on sawdust media containing 10% rice bran and used as inoculum for the bed-log inoculation. After making inoculation holes using a sterile drill on Mongolian oak bed-log (50 cm in length, 15 cm in diameter), which was pre-colonized by the shiitake strain, 1 g of the sawdust media-grown inoculum of each *Trichoderma* species was inoculated into each prepared hole. Control inoculation was performed with sterilized sawdust media. Three bed-logs and three holes per bed-log were prepared for each *Trichoderma* species inoculation. The inoculated shiitake bed-logs were incubated in a mushroom cultivation house during the summer season (average temperature 26°C, relative humidity 85%) [7]. After 54 days incubation, we removed the bark around the inoculation holes on the bed-logs to check for the presence of brown and/or black colored zones formed on the surface of the log wood by the growth and colonization of the inoculated *Trichoderma* species. To check Koch’s postulate, we tried to re-isolate the inoculated *Trichoderma* fungal species from the brown and/or black colored zones on the bed-logs.

**Results and Discussion**

The sampled shiitake bed-logs infested by *Camptomyia* pest were easily debarked and discolored to black (Fig. 1A) and/or brown (Fig. 1B). Severe infestation resulted in a dried and yellowish texture of the wood in the log and some areas of the wood were blackened (Fig. 1C). These features demonstrated that the shiitake bed-logs were not in good condition for the spreading of shiitake mycelia through the wood cells in the log that is required before the shiitake mycelia start to produce a fruiting body. In addition, the inside part of the log was not dense but loosened and easily breakable, indicating the presence of wood degradation (Fig. 1E). The SEM image of the wood tissue in Fig. 1E showed that fungal mycelia had colonized the tissues (Fig. 1F). In contrast, the microscopic image of a part of wood tissues in the undamaged bed-log showed white healthy shiitake mycelia, well colonized and evenly spread on the wood (Fig. 1D). Considering that the *Camptomyia* larvae move to eat shiitake mycelia and multiply in the wood tissues underneath the bark, their activity is likely to hinder the colonization and spreading out of shiitake mycelia through the wood cells.

*Camptomyia* larvae can degrade wood and develop only under the bark [13]. Thus, the insect larvae would not penetrate into the inside of wood cells. However, fungi are well known as wood decomposers that biochemically break down wood cells [2]. Thus, the fungi in the wood tissue are assumed to be the main source of organisms that are involved in wood decomposition. From the observations in Fig. 1, it was inferred that *Camptomyia* pest infestation accompanied wood tissue decomposition by fungi.

Wood degradation is the main reason for wood damage and damaged wood is not suitable for shiitake cultivation. Therefore, the presence of wood-degrading fungi inside of shiitake bed-logs could aggravate the damage of shiitake bed-logs infested by *Camptomyia* pest. To verify that fungi do play a role in the damage of shiitake bed-logs, we first tried to isolate them from the bed-logs samples. According to the information in Fig. 1F, fungal isolation was performed from the inside part of the oak bed-logs samples infested by *Camptomyia* pest. From the isolation work, 42 fungal isolates were obtained (Table 1). Based on microscopic observation of microstructures such as conidia and conidiophores, all 42 fungal isolates were identified as *Trichoderma*, imperfect fungi with teleomorphs belonging
to the ascomycete order Hypocreales. The 42 *Trichoderma* isolates were divided into four groups through further identification with colony morphology and morphological characters. Since *Trichoderma* is known to be a complex fungal group that is difficult to identify solely based on morphological characters, molecular analysis was additionally performed with the partial translation elongation factor 1α gene (*tef1*), which is known to be a suitable target gene for identification of *Trichoderma* spp. [17]. For the molecular analysis, each representative isolate was chosen from the four *Trichoderma* groups and coded as DUCC001, DUCC003, DUCC005, and DUCC007, respectively. By PCR with TEF728 and TEF1 primers, 651, 628, 635, and 608 bp amplicons were obtained from the four representative isolates. These PCR amplicons were sequenced and searched for homologous DNA sequences through BLAST programs. The determined nucleotide sequences of the four representative isolates DUCC001, DUCC003, DUCC005, and DUCC007 shared 99% sequence identity with *tef1* sequences of *T. citrinoviride* YNKM5010 (Accession No. JQ040469), *T. harzianum* TAMA014131 (Accession No. AB856677), *T. atroviride* 8234 (Accession No. KJ624780), and *T. deliquesces* GJS89-129 (Accession No. AY737731) registered on the GenBank DNA database. The BLAST search results agreed with the results of the four nucleotide sequences in Tricho-BLAST. Consequently, based on these results of *tef1* sequence homology, *Trichoderma* isolates DUCC001, DUCC003, DUCC005, and DUCC007 were identified as *T. citrinoviride*, *T. harzianum*, *T. atroviride*, and *T. deliquesces* (synonym *G. viride*), respectively. We deposited the determined *tef1* sequences of the four *Trichoderma* isolates to the GenBank DNA database. The *tef1* sequence of *T. harzianum* DUCC003 was deposited with its teleomorph name *Hypocrea lixii* (Accession No. HQ602998). The *tef1* sequence of *T. atroviride*

**Table 1.** *Trichoderma* species and number of isolates identified in this study from the shiitake bed-logs infested by *Camptomyia* pest.

<table>
<thead>
<tr>
<th>Source</th>
<th><em>T. harzianum</em></th>
<th><em>T. atroviride</em></th>
<th><em>T. citrinoviride</em></th>
<th><em>T. deliquesces</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mushroom farm 1</td>
<td>6</td>
<td>4</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>Mushroom farm 2</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mushroom farm 3</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>4</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>

* No isolation.

DUCC005 was deposited as Accession No. HQ603000 and that of T. citrinoviride DUCC001 as Accession No. JF700485. The tef1 sequence of T. deliquescens DUCC007 was deposited as synonym G. viride (Accession No. GU903312). Combining the results of molecular identification and morphological characters, the 42 Trichoderma isolates were identified as T. citrinoviride, T. harzianum, T. atroviride, and T. deliquescens, respectively (Table 1). Among the four Trichoderma species, T. harzianum was dominantly isolated and found on three mushroom farms. Three Trichoderma species were found in one of the three mushroom farms. These isolation data suggest that the occurrence of Trichoderma species on the oak bed-logs infested by Camptomyia pest is not confined to a single Trichoderma species and varies depending on mushroom farms.

Reportedly, T. citrinoviride and T. deliquescens were identified from shiitake bed-logs infested by mushroom flies by Kim et al. [7, 8]. Our results confirmed the previous reports. However, T. harzianum and T. atroviride have not been reported before as species associated with the mushroom fly Camptomyia. Therefore, we further studied these species morphologically using a SEM and genetically using a phylogenetic tree. The colony morphology grown on PDA and CYA at 25°C for 7 days is displayed in Fig. 2. On PDA, T. harzianum revealed a grayish green color and T. atroviride showed a white color (Table 2, Fig. 2). On CYA, both species revealed green colors. The SEM images of microstructures of the two Trichoderma species are given in Fig. 2 and their mycological characters are presented in Table 2 with comparison with known references [3, 4]. Overall, these two species morphologically matched well with the reported references. A phylogram based on the tef1 sequences clearly

**Table 2. Morphological characters of Trichoderma harzianum DUCC003 and T. atroviride DUCC005 isolated in this study from the shiitake bed-logs infested by Camptomyia pest.**

<table>
<thead>
<tr>
<th>Species</th>
<th>T. harzianum (anamorph of H. lixii) [2]</th>
<th>T. harzianum (DUCC003 this study)</th>
<th>T. atroviride (anamorph of H. atroviridis) [3]</th>
<th>T. atroviride (DUCC005 this study)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony color</td>
<td>Dark green</td>
<td>Grayish green</td>
<td>White</td>
<td>White</td>
</tr>
<tr>
<td>Conidiophores</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Color</td>
<td>Hyaline</td>
<td>Hyaline</td>
<td>Hyaline</td>
<td>Hyaline</td>
</tr>
<tr>
<td>Shape</td>
<td>Branched in a pyramidal fashion</td>
<td>Branched in a pyramidal fashion</td>
<td>Lageniform often curved</td>
<td>Lageniform often curved</td>
</tr>
<tr>
<td>Size</td>
<td>4.0–7.0 µm × 2.5–3.5 µm</td>
<td>5.0–7.0 µm × 1.7–2.6 µm</td>
<td>7.7–8.0 µm × 2.0–2.1 µm</td>
<td>8.0–10 µm × 1.4–2.0 µm</td>
</tr>
<tr>
<td>Conidia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Color</td>
<td>Pale green</td>
<td>Pale green</td>
<td>Pale green</td>
<td>Pale green</td>
</tr>
<tr>
<td>Shape</td>
<td>(sub)Spherical shape</td>
<td>(sub)Spherical shape</td>
<td>Globose</td>
<td>Globose</td>
</tr>
<tr>
<td>Size</td>
<td>2.5–3.0 µm × 2.0–2.5 µm</td>
<td>1.5–2.0 µm × 1.5–2.0 µm</td>
<td>2.8–3.5 µm × 3.0–3.8 µm</td>
<td>1.0–1.5 µm × 1.0–1.5 µm</td>
</tr>
</tbody>
</table>
displayed the position of *T. harzianum* DUCC003 and *T. atroviride* DUCC005 among the related *Trichoderma* taxa (Fig. 3). As *T. harzianum* belongs to an awkward fungal group that is not easy to identify, our results will be very useful for the identification of *T. harzianum* and *T. atroviride* from shiitake bed-logs infested by *Camptomyia* pest.

Some of the *Trichoderma* taxa possess mycoparasitic behavior towards mushrooms [18]. *T. harzianum* and *T. polysporum* are known to attack shiitake mycelia in bed-logs used for mushroom cultivation by producing antifungal substances and mycolytic enzymes [21–23]. Their attack leads to yield loss of shiitake production. To obtain evidence that the four *Trichoderma* species are antagonists that inhibit the mycelial growth of shiitake mushroom, each of the four *Trichoderma* species was dual cultured with a shiitake strain on PDA plate. The mycelia of *T. harzianum* DUCC003, *T. atroviride* DUCC005, and *T. citrinoviride* DUCC001 invaded the mycelial growth territory of the shiitake strain (Fig. 4). However, the mycelial growth territory of *T. deliquescentes* DUCC007 was aerially invaded by shiitake mycelia. These results showed that *T. harzianum*, *T. atroviride*, and *T. citrinoviride* are antagonists of shiitake mushroom. The antagonistic effect of *T. harzianum*, *T. atroviride*, and *T. citrinoviride* on shiitake strains was also reported previously [6]. This means that the presence of these antagonistic *Trichoderma* species in bed-logs for shiitake cultivation would be also harmful to the shiitake mycelium as the *Camptomyia* larvae do harm. It is expected that the coexistence of *Camptomyia* larvae and each of the antagonistic *Trichoderma* species in shiitake bed-logs will be more detrimental to the development of shiitake mushroom.

Kim et al. [6] morphologically and phylogenetically defined several *Trichoderma* spp. from the shiitake cultivation environment in Korea. In their work, the *Trichoderma* spp...
inhibited the mycelial growth of shiitake strains on both culture plates and sawdust media. These data informed that *Trichoderma* spp. are important in shiitake cultivation. However, there was no analysis about *Trichoderma* spp. isolated from the problematic *Camptomyia*-infested bed-logs. Thus, the report did not answer what caused the damage of shiitake bed-logs infested by *Camptomyia*. Additionally, their biochemical properties and colonizing ability on log have not been tested. Because the two *Camptomyia* species that infested shiitake bed-log are newly described species in Korea [19], work on the associated *Trichoderma* fungi and their enzymatic properties and colonization ability on living wood will be new and valuable information.

As some *Trichoderma* species are very good cellulase producers [15], we also evaluated their ability to produce extracellular enzymes in *T. harzianum* DUCC003, *T. atroviride* DUCC005, *T. citrinoviride* DUCC001, and *T. deliquescentes* DUCC007. Enzymes that could degrade wood components were evaluated on chromogenic media: amylase, avicelase, β-glucosidase, carboxymethyl cellulase, pectinase, xylanase, and protease. Their ability to degrade cellulose, pectin, and xylan was found in all the tested species (Table 3). All the four species showed very strong activity in extracellular protease, and production of extracellular amylase was also seen in them. There were some differences in the degree of enzyme activities among the four *Trichoderma* species. Overall, these results suggested that the four *Trichoderma* species have the ability of wood degradation by producing extracellular enzymes. Regarding the four *Trichoderma* species, an inoculation test was performed on Mongolian oak log that was pre-colonized by the mycelia of the shiitake strain. This test resulted in a brown-colored line that clearly formed around the inoculation hole (Fig. 5). The brown-colored line is a barrier formed by incompatible interactions between *L. edodes* and the four green mold *Trichoderma* species.

**Table 3.** Extracellular enzyme activity shown on chromogenic media by the four *Trichoderma* species obtained from the shiitake bed-logs infested by *Camptomyia* pest.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th><em>T. harzianum</em> DUCC003</th>
<th><em>T. atroviride</em> DUCC005</th>
<th><em>T. citrinoviride</em> DUCC001</th>
<th><em>T. deliquescentes</em> DUCC007</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase</td>
<td>S^b</td>
<td>M^c</td>
<td>VS^a</td>
<td>M</td>
</tr>
<tr>
<td>Avicelase</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>W^d</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>VS</td>
<td>M</td>
<td>S</td>
<td>VS</td>
</tr>
<tr>
<td>Carboxymethyl cellulase</td>
<td>S</td>
<td>M</td>
<td>S</td>
<td>W</td>
</tr>
<tr>
<td>Pectinase</td>
<td>M</td>
<td>W</td>
<td>M</td>
<td>W</td>
</tr>
<tr>
<td>Xylanase</td>
<td>W</td>
<td>M</td>
<td>W</td>
<td>W</td>
</tr>
<tr>
<td>Protease</td>
<td>VS</td>
<td>VS</td>
<td>VS</td>
<td>VS</td>
</tr>
</tbody>
</table>

^aVS: very strong activity (clear zone diameter > 9 cm); ^bS: strong activity (clear zone diameter 5–8 cm); ^cM: moderate activity (clear zone diameter 2–5 cm); ^dW: weak activity (clear zone diameter 0–2 cm).
are competing. Brown-colored lines formed between Trichoderma species and shiitake mycelium were also observable on culture media (Fig. 4B) [6], but no brown line was found in the control inoculation. We re-isolated the four Trichoderma species from each of the brown-colored lines. These results satisfied Koch’s postulate, indicating that the four Trichoderma species colonized and generated brown line symptom in the shiitake bed-log. This wood pathological data underlined the significance of the Trichoderma in shiitake cultivation. Together with the results of Table 3 and Figs. 4 and 5, we concluded that T. harzianum, T. atroviride, T. citrinoviride, and T. deliquescent are also fungal pests that damage oak bed-logs for shiitake mushroom cultivation. Among the four species, T. citrinoviride was also reported in a previous work [5]: its ability for colonizing on oak log has been confirmed again in this study.

In conclusion, this study confirmed that Trichoderma species exist in shiitake bed-logs infested by Camptomyia and demonstrated that they are able to invade shiitake bed-logs. This is no table information that the four Trichoderma spp. are truly damaging agents of shiitake bed-logs infested by the mushroom fly. Since T. harzianum and T. citrinoviride were isolated from the body of Camptomyia adults [12], it is assumed that Camptomyia introduced Trichoderma into the shiitake bed-logs during its infestation process. Consequently, the coexistence of Camptomyia and Trichoderma species is expected to aggravate the damage of shiitake bed-logs.

Acknowledgments

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