

Isolation and Characterization of Airborne Mushroom Damaging *Trichoderma* spp. from Indoor Air of Cultivation Houses Used for Oak Wood Mushroom Production Using Sawdust Media

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Some species of the *Trichoderma* genus are reported as the major problem in oak wood mushroom production in Korea. In spite of economic loss by the fungi, scientific information on airborne *Trichoderma* species is not much available. To generate information for disease management development we analyzed airborne *Trichoderma*. A total of 1,063 fungal isolates were purely obtained from indoor air sampling of cultivation houses used for oak wood mushroom using sawdust media. Among the obtained isolates, 248 isolates were identified as *Trichoderma* fungi including *T. harzianum*, *T. atroviride*, *T. citrinoviride*, and *T. pseudokoningii*, by morphological and molecular analysis. *T. harzianum* was dominant among the four identified species. All the four *Trichoderma* species grew fast on solid nutrient media tested (potato dextrose agar [PDA], malt extract agar [MEA], Czapek's Dox + yeast extract agar [CYA] and cornmeal dextrose agar). Compact mycelia growth and mass spore production were better on PDA and CYA. In addition, *T. harzianum* and *T. citrinoviride* formed greenish and yellowish mycelium and spores on PDA and CYA. Greenish and yellowish pigment was saturated into PDA only by *T. pseudokoningii*. These four *Trichoderma* species could produce extracellular enzymes of sawdust substrate degradation such as β -glucosidase, avicelase, CM-cellulase, amylase, pectin-

ase, xylanase, and protease. Their mycelia inhibited the growth of oak wood mushroom mycelia of two tested cultivars on dual culture assay. Among of eleven antifungal agents tested, benomyl was the best to inhibit the growth of the four *Trichoderma* species. Our results demonstrate that the airborne *Trichoderma* fungi need to be properly managed in the cultivation houses for safe mushroom production.

Keywords : green mold disease, indoor air, oak wood mushroom, pathogenicity, *Trichoderma* spp.

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Oak wood mushroom (*Lentinula edodes*, called as shiitake in Japan) is a medicinal and edible mushroom with anti-cancer and anti-obesity effects as well as with excellent nutrition, good taste and fragrance (Lee and Park, 1998; Lee et al., 2014; Nanba et al., 1987). It is a mushroom frequently consumed in East Asia, especially China, Japan and Korea. At present, the total production and output of this lucrative mushroom are consistently increasing in Korea. Recently, its production and output were 19,398 tons and 184 billion won in 2014, 23,469 tons and 219.1 billion won in 2016, and 3,984 tons and 211.9 billion won in 2017, respectively (Korea Forest Service, 2017). Its export volume in Korea was 60.59 tons (\$1.36 million dollars) in 2014, and 118 tons (\$1.84 million dollars) in 2018, respectively (Statistics of Forest Product's Trade, 2018). Domestic production of this mushroom has been performed using the log bed cultivation system and sawdust media cultivation system. Since the mushroom is sensitive to environmental changes, the use of cultivation house that could manage temperature, humidity, light, and ventilation has been

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preferred. With the development of greenhouse construction technology, the cultivation technology of the sawdust media system has greatly been improved for the mushroom production. Consequently, not only new starters of mushroom growing but also the earlier starters is getting favor to grow the mushroom using the sawdust media system. Currently, oak wood mushroom growing using the sawdust media system in cultivation house facility is increasing for commercial production across the country. This trend of choosing indoor cultivation facility would be better when we consider that climate change is affecting agricultural and horticultural production in Korea.

Trichoderma spp. were reported as the major pathogen which are harmful to oak wood mushroom cultivated in log beds (Huang et al., 1988; Jandaik and Guleria, 1999; Laixuthai et al., 1987; Liao, 1993; Lim et al., 1990). Various *Trichoderma* spp. could cause contamination in log beds substrates and sawdust media substrate, especially at high humidity condition (Laixuthai et al., 1987). *T. harzianum* was reported to the major contamination source in Singapore (Lim et al., 1990). According to Togashi's reports from Japan in 1997, *Trichoderma* spp. were isolated from indoor air of greenhouses used for oak wood cultivation, frequency of separation was analyzed (Togashi et al., 1997). The control of contamination by *Trichoderma* spp. was known to be difficult, and their contamination could cause loss of fruit body harvest amounts (Bodine, 1995; Laixuthai et al., 1987). Because *Trichoderma* spp. produced a lot of conidia that can spread easily, they became detrimental fungi in oak wood mushroom cultivation (Davidse, 1986; Eveleigh, 1985). In Korea, *T. citrinoviride* and *Gliocladium viride* (Current name: *Trichoderma deliquescens*) were reported to new recorded pathogens to oak wood mushroom (Kim et al., 2010; Kim et al., 2012b). In 2016, the damage rate of oak wood mushroom production by green mold disease of *Trichoderma* spp. took 20% of total damage rate (Korea Rural Economic Institute, 2016).

So far, *Trichoderma* problem in oak wood mushroom cultivation has been studied mainly with log beds. The presence of *Trichoderma* problems in the mushroom production with sawdust media has been recognized by the mushroom growers but not much studied on them. Therefore, this study was conducted to obtain basic information on *Trichoderma* species for sanitary management in oak wood mushroom cultivation houses. For this purpose, we performed fungal isolation, species identification, and characterization of *Trichoderma* species for 4 years from the indoor air of sawdust media-based cultivation houses.

Materials and Methods

Fungal sampling and isolation. Two cultivation houses of oak wood mushroom located at Cheongyang-gun, Chungcheongnam-do and Jangheung-gun, Jeollanam-do, were selected for air sampling. These cultivation houses have been operated by farmers who have advanced skills in the mushroom production using sawdust media. Indoor air sample of the cultivation houses were collected every month from June 2012 to February 2015 using Andersen samplers (single stage ambient viable sampler, Model 10-880, Tisch Environmental, Cleves, OH, USA) based on ISO16000-18 method. The samplers were set at a height of 1.3 m and run for 1 min with calibrated vacuum pumps (28.3 l/min) for air sampling. Air sampling was replicated at three points of the cultivation houses. Malt extract agar (MEA; Difco, BD Science, France) supplemented with streptomycin 200 µg/ml was used for air exposure in the samplers. The air exposed MEA plates in the samplers were incubated at 25°C for 5 days for fungal growth and isolation.

Fungal classification and identification. Visible colonies with different morphology grown out from the MEA plates were transferred onto new sterile MEA and incubated again at 25°C for 3-5 days. For the pure culture, single spore isolation was performed using a light stereomicroscope (SZX10, Olympus, Seoul, Korea) and spore flooded media. Microstructures of grown fungi were observed by a light microscope (Axioskop 40, Carl Zeiss, Jena, Germany) for morphological identification. *Trichoderma* fungi were classified based on morphological and molecular characteristics. Morphological identification was followed by the taxonomic key of Domsch et al. (1980), Gams and Bissett (1998), and Rifai (1969).

To classify *Trichoderma* spp., potato dextrose agar (PDA) was used for cultivation media. Colony morphology and microscopic characteristics on the PDA was observed after cultivation at 25°C for three days. For molecular identification of *Trichoderma*, we analyzed translation elongation factor 1α (TEF 1α) gene which is known as the better marker for *Trichoderma* and *Hypocrea* (sexual stage form of *Trichoderma*) identification than the internal transcribed spacer (ITS) ribosomal sequence (Samuels, 2006). Fungal genomic DNA was extracted with the drilling method described by Kim et al. (1999). TEF728 (5'-CATCGAGA-AGTTCGAGAAGG-3') and TEF1 (5'-GCCATCCTTG-GAGATACCAGC-3') primers which are targeting for the TEF 1α gene were used for PCR amplification (Dees

and Ghiorse, 2001). PCR was performed at the condition described by Kim et al. (2016). The PCR products were purified with the High Pure PCR Purification Kit (Roche, Basel, Swiss), and their nucleotide sequences were determined by Macrogen Inc. (Seoul, Korea). The obtained *tef-1 α* gene DNA sequences were deposited on the GenBank of NCBI (<http://www.ncbi.nlm.nih.gov/genbank>). *tef-1 α* gene DNA sequence of taxon related reference species were downloaded from the GenBank of NCBI. Phylogenetic analysis was conducted with a neighbor-joining method in MEGA6 program (Tamura et al., 2013). Clade reliability of phylogenetic tree was evaluated with 1,000 times bootstrap resampling. The *tef-1 α* gene sequence of *Cladosporium cladosporioides* CBS 145.35 was used for outgroup of the phylogenetic analysis. Molecular identification of *Trichoderma* was performed by putting the obtained *tef-1 α* gene sequence into the *TrichoBLAST* v.1.0 program at the site of International Subcommittee of *Trichoderma* and *Hypocrea* Taxonomy (<http://isth.info/tools/blast/index.php>).

Growth properties. Examination of mycelia growth of selected *Trichoderma* spp. was performed on media containing different nutrient substrates (Jaklitsch, 2009). Agar plugs (5 mm diameter) of PDA grown *Trichoderma* spp. were inoculated on PDA, MEA, Czapek's Dox + yeast extract agar (CYA), and cornmeal dextrose agar (CMD). After incubation at 25°C for 3 days, mycelial length was measured and morphological characteristics including colony color and texture were observed.

Extracellular enzyme activity test. Selected *Trichoderma* spp. were grown on PDA at 25°C for 2 days to be used as inoculums for extracellular enzyme activity test. For the evaluation of fungal extracellular enzyme activity, the pre-cultured *Trichoderma* spp. were transferred onto the media containing each of 0.5% CM-cellulose (Sigma, St. Louis, MO, USA), D-cellobiose (Sigma), avicel (Sigma), starch from potato (Sigma), polygalacturonic acid (MP Biomedical, France), xylan from oat spelts (Sigma) as enzymatic carbon source, 0.1% yeast nitrogen base (Difco) as its fundamental nitrogen source, and 1.5% agar powder. One point five percent Congo red dyes (Teather and Wood, 1982) was used for chromogenic reaction due to its better performance in extracellular enzyme activity detection with the tested *Trichoderma* spp. (Yoon et al., 2007). Ten percent skim milk powder (Sigma) was used for the detection of protease activity. After incubation at 25°C for 3 days, the length of clear zone formed by reaction between the extracellular enzyme secreted by the tested fungi and

chromogenic substrates were measured as enzyme activity.

Growth inhibition test of *Trichoderma* spp. against oak wood mushroom mycelia. To test the ability of *Trichoderma* spp. in inhibition of mycelia growth of oak wood mushroom strains, two kinds of mushroom cultivars, Chamaram and Sanjo 701ho, were used. The two mushroom strains were pregrown on PDA plate at 25°C for 10 days. Agar plugs of each mushroom cultivars were inoculated one side of PDA and incubated at 25°C for 7 days. Agar plugs of each *Trichoderma* species pregrown on PDA for 3 days were inoculated on the other side of the PDA plate of each mushroom cultivar grown for 5 days. Thus, each *Trichoderma* species confronted each oak wood mushroom cultivar on PDA. After dual culturing at 25°C for 14 days, the degree of *Trichoderma* spp. overlaying to oak wood mycelium was analyzed as the inhibitory effect of *Trichoderma* on the mycelia growth of the mushroom. The formation of and the degree of darkness of brown line between *Trichoderma* and the mushroom mycelia were analyzed as the endurance response of the mushroom against *Trichoderma*.

Sensitivity test of *Trichoderma* spp. to antifungal agents.

Antifungal agents (Sigma) including tebuconazole, benzimidazol, difenoconazole, hexaconazole, tricyclazole, fluquinconazole, fludioxonil, fenarimol, dichloran, benzalkonium chloride, and benomyl were tested for their effect on *Trichoderma* spp. For the sensitivity test, PDA media supplemented with each agent at three different concentration (10 µg/ml, 100 µg/ml, and 1,000 µg/ml) were prepared. Conidia suspension (1×10^6 spores/ml) was prepared in sterile water from the PDA grown test species of *Trichoderma*. One hundred microliters of conidia suspension of each *Trichoderma* species was inoculated onto each antifungal agent supplemented PDA. Three days after incubation at 25°C, the presence or absence of grown mycelia from the inoculated spores was determined by naked eyes as the criterion of sensitivity to antifungal agents.

Results and Discussion

Isolation and identification of *Trichoderma* isolates.

Through sequential air sampling for 33 months, a total of 1,063 fungal isolates were obtained from indoor air of the two oak wood mushroom cultivation houses. Among these isolated fungi, *Cladosporium* spp., *Cercospora* spp., *Epicoecum* sp., *Mortierella* spp., *Penicillium* spp., *Pestalotiopsis* sp. and several ascomycete spp. and basidiomycete spp. were found including *Trichoderma* spp. We recog-

nized *Trichoderma* fungi by observing their typical colony morphology and anamorphic structures of conidiophores and conidia. The number of *Trichoderma* isolates was 248 which took 20.16% of the total number of isolates. This percentage indicates *Trichoderma* takes one fifth of the airborne fungi in the two oak wood mushroom cultivation houses. It is considered to be a high level of *Trichoderma* presence. Farmers of the two cultivation facilities claimed that they had from time to time contamination of sawdust media by *Trichoderma* fungi despite that they tried to keep sanitary cultivation with great efforts. Especially, if *Trichoderma* contamination occurs during production of sawdust mushroom media, all the media should be discarded. In this case, the fungal damage will be very severe and huge economic loss are inevitable. They have been questioned and worried about that how the contamination happens. Our results provide evidence that there were *Trichoderma* contaminant in the air of the two cultivation houses. Thus, there has been always chance of sawdust media contamination by the *Trichoderma* buoyant in the air. That's why they had get *Trichoderma* problem occasionally.

Currently, to our knowledge, long period air monitoring data on *Trichoderma* fungi in oak mushroom cultivation houses has not been available because there has been no research report domestically and internationally on the continuous monitoring of *Trichoderma* fungi for more than 24 months at the same mushroom farms. Therefore, our result is the first and valuable data pointing out the important of airborne *Trichoderma* in indoor air. Since our data came from 33 months period monitoring, we strongly stress out that air management in cultivation houses should be improved to avoid contamination of the mushroom substrate from *Trichoderma*. Since we sampled ambient air from inside of the cultivation houses, it is questioned where they came from. They could be originated from outdoor air or indoor environment. Generally, it is known that *Trichoderma* is a soil-borne fungus (Domsch et al., 1980; Roiger et al., 1991). Thus, we could not rule out that buoyant soil dust associated with *Trichoderma* spores would be the major source of outdoor air origin. *Trichoderma* fungi also very well grow saprophytically on organic substrate of plant materials because they have enzyme system such as glucanase and cellulase (Benítez et al., 2004). Thus, air-borne *Trichoderma* spore derived from organic materials of plant debris could be another source of outdoor air origin of the cultivation houses. Regarding indoor environment, *Trichoderma* spores generated from organic materials of trashed sawdust media after mushroom harvest and/or from pre-contaminated sawdust media from media production sites could be the main source of indoor air origin in the

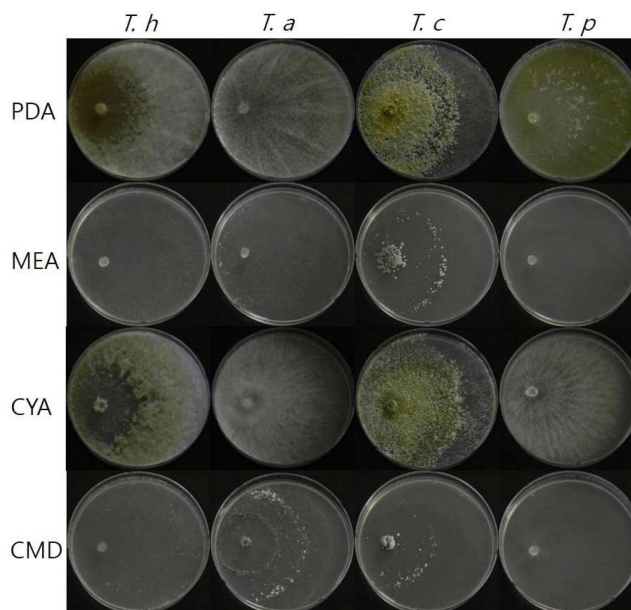


Fig. 1. Colony morphology of *Trichoderma harzianum* DUCC4018, *T. atroviride* DUCC4019, *T. citrinoviride* DUCC4020, and *T. pseudokoningii* DUCC4021 grown on nutrient different media (PDA, MEA, CYA, and CMA) at 25°C for 3 days. *T. h*, *T. harzianum* DUCC4018; *T. a*, *T. atroviride* DUCC4019; *T. c*, *T. citrinoviride* DUCC4020; *T. p*, *T. pseudokoningii* DUCC4021; PDA, potato dextrose agar; MEA, malt extract agar; CYA, Czapek's Dox + yeast extract agar; CMD, cornmeal dextrose agar.

cultivation houses.

Trichoderma genus contains diverse species and some of them could inhibit mushroom growth (Samuel, 2006). Especially, certain species of *Trichoderma* as green mold disease could give rise to serious damage resulting in mushroom crop loss (Largeteau-Mamoun et al., 2002; Wuest et al., 1996). Thus, to protect the mushroom cultivation from *Trichoderma* problem it is better to know what *Trichoderma* species go around in the air of the cultivation houses. Thus, we tried to identify the 248 *Trichoderma* isolates obtained from the air sampling of this study. Observation of growth pattern on different media and colony properties led us to classify the *Trichoderma* isolates into four groups (Fig. 1). The ratio of each *Trichoderma* group was 55.08%, 22.03%, 16.53% and 6.35%, respectively (Fig. 2). For further experiments, each representative isolate was selected from the four *Trichoderma* groups and coded as DUCC4018, DUCC4019, DUCC4020, and DUCC4021, respectively, and identified following the taxonomic key of Domsch et al. (1980) and Rifai (1969). However, identification of *Trichoderma* at species level is not easy only with morphological characters, we additionally performed molecular analysis of *tef-1a* gene which is better marker

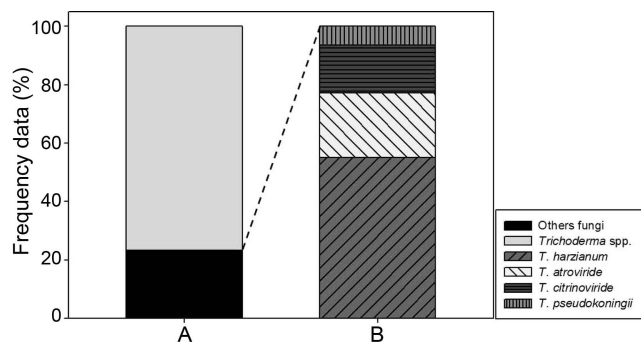


Fig. 2. The frequency rate of *Trichoderma* isolates in the total number of isolates ($n = 1,063$) from indoor air of oak wood mushroom cultivation houses in this study (A) and relative frequency rate of the isolates of each identified *Trichoderma* species among the total *Trichoderma* isolates ($n = 248$) (B).

than ITS DNA for *Trichoderma* species identification. Using TEF1 primer pairs, 529 bp, 1,249 bp, 845 bp, and 555 bp sizes of PCR amplicons were obtained from each of the four representative isolates. This result implied that the four representative isolates would be genetically different species. To verify this implication, BLAST analysis

was performed. BLAST search in the GenBank database revealed that the determined nucleotide sequences of DUCC4018, DUCC4019, DUCC4020 and DUCC4021 isolates share 99% sequence identity with *Trichoderma harzianum* TMEX33 (accession No. JX650134), *T. atroviride* CBS 693.94 (accession No. KJ786838), *T. citrinoviride* DAOM 172792 (accession No. EU280036), and *T. pseudokoningii* GJS 99-149 (accession No. JN175589), respectively. Sequence search results in the *Tricho*BLAST are agreed with the BLAST search results in the GenBank. Thus, we conclusively identified the four *Trichoderma* isolates, DUCC4018, DUCC4019, DUCC4020, and DUCC4021 as *T. harzianum*, *T. atroviride*, *T. citrinoviride*, *T. pseudokoningii*, respectively. The *tef-1 α* gene sequences of DUCC4018 (accession no. KX431214), DUCC4019 (accession No. KX431215), DUCC4020 (accession no. KX431216), and DUCC4021 (accession No. KX431217) isolates were deposited to the GenBank DNA database. *T. harzianum* DUCC4018, *T. atroviride* DUCC4019, *T. citrinoviride* DUCC4020, and *T. pseudokoningii* DUCC4021 from this study clearly showed their phylogenetic position with the known reference species in the phylogenetic tree

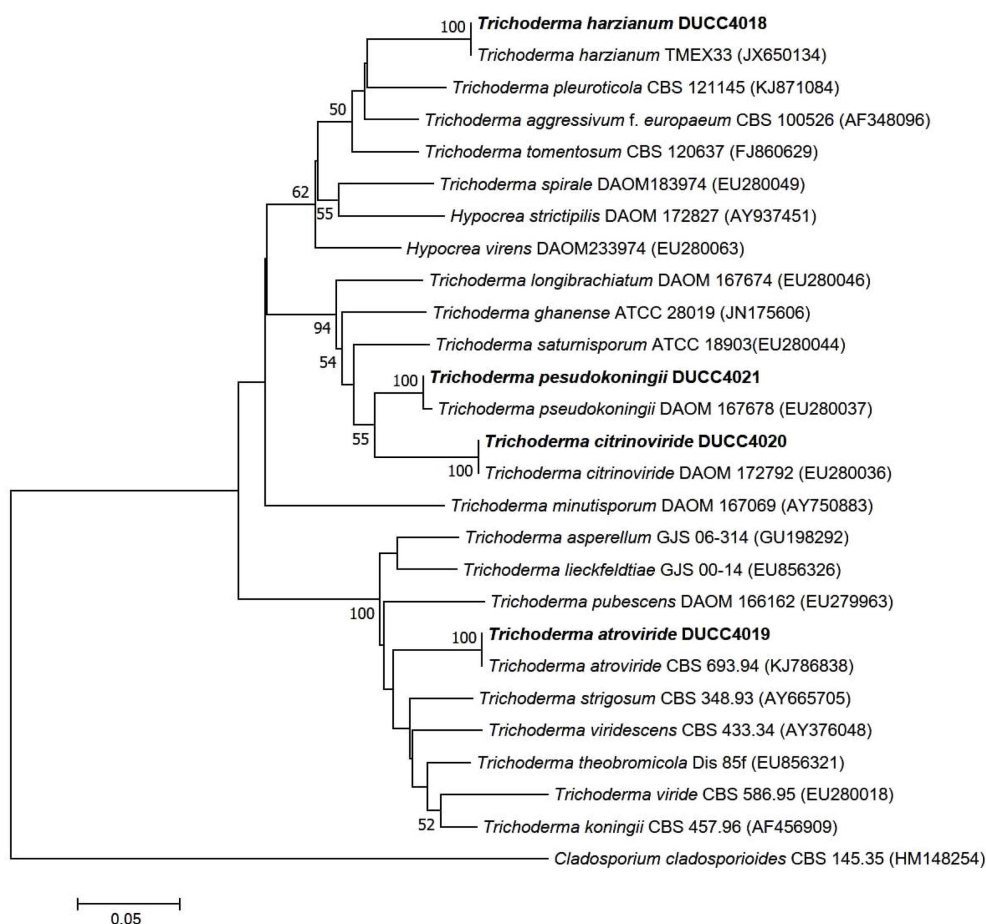


Fig. 3. Phylogenetic position of *Trichoderma harzianum* DUCC4018, *T. atroviride* DUCC4019, *T. citrinoviride* DUCC4020, and *T. pseudokoningii* DUCC4021 isolated from indoor air of oak wood mushroom cultivation houses. Phylogenetic tree was constructed based on *tef-1 α* gene sequences by the neighbor-joining method in Mega program. Bootstrap values based on 1,000 replications are shown above the node.

constructed with the *tef-1 α* sequences (Fig. 3). According to the identification results, we obtained information that there were four *Trichoderma* species in the air of the two oak wood cultivation houses. And the portion of *Trichoderma* species in the two cultivation houses was 39% in Cheonyang and 61% in Jangheung, respectively. In Cheonyang cultivation house *T. harzianum*, *T. atroviride*, and *T. citrinoviride* were detected. While, in Jangheung cultivation house, *T. harzianum*, *T. atroviride*, *T. citrinoviride*, and *T. pseudokoningii*. Thus, more portion and number of species were found in Jangheung cultivation house than Cheongyang cultivation house. In the future, it would be better to monitor more number of cultivation houses to get idea that whether more diverse *Trichoderma* species go around indoor air of cultivation houses. These four *Trichoderma* species are found to be known agents that cause damage oak wood mushroom cultivation (Kim et al., 2012a; Pukahuta et al., 2000). And among these four *Trichoderma* species, dominant species is *T. harzianum* which is well known agent of green mold disease in button mushroom and oyster mushroom (Komoń-Zelazowska et al., 2007; Wuest et al., 1996). In addition, it is noticeable that *T. harzianum*, *T. atroviride*, and *T. citrinoviride* are mushroom fly carried species that greatly damaged oak mushroom production in Korea (Kim et al., 2016). Therefore, it is strongly recommended to develop a method that efficiently manages indoor air in the cultivation houses.

Colony morphology and growth rate according to different substrates. *T. harzianum*, *T. atroviride*, and *T. citrinoviride* have been reported in Korea regarding their morphology and pathogenicity to shiitake mushrooms (Kim et al., 2012a; Kim et al., 2016). But comparative description between these species has not been described. Thus, we compared *T. harzianum* DUCC4018, *T. atroviride* DUCC4019, *T. citrinoviride* DUCC4020, and *T. pseudokoningii* DUCC4021 on PDA, MEA, CYA, and CMD (Fig. 1). Mycelium of *T. pseudokoningii* DUCC4021 matted and embedded yellow pigment on whole media. *T. pseudokoningii* DUCC4021 formed white colony, matted, and showed floccose hyphal growth on PDA. On MEA and CMD, its mycelia matted with colorless. But on CYA, its mycelium was white and floccose. Mycelium density on CYA was low in the middle part than other parts. After 3 days of culturing at 25°C, the length of grown hypha of *T. pseudokoningii* DUCC4021 were 63 mm on PDA and CMD, 62.25 mm on MEA, 49.5 mm on CYA (Fig. 4). *T. harzianum* DUCC4018 and *T. citrinoviride* DUCC4020 grew 63mm on four different media for 3 days. *T. atroviride* DUCC4019 grew 50.75 mm on CMD and over than 60

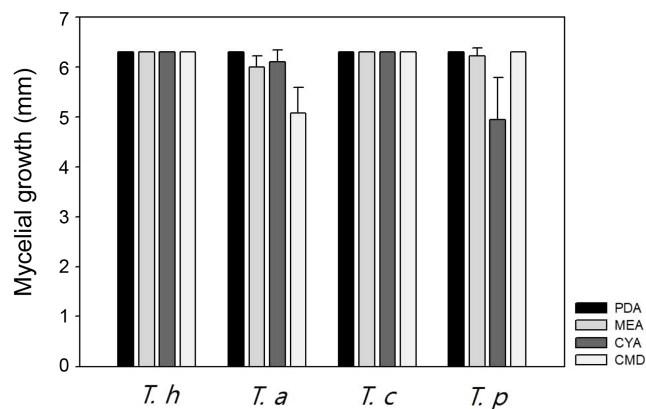


Fig. 4. Mycelial growth of *Trichoderma harzianum* DUCC4018, *T. atroviride* DUCC4019, *T. citrinoviride* DUCC4020, and *T. pseudokoningii* DUCC4021 on different nutrient media (PDA, MEA, CYA, and CMA). *T. h.*, *T. harzianum* DUCC4018; *T. a.*, *T. atroviride* DUCC4019; *T. c.*, *T. citrinoviride* DUCC4020; *T. p.*, *T. pseudokoningii* DUCC4021; PDA, potato dextrose agar; MEA, malt extract agar; CYA, Czapek's Dox + yeast extract agar; CMD, cornmeal dextrose agar.

mm on PDA, MEA, and CYA. These results showed that the four *Trichoderma* species different growth physiology and colony morphology. Thus, we could apply these results for the quick differentiation of these four species from indoor air samples of cultivation houses.

Extracellular enzyme activity test. To further understand at the biochemical level, the four *Trichoderma* species were examined for their ability to produce seven kinds of extracellular enzymes on chromogenic media (Fig. 5). Size of clear zone of enzyme activity produced by *T. harzianum* DUCC4018 was 55.22 mm (β -glucosidase), 55.11 mm (avicelase), 36.74 mm (CM-cellulase), 36.54 mm (amylase), 36.74 mm (pectinase), 29.89 mm (xylanase), and 46.12 mm (protease), respectively. Size of clear zone produced by *T. atroviride* DUCC4019 was 40.16 mm (β -glucosidase), 32.45 mm (avicelase), 38.88 mm (CM-cellulase), 61.29 mm (amylase), 38.88 mm (pectinase), 12.45 mm (xylanase), and 60.01 mm (protease) (Fig. 5). Size of clear zone produced by *T. citrinoviride* DUCC4020 was 41.56 mm (β -glucosidase), 33.04 mm (avicelase), 39.11 mm (CM-cellulase), 30.59 mm (amylase), 39.11 mm (pectinase), 13.85 mm (xylanase), and 44.60 mm (protease). Size of clear zone produced by *T. pseudokoningii* DUCC4021 was 61.18 mm (β -glucosidase), 27.32 mm (avicelase), 42.26 mm (CM-cellulase), 49.27 mm (amylase), 42.26 mm (pectinase), 11.79 mm (xylanase), and 58.61 mm (protease). These results demonstrate that the four representative isolates of airborne *Trichoderma* species are able to produce

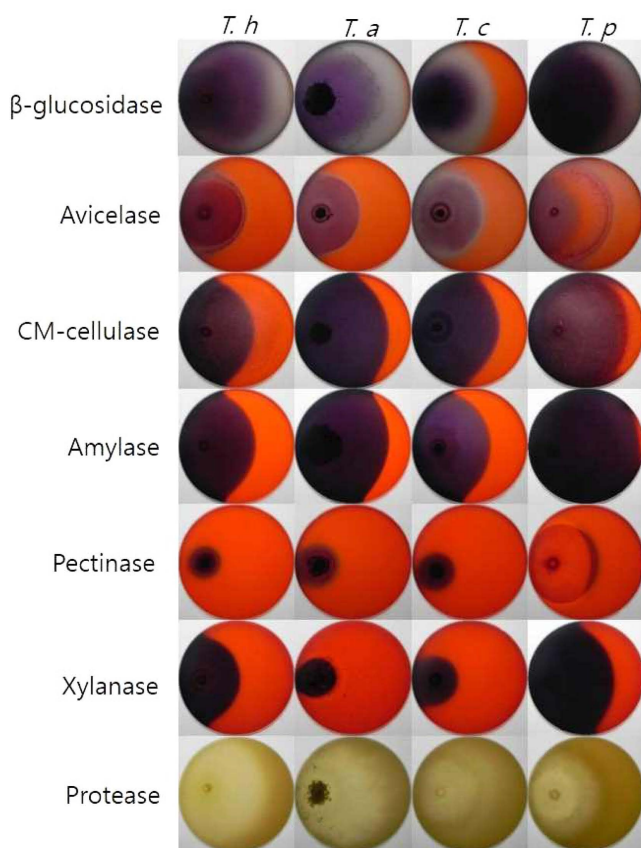


Fig. 5. Examples of extracellular enzyme activities shown by *Trichoderma harzianum* DUCC4018, *T. atroviride* DUCC4019, *T. citrinoviride* DUCC4020, and *T. pseudokoningii* DUCC4021 on chromogenic media. *T. h*, *T. harzianum* DUCC4018; *T. a*, *T. atroviride* DUCC4019; *T. c*, *T. citrinoviride* DUCC4020; *T. p*, *T. pseudokoningii* DUCC4021.

extracellular enzymes. Since sawdust media for oak wood mushroom cultivation contain woody components and carbohydrates from rice bran, the examined extracellular

enzymes could well hydrolyze sawdust media as their substrate. Thus, it is expected that these four extracellular enzyme-producing airborne *Trichoderma* could easily colonize and damage the substrates of sawdust media causing contamination problems. *Trichoderma* contamination of sawdust media through air means they could occur not only at the early stage of the cultivation process, especially during spawn running period, but also during fruit body cropping period. Therefore, the potential of huge losses of mushroom crops are considered to be highly possible anytime (Jandaik and Guleria, 1999).

Dual culture with oak wood mushroom strains. Antagonism of *Trichoderma* spp. with oak wood mushroom mycelium is related enzyme activity. This enzyme was reported to lysis the cell wall of oak wood mushroom mycelium. And *Trichoderma* spp. produced colorless antifungal metabolites (Ishikawa et al., 1980; Tokimoto and Komatsu, 1979). But shiitake mycelia endure to *Trichoderma* spp. infection, and brown confronted line was created between *Trichoderma* spp. mycelium and shiitake (oak woof mushroom) mycelium (Ishikawa et al., 1980; Kawamura et al., 1980; Tokimoto and Komatsu, 1979). Therefore, in addition to their ability to degrade the substrate of mushroom cultivation media, we further examined whether the four airborne *Trichoderma* species could inhibit the growth of oak wood mushroom strains using dual culture assay on solid media which has been successively used for interactions between *Trichoderma* and oak wood mushroom strain (*Lentinula edodes*) (Kim et al., 2012a; Wang et al., 2016). Mushroom competitors of *Trichoderma* hinder the colonization of oak wood mushroom strain onto sawdust media or log bed substrate, resulting in no further growth of the mushroom strain. Consequently, fruit body does not form and mushroom production reduces.

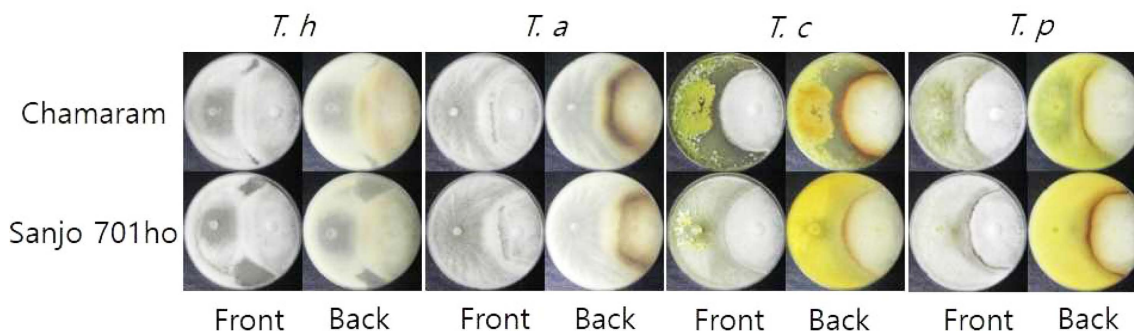


Fig. 6. Dual culture results of *Trichoderma harzianum* DUCC4018, *T. atroviride* DUCC4019, *T. citrinoviride* DUCC4020, and *T. pseudokoningii* DUCC4021 with Korean oak wood mushroom cultivars (Chamaram and Sanjo 701ho) on PDA. *T. h*, *T. harzianum* DUCC4018; *T. a*, *T. atroviride* DUCC4019; *T. c*, *T. citrinoviride* DUCC4020; *T. p*, *T. pseudokoningii* DUCC4021; PDA, potato dextrose agar. Dual cultures were incubated at 25°C for 10 days.

All four *Trichoderma* species showed antagonistic behavior against two mushroom strains, Chamaram and Sanjo 701ho, by invading the mycelial blocks of the two mushroom strains on PDA (Fig. 6). Enduring reaction by the mycelia of two mushroom strains to invasion of the four *Trichoderma* spp. produced brown confrontation line between each *Trichoderma* species mycelium and oak wood mushroom mycelium. The size, color, and sharpness of the reaction derived confrontation line varied depending on *Trichoderma* species, indicating there is difference in the ability of invasion among the four *Trichoderma* spe-

cies. The degree of overlap area by the four *Trichoderma* species' invasion to the two mushroom strains was the highest by *T. harzianum* DUCC4018 (30 mm) and *T. atroviride* DUCC4019 (30 mm), followed by *T. citrinoviride* DUCC4020 (20 mm) and *T. pseudokoningii* DUCC4021 (10 mm). These data implied that all the *Trichoderma* isolates of the four species from indoor air source are likely competitors of oak wood mushroom strains. *T. harzianum*, *T. atroviride*, and *T. citrinoviride* were reported as competitors of oak wood mushroom strains in Korea (Kim et al., 2012a). Thus, based on our results in Figs. 5 and 6, we

Table 1. Degree of growth inhibition by various fungicides to the four *Trichoderma* species isolated from indoor air of oak wood cultivation houses

Fungicide	Concentration ($\mu\text{g/ml}$)	<i>T. harzianum</i> DUCC4018	<i>T. atroviride</i> DUCC4019	<i>T. citrinoviride</i> DUCC4020	<i>T. pseudokoningii</i> DUCC4021
Tebuconazole	10	+	+	+	+
	100	+	+	—	—
	1,000	+	+	—	—
Benzimidazol	10	+	+	+	+
	100	+	+	+	+
	1,000	+	+	+	+
Difenoconazole	10	+	+	+	+
	100	+	+	+	+
	1,000	+	+	—	—
Hexaconazole	10	+	+	+	+
	100	+	+	+	+
	1,000	+	+	—	—
Tricyclazole	10	+	+	+	+
	100	+	+	+	+
	1,000	+	+	+	+
Fluquinconazole	10	+	+	+	+
	100	+	+	+	+
	1,000	+	+	—	+
Fludioxonil	10	+	—	—	—
	100	+	—	—	—
	1,000	+	—	—	—
Fenarimol	10	+	+	+	+
	100	+	+	+	+
	1,000	+	+	+	+
Dichloran	10	+	+	+	+
	100	+	+	+	+
	1,000	+	+	+	+
Benzalkonium chloride	10	+	+	+	+
	100	+	+	+	+
	1,000	+	—	—	—
Benomyl	10	—	—	—	—
	100	—	—	—	—
	1,000	—	—	—	—

+, test culture grew on the media containing the fungicide; —, test culture didn't grow on the media containing the fungicide.

would like to report *T. pseudokoningii* as a new competitor of oak wood mushroom strains in sawdust media cultivation in Korea.

Antifungal agents sensitivity test of *Trichoderma* spp.

To help the potent choice of sanitary compounds that could be used for mushroom cultivation facility as preventive measures, the sensitivity of four *Trichoderma* species were assessed to twelve antifungal agents; tebuconazole, benzimidazol, difenoconazole, hexaconazole, tricyclazole, fluquinconazole, fludioxonil, fenarimol, dichloran, benzalkonium chloride, and benomyl. *T. harzianum* DUCC4018 could grow at all concentration of test antifungal agents, except every concentration of benomyl (10 µg/ml, 100 µg/ml, and 1,000 µg/ml) (Table 1). *T. atroviride* DUCC4019 was sensitive to all concentration of fludioxonil and benomyl, and 1,000 µg/ml of benzalkonium chloride. *T. citrinoviride* DUCC4020 showed broad sensitivity to tebuconazole (10 µg/ml, 100 µg/ml), 1,000 µg/mL of difenoconazole, hexaconazole, fluquinconazole and benzalkonium chloride and every concentration of fludioxonil and benomyl. *T. pseudokoningii* DUCC4021 also showed sensitivity to tebuconazole (10 µg/ml, 100 µg/ml), 1,000 µg/mL of difenoconazole, hexaconazole, and benzalkonium chloride and every concentration of fludioxonil and benomyl. These results show there is difference in the sensitivity to antifungal agent among the four *Trichoderma* species. Overall, among of tested antifungal agents, benomyl inhibited mycelial growth and conidia germination of the four *Trichoderma* species at all tested concentrations. Benomyl was reported to inhibit assembly of fungal microtubules (Davidse, 1986), rapidly degrade into carbendazim in aqueous (Tang et al., 1992) or organic (Chiba and Cherniak, 1978) solution, or induce hydroxylation in the microsomal monooxygenase system (Danielson and Davey, 1973). While, mushroom strains of Chamaram and Sanjo 701ho could grow at all the fungicide concentration (data not shown). Thus, we found benomyl is the best choice to disinfect the oak wood mushroom cultivation facility to deter *Trichoderma* spp. contamination.

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