

## Two New Species of *Trichoderma* Associated with Green Mold of Oyster Mushroom Cultivation in Korea

Myung Soo Park<sup>1</sup>, Kyung Sook Bae<sup>2</sup> and Seung Hun Yu<sup>1\*</sup>

<sup>1</sup>Department of Applied Biology, College of Agriculture and Life Sciences, Chungnam National University, Daejeon 305-764, Korea

<sup>2</sup>Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-333, Korea

(Received April 14, 2006)

This paper describes and illustrates two new species, *Trichoderma pleurotum* and *T. pleurotica*, associated with green mold disease of oyster mushroom in Korea.

**KEYWORDS:** Green mold, Morphology, *Pleurotus*, Taxonomy

Oyster mushroom (*Pleurotus ostreatus*) production in Korea has increased rapidly since the early 1980s. It is now one of the most valuable horticultural enterprises in Korea. During the last 10 years, however, commercial production of oyster mushroom has been seriously affected by green mold epidemics. The typical symptoms of the green mold are the appearance of green fungal sporulation on oyster mushroom substrates. In severe outbreaks, no mushrooms are produced from the contaminated beds (Park *et al.*, 2005a; Yu, 2001).

To identify the *Trichoderma* species associated with the green mold epidemics of commercially grown oyster mushroom in Korea, we collected more than one hundred isolates of *Trichoderma* from oyster mushroom substrates throughout Korea over a period of 6 years from 1997 to 2002 (Park *et al.*, 2004, 2005a; Yu, 2001). The isolates were classified into 7 distinct species based on morphological and cultural characteristics, and two of the 7 species were revealed as new taxa (Park *et al.*, 2003, 2004, 2005a). Phylogenetic analysis of the ITS, the translating elongation factor 1 alpha gene (EF-1 $\alpha$ ) and RNA polymerase II gene (RPB2) sequences of the two taxa also supported their status as new to science (Park *et al.*, 2003, 2004, 2005b). However, we have not reported new epithets and Latin diagnosis for the two fungal species. In this paper the two new species are described as *Trichoderma pleurotum* and *T. pleurotica*, respectively.

### Materials and Methods

*Trichoderma* strains, CNUMH 501 and CNUMH 601, were isolated from green molded waste cotton substrates at oyster mushroom farms in Chuncheon, Gangwon Province, Korea, April, 1998, and in Paju, Gyeonggi Province,

Korea, May, 1999, respectively. Colony characteristics and growth rates of each species were examined from their cultures grown in darkness at 25°C for 7 days on PDA. Micromorphological data were examined on the cultures grown on cornmeal dextrose agar (CMDA) and PDA for 7 days at 25°C. The examination and measurements of conidiophores and conidia were made from slide preparations stained with 3% KOH. Differential contrast microscopy was used for observation and 50 units of morphological characters were measured. The morphology of each species was also illustrated with the aid of a drawing tube.

### Taxonomy

*Trichoderma pleurotum* S. H. Yu & M. S. Park, sp. nov.

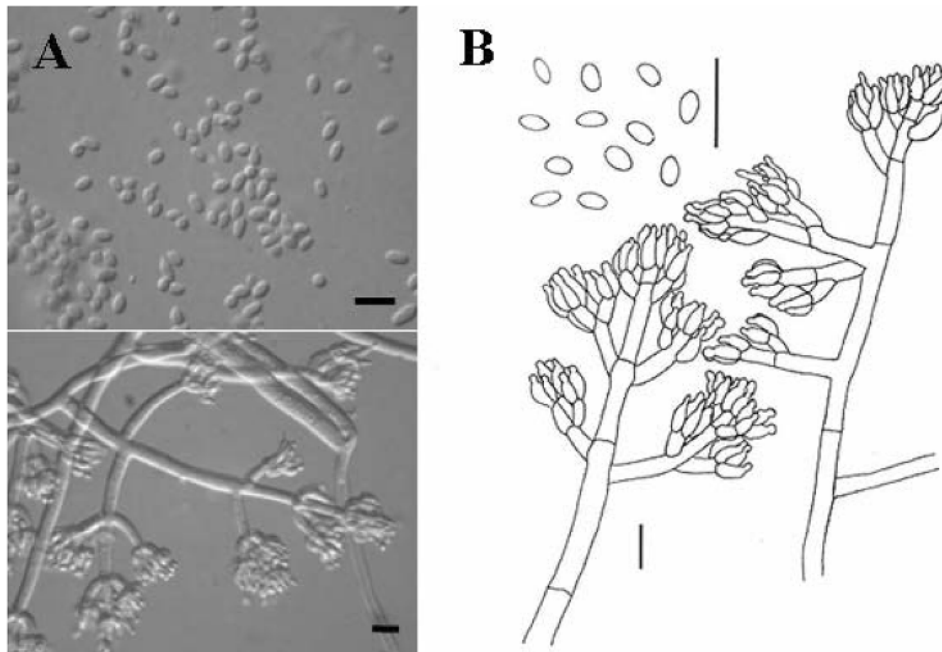
Fig. 1.

*Coloniae in agar succroso solanaceo sat rapide crescentes ad, 52~60 mm diametro post 3 dies (25 C). Conidiophorae pyramidae verticillata (typo Gliocladium), Phialides plerumque ampulliformes raro lageniformes, plerumque 4~7 subverticillatae, 4.2~7.0  $\times$  2.0~4.0  $\mu$ m. Conidia globosa vel ellipsoidea, laevia, 2.8~4.2  $\times$  1.6~2.2  $\mu$ m, in medio 3.4  $\times$  2.0  $\mu$ m. Chlamydosporae subglobosae vel ellipsoideae, laeves, 5.0~10.0  $\mu$ m diam. Simile *Trichodermati virens*, sed conidia magis parva et per sequentem "ITS" "EF-1 $\alpha$ " et "RPB2" gene distinguibile.*

**Holotype:** dried culture specimen on PDA, isolated from the waste cotton substrate of oyster mushroom, Chuncheon, Gangwon Province, Korea, April 1998, S. H. Yu, CNUMH 501, deposited in the Mycological Herbarium, Chungnam National University (CNUMH), Korea, culture ex-type KCTC 26314.

**Additional material examined:** dried culture specimen on PDA, isolated from the waste cotton substrate of oys-

\*Corresponding author <E-mail: shunyu@cnu.ac.kr>



**Fig. 1.** Micrographs (A) and drawings (B) of conidiophores and conidia of *Trichoderma pleurotum*. Scale bar = 10  $\mu$ m.

ter mushroom, Boeun, Chungbuk Province, Korea, Oct. 1998, S. H. Yu, CNUMH 523.

**Etymology:** from the host oyster mushroom *Pleurotus*.

**Colonies** (on PDA at 25°C after 3 days) growing rapidly, 52–60 mm in diam. **Conidiation** sparse and powdery; greenish white or grayish green, becoming darker at age. **Conidiophores** usually simply branched; primary branches tended to be held at nearly right angles, apices of conidiophores and primary branches usually with a *Gliocladium*-like head comprised of 2–4 branches terminated by crowded whorls of phialides; relatively broad. **Phialides** mostly ampulliform, infrequently lageniform, mostly arising in crowded whorls (verticils) of 4–7,  $4.2\text{--}7.0 \times 2.0\text{--}4.0 \mu\text{m}$ . **Conidia** ellipsoidal to obovoid,  $2.8\text{--}4.2 \times 1.6\text{--}2.2 \mu\text{m}$  (av.  $3.4 \times 2.0 \mu\text{m}$ ), smooth-walled, pale green. **Chlamydospores** subglobose to ellipsoidal, smooth-walled,  $5.0\text{--}10.0 \mu\text{m}$  diam.

**Comments:** *T. pleurotum* resembles *T. virens* in penicillate branching patterns of conidiophores and phialids, but can be distinguished readily from the latter by its predominant conidiation, many divided branches, more gathering of the fingers to top and more phialids to the apex. Additionally, the conidia of the new species are smaller ( $2.8\text{--}4.2 \times 1.6\text{--}2.2 \mu\text{m}$ ) compared to the conidia of *T. virens* ( $4.3\text{--}5.6 \times 3.2\text{--}4.4 \mu\text{m}$ ) and the conidiophores of the new species are broader ( $5\text{--}8 \mu\text{m}$  in diameter) than those of *T. virens* ( $3\text{--}6 \mu\text{m}$  in diameter). The molecular trees supporting circumscription of the taxa as new was also provided by the authors (Park *et al.*, 2004, 2005b). The species was

the most predominant species and the virulence of the species was significantly greater than that of the other *Trichoderma* species found in oyster mushroom environment (Park *et al.*, 2005a).

***Trichoderma pleuroticola* S. H. Yu & M. S. Park sp. nov.**

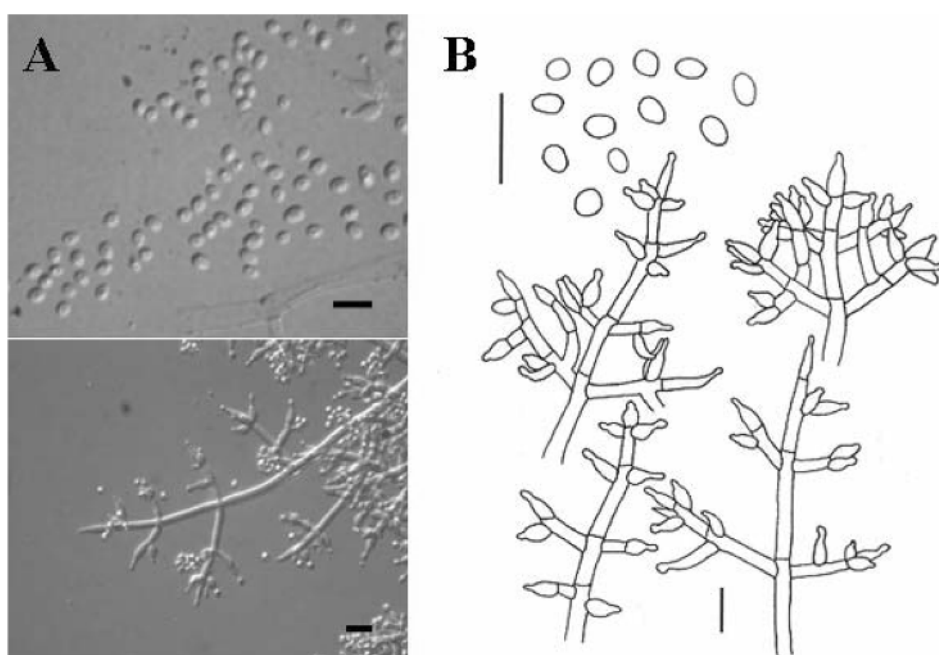
**Fig. 2.**

*Coloniae in agar succroso solanaceo sat rapide crescentes ad, 52–60  $\mu$ m diametro post 3 dies (25 C). Conidiophorae pyramidae verticillata. Phialides ampulliformes vel lageniformes, plerumque 2–4 subverticillatae raro solitariae,  $5.5\text{--}11.0 \times 2.6\text{--}4.0 \mu\text{m}$ . Conidia continua, subgloboseae vel lato ellipsoideae, laevia,  $2.9\text{--}4.5 \times 2.4\text{--}3.5 \mu\text{m}$ . in medio  $3.3 \times 2.7 \mu\text{m}$ . Chlamydosporae subgloboseae vel ellipsoideae,  $5.0\text{--}10.0 \mu\text{m}$  diam. Simile Trichodermati harziani, sed phialides magis longae et per sequentem “ITS” “EF-1 $\alpha$ ” et “RPB2” gene distinguibile.*

**Holotype:** dried culture specimen on PDA, isolated from the waste cotton substrate of oyster mushroom, Paju, Gyonggi Province, Korea, May 1999, S. H. Yu, CNUMH 601, deposited in the Mycological Herbarium, Chungnam National University, Korea, culture ex-type KCTC 26315.

**Additional material examined:** dried culture specimen on PDA, isolated from rice straw substrate of oyster mushroom, Inje, Gangwon Province, Korea, May 1999, S. H. Yu, CNUMH 571.

**Etymology:** from the Latin *Pleurotus*, the epithet of the host oyster mushroom.



**Fig. 2.** Micrographs (A) and drawings (B) of conidiophores and conidia of *Trichoderma pleuroticola*. Scale bar = 10  $\mu$ m.

**Colonies** (on PDA at 25°C after 3 days) growing rapidly, 60-65 mm in diam. **Conidiation** effuse, fasciculate, small pustules coalesced and forming broad concentric rings; initially greenish gray turning grayish-green to dark green. **Conidiophores** tending to be regularly verticillate and forming a more or less pyramidal structure. **Phialides** ampulliform to lageniform, mostly 3-4 verticillate or paired, occasionally arising singly, 5.5-11.0  $\times$  2.6-4.0  $\mu$ m. **Conidia** one celled, subglobose to broadly ellipsoidal, often pointed at the base, 2.9-4.5  $\times$  2.4-3.5  $\mu$ m (av. 3.3  $\times$  2.7  $\mu$ m), smooth-walled, pale green. **Chlamydospores** subglobose to ellipsoidal, 5.0-10.0  $\mu$ m diam.

**Comments:** *T. pleuroticola* resembles *T. harzianum* in the morphology of conidiophores and branching patterns but differs in the length of phialides and conidial shape; in *T. pleuroticola*, the phialides are longer (5.5-11.0  $\times$  2.6-4.0  $\mu$ m) compared to 3.5-7.6  $\times$  1.8-3.4  $\mu$ m in *T. harzianum*; and *T. pleuroticola* has subglobose to broadly ellipsoidal conidia, while *T. harzianum* has globose to broadly ellipsoidal conidia. Phylogenetic analysis of ITS, EF-1 $\alpha$  and RPB2 gene sequences also revealed that *T. pleuroticola* was phylogenetically distinct not only from *T. harzianum* but also from other previously reported *Trichoderma* species (Park *et al.*, 2004, 2005b).

### Acknowledgement

This study was supported by a grant from the Agricul-

tural Research Promoting Center, Ministry of Agriculture and Forestry, Korea. The authors wish to thank Dr. H. B. Lee (Chonnam National University) for his comments on the manuscript. Dr. H. D. Shin (Korea University) is also thanked for helping the Latin diagnosis.

### References

- Park, M. S., Bae, K. S. and Yu, S. H. 2003. Molecular and morphological analysis of *Trichoderma* isolates associated with green mold epidemic of oyster mushroom in Korea (Abstract). Programme & Abstract, the Sixth China-Korea Joint Symposium on Mycology (Wuhan, China): 16.
- \_\_\_\_\_, \_\_\_\_\_ and \_\_\_\_\_. 2004. Morphological and molecular analysis of *Trichoderma* species associated with green mold epidemic of oyster mushroom in Korea. New Challenges in Mushroom Science. Proceedings of the 3rd Meeting of Far East Asia for Collaboration on Edible Fungi Research 143-158.
- \_\_\_\_\_, Seo, G. S., Lee, K. H., Bae, K. S. and Yu, S. H. 2005a. Morphological and cultural characterization of *Trichoderma* spp. associated with green mold of oyster mushroom in Korea. *Plant Pathol. J.* **21**: 221-228.
- \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_ and \_\_\_\_\_. 2005b. Characterization of *Trichoderma* spp. associated with green mold of oyster mushroom by PCR-RFLP and sequence analysis of ITS regions of rDNA. *Plant Pathol. J.* **21**: 229-236.
- Yu, S. H. 2001. Integrated Control of Oyster Mushroom Green Mould. Research Report Submitted to the Ministry of Agriculture and Forestry, Korea. 157pp.