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# Breeding and Screening of *Lentinula edodes* Strains Resistant to *Trichoderma* spp.

**Hye-Min Lee*, Won-Chull Bak, Bong-Hun Lee, Hyun Park and Kang-Hyeon Ka**

*Division of Wood Chemistry and Microbiology, Korea Forest Research Institute, Seoul 130-712, Korea*

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***Trichoderma* spp. cause large crop losses of the cultivated shiitake mushroom, *Lentinula edodes*. We bred several shiitake strains that are resistant to *Trichoderma* spp. using di-mon mating to establish a useful method for controlling the greenmold disease. We examined the competitive ability of *L. edodes* against *Trichoderma* spp. using a dual culture system to select resistant strains. By screening *Trichoderma*-resistant strains, we found that among 11 parental strains, 4 strains, including KFRI 36, were confirmed resistant strains. They showed especially strong resistance to *T. harzianum*, which formed deadlock after mycelial contact and then invaded into the territory of *T. harzianum*. KFRI 171 also showed resistance to *T. atroviride* strains. Among 13 strains, which were made by hybridization of shiitake strains, 5 were confirmed to be resistant to *Trichoderma*, including KFRI 58-1. Their resistance was not correlated to the resistant activity of their parents' strains. Two strains lose resistance and two strains acquire resistance compared to their parents' strains. In SEM observation, the mycelium of *L. edodes* at the interaction zone of *Lentinula-Trichoderma* was rugged and swollen by *T. harzianum*.**

**KEYWORD : Hybrid strains, *Lentinula edodes*, Screening of resistant strains, *Trichoderma* species**

The shiitake mushroom, *Lenitula edodes*, is widely cultivated and managed throughout Korea. However, a wide range of pests and disease occur in cultivation. Mushroom yields and quality may be reduced by diseases caused by *Diatrype* sp., *Hypocrea* sp., *Nitschkia* sp. (Bak and Kwon, 2005). Among them, fungal species in the genus *Trichoderma* are commonly existed in soil, which used biocontrol agents of some phytopathogenic fungi. They produce a large range of secondary metabolites which have inhibitory activities, direct mycoparasitism as well as lysis and degradation of cell wall by enzymes (Howell, 2003). However, *Trichoderma* species, such as *T. harzianum* and *T. polysporum*, often attack and kill shiitake mycelium in bed-logs for mushroom by producing antifungal substances and mycolytic enzymes (Seaby, 1998; Tokimoto, 1985; Ulhoa and Perberdy, 1992), thereby reducing the yield of shiitake cultivation. However, control of these diseases was not effective because of the lack of effective control methods. Shiitake cultures produce at least five straight-chain alcohols, which could act as antifungal substances (Ishikawa *et al*., 2001) and extracellular enzymes, which could help to adapt environmental stress or antagonists (Mata and Savoie, 1998). These substances may play a role in the resistance of *Trichoderma* spp. through their antifungal effects (Savoie and Mata, 2003; Tokimoto *et al*., 1987). These days, breeding of shiitake strain is a useful way to acquire strains that are resistant to *Trichoderma* spp. It can increase their resistance activity by insertion of resistance genes, which produce some antifungal substances (Tokimoto and Komatsu, 1995). In this study, we bred strains of shiitake that are resistant to *Trichoderma* spp. by di-mon breeding; dikaryon mycelium contact with another monokaryon mycelium that monokaryon is adapted to a donor for cytoplasmic substances to dikaryon mycelium. We then tested them against five strains of *Trichoderma* spp. to confirm their resistant activities.

*Corresponding author <E-mail : hye8301@hanamil.net>

## Materials and Methods

**Test organisms.** *Lentinula edodes* strains were collected from various sources and maintained at the Korea Forest Research Institute (KFRI), Seoul, Korea. The selected shiitake strains (dikaryons, KFRI 36, 38, 57, 58, 59, 171, 182, 183, 192, 193, 194) were bred with monokaryons (KFRI 535, 536), which were isolated from the spores of the KFRI 405 fruiting body. The newly bred shiitake strains were obtained by di-mon breeding method (Table 1). *Trichoderma* spp., were isolated from KFRI shiitake cultivation and five strains were used in this experiments. All of the fungal cultures were incubated on potato dextrose agar (PDA, Gellix™, Korea) at 23°C for 10 days and used as inoculum. And all of the experiments carried out five replicates.

**Dual culture of *Lentinula edodes* and *Trichoderma* spp. on agar medium.** Competitive interactions between shiitake and mycoparasitic fungi were studied in dual-culture experiments on a PDA *in vitro* system. In each experiment, 5 mm diameter mycelial disks, which trans-

**Table 1.** Hybrid strains made by di-mon mating method

| KFRI No.[a] | | |
|---|---|---|
| mating strains | | hybrid strains[c] |
| monokaryon[b] | dikaryon | |
| 535 | 36 | 36-1 |
| | 38 | 38-1 |
| | 57 | 57-1 |
| | 58 | 58-1 |
| | 59 | 59-1 |
| | 171 | 171-1 |
| | 182 | 182-1 |
| | 183 | 183-1 |
| | 192 | 192-1 |
| | 193 | 193-1 |
| | 194 | 194-1 |
| 536 | 38 | 38-2 |
| | 182 | 182-2 |

[a]*L. edodes* strains maintained in Korea Forest Research Institute
[b]Monokaryons derived from spores of KFRI 405
[c]Newly made *L. edodes* strains by di-mon mating

ferred to the shiitake and *Trichoderma* spp. cultures, placed on the PDA apart from 30 mm. The shiitake inoculum were prepared 7 days before *Trichoderma* inoculation because of its stabilization before *Trichoderma*'s attack. The shiitake-*Trichoderma* paired in all possible combinations and five replicates were carried out all the experiments. The fungal cultures were incubated at 23°C and measured their invading zone, after 7 and 30 days later. The invading zone were divided into 5 types as follow: No resistant activity, *L. edodes* was completely invaded by *Trichoderma* spp.; Deadlock, both *L. edodes* and *Trichoderma* spp. stop growing after mycelial contact and formed strong antithetic zone line; Weakly resistant, *L. edodes* partially overgrew the territory of *Trichoderma* spp.; Moderately resistant, *L. edodes* overgrew up to the *Trichoderma* spp. inoculation site; Strong resistant, *L. edodes* completely overgrew the territory of *Trichoderma* spp.

**Antagonism interactions of *Lentinula edodes* against *Trichoderma* spp.** To observe antagonistic action of *L. edodes* on *Trichoderma* spp., the two fungi were co-cultured on PDA. After 1 month of incubation, a strong antithetic line was formed on the agar. The *L. edodes* resistant to *Trichoderma* spp. were observed by light and scanning electron microscopy. The interacting zones between *L. edodes* and *Trichoderma* spp. were cut and prepared for scanning electron microscopic observation (Jacob *et al*., 1996).

## Results and Discussion

**Screening of resistance strains against *Trichoderma* spp.** Among 11 *L. edodes* strains, only 4 (KFRI 36, 38, 58, 171) were resistant to *Trichoderma harzianum* (Table 2). At the initial time of incubation, most of *L. edodes* strains were slightly invaded or formed deadlock with *Trichoderma* spp. After one month, *L. edodes* was partially or completely invaded by *Trichoderma* spp. in most pairings (Fig. 1). However, KFRI 36, 38, 58, 171 showed resistance to *T. harzianum* and overgrew the territory of mycoparasitic fungi. KFRI 171 also showed resistance to one of *T. atroviride* strains. KFRI 58 had a similar competitive activity against *Trichoderma* spp. They stopped

**Table 2.** Resistance of *L. edodes* strains against *Trichoderma* species

| KFRI No. | Resistance | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *T. harzianum* | | *T. atroviride* | | | | *T. reesei* | | | |
| | biotype T-8 | | biotype T-4 | | biotype T-16 | | biotype T-10 | | biotype T-15 | |
| | A | B | A | B | A | B | A | B | A | B |
| 36 | ±[a] | ++ | – | – | ± | – | – | – | – | – |
| 38 | ± | ++ | – | – | – | – | – | – | – | – |
| 57 | – | – | – | – | – | – | ± | – | – | – |
| 58 | ± | +++ | ± | ± | ± | ± | ± | – | – | – |
| 59 | ± | ± | – | – | ± | ± | – | – | – | – |
| 171 | ± | +++ | – | – | ± | ++ | – | – | – | – |
| 182 | – | – | – | – | – | – | – | – | – | – |
| 183 | – | – | – | – | – | – | – | – | – | – |
| 192 | – | – | – | – | – | – | – | – | – | – |
| 193 | – | – | – | – | – | – | – | – | – | – |
| 194 | – | – | – | – | – | – | – | – | – | – |

[a]±: Deadlock, both *L. edodes* and *Trichoderma* spp. stop growing at antithetic zone line, –: No resistant activity, *L. edodes* was invaded by *Trichoderma* spp., +: Weakly resistant, *L. edodes* partially overgrew the territory of *Trichoderma* spp., ++: Moderately resistant, *L. edodes* overgrew up to the *Trichoderma* spp. inoculation site, +++: Strong resistant, *L. edodes* completely overgrew the territory of *Trichoderma* spp., A: observed 7 days after incubation with *Trichoderma* spp., B: observed 30 days after incubation with *Trichoderma* spp.

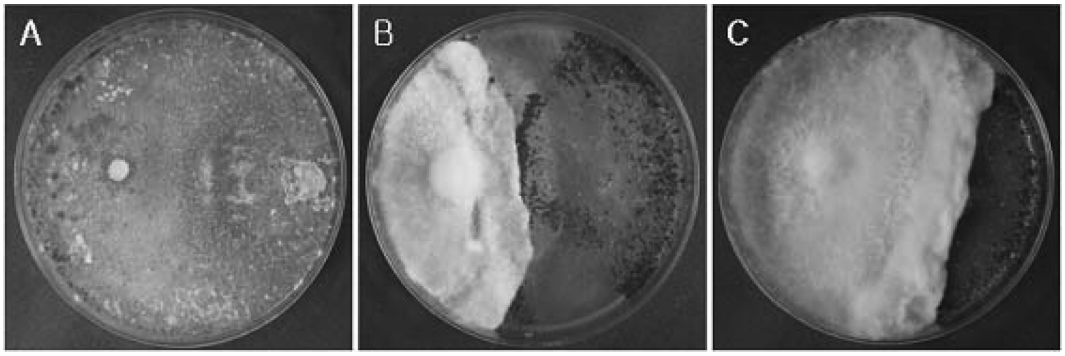


**Fig. 1.** Resistance of *L. edodes* to *T. harzianum* (biotype T-8) on PDA (right: *T. harzianum*, left: *L. edodes*). *T. harzianum* invaded into territory of *L. edodes* (A). Both *L. edodes* and *T. harzianum* stop growing, and formed antithetic line (B). *L. edodes* resistant to *T. harzianum*, and overgrew into territory of *T. harzianum* (C).

growing with the formation of strong antithetic zone lines with *T. harzianum* and *T. atroviride* after one month. These resistant strains need to be studied further in bed-log culture and then might be used for greenmold disease resistant strains. *L. edodes* had some antifungal activities, including antioxidant and enzyme activities. These anti-fungal activities may be increased their resistant activities against some mushroom pathogenic fungi, for example *Trichoderma* spp. (Savoie *et al.*, 1998; Savoie and Mata, 2003). The understanding of these particular interaction may be a useful key to the control of greenmold disease during shiitake cultivation and needs further investigation.

**Screening of resistance strains against *Trichoderma* spp. made by di-mon mating.** Thirteen hybrid shiitake strains were bred by di-mon breeding method (Table 1). Most of the hybrid strains formed deadlock or invading zone at the initial time of incubation, like their parent strains. Among them, only 5 strains showed resistant to *Trichoderma* spp. KFRI 57-1 and 59-1 had resistance activity against *T. reesei* and *T. harzianum*, respectively. However, KFRI 38-1, 38-2 and 36-1 lost the resistance activity of their parent strains against *T. harzianum*. KFRI 58-1 had resistance activity against *T. atroviride* but lost their resistance activity against *T. harzianum*, compared to their parent strains. These results may be associated with insertion of foreign genes of monokaryons derived from basidiospores. On breeding of shiitake strains resistant to *Trichoderma* spp., Tokimoto and Komatsu (1995) observed that the resistance was revealed dominantly. However, in

**Table 3.** Resistance of hybrid *L. edodes* strains against *Trichoderma* species

| KFRI No. | Resistance[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *T. harziaum* | | *T. atroviride* | | | | *T. reesei* | | | |
| | biotype T-8 | | biotype T-4 | | biotype T-16 | | biotype T-10 | | biotype T-15 | |
| | A | B | A | B | A | B | A | B | A | B |
| 36-1 | − | − | − | − | − | − | ± | − | − | − |
| 38-1 | − | − | − | − | − | − | − | − | ± | − |
| 38-2 | ± | ± | − | − | ± | ± | ± | − | − | − |
| 57-1 | − | − | − | − | − | − | − | + | − | − |
| 58-1 | ± | ± | ± | ++ | − | − | ± | ± | − | − |
| 59-1 | ± | ++ | − | − | − | − | − | − | − | − |
| 171-1 | − | ++ | ± | − | − | − | − | − | − | − |
| 182-1 | − | − | − | − | − | − | ± | − | ± | − |
| 182-2 | ± | + | ± | − | − | − | − | − | − | − |
| 183-1 | − | − | − | − | − | − | ± | − | − | − |
| 192-1 | − | − | − | − | − | − | ± | ± | − | − |
| 193-1 | − | − | − | − | − | − | − | − | − | − |
| 194-1 | − | − | − | − | − | − | − | − | − | − |

[a]±: Deadlock, both *L. edodes* and *Trichoderma* spp. stop growing at antithetic zone line, −: No resistant activity, *L. edodes* was invaded by *Trichoderma* spp., +: Weakly resistant, *L. edodes* partially overgrew the territory of *Trichoderma* spp., ++: Moderately resistant, *L. edodes* overgrew up to the *Trichoderma* spp., inoculation site, +++: Strong resistant, *L. edodes* completely overgrew the territory of *Trichoderma* spp., A: observed 7 days after incubation with *Trichoderma* spp., B: observed 30 days after incubation with *Trichoderma* spp.

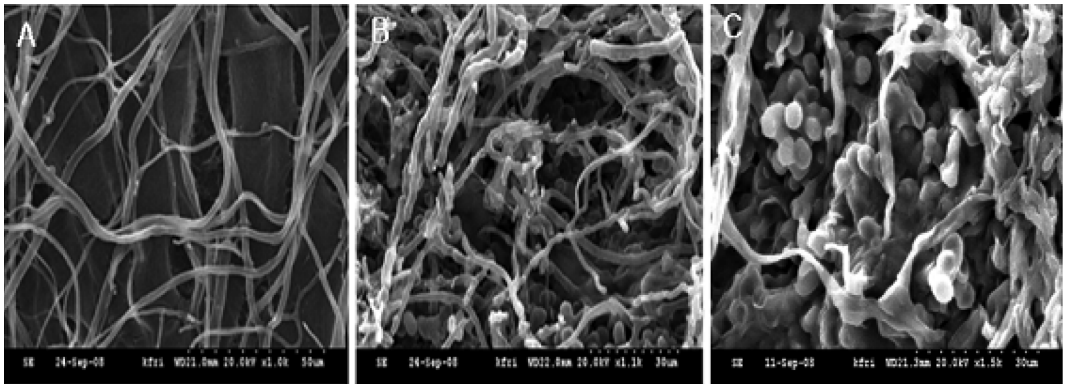


**Fig. 2.** Hyphal interactions of *L. edodes* and *T. harzianum* (biotype T-8). Hyphal cells of *L. edodes*. (A) Rugged, (B) swollen and (C) influenced by *T. harzianum*.

the new hybrid strains of *L. edodes* in our study, resistance to *Trichoderma* spp. was not dominant inherited. The mechanism of these resistant gene transfers might be the key in making resistant strains and needs further investigation.

**Interaction between shiitake and mycoparasitic fungi *in vitro* system.** In the interaction zone between *L. edodes* and *Trichoderma* spp., the hyphal cell of *L. edodes* was distorted with local swellings and was inhibited by *Trichoderma* spp. In untreated *L. edodes*, the hyphal cell was smooth, straight and normally-growing (Fig. 2). On the *Pleurotus-Trichoderma* interaction, Reper and Penninckx (1987) observed that mycoparasitic fungus affected the growth of *P. ostreatus* mainly by producing a volatile toxin which killed *P. ostreatus*. In our study, *L. edodes* mycelium were resistant to *Trichoderma* spp. mechanically by forming brown barrages in the antithetic zone line. Through SEM observation, we determined that the hyphal cells of antithetic zone line were swollen and thickened. This could be associated with the formation of melanin compounds that protect hyphal cells, making them resistant to mycoparasitic fungi attack. Some studies suggest that melanin formation is part of a defensive response against mycelial invasion and that these compounds help fungi to adapt to environmental stress (Rayne *et al*., 1994; Badalyan *et al*., 2004). These could be associated with release of volatile and non-volatile metabolites produced by *Trichoderma* spp. (Howell, 2003).

## References

Badalyan, S. M., Innocenti, G. and Garibyan, N. G. 2004. Interactions between xylotrophic mushrooms and mycoparasitic fungi in dual-culture experiments. *Phytopathol. Mediterr.* 43:44-48.

Bak, W. C. and Kwon, H. J. 2005. Biology and control of pests and diseases in shiitake log cultivation. Mushroom Grower's Handbook, 2nd edition, pp. 152-161. Mushworld.

Howell, C. R. 2003. Mechanism employed by *Trichoderma* species in the biological control of plant disease: The history and evolution of current concepts. *Plant Dis.* 87:4-10.

Ishikawa, N. K., Kasuya, M. C. M., Cristina, M. and Vanetti, M. C. D. 2001. Antibacterial activity of *Lentinula edodes* grown in liquid medium. *Braz. J. Microbiol.* 32:206-210.

Jacob, I., Ana, M. and AndIlan, C. 1996. Hyphal interaction between *Trichoderma harzianum* and *Sclerotia sclerotiorum* and its role in biological control. *Soil Biol. Biochem*. 28:757-763.

Mata, G. and Savoie, J. M. 1998. Extracellular enzyme activities in six *Lentinula edodes* strains during cultivation in wheat straw. *J. Microbiol. Biotechnol.* 14:513-519.

Rayner, A. D. M., Griffith, G. S. and Wildman, H. G. 1994. Induction of metabolic and morphogenetic changes during mycelia interactions among species of higher fungi. *Trans. Biochem. Soc.* 22:389-394.

Reper, C. and Penninckx, M. J. 1987. Inhibition of *Pleurotus ostreatus* growth and fructification by a diffusible toxin from *Trichoderma hamatum*. *Lebensm.-Wiss. Technol.* 20:291-292.

Savoie, J. M., Mata, G. and Billette, C. 1998. Extracellular laccase production during hyphal interactions between *Trichoderma* sp. and shiitake, *Lentinula edodes*. *Appl. Microbiol. Biotech.* 49:589-593.

Savoie, J. M. and Mata, G. 2003. *Trichoderma harzianum* metabolites pre-adapt mushrooms to *Trichoderma aggressive* antagonism. *Mycologia* 95:191-199.

Seaby, D. 1998. *Trichoderma* as a weed mould or pathogen in mushroom cultivation: Trichoderma and Gliocladium. Vol. 2, pp.267-272. CRC Press. U.S.A.

Tokimoto, K. 1985. Physiological studies on antagonism between *Lentinula edodes* and *Trichoderma* spp. in bedlogs of the former (in Japanese). *Rep. Tottori Mycol. Inst.* 23:1-54.

Tokimoto, K. Fujita, T., Takeda, Y. and Takaishi, Y. 1987. Increased or induced formation of antifungal substances in cultures of *Lentinula edodes* by the attack of *Trichoderma* spp. *Proc. Jp. Acad.* 63:277-280.

Tokimoto, K. and Komatsu, M. 1995. Selection and breeding of shiitake strains resistant to *Trichoderma* spp. *Can. J. Bot.* 73:S962-S966.

Ulhoa, C. J. and Peberdy, J. F. 1992. Purification and some properties of the extracellular chitinase produced by *Trichoderma harzianum*. *Enzyme Microb. Technol*. 14:236-240.