Mycobiology 31(3): 139-144 (2003)

Physiological Characteristics of Green Mold (*Trichoderma* spp.) Isolated from Oyster Mushroom (*Pleurotus* spp.)

In-Young Choi*, Gi-Tae Joung, Joung Ryu, Joung-Sik Choi and Yeong-Geun, Choi

Jeollabuk-do Agricultural Research and Extension Services, Iksan 570-704, Korea
(Received April 18, 2003)

This study was conducted to investigate physiological characteristics of *Trichoderma* spp. isolated from *Pleurotus* spp. Damage tests of *Pleurotus* spp. and mycotoxins tests of *Trichoderma* spp. were also done. The optimal growth temperature of *Trichoderma* spp. was 27~30°C. Although, *T. longibrachiatum* was able to grow at 37°C and grew 30~40 times faster than *Pleurotus*. The colony colour on PDA medium of *T.* cf. *virens* was yellowish green, *T. longibrachiatum* was yellow, and *T. harzianum* was turning to bright green. In damage tests of *Pleurotus* by *Trichoderma*, *T.* cf. *virens* caused the most severe damage to *Pleurotus*. *T. longibrachiatum* and *T. harzianum* caused less damage on *Pleurotus* but were able to cause greater damage to *P. eryngii*. One of the mushroom cultivars, *P. ostreatus* 8 was the most resistant to all *Trichoderma* spp.. Chitinolytic mycotoxin released by *Trichoderma* spp. caused 52.7% damage to *Pleurotus*. Mycotoxins released by *T. longibrachiatum* caused the greatest damaged (78.6%) on *P. eryngii*.

KEYWORDS: Interaction, Mycotoxin, *Pleurotus* spp., *Trichoderma* spp.

Pleurotus ostreatus is the most popular oyster mushroom grown in Korea (Bae *et al.*, 1996; Kang *et al.*, 2001; Sung *et al.*, 1999) cultivated by over 60% of mushroom growers (Oh *et al.*, 2000; Sung *et al.*, 1999). After its initial cultivation, techniques were standardized using cotton waste and rice straw based substrates, it became the most popular variety among mushroom growers in Korea. Unfortunately this mushroom is subject to many vagaries of nature *viz*, pests and diseases that adversely affect its production and productivity. Among the fungal pathogens, *Hyphomycetous* fungi including *Trichoderma* are the most common (Oh *et al.*, 2003). One of the major diseases of the button mushroom (*Agaricus bisporus*) worldwide is caused by *T. harzianum* (Bayer *et al.*, 2000). Green mold epidemics have been reported in the U.S.A., Canada, South America, Asia, Australia and European Countries (Grogan *et al.*, 2000; Muthumeenakshi *et al.*, 1994; Samuels *et al.*, 2002). Disease samples from mushroom farms were collected by many workers in the past and several species of *Trichoderma* were identified including *T. harzianum*, *T. longibrachiatum*, *T. virens* and *Trichoderma* sp. (Danesh *et al.*, 2000; Muthumeenakshi *et al.*, 1994; Samuels *et al.*, 2002).

Trichoderma initially produces a dense pure white mycelium which resembles mushroom mycelium therefore they are very difficult to distinguish. Mycelial mat on the casing layer gradually turns to a green colour because of the heavy sporulation of the causal agent producing a characteristic symptom of the disease (Danesh *et al.*, 2000). *Trichoderma* colonized in mushroom compost competes with mushroom mycelium for space and nutrients and results in large areas of the growing beds not producing mushroom fruit bodies (Bayer *et al.*, 2000; Samuels, 1996). Green mold is characterized by large areas of dense green sporulation on the compost and casing surface resulting in a dramatic reduction in mushroom yield (Anderson *et al.*, 2000). Mycotoxin produced by fungi is a secondary substance which causes damage to humans and animals as well as retarding the growth of mushroom mycelium. The mycotoxins produced by *Trichoderma* were reported and identified as gliotoxin, viridin, trichodermin and peptide type (Kim, 1985). Also, *Trichoderma* species secrete hydrolytic enzymes including chitinases, *β*-glucanases and cellulases which are kind of mycotoxins and lyse the fungal cell walls and are thought to play a role in the mycoparasitic activity of this fungus (Goltapeh and Danesh, 2000).

Earlier studies on *Trichoderma* were mainly focused on morphological and microbiological characteristics associated with the commercially grown button mushroom *Agaricus bisporus* (Kubicek and Harman, 1998; Samuels *et al.*, 2002; Samuels *et al.*, 1994). However, the analysis of damage type, especially the host-pathogen interaction at cellular level is still not fully understood in *Pleurotus*. Furthermore, the effects of green mold with oyster mushroom cultivars have not been investigated. The objective of this study was to provide mushroom growers with information on the physiological characteristics of *Trichoderma* and the resistance of various mushroom cultivars to *Trichoderma*. The information could be used to develop manage the infection of mushroom crops by *Trichoderma*.

*Corresponding author <E-mail: choiiy@lycos.co.kr>

Materials and Methods

Fungal isolates. For the study, disease samples from mushroom farms were collected and several species of *Trichoderma viz* : *T.* cf. *virens*, *T. longibrachiatum* and *T. harzianum* were isolated from *Pleurotus ostreatus* beds which were mostly composed of pasteurized rice straw (Choi *et al.*, 2003). The 7 cultivars of oyster mushrooms used for pathogenicity tests, were obtained from KACC (Korean Agricultural Collection, National Institute of Agricultural Science and Technology, Suwon, Korea).

Mycelial growth and colony characteristics of *Pleurotus ostreatus* and *Trichoderma* spp. The growth patterns of 3 *Trichoderma* spp. and *Pleurotus ostreatus* were studied on PDA (Potato dextrose agar, Difco) incubated at different temperatures. Plates were incubated at 15~30°C which were thought to be optimal for growing oyster mushroom with a 5 increment. Temperatures of 5 and 37°C were also included as treatments to examine the possible growth limiting temperatures. The mycelial growth of the mushroom cultivar *Pleurotus ostreatus* was compared with that of the *Trichoderma* species at 25°C on a daily basis for 3 days. The colony colour of the 3 *Trichoderma* species was investigated using growth after 10 days from inoculation by spectrophotometer (Minolta CM-3500d, Japan) with zero calibration CM-A124 box and white calibration CM-A120 box.

Damage tests of *Pleurotus ostreatus* by *Trichoderma* species. The 7 cultivars of oyster mushroom obtained from the KACC were used for the damage tests. Each mushroom cultivar was cross-cultured with each *Trichoderma* species on PDA medium for 6 days. Tip cultures were taken from the *Trichoderma* and oyster mushroom mycelia using a cork-borer (No. 2) and inoculated on PDA 60 mm apart. The resistance tests were done by measuring the growth length of oyster mushroom against *Trichoderma*. The colonization of *Pleurotus* spp., damage to *Pleurotus* spp. and resistance of *Pleurotus* species to *Trichoderma* were calculated as follows. Colonization of *Pleurotus* spp. by *Trichoderma* (%) = (length of overlap culture/culture length of *Pleurotus* spp.) × 100. Damage to *Pleurotus* spp. by *Trichoderma* (%) = 100 − (growth length of *Pleurotus* spp./growth length of control *Pleurotus* spp.) × 100. Resistance of *Pleurotus* spp. to *Trichoderma* (%) = (growth length of *Pleurotus* spp./length of inoculum) × 100.

Mycotoxins test of *Trichoderma* spp. One of the most common causes of damage by *Trichoderma* is the lysis of the mushroom cell by hydrolytic enzymes secreted during the cultivation of oyster mushroom. Therefore, for the mycotoxins inhibition test of each mushroom cultivar, *T.* cf. *virens*, *T. longibrachiatum* and *T. harzianum* were cultured on PDA medium on cellophane for 3 days. The cellophane with *Trichoderma* spp. mycelium was removed, and the PDA medium now contained the mycotoxins which had leaked through the cellophane. The tips of each mushroom cultivar were punched out using a cork borer (No. 2) and plated onto the PDA media. Each cultivar was cultured on each medium for 6 days and the growth of mushroom mycelium was measured. After six days, the extent of growth inhibition of *Pleurotus* species was measured in relation to *Pleurotus* species grown on control PDA medium without mycotoxins.

Results

Effect of temperature on mycelial growth and colony colour. The optimal growth temperature of *Trichoderma* species *T.* cf. *virens* and *T. harzianum* and *Pleurotus ostreatus* was 25°C. *T. longibrachiatum* grew faster at temperatures under 37°C compared to the other *Trichoderma* species tested and also continued to grow slowly at 37°C (Fig. 1). *T.* cf. *virens*, *T. harzianum* and *Pleurotus ostreatus* did not grow at 37°C. The growth of *Pleurotus* was slower than that of the *Trichoderma* spp. and growth was recorded after 7 days at each temperature. Mycelial growth of *T.* cf. *virens*, *T. longibrachiatum*, *T. harzianum* and *Pleurotus ostreatus* was recorded daily over 3 days on PDA medium at 25°C. *Pleurotus ostreatus* grew only 2±1 mm in the 3 days compared to the mycelial growth of *T. longibrachiatum* which grew 80±2 mm in 3 days which was 30~40 times faster than *P. ostreatus*. *T.* cf. *virens* and *T. harzianum* grew at a similar rate and grew 55 mm in the 3 days (Fig. 2).

The colony colour of the 3 *Trichoderma* species isolated from *Pleurotus* spp. on PDA medium were; *T.* cf. *virens* was white, turning yellowish green in the centre. *T. longibrachiatum* had yellow conidial areas and had a conidial crust forming with dense conidiation in older cul-

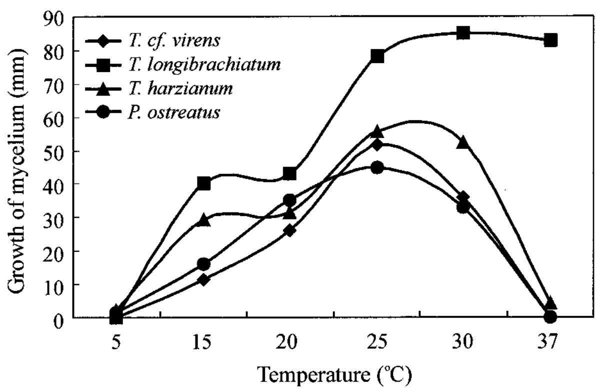

Fig. 1. The effect of mycelial growth on temperature (°C) of *Trichoderma* spp. after 3 days of inoculation on PDA, but *Pleurotus ostreatus* was investigated after 7 days.

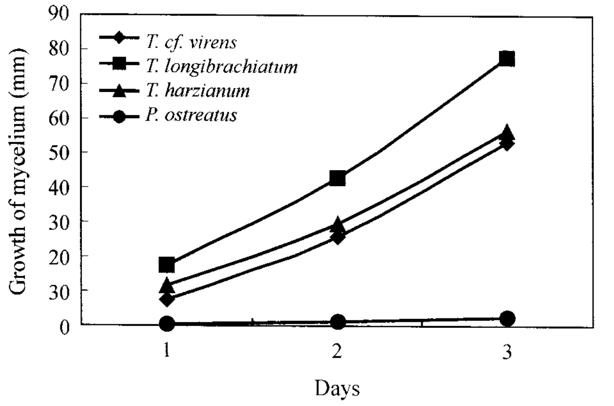

Fig. 2. Mycelial growth at 1, 2, and 3 days of *Trichoderma* spp. and *Pleurotus ostreatus* at 25°C on PDA.

Table 1. The colony colour of *Trichoderma* spp. isolated from *Pleurotus* spp. on PDA[a]

Species	L	a	b
T. cf. *virens*	47.80	−1.08	4.12
T. longibrachatum	42.28	−0.30	7.17
T. hazianum	39.05	2.36	4.02
Control (PDA)	43.14	−0.62	−0.83

[a]: After 10 days using spectrophotometer. L: lightness, a: redness, b: yellowness.

tures and *T. harzianum* was conidiation concentric, had a whitish yellow conidial area which turned dull green and finally dull brown (Table 1).

Damage test of *Pleurotus* spp. The selection of resistance cultivars of oyster mushroom through damage tests between *Trichoderma* spp. and *Pleurotus* spp. is increasing income of mushroom growers. The pathogenicity of pathogen tests on each mushroom cultivar. Tested mushroom cultivars had 100% colonization of *Pleurotus* spp. and damage to *Pleurotus* spp. by *T.* cf. *virens*. The resistance of *Pleurotus* spp. was 0% showing that none of the cultivars tested were resistant to the *T.* cf. *virens* (Table 2, Fig. 3). The damage caused to *P. ostreatus*8 and *P. ostreatus*1 by *T. longibrachiatum* and *T. harzianum* had a low percentage colonization of *Pleurotus* spp. and damage to *Pleurotus* spp., and high percentage resistance of *Pleurotus* spp. had more resistance than others. However, *P. eryngii* which is a bottle cultivation mushroom was not the most resistant of the tested cultivars and sensitive of *T. longibrachiatum* and *T. harzianum* was like results of *T.* cf. *virens* (Tables 3, 4). *T.* cf. *virens* caused the most damage in the *Trichoderma* spp. and *P. ostreatus*8 and *P. ostreatus*1 were the most resistant of the mushroom cultivars tested.

Mycotoxins test of *Trichoderma* spp. *Trichoderma* compete with the mushroom mycelium for nutrients from the compost. *Trichoderma* also attack the mushroom by

Table 2. Pathogenicity test conducted after dual-culture between *Trichoderma* cf. *virens* and *Pleurotus* spp. after 6 days growth on PDA

Cultivar	Mushroom growth (mm)	*Trichoderma* growth (mm)	Colonization of *Pleurotus* spp. (%)[a]	Damage to *Pleurotus* spp. (%)[b]	Resistance of *Pleurotus* spp.(%)[c]
*P. sajor-caju*1	24.3	56.0	100	100	0
*P. sajor-caju*2	30.6	53.3	100	100	0
*P. ostreatus*1	23.3	52.0	100	100	0
*P. ostreatus*2	35.7	58.3	100	100	0
*P. ostreatus*3	31.7	57.3	100	100	0
*P. ostreatus*8	37.0	57.7	100	100	0
P. eryngii	21.0	53.0	100	100	0

[a]Colonization of *Pleurotus* spp. by *Trichoderma* (%) = (length of overlap culture/culture length of *Pleurotus* spp.) x 100.
[b]Damage to *Pleurotus* spp. by *Trichoderma* (%) = 100 − (growth length of *Pleurotus* spp./growth length of control *Pleurotus* spp.) x 100.
[c]Resistance of *Pleurotus* spp. by *Trichoderma* (%) = (growth length of *Pleurotus* spp./length of inoculum) x 100.

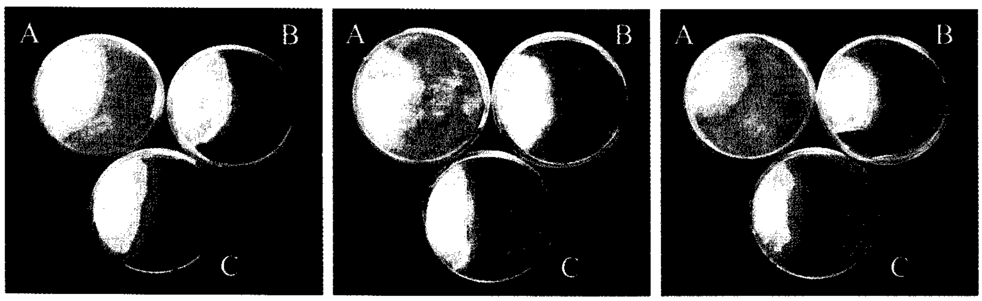

Fig. 3. Pathogenicity test in dual-culture plates of *Trichoderma* spp. and *Pleurotus* spp after 6 days growth on PDA. A: *P. ostreatus*8, B: *P. sajor-caju*2, C: *P. sajor-caju*1.

Table 3. Pathogenicity test conducted with dual-culture between *Trichoderma longibrachiatum* and *Pleurotus* spp. at 6 days after growth on PDA.

Cultivar	Mushroom growth (mm)	*Trichoderma* growth (mm)	Colonization of *Pleurotus* spp. (%)[a]	Damage to *Pleurotus* spp. (%)[b]	Resistance of *Pleurotus* spp. (%)[c]
*P. sajor-caju*1	23.7	41.3	45.0	61.4	27.0
*P. sajor-caju*2	22.3	37.0	40.4	61.6	27.8
*P. ostreatus*1	27.3	39.0	34.8	60.0	31.7
*P. ostreatus*2	27.0	43.7	46.9	68.9	24.1
*P. ostreatus*3	32.0	42.3	39.6	52.5	30.8
*P. ostreatus*8	34.0	34.3	22.5	46.7	39.4
P. eryngii	16.0	59.0	100	100	0

[abc]Described at Table 2.

Table 4. Pathogenicity test conducted with dual-culture between *Trichoderma harzianum* and *Pleurotus* spp. at 6 days after growth on PDA

Cultivar	Mushroom growth (mm)	*Trichoderma* growth (mm)	Colonization of *Pleurotus* spp. (%)[a]	Damage to *Pleurotus* spp. (%)[b]	Resistance of *Pleurotus* spp. (%)[c]
*P. sajor-caju*1	24.0	43.5	56.3	64.5	24.9
*P. sajor-caju*2	25.0	43.3	46.7	61.7	27.0
*P. ostreatus*1	25.7	40.7	42.2	65.9	27.3
*P. ostreatus*2	28.0	41.3	47.6	65.2	27.9
*P. ostreatus*3	31.3	45.7	67.1	69.2	22.0
*P. ostreatus*8	32.5	37.5	38.4	51.8	35.7
P. eryngii	23.3	55.7	100	77.8	0

[abc]Described at Table 2.

Table 5. Inhibition was investigated with culture of *Pleurotus* spp. for 6 days on PDA including mycotoxins

Cultivar	*T.* cf. *virens*	*T. longibrachiatum*	*T. harzianum*	Means (%)
*P. sajour-caju*1	38.3	77.3	50.0	55.2
*P. sajour-caju*2	51.4	62.9	48.6	54.3
*P. ostreatus*1	47.1	64.7	32.4	48.0
*P. ostreatus*2	32.6	52.2	34.8	40.0
*P. ostreatus*3	45.3	55.3	56.2	52.3
*P. ostreatus*8	34.3	68.3	48.3	50.3
P. eryngii	57.1	78.6	71.4	69.0
Means (%)	43.7	65.5	48.8	52.7

[a]Inhibition of *Pleurotus* spp. growth (%) = 100 − (mycelial growth on medium including mycotoxins/mycelial growth of control *Pleurotus* spp.) × 100.

hydrolytic enzyme secretion which lyses the fungal cells. Hence, we investigated the effects mycotoxins on each mushroom cultivar. The damage to the mushroom by the mycotoxins secreted by *Trichoderma* was collectively 52.7%. *P. eryngii* was damaged about 69%. Especially it by *T. longibrachiatum* was 78.6% that has the highest of tested *Trichoderma*. As influence about mushroom cultivars with *Trichoderma* species, mycotoxins secreted by *T. longibrachiatum* given damage of average 65.5% (Table 5).

Discussion

The results of the tests to examine mycelial growth of *Trichoderma* spp. and *Pleurotus* showed that growth of the *Trichoderma* pathogen was faster at temperatures between 20~30°C. The optimal growth of *Pleurotus* was 25°C, which suggested that optimizing the growth temperature of the mushroom crop might result in greater damage to the crop if any *Trichoderma* are present during culture or cultivation. *T. longibrachiatum* was also able to grow at temperatures above 30°C and grew faster than the other *Trichoderma* species and would therefore cause more damage. The accelerated mycelial growth of the *Trichoderma* spp. compared to that of *Pleurotus* over 3 days showed that the *Trichoderma* species were able to establish a substantial amount of growth before *Pleurotus ostreatus* mycelium had began to grow. If this occurs during cultivation where *Trichoderma* is present in the compost, the mushroom crop could be destroyed immediately.

Therefore attempts should be made to ensure that compost and the mushroom units are free from *Trichoderma* before cultivation.

In order for growers to control mushroom disease by *Trichoderma* species it was necessary to investigate the optimal growth temperature of *Trichoderma* and the respective damage of the mushroom crops. The present results suggested that more damage would be caused at temperatures up to 30°C. Therefore, temperature control during mushroom cultivation is critical. For the growing pattern of *Trichoderma*, Denesh *et al.* (2000) reported that growth patterns at 25°C of *T. longibrachiatum*, *T. harzianum*, *T. virens* and *Trichoderma* sp. collected from mushroom farms showed significant differences in the type of growth and sporulation patterns between various species and isolates.

The damage tests results showed that all the 7 cultivars of mushroom tested were susceptible to all 3 species of *Trichoderma*. Resistance would have been an effective method of control but resistant varieties are not available at present. Two cultivars of *Pleurotus ostreatus*8 and *P. ostreatus*1 were the least susceptible of the cultivars tested to all 3 species of *Trichoderma*. However, it is not known whether this resistance would be of substantial benefit against *Trichoderma* infection during cultivation. Although *T. longibrachiatum* had produced the fastest growth and was able to grow at temperatures above 37°C, it did not cause the most damage to all cultivars of *Pleurotus*. *T.* cf. *virens* caused the most damage to all 7 cultivars of *Pleurotus*. Especially, *P. eryngii* which is popular in bottle cultivation was expected to be susceptible to three *Trichoderma* species, and special cultivation practices are needed. The most serious outbreak of *Trichoderma* species on mushroom crops was reported by biotyoe Th-2 of *T. harzianum*, in Ireland in 1985~1986 and resulted in losses about 3~4 million pounds in mushroom industries in U.K. and Ireland (Fletcher, 1990).

The mycotoxin tests showed that the mycotoxin produced by *T. longibrachiatum* caused severe damage to 5 of the 7 mushroom cultivars tested. *T.* cf. *virens* had been shown to cause the most severe damage to all 7 cultivars during the damage tests. Growers have to make sure that contamination after 7~10 days by *Trichoderma* spp. does not occur particularly. Kim (1985) reported that he could forecast the damage of mushrooms by mycotoxins secreted by *T. viride* on the growth of mushroom. About mycotoxins, *Penicillium* has been reported to produce about 40 mycotoxins such as citirnin, patulin, penicillic acid and cyclopiazonic acid, causing damage to persons and animals (Paik *et al.*, 2000).

Acknowledgment

This work was supported by the Post-doctoral Fellowship Program of Korea Science & Engineering Foundation (KOSEF).

References

Anderson, M. G., Beyer, D. M. and Wuest, P. J. 2000. Using spawn strain resistance to manage *Trichoderma* green mold. In *Science and Cultivation of Edible fungi: Mushroom Science* **XV(2)**: 641-644.

Bae, S. C., Seong, K. Y., Lee, S. W., Go, S. J., Eun, M. Y. and Rhee, I. K. 1996. Phylogenetic relationships among *Pleurotus* species inferred from sequence data of PCR amplified ITS region in ribosomal DNA. *Korean J. Plant Pathol.* **24**: 155-165.

Bayer, D. M., Wuest, P. J. and Kremser, J. J. 2000. Evaluation of epidermilolgical factors and mushroom substrate characteristics influencing the occurrence and development of *Trichoderma* green mold. In *Science and Cultivation of Edible fungi: Mushroom Science* **XV(2)**: 633-640.

Choi, I. Y., Hong, S. B. and Yadav, M. C. 2003. Molecular and morphological characterization of green mold, *Trichoderma* spp. isolated from oyster mushroom. *Mycobiology* **31**: 74-80.

Danesh, Y. R., Goltapeh, E. M. and Rohani, H. 2000. Identification of *Trichoderma* species causing green mold in button mushroom farms, distribution and their relative abundance. In *Science and Cultivation of Edible fungi: Mushroom Science* **XV(2)**: 653-659.

Fletcher, J. T. 1990. *Trichoderma* and *Penicillium* diseases of *Agaricus bisporus*. A Literature Review for The Horticultural Development Council. London. ADAS.

Goltapeh, E. M. and Danesh, Y. R. 2000. Studies on interaction between *Trichoderma* species and *Agaricus bisporus* mycelium. In *Science and Cultivation of Edible fungi: Mushroom Science* **XV(2)**: 661-666.

Grogan, H. M., Scruby, A. and Harvey, L. 2000. Moulds in spawn-run compost and their effect on mushroom production. In *Science and Cultivation of Edible fungi: Mushroom Science* **XV(2)**: 609-615.

Kang, H. W., Park, D. S., Park, Y. J., You, C. H., Lee, B. M. Eun, M. Y. and Go, S. J. 2001. Genomic differentiation among oyster mushroom cultivars released in Korea by URP-PCR fingerprinting. *Mycobiology* **29**: 85-89.

Kim, M. K. 1985. Influences of antibiotic components produced by *Trichoderma* spp. to oyster mushroom. *Kor. J. Mycol.* **13**: 105-109.

Kubicek, C. P. and Harman, G. E. 1998. *Trichoderma* and *Gliocladium* Vol. 1. Taylor and Francis Ltd. London. 270pp.

Muthumeenakshi, S., Mills, P. R., Brown, A. E. and Seaby, D. A. 1994. Intraspecific molecular variation among *Trichoderma harzianum* isolates colonizing mushroom compost in the British Isles. *Microbiology* **140**: 769-777.

Oh, S. J., Kong, W. S. and Kim, H. K. 2000. Studies on the effect of vinyl covering on *Pleurotus* spp. cultivation-Improved picking efficiency of *P. ostreatus* and *P. sajor-caju*. In *Science and Cultivation of Edible fungi : Mushroom Science* **XV(2)**: 949-953.

_____, Park, J. S., Lee, D. C. and Shin, P. G. 2003. Studies on the effect of vinyl mulching on *Pleurotus* cultivation - Control of mushroom disease on *P. ostreatus* (II). *Micobiology* **31**: 50-53.

Paik, S. B., Chung, I. M., Yu, S. H. and Kim, E. Y. 2000. Survey and control of the occurrence of mycotoxins from post-harvest

fruits. *Kor. J. Mycol.* **28**: 49-54.
Samuels, G. J., Petrini, O. and Manguin, S. 1994. Morphological and macromolecular characterization of *Hypocrea schweinitzii* and its *Trichoderma* anamorph. *Mycologia* **86**: 421-435.
_____. 1996. *Trichoderma*: a review of biology and systematics of the genus. *Mycol. Res.* **100**: 923-935.
_____, Dodd, S. L., Gams, W., Castlebury, L. A. and Petrini. O. 2002. *Trichoderma* species associated with the green mold epidemic of commercially grown *Agaricus bisporus. Mycologia* **94**: 146-170.
Sung, J. M., Moon, H. W. and Park, D. S. 1999. Growth condition of liquid culture by *Pleurotus ostreatus*. *Kor. J. Mycol.* **27**: 1-9.