

Species Diversity of *Trichoderma* Contaminating Shiitake Production Houses in Thailand

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ABSTRACT

From 38 samples of green fungal contaminated wood logs, sawdust in polypropylene bags and other related materials used for shiitake (*Lentinula edodes* (Berk.) Pegler) production collected from five shiitake production houses, 43 isolates of fungi produced green spores were obtained. Twenty seven of which belonged to genus *Trichoderma* (62.8%), 9 were *Gliocladium* (20.9%), 4 were *Penicillium* (9.3%) and 3 were *Aspergillus* (7.0%). Among *Trichoderma*, 22 isolates (81.5%) were identified as *T. harzianum*, 2 isolates (7.4%) were *T. piluliferum* and 1 isolate each (3.7%) was *T. aureoviride*, *T. koningii* and *T. pseudokoningii*. It was obvious that *T. harzianum* was the predominant species and widely distributed in shiitake production houses compared with the other 4 species. All isolates of *T. harzianum* did not show any resistance to a fungicide, benomyl.

Key words: *Trichoderma*, *T. harzianum*, species diversity, *Lentinula edodes*, shiitake

INTRODUCTION

One of the most common contaminating fungi causing serious damage in the production of shiitake, *Lentinula edodes* (Berk.) Pegler, when cultivation is carried out either on wood logs or on supplemented sawdust in polypropylene bags is *Trichoderma* (Huang *et al.*, 1988; Ito, 1978; Laixuthai *et al.*, 1987; Liao, 1993; Lim *et al.*, 1990). In the later case, when spawn bags were moistening, *Trichoderma* spp. and *Penicillium* spp. were found within seven days after the first harvest of mushrooms (Laixuthai *et al.*, 1987). Ito (1987) reported that notable fungi that contaminated on logs cultivated *L. edodes* in Japan were various

fungi including several species of *Trichoderma*. *Trichoderma* was also reported as one of the most damaging fungal genera in competition with *L. edodes* mycelium in laying yards (Hayes, 1985). *T. harzianum* had caused a significant loss of mushroom in India (Pandy and Tewari, 1990). In Singapore, the most common fungal contaminants found in shiitake cultivation in supplemented sawdust in polypropylene bags were also *Trichoderma* spp., especially *T. harzianum* (Lim *et al.*, 1990). Togashi *et al.* (1997) found that *Trichoderma* spp. was an air-borne fungal flora of 24 fruiting houses for the sawdust-based cultivation of *L. edodes* though the frequency was not high. Outbreak of these fungal species were difficult to

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control, resulted in a severe yield reduction in shiitake production (Bodine, 1995; Laixuthai *et al.*, 1987). *Trichoderma* spp. not only grows rapidly in versatile conditions and utilizes various kinds of substrates (Eveleigh, 1985), but also produce tiny spores that easily disperse. Control of *Trichoderma* spp. in mushroom production using benomyl, a fungicide, has been practiced and reported elsewhere (Fukui *et al.*, 1974; Huang *et al.*, 1988; Liao, 1985). Although benomyl is harmful to humans and environment, long term treatment will select benomyl resistant progeny of *Trichoderma* spp.

This paper reported the result of investigation to confirm that the most potent fungal contaminants during production of shiitake were *Trichoderma* spp. and the most common contaminant species of *Trichoderma* was also determined. The information obtained would be useful for the effective control of fungal contamination in shiitake production houses in Thailand.

MATERIALS AND METHODS

Isolation of green fungi

Green fungal contaminated wood logs, supplemented sawdust in polypropylene bags and other related materials from shiitake production houses were collected. Small amounts of conidial mass were streaked on a sterile filter paper (Whatman no.42) which was moistened using 2 ml of nutrient solution (1 g KH_2PO_4 , 0.1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.1 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in 1 l of distilled water) in sterile plate and incubated at room temperature for one week. Conidial mass of green fungal colony appearing on filter paper was restreaked on a Petri dish containing malt extract agar (20 g malt extract and 20 g agar in 1 l of distilled water, pH 5.6) and then incubated at room temperature until pure fungal colony was obtained.

Identification of green fungi

Genus identification of green fungus was undertaken using the method of Domsch *et al.* (1980). *Trichoderma* isolates were identified at species level following the taxonomic key of the genus *Trichoderma* (Rifai, 1969). The fungus was cultivated on malt extract agar at 25°C for 48 h in the dark and then allowed to sporulate in the light at room temperature. Size, shape and position of phialides were observed after 5-7 days of cultivation and ornamentation of conidia was determined at 15 days. Microscopic examination was carried out by mounting the culture in lactophenol cotton-blue, but for size measurement water was used as mounting fluid.

Resistance to benomyl

The resistance of fungus to benomyl was determined by the size of colony on malt extract agar containing 0, 0.5, 1 and 2 mg of benomyl /l at 25 °C for 48 h. Four replications were made for each isolate and treatment.

RESULTS AND DISCUSSION

Isolation and identification of green fungi

Thirty eight samples of green fungal contaminated wood logs, supplemented sawdust in polypropylene bags and other related materials, such as wood chip, sorghum seed and tree barks, were collected from five mushroom production houses in Thailand. The samples collected from Doi Pui mushroom production house in Chiang Mai province consisted of 13 samples of wood log and 1 sample of supplemented sawdust in polypropylene bag, 1 sample of tree bark and 1 sample of fallen tree bark. From the other two mushroom production houses in Chiangmai province, Teen Tok and Ang Khang, five samples of log and two samples of wood ship were obtained, respectively. Ten samples of supplemented sawdust

in polypropylene bag were derived from Chernghai Co. Ltd., a shiitake production company in Chiang Rai province. Five samples collected from Arunyik Mushroom Center in Nakhon Pathom province consisted of 3 samples of supplemented sawdust in polypropylene bag and 2 samples of sorghum seed. A total of 43 isolates of fungi produced green color spores were obtained. Twenty six isolates were derived from 16 samples collected from Doi Pui mushroom production house. Five isolates were obtained from 5 samples collected from Teen Tok mushroom production house, 1 isolate from 2 samples of Ang Khang mushroom production

house, 7 isolates from 10 samples collected from Chernghai Co. Ltd. and 4 isolates from 5 samples of Arunyik Mushroom Center (Table 1).

Following the taxonomic key of Domsch *et al.* (1980), the result showed in Table 2 that 27 isolates of which belonged to genus *Trichoderma* (62.8%), 9 to *Gliocladium* (20.9%), 4 to *Penicillium* (9.3%) and 3 to *Aspergillus* (7.0%). The results confirmed that *Trichoderma* spp. were the common contaminants found in shiitake production houses as reported in China (Pandy and Tewari, 1990), Japan (Ito, 1978), Singapore (Lim *et al.*, 1990) and Thailand (Laixuthai *et al.*, 1987).

Table 1 Isolation of green fungi from various contaminated materials collected from five shiitake production houses.

Place of sample collection	Contaminated materials	No. of samples	Isolate code	No. of isolates
Doi Pui, Chiang Mai province	Supplemented sawdust in bag	1	GB-11, PB-1, TB-1, TB-3, TB-13	5
	wood log	13	GB-2, GL-1, GL-10, GL-12, GL-8, PL-8, TL-9, TL-11, TL-13, TL-14, TL-15, TL-16, TL-17, TL-18, TL-19, TL-20, TL-21,	17
	Tree bark	1	AE-3, TE-3	2
	Fallen tree bark	1	AE-2, GE-2	2
Teen Tok, Chiang Mai province	Wood log	5	GL-3, TL-4, TL-5, TL-6, TL-7	5
Ang Khang, Chiang Mai province	Wood chips	2	TL-2	1
Chernghai Co., Ltd., Chiang Rai province	Supplemented sawdust in bag	10	GB-10, PB-12, TB-6, TB-7, TB-8, TB-14, TB-9	7
Arunyik Mushroom Center, Nakhon Pathom province	Sorghum seed	2	AB-4, TB-4	2
	Supplemented sawdust in bag	3	PB-5, TB-5	2

Trichoderma isolates were identified at species level based on microscopic and microscopic characteristics following the taxonomic key of Domach *et al.* (1980) and Rifai (1969). Among 27

Trichoderma isolates, 22 (81.5%) of which were *T. harzianum*, 2 (7.4%) were *T. piluliferum* and 1 (3.7%) each was *T. aureoviride*, *T. koningii* and *T. pseudokoningii* (Table 3). Figure 1 show the

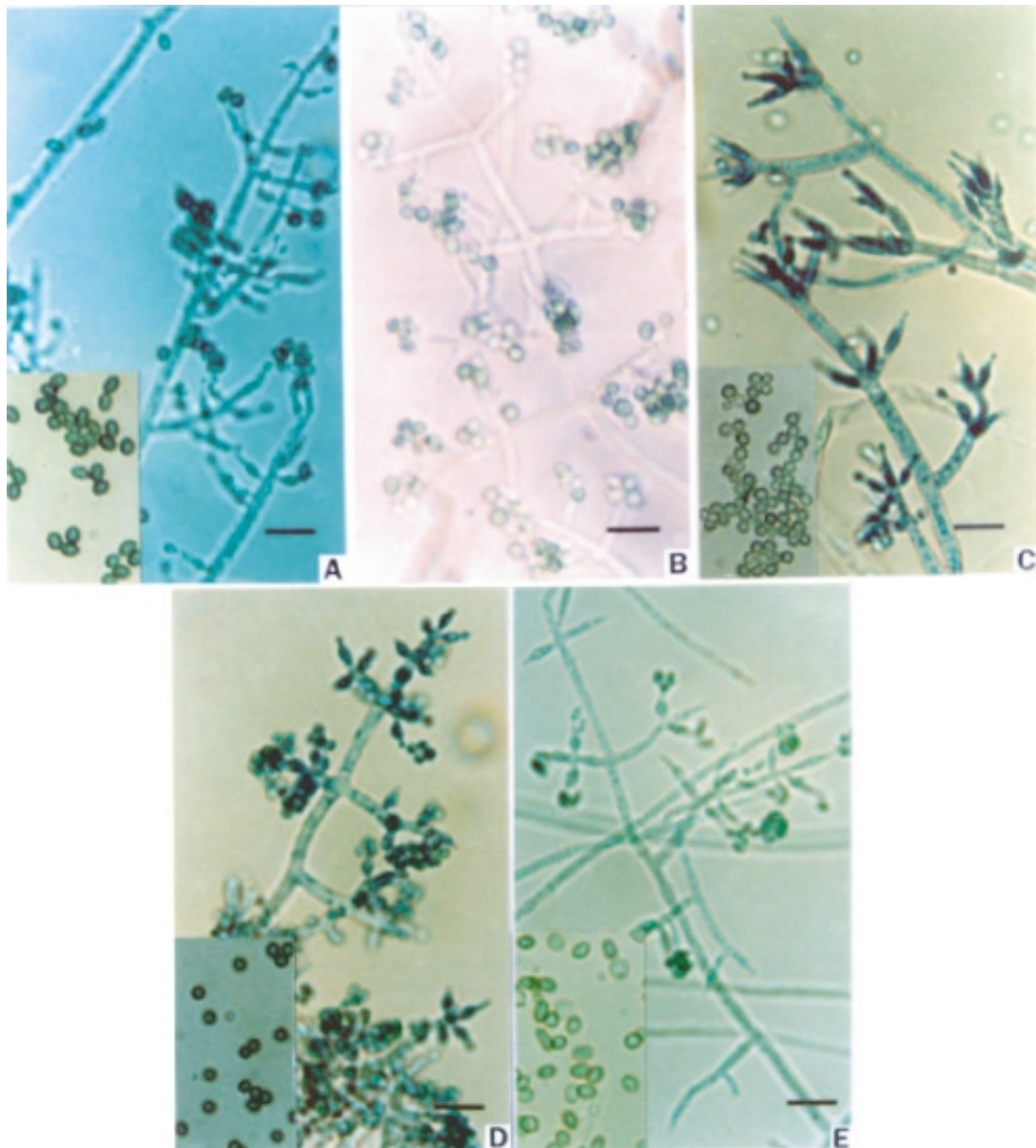


Figure 1 Morphology of phialides and conidia of *Trichoderma aureoviride*, TL-2 (A), *T. harzianum*, TB-3 (B), *T. koningii*, TB-4 (C), *T. piluliferum*, TB-9 (D) and *T. pseudokoningii*, TL-9 (E). Bar was 10 μ m.

Table 2 Identification of green fungal isolates at genus level.

Genus	Isolate code	Number of isolates	%
<i>Trichoderma</i>	TB-1, TB-3, TB-4, TB-5, TB-6, TB-7, TB-8, TB-9, TB-13, TB-14, TE-3, TL-2, TL-4, TL-5, TL-6, TL-7, TL-9, TL-11, TL-13, TL-14, TL-15, TL-16, TL-17, TL-18, TL-19, TL-20, TL-21,	27	62.8
<i>Gliocladium</i>	GB-2, GB-10, GB-11, GE-2, GL-1, GL-3, GL-8, GL10, GL-12	9	20.9
<i>Penicillium</i>	PB-1, PB-5, PB-12, PL-8	4	9.3
<i>Aspergillus</i>	AB-4, AE-2, AE-3	3	7
Total		43	100

Table 3 Identification of *Trichoderma* isolates at species level.

<i>Trichoderma</i> species	Place of sample collection	Number of isolates	Isolate code	%
<i>T. harzianum</i>	Doi Pui Chiang Mai province	22 (13)	TB-1, TB-3, TB-13, TE-3, TL-11, TL-13, TL-14, TL-16, TL-17, TL-18, TL-19, TL-20, TL-21	81.5
	Teen Tok Chiang Mai province	(4)	TL-4, TL-5, TL-6, TL-7	
	Ang Khang Chiang Mai province	(4)	TB-6, TB-7, TB-8, TB-14	
	Arunyik, Nakhon Pathom province	(1)	TB-5	
<i>T. piluliferum</i>	Chernghai Co., Ltd., Chiang Rai province	2 (1)	TB-9	7.4
	Doi Pui Chiang Mai province	(1)	TL-15	
<i>T. aureoviride</i>	Ang Khang, Chiang Mai province	1	TL-2	3.7
<i>T. koningii</i>	Arunyik, Nakhon Pathom province	1	TB-4	3.7
<i>T. pseudokoningii</i>	Doi Pui, Chiang Mai province	1	TL-9	3.7
Total		27		100

morphology of phialides and conidia of each representative *Trichoderma* species. Our finding was similar to the result of Laixuthai *et al.* (1987), who found *T. aureoviride*, *T. harzianum*, *T. koningii*, *T. hamatum* and *T. polysporum* during the production of shiitake on supplemented sawdust in polypropylene bag in Thailand. The result showed that *T. harzianum* could be isolated from contaminated materials collected from 4 shiitake production houses. Furthermore, among 15 isolates of *Trichoderma* obtained from Doi Pui 13 isolates (86.67%) were *T. harzianum*. These indicated the dominance and widely distribution of *T. harzianum* in the environment of shiitake production.

Benomyl resistance of *T. harzianum*

Benomyl is widely used to inhibit *Trichoderma* spp. during shiitake production in many countries including Thailand (Ogawa *et al.*, 1975; Pandey and Tewari, 1990; Triratana *et al.*, 1991). However, Bodine (1995) reported the ineffective control of *T. harzianum* by benomyl.

This may be resulted from the development of resistant strain after using benomyl for a long period. All 22 isolates of *T. harzianum* were determined for their resistance to benomyl by cultivation on medium containing benomyl at 0.5, 1 and 2 mg/l. Results revealed that there were no isolate able to grow on medium containing 2 mg/l benomyl. On medium containing 1 mg/l benomyl, only three isolates, TB-1, TB-3 and TL-16, could grow. However, their growths were strongly retarded (Figure 2). Colony diameters of these three isolates on this treatment were 11.75, 6.9 and 5.2 mm, corresponding to 26.6, 11.3 and 12.8% of the colony diameters of the control, respectively. On the medium containing 0.5 mg/l benomyl, only isolate TL-7 could not grow, while all other isolates showed different degree of growth. TB-1, TL-11 and TL-16 exhibited higher resistance to 0.5 mg/l benomyl as their colony diameters were 60.3, 62.4 and 67.5% of the colony diameters of the control, respectively. TL-13, TL-19 and TL-20 showed much decrease in their growth, their colony diameter

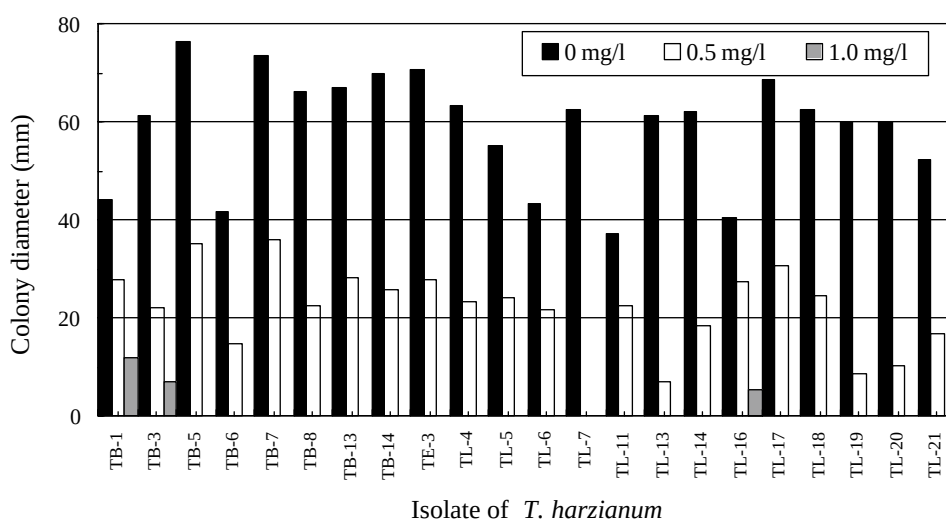


Figure 2 Growth of 22 isolates of *T. harzianum* on malt extract agar containing 0, 0.5 and 1 mg/l benomyl at 25°C for 48 h. Growth was measured as colony diameter (mm) and the diameters were averaged from four replications.

were only 11.6, 14.6 and 17.4% of the diameters of the control, respectively. Colony diameters of the remaining isolates were in the range of 29.6-50.5% of the control. It could be concluded that all isolates of *T. harzianum* were sensitive to benomyl at various degrees. This is probable due to the fact that benomyl has not been used for preventing fungal contamination in mushroom production in Thailand for a long period. Therefore, benomyl resistant strains have not been developed. Our finding agreed with Fukai *et al.* (1974) who reported that mycelial growth of *T. viride* in agar medium was inhibited by 0.25 mg/l benomyl. Also, Togashi *et al.* (1996) found that the mycelial growth of *Trichoderma* spp. collected as air-borne fungi was inhibited completely on medium containing high concentration of benomyl (200 mg/l).

CONCLUSION

In this study *Trichoderma* was confirmed to be a fungal contaminant in shiitake production in Thailand. *T. harzianum* was the dominant species and widely distributed in the environment of shiitake production house. The other four isolated species were *T. piluliferum*, *T. aureoviride*, *T. koningii* and *T. pseudokoningii*. Due to the fact that benomyl has not been used for preventing fungal contamination in mushroom production in Thailand for a long period, all isolates of *T. harzianum* did not show any resistance to benomyl.

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