Plant Pathology Bulletin 19: 261-270, 2010

Antimicrobial Activity of Edible Mushroom Culture Filtrates on Plant Pathogens

Jin-Tong Chen [1, 2] and Jenn-Wen Huang [1, 3]

[1] Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan

[2] Plant Pathology Division, Taiwan Agricultural Research Institute, Council of Agriculture, Wufeng, Taichung, Taiwan

[3] Corresponding author, E-mail: jwhuang@dragon.nchu.edu.tw; Fax: +886-4-2285-1676

Accepted for publication: August 24, 2011

ABSTRACT

Chen, J. T. and Huang, J. W. 2011. Antimicrobial activity of edible mushroom culture filtrates on plant pathogens. Plant Pathol. Bull. 19: 261-270.

The culture filtrates of 27 edible mushrooms were screened for antimicrobial activity against the plant pathogens. The culture filtrates of *Lentinula edodes* and *Clitocybe nuda* were able to completely inhibit spore germination of *Colletotrichum higginsianum*. Three culture filtrates contained substances that had the capacity to completely inhibit spore germination of *Alternaria brassicicola* were *Ganoderma lucidum*, *L. edodes* and *C. nuda*. The culture filtrates of *Coprinus comatus*, *L. edodes*, *Tremella aurantialba* and *C. nuda* showed complete suppression of spore germination of *Phytophthora capsici*. Only the culture filtrate of *C. nuda* moderately inhibited spore germination of *Fusarium oxysporum* f. sp. *lactucae*. The paper-disc agar-diffusion method was used to test the effect of the 27 culture filtrates from the 14 species of edible mushrooms examined, 18 culture filtrates inhibited the growth of bacteria. Four culture filtrates strongly inhibited the growth of *Acidovorax avenae* subsp. *citrulli*. Only the culture filtrate of *Agrocybe cylindracea* showed a clear inhibition zone against *Pectobacterium carotovorum* subsp. *carotovorum*. The culture filtrates of *A. cylindracea*, *Grifola frondosa* and *L. edodes* showed various sizes of growth inhibition zones against *Ralstonia solanacearum*. Six culture filtrates inhibited the growth of *Xanthomonas oryzae* pv. *oryzae*, 2 culture filtrates inhibited the growth of *Erwinia chrysanthemi*, and 13 culture filtrates inhibited *X. campestris* pv. *campestris* and *X. axonopodis* pv. *vesicatoria*. None of the culture filtrates was able to inhibit mycelial growth of *C. higginsianum*, *R. solani*, *P. aphanidermatum*, and *F. oxysporum* f. sp. *lactucae*. The culture filtrates of *C. nuda* and *C. comatus* effectively reduced the disease severity of Phytophthora blight of pepper caused by *P. capsici*. These results suggest that substances from edible mushrooms have the potential to be developed into biocontrol agents for the control of plant diseases.

Keywords: antimicrobial activity, culture filtrate, *Clitocybe nuda*, plant pathogen, *Phytophthora capsici*

INTRODUCTION

Synthetic pesticides have been used extensively in agriculture for pest and disease control during the past few decades [12, 13]. Widespread use of highly toxic synthetic pesticides in crop production is harmful to the environment, ecosystems, and animal and human health [21]. Therefore,

non-chemical control of crop pests has received great public attention worldwide. The use of natural products for pest control is ideal for sustainable agricultural crop production with minimum damage to the environment [26].

Basidiomycetes produce a large number of biologically active compounds [4] that show antibacterial, antifungal, antiviral, cytotoxic or hallucinogenic activities [5, 10, 11, 14, 18]. Several compounds isolated from wild mushrooms inhibited the growth of a large spectrum of saprophytic and phytopathogenic fungi [1-3]. Florey *et al.* [9] detected different antibiotic activities in either fruiting bodies or mycelial cultures of over 2,000 fungal species. Edible fungi, such as *Agaricus bisporus*, *Lentinula edodes*, *Auricularia auricula* and many *Pleurotus* species, also showed antagonistic effects against human pathogenic bacteria, fungi, viruses and cancer cells [15, 25, 29]. Many extensive clinical studies, primarily in Japan, have clearly demonstrated that a number of mushroom species have medicinal usefulness for the prevention and treatment of tumors, viral and bacterial diseases, hypercholesterolemia and blood platelet aggregation [6, 7, 16].

However, most previous investigations have focused on therapeutics and less on the control of plant diseases. Only a few reports of the effect of culture filtrates of edible mushrooms on the growth of plant pathogenic bacteria or fungi [20, 22]. The objectives of this study were to evaluate the antimicrobial activities of culture filtrates of edible mushrooms from Taiwan and to demonstrate their potential usefulness for controlling plant diseases.

MATERIALS AND METHODS

Sources of bacterial and fungal pathogens

The seven plant pathogenic bacteria (Table 1) used in this study were isolated from infected plants and purified by single colony growth on nutrient agar (NA; 1% peptone, 0.5% NaCl, 0.3% beef extract, and 2.5% agar). Pure cultures of *Acidovorax avenae* subsp. *citrulli* (Schaad *et al.*) Willems *et al.* (the causal agent of bacterial fruit blotch of watermelon) (strain AAC33), *Pectobacterium carotovorum* subsp. *carotovorum* (Jones) Hauben *et al.* (the causal agent of soft rot of calla lily) (strain Zl1), *Erwinia chrysanthemi* Burkholder *et al.* (the causal agent of soft rot of calla lily) (strain Cas7), *Ralstonia solanacearum* (Smith) Yabuuchi *et al.* (the causal agent of wax apple bacterial wilt) (strain PS152), *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson (the causal agent of black rot of cabbage) (strain XCC79), *X. oryzae* pv. *oryzae* (Ishiyama) Dye (the causal agent of bacterial blight of rice) (strain XF89-6) and *X. axonopodis* pv. *vesicatoria* (Doidge) Dowson (the causal agent of bacterial spot of pepper)

Table 1. List of fungal and bacterial plant pathogens used in this study and their hosts

Phytopathogen	Strain	Host plant	Source[a]
Fungi and oomycetes			
Alternaria brassicicola	ABA01	Chinese cabbage	1
Colletotrichum higginsianum	PA01	Chinese cabbage	1
Fusarium oxysporum f. sp. *lactucae*	LFO 1-13	Lettuce	1
Phytophthora capsici	PCM81	Pepper	2
Pythium aphanidermatum	PAM	Cucumber	1
Rhizoctonia solani	AG1	Rice	1
Bactertia			
Acidovorax avenae subsp. *citrulli*	AAC33	Watermelon	3
Pectobacterium carotovorum subsp. *carotovorum*	Zl1	Calla lily	3
Erwinia chrysanthemi	ECH	Calla lily	3
Ralstonia solanacearum	PS152	Wax apple	3
Xanthomonas axonopodis pv. *vesicatoria*	XV64	Pepper	3
Xanthomonas campestris pv. *campestris*	XCC79	Cabbage	3
Xanthomonas oryzae pv. *oryzae*	XF89-6	Rice	4

[a] Source of plant pathogens: 1, from the Laboratory of Plant Disease Management, National Chung Hsing University (NCHU), Taiwan; 2, from the Laboratory of Phytophthora Bology, NCHU, Taiwan; 3, from the Laboratory of Plant Bacterial Diseases, NCHU, Taiwan; and 4, from the Taiwan Agricultural Research Institute, Wufeng, Taichung, Taiwan.

(strain XV64) were maintained on NA in the laboratory. Before use, all bacterial strains were cultured in nutrient broth (Difco, USA) shaken at 150 rpm on a rotary shaker (Firstek, Taiwan) at 30℃. After 24-48 hr, the bacteria were harvested by centrifugation (6000 rpm at 10℃ for 10 min) and resuspended in sterile distilled water (SDW). The concentrations were adjusted to an optical density at 600 nm (OD_{600}) of 0.3 (which is equivalent to 10^8 CFU/ml) using a spectrophotometer (Biotech photometer, U.K.) for each treatment. The plant pathogenic fungi used in this study are listed in Table 1. They were isolated from infected plant tissues and cultured on PDA plates. Spore of *Alternaria brassicicola* (Schwein.) Wiltshire (isolate ABA01), *Colletotrichum higginsianum* Sacc. in Higgins (isolate PA01) and *Fusarium oxysporum* f. sp. *lactucae* (Matuo and Motohashi) (isolate LFO1-13) were cultured on PDA at 24℃ under 12 hr diurnal illumination for 14 days. Sporangia of *Phytophthora capsici* isolate PCM81 were produced by growing the fungi on 20% V8 agar (20% V8 juice, 0.03% $CaCO_3$, and 2% agar) at 24℃ with a 12 hr light-and-dark cycle and cool white fluorescent irradiation for 7 days. For the release of zoospores from the sporangia, the culture blocks (ca. 10 mm×10 mm) were cut in a 9 cm Petri dish with 15 ml of SDW. After incubation at 24℃ under light for 2 days, the water was replaced with 15 ml of SDW, and the Petri dish was placed at 4℃ for 1 hr. For testing spore inhibition, the concentration of the spore suspensions was adjusted to 10^5 spores/ml with a Pipetman microliter pipet [17]. *C. higginsianum* was adjusted to 10^4 spores/ml to avoid self-inhibition [19].

Edible mushroom strains

Agaricus bisporus strains A5033, MS and F4KN; *Agaricus subrufescens* strains AB4, BZ4 and WB; *Agrocybe cylindracea* strain H911; *Agrocybe chaxingu* strains BH45, LUB, LUW and TM01; *Coprinus comatus* strains CC1 and CC2; *Flammulina velutipes* strain F1; *Ganoderma lucidum* strain 1-14; *Grifola frondosa* strain G1; *Lentinula edodes* strain L1; *Clitocybe nuda* strains 999, LNG, LNE2, LA82 and LA84; *Pleurotus citrinopileatus* strain PC and *Pleurotus eryngii* strains B11 and B12 used in this study, were obtained from the Taiwan Agricultural Research Institute. The edible mushrooms were maintained on potato dextrose agar (PDA; Difco) and rice straw compost extract agar (CEA), which consisted of 80% compost extract, 20% corn meal and 2.5% agar.

Culture filtrates of edible mushrooms

A mushroom fungus cultural block (10 mm×10 mm ×3 mm) was used to inoculate 100 ml of sterile potato dextrose broth (PDB, Difco) in a 500 ml Erlenmeyer flask, which was incubated at 24℃ for 21 days on a shaker (120 rpm). The culture fluid was harvested by filtration through a Whatman No. 1 filter paper and a 0.22 μm filter (Millipore, USA) and then stored at -20℃.

Spore germination tests

To test the mushroom culture filtrates for the ability to inhibit spore germination, 10 μl of spore suspension was mixed with an equal volume of mushroom culture filtrate in a well of a sterile eight-well slide. The slides were kept moist by placing them on L-shaped glass rods on moistened paper towels in 9 cm plastic Petri dishes sealed with parafilm. A spore suspension mixed with PDB was used as a control. Germination was recorded after incubation at 24℃ for 8 hr, and 100 spores were counted for each of four replicates. All experiments were repeated twice.

Effect of mushroom culture filtrates on the mycelial growth of fungi

The effect of mushroom culture filtrates on the mycelial growth of *A. brassicicola*, *C. higginsianum*, *F. oxysporum* f. sp. *lactucae*, *Rhizoctonia solani* Kühn (isolate AG1), *P. capsici* and *Pythium aphanidermatum* (Edson) Fitzp. (isolate PAM) were determined using PDA plate. Two hundred microliters of each culture filtrate was used to flood the surface of a PDA dish (9 cm diameter) to form a thin layer. The culture filtrate was replaced with 200 μl of SDW as a control. Ten-day-old cultural disks (7 mm diameter) of phytopathogenic fungi were placed in the center of the PDA dish containing culture filtrate. The dishes were incubated at 28℃ for 24 hr for *P. aphanidermatum*, 24℃ for 48 hr for *R. solani*, and 24℃ for 7 days for *C. higginsianum*, *F. oxysporum* f. sp. *lactucae* and *P. capsici* before the colony diameter was measured. The effect of the mushroom culture filtrates was calculated according to the formula of Pandey and Dubey. [23]: Inhibition (%) = [(colony diameter of control - colony diameter of treatment)/colony diameter of control] x 100. Four replicates in each treatment were used, and experiments were repeated twice.

Effect of mushroom culture filtrates on the growth of bacteria

Effects of mushroom culture filtrates on bacterial growth were tested in Petri dishes containing NA. Soft agar (0.8%) at 48℃ was inoculated with a bacterial broth culture (10^6 to 10^8 CFU/ ml) of the tested organism and poured over the NA plates. Three filter paper disks (8 mm in diameter) (Advantec, Japan) were immersed in each mushroom culture filtrate and placed on the inoculated plate. Filter paper disks soaked in sterile PDB were used as a control. The plates were incubated at 30℃ for 48 hr, and the difference in the zones of growth inhibition were recorded. Four replicates were used, and all experiments were repeated twice.

Effect of mushroom culture filtrates on Phytophthora blight of pepper

Five-week-old sweet pepper (*Capsicum annuum* L.) cv. Trim-Star (Known-You Seed Co., Taiwan) plants with four to five fully expanded leaves were sprayed to runoff with culture filtrate of *C. nuda* (LA82), *C. comatus* (CC1) or *L. edodes* (L1). Control plants were similarly sprayed with PDB. The plants were sprayed three times over a three-day period. One day after the last leaf spray, each leaf was inoculated with four 2 μl drops of a spore suspension of *P. capsici* along the edge of the leaf, and a 10 μl drop of 1% V8 juice agar at 55-60℃ was added to each inoculum drop [30]. After incubation in moist chambers at 24℃ for 5 days, disease symptoms were evaluated based on a 0-5 scale: 0 = no visible disease symptoms; 1 = slightly wilted leaves with brownish lesions beginning to appear on the stems; 2 = 30-50% of plant diseased; 3 = 51-70% of plant diseased; 4 = 71-90% of plant diseased and 5 = dead plant. The disease severity was calculated using the formula of Sunwoo *et al.* [28]. Three leaves per plant were inoculated, and four plants were used for each treatment. Experiments were repeated twice.

RESULTS

Effect of culture filtrates of edible mushrooms on the spore germination of fungal pathogens

Among the 27 culture filtrates from different mushrooms, 3 culture filtrates contained substances that were able to completely inhibit sporel germination of *A. brassicicola* were *G. lucidum* (1-14), *L. edodes* (L1) and *C. nuda* (LA82). The other 24 culture filtrates were innocuous to *A. brassicicola*, which showed 69.0-97.8% germination (Table 2). Culture filtrates of *L. edodes* (L1) and *C. nuda* (LA82) were also able to completely inhibit sporel germination of *C. higginsianum*. The other 25 culture filtrates were ineffective for the suppression of spore germination of *C. higginsianum*, which showed 72.4-92.8% germination (Table 2). Only the culture filtrate of *C. nuda* (LA82) showed moderate inhibition of spore germination of *F. oxysporum* f. sp. *Lactucae* and reduced the germination rate from 99.8% in the PDB control to 61.6%. Other culture filtrates were ineffective (Table 2). The culture filtrates of *C. comatus* (CC1, CC2), *L. edodes* (L1), *T. aurantialba* (Ta) and *C. nuda* (LNE2, LA82) showed complete suppression of the spore germination of *P. capsici* (Table 2, Fig. 1), while the culture filtrates of *C. nuda* (LNG) and *P. eryngii* (B11) were could reduce the germination rate to 26.4-34.4%. The other culture filtrates were innocuous to *P. capsici*. The culture filtrates of *C. nuda* (LA82) and *L. edodes* (L1) were able to inhibit all four plant pathogenic microorganisms tested.

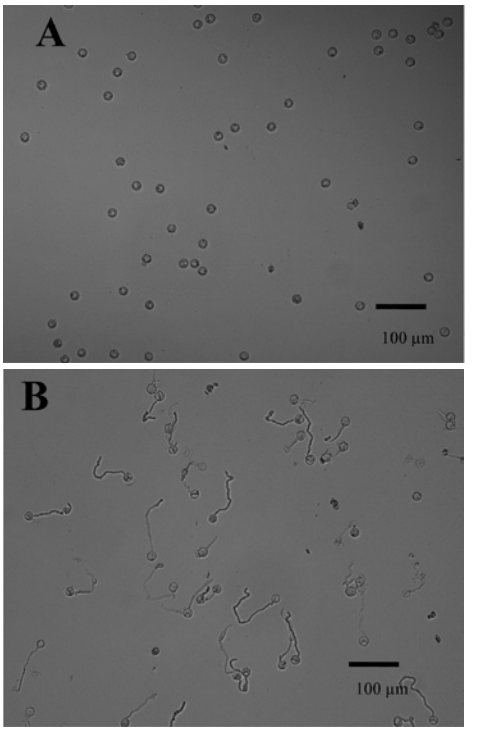

Fig. 1. Effect of culture filtrate of *Clitocybe nuda* strain LA82 on spore germination of *Phytophthora capsici*. (A) The zoospores of *P. capsici* were treated with culture filtrate of *C. nuda* (LA82); (B) PDB broth (control).

Table 2. Effects of the culture filtrates of 27 strains of 14 species of edible mushrooms on the spore germination of four plant pathogens[a]

Scientific name (strain)[b]	Spore germination (%)[c]			
	ABA01	PA01	LFO1-13	PCM81
Agaricus bisporus (A5033)	83.6	75.0	100.0	99.4
Agaricus bisporus (F4KN)	88.4	85.0	100.0	99.2
Agaricus bisporus (MS)	90.0	88.0	100.0	98.8
Agaricus subrufescens (AB4)	97.0	79.0	99.2	100.0
Agaricus subrufescens (BZ40)	92.6	82.2	100.0	95.8
Agaricus subrufescens (WB)	91.0	92.0	99.8	97.4
Agrocybe chaxingu (BH45)	90.0	75.8	100.0	98.0
Agrocybe chaxingu (LUB)	90.0	81.0	99.0	100.0
Agrocybe chaxingu (LUW)	89.8	86.0	97.6	99.4
Agrocybe chaxingu (TM01)	94.2	88.2	100.0	99.6
Agrocybe cylindracea (H911)	90.4	83.8	93.4	98.8
Clitocybe nuda (999)	74.4	92.0	100.0	54.2
Clitocybe nuda (LA82)	0.0	0.0	61.6	0.0
Clitocybe nuda (LA84)	74.6	84.0	100	73.0
Clitocybe nuda (LNE2)	77.4	89.0	99.4	0.1
Clitocybe nuda (LNG)	84.2	85.6	99.0	26.4
Coprinus comatus (CC1)	97.0	92.8	100.0	0.0
Coprinus comatus (CC2)	97.8	81.4	95.8	0.0
Flammulina velutipes (F1)	93.0	85.8	100.0	99.6
Ganoderma lucidum (1-14)	0.0	76.0	100.0	97.2
Grifola frondosa (G1)	83.4	90.8	100.0	97.0
Lentinula edodes (L1)	0.0	0.0	98.0	0.0
Pleurotus citrinopileatus (PC)	94.8	84.2	100.0	99.0
Pleurotus eryngii (B11)	69.0	84.0	98.0	34.4
Pleurotus eryngii (B12)	83.6	72.4	100.0	100.0
Tremella aurantialba (Ta)	89.8	85.4	100.0	0.0
Tremella fuciformis (Tf)	95.6	83.0	99.4	99.4
PDB (control)	98.4	87.2	99.8	98.8

[a] ABA01: *Alternaria brassicicola*, PA01: *Colletotrichum higginsianum*, LFO 1-13: *Fusarium oxysporum* f. sp. *lactucae*, PCM81: *Phytophthora capsici*.

[b] Mushroom fungi were cultured in potato dextrose broth under shaking at 120 rpm for 21 days at 24°C.

[c] Four replicates were used for each mushroom and 100 spores were counted for each replicate. Percentage of germination was based on the average of two tests.

Effect of mushroom culture filtrates on growth of fungal pathogens

The effects of the culture filtrates of 27 mushroom isolates on mycelial growth of *A. brassicicola*, *C. higginsianum*, *F. oxysporum* f. sp. *lactucae*, *R. solani*, *P. capsici* and *P. aphanidermatum* are shown in Table 3. Among the 27 culture filtrates tested, 13 were able to slightly suppress mycelial growth of *A. brassicicola*. For example, the *Agaricus bisporus* (F4KN) culture filtrate was able to reduce the mycelial growth rate to 37.3% (Table 3). The other culture filtrates were ineffective for the suppression of mycelial growth of *A. brassicicola*. The culture filtrates were able to weakly inhibit mycelial growth of *C. higginsianum*, *F. oxysporum* f. sp. *lactucae* and *P. capsici*. None of the culture filtrates showed inhibition of mycelial growth of *R. solani* and *P. aphanidermatum*.

Effect of mushroom culture filtrates on the growth of bacteria

The 27 culture filtrates showed different degrees of suppression of the bacteria tested. Four culture filtrates were able to strongly inhibit the growth of *A. avenae* subsp. *citrulli* (AAC33) (Table 4). Only the culture filtrate of *A. cylindracea* (H911) showed a clear inhibition zone of 14.8

mm on NA against *P. carotovorum* subsp. *carotovorum* (Zl1) (Table 4). The others were not able to inhibit this bacterium. The culture filtrates of *A. cylindracea* (H911) and *L. edodes* (L1) inhibited the growth of the bacterial plant pathogen *E. chrysanthemi* (ECH). The culture filtrates of *A. cylindracea* (H911), *G. frondosa* (G1) and *L. edodes* (L1) showed various zones of growth inhibition against *R. solanacearum* (PS152). Culture filtrates from 13 of the 27 mushroom isolates showed inhibitory effects on the growth of *X. campestris* pv. *campestris* (XCC79). The inhibition zones ranged from 10.5 to 25.0 mm (Table 4, Fig. 2). Among the 27 culture filtrates tested, 13 showed inhibitory effects on the growth of *X. axonopodis* pv. *vesicatoria* (XV64) (Table 4). The culture filtrate of *A. cylindracea* (H911) inhibited all of the bacterium tested, while the culture filtrate of *L. edodes* (L1) was able to inhibit all bacteria except *P. carotovorum* subsp. *carotovorum* (Zl1).

Effect of edible mushroom culture filtrates on Phytophthora blight of pepper

Mushroom culture filtrates that showed the strongest inhibitory effects on the spore germination of *P. capsici* were further assessed for effectiveness in the control of disease caused by this pathogen. The results showed that culture filtrates of *C. comatus* (CC1) and *C. nuda* (LA82) with foliar spray reduced the disease severity from 90% for

Table 3. Effects of the culture filtrates of 27 strains of 14 species of edible mushrooms on the mycelial growth of six plant pathogens[a]

Scientific name (strain)[b]	Inhibition of mycelial growth %[c]					
	ABA01	PA01	LFO1-13	AG1	PAM	PCM81
Agaricus bisporus (A5033)	17.6	0.0	0.0	0.0	0.0	0.0
Agaricus bisporus (F4KN)	37.3	0.0	0.0	0.0	0.0	23.2
Agaricus bisporus (MS)	18.8	0.0	0.0	0.0	0.0	0.0
Agaricus subrufescens (AB4)	20.0	0.0	11.6	0.0	0.0	4.4
Agaricus subrufescens (BZ40)	0.0	0.0	0.0	0.0	0.0	0.0
Agaricus subrufescens (WB)	14.1	0.0	0.0	0.0	0.0	0.0
Agrocybe chaxingu (BH45)	24.7	0.0	0.0	0.0	0.0	0.0
Agrocybe chaxingu (LUB)	0.0	0.0	0.0	0.0	0.0	0.0
Agrocybe chaxingu (LUW)	0.0	0.0	0.0	0.0	0.0	0.0
Agrocybe chaxingu (TM01)	0.0	0.0	0.0	0.0	0.0	0.0
Agrocybe cylindracea (H911)	16.4	0.0	15.6	0.0	0.0	0.0
Clitocybe nuda (999)	0.0	0.0	0.0	0.0	0.0	0.0
Clitocybe nuda (LA82)	7.6	6.8	0.0	0.0	0.0	0.0
Clitocybe nuda (LA84)	0.0	5.1	0.0	0.0	0.0	0.0
Clitocybe nuda (LNE2)	1.8	0.0	0.0	0.0	0.0	0.0
Clitocybe nuda (LNG)	0.0	3.4	0.0	0.0	0.0	0.0
Coprinus comatus (CC1)	22.5	0.0	0.0	0.0	0.0	0.0
Coprinus comatus (CC2)	0.0	0.0	0.0	0.0	0.0	0.0
Flammulina velutipes (F1)	0.0	0.0	0.0	0.0	0.0	0.0
Ganoderma lucidum (1-14)	0.0	0.0	8.6	0.0	0.0	0.0
Grifola frondosa (G1)	0.0	4.2	19.6	0.0	0.0	0.0
Lentinula edodes (L1)	0.0	0.0	20.5	0.0	0.0	0.0
Pleurotus citrinopileatus (PC)	0.0	0.0	0.0	0.0	0.0	0.0
Pleurotus eryngii (B11)	6.2	0.0	0.0	0.0	0.0	0.0
Pleurotus eryngii (B12)	15.6	0.0	0.0	0.0	0.0	10.8
Tremella aurantialba (Ta)	0.0	0.0	0.0	0.0	0.0	0.0
Tremella fuciformis (Tf)	24.4	0.0	8.1	0.0	0.0	0.0
PDB (control)	0.0	0.0	0.0	0.0	0.0	0.0

[a] ABA01: *Alternaria brassicicola*, PA01: *Colletotrichum higginsianum*, LFO1-13: *Fusarium oxysporum* f. sp. *lactucae*, PCM81: *Phytophthora capsici*, PAM: *Pythium aphanidermatum*, AG1: *Rhizoctonia solani*.

[b] Mushroom fungi were cultured in potato dextrose broth under shaking at 120 rpm for 21 days at 24°C.

[c] Inhibition of mycelial growth (%) = (colony size of control – colony size of treatment)/ colony size of control × 100. All data were means from four replicates.

Table 4. Effect of culture filtrates of 27 strains in 14 species of edible mushrooms on growth of seven plant bacterial pathogens[a]

Strain	Inhibition zone (mm)[b]						
	AAC33	Z11	ECH	PS152	XF89-6	XCC79	XV64
Agaricus bisporus (A5033)	0.0	0.0	0.0	0.0	0.0	15.9	0.0
Agaricus bisporus (MS)	0.0	0.0	0.0	0.0	0.0	15.6	0.0
Agaricus bisporus (F4KN)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Agaricus subrufescens (AB4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Agaricus subrufescens (BZ40)	0.0	0.0	0.0	0.0	0.0	0.0	9.8
Agaricus subrufescens (WB)	0.0	0.0	0.0	0.0	0.0	0.0	9.9
Agrocybe cylindracea (H911)	17.0	14.8	22.6	19.1	18.3	17.6	9.5
Agrocybe chaxingu (TM01)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Agrocybe chaxingu (LUB)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Agrocybe chaxingu (LUW)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Agrocybe chaxingu (BH45)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Clitocybe nuda (999)	0.0	0.0	0.0	0.0	14.6	22.6	8.5
Clitocybe nuda (LNG)	0.0	0.0	0.0	0.0	0.0	18.7	9.8
Clitocybe nuda (LNE2)	0.0	0.0	0.0	0.0	0.0	20.7	10.1
Clitocybe nuda (LA82)	0.0	0.0	0.0	0.0	0.0	20.0	11.0
Clitocybe nuda (LA84)	0.0	0.0	0.0	0.0	10.1	15.7	0.0
Coprinus comatus (CC1)	0.0	0.0	0.0	0.0	0.0	15.7	12.3
Coprinus comatus (CC2)	0.0	0.0	0.0	0.0	13.5	10.5	10.6
Flammulina velutipes (F1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ganoderma lucidum (1-14)	20.5	0.0	0.0	0.0	0.0	18.6	0.0
Grifola frondosa (G1)	22.0	0.0	0.0	15.3	0.0	18.1	9.8
Lentinula edodes (L1)	26.6	0.0	15.2	15.6	15.0	25.0	10.4
Pleurotus citrinopileatus (PC)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pleurotus eryngii (B11)	0.0	0.0	0.0	0.0	0.0	0.0	10.9
Pleurotus eryngii (B12)	0.0	0.0	0.0	0.0	0.0	0.0	9.0
Tremella aurantialba (Ta)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tremella fuciformis (Tf)	0.0	0.0	0.0	0.0	11.0	0.0	0.0
CK	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] AAC33: *Acidovorax avenae* subsp. *citrulli*, Z11: *Pectobacterium carotovorum* subsp. *carotovorum*, ECH: *Erwinia chrysanthemi*, PS152: *Ralstonia solanacearum*, XV64: *Xanthomonas axonopodis* pv. *vesicatoria*, XCC79: *Xanthomonas campestris* pv. *campestris*, XF89-6: *Xanthomonas oryzae* pv. *oryzae*.

[b] Values were means of four replicates for each treatment.

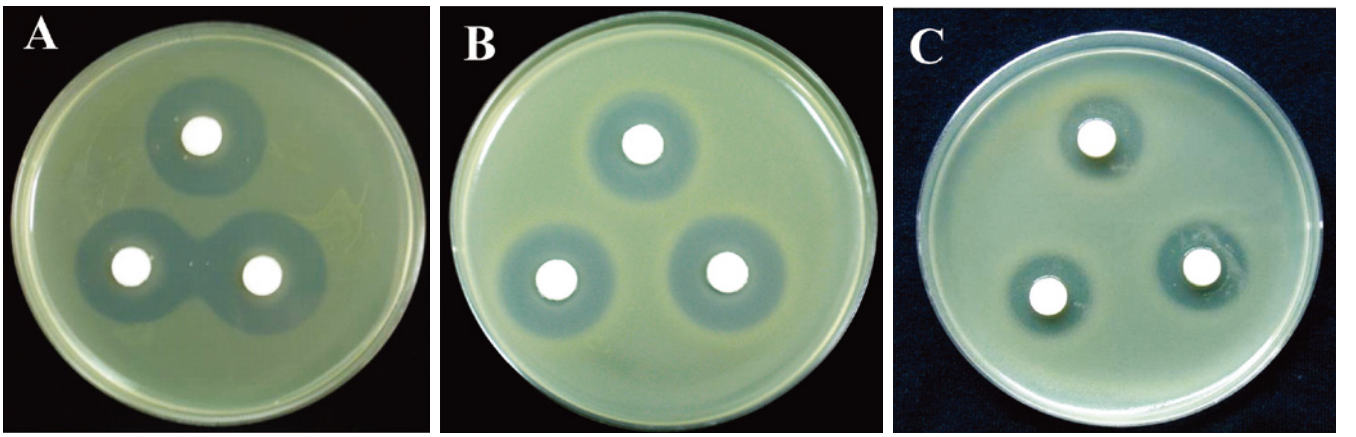

Fig. 2. Clear zones of *Xanthomonas campestris* pv. *campestris* (XCC79) inhibited by culture filtrates of (A) *Lentinula edodes* (L1); (B) *Clitocybe nuda* (LA82); and (C) *Grifola frondosa* (G1).

the control to 7.6% and 2.2%, respectively (Table 5, Fig. 3). The culture filtrate of *L. edodes* (L1) did not suppress Phytophthora blight of pepper caused by *P. capsici*.

DISCUSSION

Of the 27 culture filtrates from 14 species of edible mushrooms examined, 11 culture filtrates inhibited spore germination but not mycelial growth of tested fungal pathogens, and 18 culture filtrates inhibited the growth of pathogenic bacteria. However, the antimicrobial effects of the culture filtrates depended on the strains and species of mushroom and the species of microorganism. For example, the mushroom culture filtrate from strain LA82, but not 999, LA84, LNE2, or LNG, of *C. nuda* was highly effective for the inhibition of spore germination of *A. brassicicola*, *C. higginsianum* and *P. capsici*. Previous reports showed that different strains from the same species produced different amounts of antimicrobial compounds [24, 27].

Table 5. Effects of mushroom culture filtrates on the disease severity of Phytophthora blight of peppers caused by *Phytophthora capsici*

Mushroom fungus	Strain	Disease severity (%)[a]
Clitocybe nuda	LA82	2.2±1.5
Coprinus comatus	CC1	7.6±5.2
Lentinula edodes	L1	90.0±6.7
PDB (CK)		90.0±5.1

[a] Values were means ± standard deviations of four replicates for each treatment. The experiment was repeated twice.

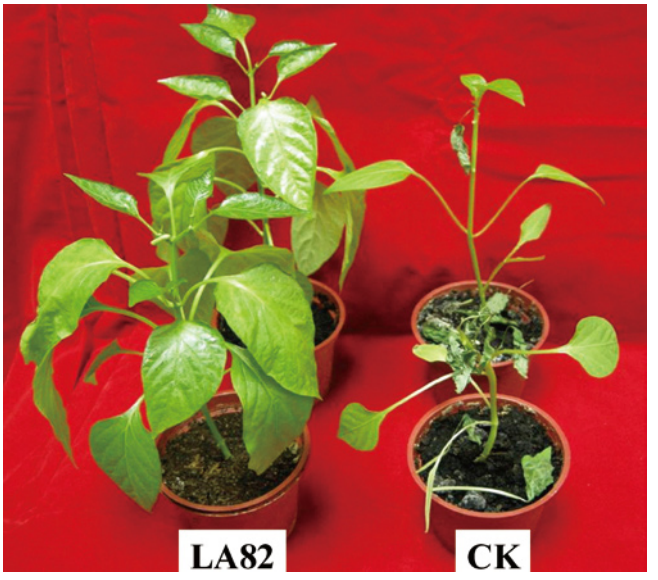

Fig. 3. Effect of culture filtrate of *Clitocybe nuda* strain LA82 on disease severity of Phytophthora blight of pepper caused by *Phytophthora capsici*.

The culture filtrate of *L. edodes* appeared to inhibit numerous species of microorganisms. Besides its inhibitory effects on *A. brassicicola*, *C. higginsianum*, *P. capsici*, *A. avenae* subsp. *citrulli*, *E. chrysanthemi*, *R. solanacearum*, *X. campestris* pv. *campestris*, *X. oryzae* pv. *oryzae* and *X. axonopodis* pv. *vesicatoria* observed in this study, the mycelial leachate of *L. edodes* was also reported to contain substances that suppressed other plant pathogens, such as *Pseudomonas syringae* pv. *glycinea*, *P. syringae* pv. *tabaci*, *Xanthomonas campestris* pv. *glycines*, *Erwinia amylovora* and *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* [22]. Although the culture filtrate of *L. edodes* (L1) was effective for the inhibition of zoospore germination of *P. capsici*, it was not effective against Phytophthora blight of pepper caused by *P. capsici* (Table 5).

The wood blewit has been considered by many authorities as belonging to the *Clitocybe* segregate genus *Lepista*. The culture filtrate of *C. nuda* (LA82) showed strong antimicrobial activity on numerous plant pathogenic fungi and bacteria in this study. The sporophore or mycelial culture of *C. nuda* has also been reported to be effective against human clinical pathogens, such as *Staphylococcus aureus* [8, 20, 27] and *Candida albicans* [8, 27]. Pepper leaves treated with the culture filtrates of *C. nuda* (LA82) and *C. comatus* (CC1) effectively reduced the disease severity of Phytophthora blight of pepper caused by *P. capsici*. To our knowledge, this is the first report of the control of a plant disease by culture filtrates of *C. nuda* and *C. comatus*.

ACKNOWLEDGEMENTS

We thank Professor K. C. Tzeng (National Chung Hsing University, Taichung, Taiwan) for supplying the isolates of bacterial plant pathogens.

LITERATURE CITED

1. Anke, T. 1989. Basidiomycetes: a source for new bioactive secondary metabolites. Prog. Ind. Microbiol. 27: 51-66.
2. Anke, T. 1995. The antifungal strobilurins and their possible ecological role. Can. J. Bot. 73: 940-945.
3. Anke, T. 1997. Strobilurins. Pages 206-212 *in*: Fungal

Biotechnology. T. Anke ed. Chapman and Hall, London. 409 pp.
4. Arnone, A., Nasini, G., and Vajna, de P. O. 1997. Marasmane sesquiterpenes from the basidiomycete *Clitocybe hydrogramma*. Phytochemistry 46: 1099-1101.
5. Benedict, R. G. and Brady, L. R. 1972. Antimicrobial activity of mushroom metabolites. J. Pharm. Sci. 61: 1820-1822.
6. Breene, W. M. 1990. Nutritional and medicinal value of specialty mushrooms. J. Food Protec. 53: 883-894.
7. Chang, S. T., Buswell, J. A., and Miles, P. G. 1991. Genetics and Breeding of Edible Mushrooms. SA. Amsterdam, Gordon and Breach Science Press. 324 pp.
8. Dulger, B., Ergul, C. C., and Gucin, F. 2002. Antimicrobial activity of the macrofungus *Lepista nuda*. Fitoterapia 73: 695-697.
9. Florey, H. W., Chain, E., Heatley, N. G., Jennings, M. A., Sanders, A. G., Abraham, E. P., and Florey, M. E. 1949. Antibiotics. Oxford University Press, London. 554 pp.
10. Hautzel, R. and Anke, T. 1990. Screening of basidiomycetes and ascomycetes for plant growth regulating substances. Introduction of the gibberellic acid induced de-novo synthesis of hydrolytic enzymes in embryoless seeds of *Triticum aestivum* as test system. Z. Naturforsch 45: 1093-1098.
11. Heim, R. 1963. Les Champignons Toxiques et Hallucinogenes. Boubee Press, Paris. 327 pp.
12. Hewitt, H. G. 1998. Fungicides in Crop Protection. CABI Publishing, Wallingford, Oxon, UK. 232 pp.
13. Hickey, K. D. 1986. Methods for Evaluating Pesticides for Control of Plant Pathogens. APS Press, MN, USA. 312 pp.
14. Janssens, L., De Pooter, H. L., Schamp, N. M., and Vandamme, E. J. 1992. Production of flavors by microorganisms. Proc. Biochem. 27: 195-215.
15. Jonathan, S. G. and Fasidi, I. O. 2003. Antimicrobial activities of two Nigerian edible macro fungi -*Lycoperdon pusilum* (Bat. Ex) and *Lycoperdon giganteus* (Pers). Afr. J. Biomed. Res. 6: 85-90.
16. Jong, S. C. and Birmingham, J. M. 1993. Medicinal and therapeutic value of the shiitake mushroom. Adv. Appl. Microbiol. 39: 153-184.
17. Ko, W. H., Chase, L., and Kunimoto, R. 1973. A microsyringe method for determining concentration of fungal propagules. Phytopathology 63: 1206-1207.
18. Korzybski, T., Kowszyk, G. Z., and Kurylowicz, W. 1967. Antifungal antibiotics (polyenes). Pages 450-715 *in*: Antibiotics: Origin, Nature and Properties. T. Korzybski ed. Translated by E. Paryski, Pergamon Press, Oxford. 2269 pp.
19. Lingappa, B. T., Lingappa, Y., and Bell, E. 1973. A self inhibitor of protein synthesis in the conidia of *Glomerella cingulata*. Arch. Microbiol. 94: 97-107.
20. Mustafa, Y. and Bilgili, F. 2006. Antimicrobial activities of fruit bodies and/or mycelial cultures of some mushroom isolates. Pharm. Biol. 44: 660-667.
21. Muto, M., Takahashi, H., Ishihara, K., Yuasa, H., and Huang, J. W. 2005. Antimicrobial activity of medicinal plant extracts used by indigenous people in Taiwan. Plant Pathol. Bull. 14: 13-24.
22. Pacumbaba, R. P., Beyl, C. A., and Pacumbaba, R. O. Jr. 1999. Shiitake mycelial leachate suppresses growth of some bacterial species and symptoms of bacterial wilt of tomato and limabean in vitro. Plant Dis. 83: 20-23.
23. Pandey, V. N. and Dubey, N. K. 1994. Antifungal potential of leaves and essential oils from higher plants against soil phytopathogens. Soil Biol. Biochem. 26: 1417-1421.
24. Sidorova, I. I. and Velikanov, L. L. 2000. Bioactive substances of agaricoid basidiomycetes and their possible role in regulation of myco-and microbiota structure in soils of forest ecosystems. II. Antibiotic activity in cultures of litter saprotrophic mushroom *Lepista nuda*. Mikol. Fitopatol. 34: 10-16.
25. Stamets, P. 1993. Growing Gourmet and Medicinal Mushrooms. Edited by C. A. Berkeley, Ten Speed Press. 552 pp.
26. Stanley, M. S., Callow, M. E., Perry, R., Alberte, R. S., Smith, R., and Callow, J. A. 2002. Inhibition of fungal spore adhesion by zosteric acid as the basis for a novel, nontoxic crop protection technology. Phytopathology 92: 378-383.
27. Suay, I., Arenal, F., Asensio, F. J., Basilio, A., Cabello, M. A., Díez, M. T., García, J. B., Val, A. G., Gorrochategui, J., Hernández, P., Peláez, F., and Vicente, M. F. 2000. Screening of basidiomycetes for antimicrobial activities. Antonie Leeuwenhoek 78: 129-139.
28. Sunwoo, J. Y., Lee, Y. K., and Hwang, B. K. 1996. Induced resistance against *Phytophthora capsici* in pepper plants in response to DL-β-amino-n-butyric acid. Eur. J. Plant Pathol. 102: 663- 670.
29. Tochikura, T. S., Nakashima, H., Ohashi, Y., and Yamamoto, N. 1988. Inhibition (in vitro) of replication and of the cytopathic effect of human immunodeficiency virus by an extract of the culture medium of *Lentinus edodes* mycelia. Med. Microbiol. Imm. 177: 235-244.
30. Xu, X. L. and Ko, W. H. 1998. A quantitative confined inoculation method for studies of pathogenicity of fungi on plants. Bot. Bull. Acad. Sin. 39: 187-190.

摘　要

陳錦桐[1, 2]、黃振文[1, 3]. 2010. 食用菇培養濾液對植物病原菌的抗菌活性. 植病會刊 19: 261-270.
([1]國立中興大學植物病理學系；[2]行政院農業委員會農業試驗所植物病理組；[3]聯絡作者，電子郵件：jwhuang@dragon.nchu.edu.tw；傳眞：+886-4-2285-1676)

評估食用菇類 27 個菌株之培養濾液的抗植物病原菌活性，香菇 (*Lentinula edodes*) 與紫丁香蘑 (*Clitocybe nuda*) 菌株之培養濾液可完全地抑制白菜炭疽病菌 (*Colletotrichum higginsianum*) 的孢子發芽，而靈芝 (*Ganoderma lucidium*)、香菇與紫丁香蘑菌株的培養濾液具有完全抑制白菜黑斑病菌 (*Alternaria brassicicola*) 孢子發芽的功效；紫丁香蘑、雞腿蘑 (*Coprinus comatus*)、香菇及金耳 (*Tremella aurantialba*) 等四個菌株培養濾液可完全抑制番椒疫病菌 (*Phytophthora capsici*) 的游走孢子發芽，所有培養濾液中只有紫丁香蘑具有中度抑制萵苣萎凋病菌 (*Fusarium oxysporum* f. sp. *lactucae*) 的分生孢子發芽的效果，其餘皆無效。以濾紙圓片法測試對植物病原細菌的抑制效果，發現有 18 個食用菌培養濾液具有抑制效果，有四個菌株培養液可顯著抑制細菌性果斑病菌 (*Acidovorax avenae* subsp. *citrulli*) 的細胞增殖；柳松菇菌 (*Agrocybe cylindracea*) 培養濾液也具抑制海芋軟腐細菌 (*Pectobacterium carotovorum* subsp. *carotovorum*) 效果；舞菇 (*Grifola frondosa*)、柳松菇與香菇的培養濾液則可抑制蓮霧青枯病菌 (*Ralstonia solanacearum*)；有6個菌株的培養濾液可抑制水稻白葉枯病菌 (*Xanthomonas oryzae* pv. *oryzae*) 的生長；有 13 個菌株的培養濾液可抑制十字花科黑腐病菌 (*X. campestris* pv. *campestris*) 和茄科細菌性斑點病菌 (*X. axonopodis* pv. *vesicatoria*)。惟前述所有培養濾液均無法有效抑制 *C. higginsianum*、*F. oxysporum* f. sp. *lactucae*、*Rhizoctonia solani* 及 *Pythium aphanidermatium* 等菌之菌絲生長。由溫室試驗的結果發現噴佈紫丁香蘑與雞腿菇菌株的培養濾液，可有效降低番椒疫病的罹病度。綜合本研究的初步成果，證明食用菇類的培養濾液具有研發成爲植物保護製劑的潛力。

關鍵詞：抗菌活性、培養濾液、食用菇類、紫丁香蘑、植物病原菌、番椒疫病菌 (*Phytophthora capsici*)