Morphology and Distribution of *Talaromyces flavus* from Soil and Potential Use as a Biological Control Agent against Plant Pathogenic Fungi

T. Dethoup[1], L. Manoch[1,]*, N. Visarathanonth[1], C. Chamswarng[2] and A. Kijjoa[3]

[1]*Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand*
[2]*Department of Plant Pathology, Faculty of Agriculture Kamphaeng Saen, Kasetsart University, Nakhon Pathom 73140, Thailand*
[3]*ICBAS- Instituto de Ciencias Biomedicas de Abel Salazar- CIIMAR, Universidade do Porto, Porto, Portugal*

**Corresponding author. Email: agrlkm@ku.ac.th*

Abstract

Using various isolation methods, 122 isolates of *Talaromyces flavus* were recorded from 45 soil samples in 38 provinces of Thailand. Morphological features of this fungus were observed, such as colony growth and color on different agar media. Microscopic characteristics were examined by stereo-, light-, and scanning electron microscopy. Isolates were found in both non-agricultural and agricultural soil in Chiang Mai and Mae Hong Son provinces following heat and alcohol treatments. Twenty isolates of *T. flavus* were selected for antagonistic tests against 15 species of plant pathogenic fungi *in vitro* and in the greenhouse. All of the selected isolates of *T. flavus* inhibited the mycelial growth of *Phytophthora palmivora*, *P. parasitica*, *Peronophythora litchii*, *Colletotrichum capsici*, *C. gloeosporioides*, *Pestalotiopsis guepinii*, *Phyllosticta* sp., *Curvularia lunata*, *Helminthosporium maydis*, *H. oryzae* and *Fusarium oxysporum*. However, none of the isolates controlled *Pythium aphanidermatum*, *Lasiodiplodia theobromae*, *Rhizoctonia solani* and *Sclerotium rolfsii in vitro.* However, in the greenhouse experiment, 20 isolates of *T. flavus* controlled stem rot of mungbean, caused by *S. rolfsii*, 7 and 14 days inoculation, and 6 isolates gave control up to 30 days inoculation.

Keywords: trichocomaceae, soil fungi, taxonomy, ecology, antagonistic test, plant parasitic fungi

Introduction

Talaromyces flavus (Klöcker) Stolk and Samson (conidial state *Penicillium vermiculatum* Dangeard) belongs to the Family Trichocomaceae of the Ascomycota. It is the type species of the *Talaromyces* and was first reported from soil of paddy fields in West Bengal, India by C.R. Benjamin (Stolk and Samson, 1972). This fungus is perhaps the commonest species of its genus with a worldwide distribution and is commonly isolated from soil and organic substrates in warmer regions (Domsch et al., 1993a, b). Pitt and Hocking (1997) stated that it occurred in food as a contaminant rather than a spoilage fungus. Udagawa et al. (1996) reported that *T. flavus* was predominantly isolated from house dust in Kobe City using the alcohol and heat treatment methods.

Some strains of *T. flavus* can produce novel bioactive compounds, such as actofunicone, deoxy-funicone and vermistatin, which reinforce the anti *Candida albicans* activity of miconazole (Arai et al., 2002). Funicone showed inhibition against a human pathogenic fungus, *Aspergillus fumigatus* (Komai et al., 2004). *T. flavus* has also been reported as biological control agent against several plant pathogenic fungi, such as *Verticillium dahliae*, causing wilt of eggplant in Israel (Fahima and Henis, 1995; Madi et al., 1997), *Sclerotinia sclerotiorum*, causing white mold of dry bean in Canada (McLaren et al., 1986) and *Sclerotium rolfsii*, causing bean stem rot in Israel (Fravel, 1996; Madi et al., 1997). The mechanisms for biocontrol included mycoparasitism (McLaren et al., 1986; Fahima et al., 1992; Madi et al., 1997), antibiosis (Kim et al., 1988; Stosz et al., 1996) and competition (Marois et al., 1982, 1984). In Thailand, several strains of *T. flavus* have been reported from soil and termite mounds in Trat, Sakon Nakhon, Bangkok and Mae Hong Son using the heat and alcohol treatment methods (Manoch, 2004).

The aims of this study were 1) to study the morphology and distribution of *T. flavus* from various soil samples from different locations, and 2) to test for antagonistic activity of *T. flavus* against 15 species of plant pathogenic fungi *in vitro* and in the greenhouse.

Materials and Methods

Fungal Isolation and Identification

Forty-five soil samples were collected from 38 provinces of Thailand from March 2002 to August 2004 (Figure 1). The soil plate, dilution plate, heat, alcohol treatment methods, and Gochenaur's glucose ammonium nitrate agar (Gochenaur, 1964) (1 g NH_4NO_3, 1 g K_2HPO_4, 0.5 g $MgSO_4.7H_2O$, 0.03 g rose bengal, 1 g yeast extract, 5 g glucose, 15 g agar, 4 mL streptomycin solution, 1 L distilled water) were employed. Identification was based on morphological characteristics, such as colony growth pattern, color, texture on malt extract agar (MEA), Czapek's agar (CZA), Czapek yeast agar (CYA), oatmeal agar (OA), cornmeal agar (CMA) and 25% glycerol nitrate agar (G25N), after 7 days incubation at 25^{o}C. For judgment of conidial and colony colours, Rayner's "A Mycological Colour Chart" (1970) was used. Microscopic characteristics were examined under stereo-, light-, and scanning electron microscopes. A preparative needle was used to pick up the penicilli and ascogonial initials from the agar surface using a stereo microscope. These were mounted in sterile distilled water and lactophenol on a slide and examined under a light microscope. Cleistothecia, asci and ascospores were mature following 10-14 days incubation. Camera lucida drawings of penicilli, conidia, ascomatal initials, asci and ascospores were made. Ascospore ornamentation was examined under a scanning electron microscope prepared as follows, ripe ascomata were transferred from a dry culture with a preparative needle and placed on double-stick scotch brand tape on aluminum stubs. The specimens were coated with gold for 5-7 min and examined in a JEOL JSM 5600 LV scanning electron microscope (Manoch et al., 2004). A single ascospore isolation technique was used. A hyphal tip from a single spore was transferred to a slant of potato dextrose agar (PDA) and kept as a pure culture in a culture collection at the Department of Plant Pathology, Faculty of Agriculture, Kasetsart University Fungal Collection (KUFC).

Table 1 Fifteen species of plant pathogenic fungi from various diseased fruits and vegetables used for antagonistic activity tests.

Plant pathogenic fungi	Host plant
Phytophthora palmivora	*Durio zibethinus* (durian)
Phytophthora parasitica	*Citrus reticulata* (orange)
Peronophythora litchii	*Litchi chinensis* (longan)
Pythium aphanidermatum	*Cucumis sativus* (cucumber)
Colletotrichum capsici	*Capsicum annuum* (chili)
C. gloeosporioides	*Pyrus pyrifolia* (pear)
Lasiodiplodia theobromae	*Garcinia mangostana* (mangosteen)
Pestalotiopsis guepinii	*Psidium guajava* (guava)
Phyllosticta sp.	*Pyrus pyrifolia* (pear)
Curvularia lunata	*Zea mays* (corn)
Fusarium oxysporum	*Lycopersicon esculentum* (tomato)
Helminthosporium maydis	*Zea mays* (corn)
Helminthosporium oryzae	*Oryza sativa* (rice)
Rhizoctonia solani	*Oryza sativa* (rice)
Sclerotium rolfsii	*Vigna radiata* (mungbean)

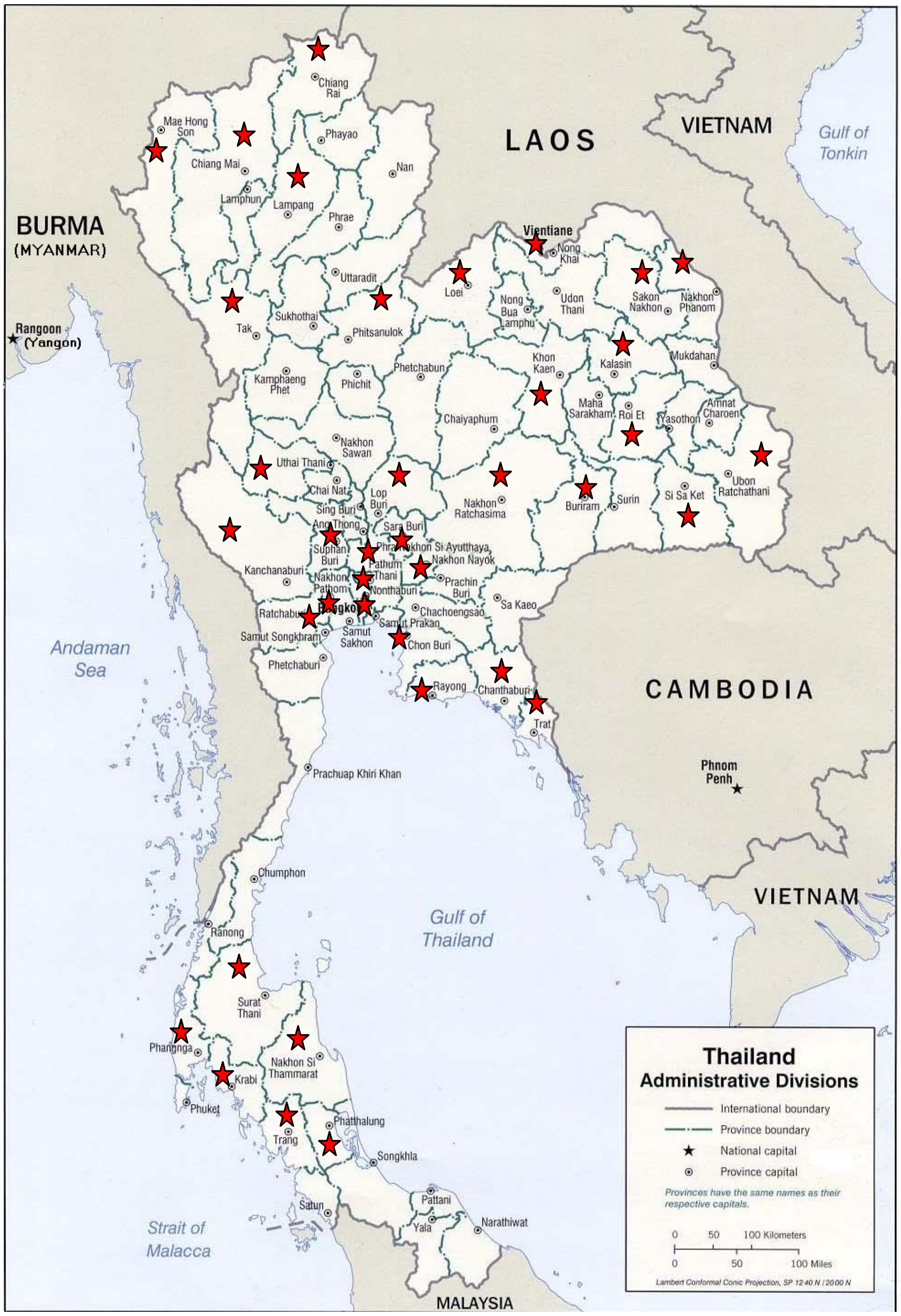

Figure 1 Map of Thailand indicating the collection sites of the soil samples from 38 provinces.

Antagonistic Tests of *Talaromyces flavus in vitro*

Twenty isolates of *T. flavus* were selected to test for antagonistic activity against 15 species of plant pathogenic fungi (Tables 1 and 2). They were cultivated as dual cultures on PDA for 14 days at 28°C. The young mycelium from the colony margin of *T. flavus* and the particular plant pathogenic fungus were cut with sterile cork borer (0.8 cm diam) and placed on PDA, 7 cm apart. All Petri dishes were incubated at room temperature (28°C) for 14 days. The inhibition levels were calculated by using the formula: G_1-G_2 /G_1 × 100 where G_1 = colony radius of the plant pathogenic fungi in the control and G_2 = colony radius of plant pathogenic fungi in the dual culture test (Intana et al., 2003). Each treatment was performed with three replicates.

Antagonistic Tests of *Talaromyces flavus* in the Greenhouse

A modification of Madi et al. (1997) method for antagonistic tests of *T. flavus* in the greenhouse was conducted. Dried mungbean seeds (*Vigna radiata* (L.)R. Wilczek) were surface-disinfested with 10% sodium hyperclorite for 5 min, rinsed 3 times with sterile water and immersed in an ascospore suspension (10^6 mL) of *T. flavus* strains for 24 h. Three 10 cm plastic pots were filled with 400 g of garden soil. Ten bean seeds were placed on the soil surface in each pot and two sclerotia were placed 0.5-1 cm apart from each bean seed. Seeds were covered with 150 g of soil, and the pot was incubated in the greenhouse at temperatures ranging from 28 to 30^{o}C. Disease symptoms were recorded at 7 and 14 days after planting. There were three treatments in the experiment: mungbean + *S. rolfsii* + *Trichoderma harzianum*, mungbean + distilled water and mungbean + *S. rolfsii*. Disease reduction by *T. flavus* treatment against plant pathogenic fungi was described previously by Madi et al. (1997). The experiment was conducted three times, with 3 replicates per treatment. Each pot was served as a replicate, and the data were pooled for analysis.

Table 2 Twenty isolates of *Talaromyces flavus* isolated from various locations used for antagonistic tests against plant pathogenic fungi.

T. flavus strain KUFC	Location	Method[1/]	Collecting date
3334	Forest soil, Mae Hong Son	ht	3 Dec. 2003
3363	Watermelon field soil, Chon Buri	alc	2 Jan. 2002
3381	Chili garden soil, Kasetsart Univ., Bangkok	ht	11 Feb. 2002
3388	Mango field soil, Chiang Mai	sp	17 July 2004
3395	Rice paddy field soil, Suphan Buri	alc	5 Jan. 2002
3397	Cucumber garden soil, Nonthaburi	ht	9 May 2003
3400	Corn field soil, Chiang Mai	alc	17 July 2004
3446	Rice paddy field soil, Chiang Mai	alc	29 Jan. 2002
3450	Longan field soil, Chiang Rai	ht	16 Jan. 2004
3473	Non-agricultural soil, Krabi	alc	10 Aug. 2003
3483	Tomato garden soil, Nonthaburi	ht	9 May 2003
3485	Mungbean field soil, Nakhon Ratchasima	ht	25 Oct. 2002
3501	Cucumber garden soil, Chiang Mai	alc	17 July 2004
3506	Rice paddy field soil, Bangkok	ht	11 Feb. 2002
3508	Cabbage garden soil, Chiang Mai	alc	17 July 2004
3523	Cucumber garden soil, Kanchanaburi	sp	30 June 2002
3525	Kale garden soil, Nonthaburi	ht	9 May 2003
3528[2/]	Mungbean field soil, Chon Buri	ht	2 Jan. 2002
3530[2/]	Durian field soil, Chanthaburi	dp	24 Nov. 2004
3550	Forest soil, Sakon Nakhon	ht	16 Sep. 2001

[1/] ht = heat treatment method, alc = alcohol treatment method, sp = soil plate technique, dp = dilution plate technique.
[2/] indicate the most effective strains for antagonistic activity tests.

Results and Discussion

One hundred and twenty-two isolates of *T. flavus* were found in 45 soil samples from 38 provinces (Table 3, Figure 1). Most isolates of *T. flavus* were derived from heat treatment (64 isolates) and alcohol treatment methods (38) followed by the soil plate (14) and dilution plate methods (6) (Table 3). Similar results were recorded by Udagawa (1996) when he searched for *Neosartorya* species from house dust in Kobe, using a heat treatment method a number of *T. flavus* were isolated. This fungus has been recorded as a heat resistant fungus in food products (Udagawa, 2000). We made most isolations using heat and alcohol treatment techniques, in contrast with fewer isolates derived from the soil plate and dilution plant methods. Isolates were most commonly found in non-agricultural soil (56 isolates) followed by agricultural soil (51) and forest soil (15) (Table 3). Isolates were obtained most frequently from Chiang Mai, Mae Hong Son, Krabi, and Bangkok. In Trat province, *T. flavus* was predominantly found from nonfertile, dry soil by the roadside.

Stolk and Samson (1972) stated that *T. flavus* is an extremely variable species. Different strains may vary in colour, in the amount of red pigment produced, in the number of penicilli and in size of the ascospores. We also found the variation among different *T. flavus* isolates (Figures 2L, 2M, 2N, 2O, 2P). Differences in the shape, size, and ornamentation of ascospores were also noted.

Talaromyces flavus **(KlÖcker) Stolk and Samson** (Figure 2)

Reference: Stolk and Samson (1972)

Specimens examined: KUFC 3334 forest soil, Mae Hong Son; KUFC 3363 watermelon field soil, Chon Buri; KUFC 3381 chili garden soil, Kasetsart Univ., Bangkok; KUFC 3388 mango field soil, Chiang Mai; KUFC 3395 rice paddy field soil, Suphan Buri; KUFC 3397 cucumber garden soil, Nonthaburi; KUFC 3400 corn field soil, Chiang Mai; KUFC 3485 mungbean field soil, Nakhon Ratchasima; KUFC 3446 rice paddy field soil, Chiang Mai; KUFC 3450 longan field soil, Chiang Rai; KUFC 3473 non-agricultural soil, Krabi; KUFC 3483 tomato garden soil, Nonthaburi; KUFC 3501 cucumber garden soil, Chiang Mai; KUFC 3506 rice paddy field soil, Bangkok; KUFC 3508 cabbage garden soil, Chiang Mai; KUFC 3523 cucumber garden soil, Kanchanaburi; KUFC 3525 kale garden soil, Nonthaburi; KUFC 3528 mungbean field soil, Chon Buri; KUFC 3530 durian field soil, Chanthaburi; KUFC 3550 forest soil, Sakon Nakhon.

Morphological characteristic of *T. flavus* isolate KUFC 3530: Colonies on CZA growing slowly, attaining a diameter of 2.5-2.8 cm in 7 days at 25°C, plane, more or less funiculose, consisting of basal felt with Pure Yellow (R14) aerial mycelium, producing numerous developing ascomata on the felt after 10 days, Light Yellow or Pure Yellow (R14); conidiogenesis sparse, inconspicuous; exudates sparse, Pale Orange; reverse Red (R2) but white at margin (Figure 2A).

Colonies on CYA spreading broadly, attaining a diameter of 5.5-6.0 cm in 7 days at 25°C, velvety, plane, zonate, consisting of a thin basal felt, producing abundant young ascomata in a layer on the felt; conidiogenesis sparse, Sulphur Yellow (R15) to Pure Yellow (R14); exudates Pale Orange at center; reverse ranging from Yellow (R14), Orange (R7) to Umber (R9) (Figure 2B).

Colonies on MEA growing rapidly, attaining a diameter of 5.0-5.5 cm in 7 at 25°C, consisting of basal felt in which numerous developing ascomata occur on the entire surface, usually forming a continuous thick layer, conspicuous yellow, ranging from Sulphur Yellow (R15) to Pure Yellow (R14), but in some strains, e.g. KUFC 4409 producing a conspicuous pinkish shade of Rosy Vinaceous (R58) grew moderately rapidly, 4.0-4.5 cm within 14 days and produced paler yellow ascomata (Figure 2N). Conidial sparse and intermixed with aerial hyphae; exudates limited; reverse conspicuous brown, ranging from Pale Brown to Umber (R9) (Figure 2C).

Colonies on CMA growing rapidly, reaching 4.0-5.0 cm in diameter in 7 days at 25°C, plane, funiculose, producing abundant ascomata on the agar surface, Light Yellow or Pure Yellow (R14); conidiogenesis moderate; exudates scattered, as small clear drops; reverse uncolored (Figure 2D).

Colonies on OA spreading broadly, reaching 5.0–5.5 cm in diameter in 7 days at 25°C, velvety to more or less funiculose, consisting of a thin basal felt, yellow, ascomata slowly developing on the felt, Sulphur Yellow (R15); conidiogenesis limited;

Table 3 Distribution of *Talaromyces flavus* from various locations using different isolation methods.

KUFC[1/]	Isolation method[2/]	Source[3/]	Location	KUFC[1/]	Isolation method[2/]	Source[3/]	Location
3331	ht	A	Chon Buri	3400	alc	A	Chiang Mai
3332	sp	A	Chon Buri	3402	ht	NA	Nakhon Phanom
3334	ht	F	Mae Hong Son	3403	ht	NA	Suphan Buri
3335	ht	F	Kalasin	3405	ht	A	Trat
3336	ht	NA	Chiang Mai	3406	ht	NA	Trat
3337	ht	A	Bangkok	3407	alc	A	Nakhon Ratchasima
3338	alc	NA	Chon Buri	3408	ht	NA	Nakhon Ratchasima
3340	alc	NA	Chon Buri	3409	ht	F	Nakhon Si Thammarat
3341	sp	NA	Chon Buri	3411	ht	F	Nakhon Si Thammarat
3344	alc	A	Lop Buri	3412	sp	F	Phangnga
3358	ht	NA	Chiang Rai	3414	alc	F	Chiang Mai
3360	ht	F	Chiang Rai	3415	alc	A	Trang
3361	alc	NA	Lampang	3416	alc	A	Trang
3362	sp	NA	Chiang Mai	3418	ht	A	Rayong
3363	alc	A	Chon Buri	3419	ht	A	Krabi
3369	ht	F	Mae Hong Son	3420	alc	NA	Surat Thani
3369	ht	F	Mae Hong Son	3424	sp	NA	Chiang Mai
3370	ht	F	Chiang Mai	3426	ht	A	Saraburi
3375	ht	A	Chiang Mai	3427	dp	NA	Khon Kaen
3376	sp	NA	Buri Rum	3429	dp	NA	Sakon Nakhon
3381	ht	A	Bangkok	3430	dp	NA	Sakon Nakhon
3382	alc	NA	Tak	3431	dp	NA	Saraburi
3383	alc	NA	Tak	3432	alc	NA	Saraburi
3388	sp	A	Chiang Mai	3433	alc	A	Nakhon Pathom
3389	alc	NA	Phitsanulok	3434	sp	A	Nakhon Pathom
3390	alc	NA	Phitsanulok	3435	ht	NA	Ubol Ratchathani
3391	alc	A	Loei	3436	ht	NA	Ubol Ratchathani
3392	alc	NA	Loei	3442	ht	NA	Chiang Mai
3394	ht	NA	Loei	3445	ht	NA	Si Sa Ket
3395	alc	A	Suphan Buri	3446	alc	A	Chiang Mai
3397	ht	A	Nonthaburi	3447	alc	NA	Mae Hong Son
3450	ht	A	Chiang Rai	3539	alc	NA	Ratchaburi
3456	ht	NA	Nakhon Nayok	3541	alc	A	Ratchaburi
3457	ht	A	Ayutthaya	3542	ht	NA	Rayong
3458	ht	NA	Roi Et	3543	ht	A	Trat
3459	ht	NA	Roi Et	3544	ht	NA	Trat
3466	ht	NA	Roi Et	3549	ht	A	Surat Thani
3467	ht	A	Nakhon Ratchasima	3550	ht	F	Sakon Nakhon
3473	alc	NA	Krabi	3554	ht	F	Khon Kaen
3474	sp	A	Loei	3555	ht	F	Chiang Mai
3467	ht	A	Nakhon Ratchasima	3556	dp	F	Chiang Mai
3468	ht	NA	Chiang Mai	3557	ht	A	Trang
3469	ht	NA	Chiang Mai	3558	ht	A	Trang
3483	ht	A	Nonthaburi	3559	ht	NA	Surat Thani
3485	ht	A	Nakhon Ratchasima	3605	ht	NA	Bangkok
3491	ht	NA	Bangkok	3606	ht	NA	Krabi
3492	ht	NA	Bangkok	3608	ht	NA	Krabi
3501	alc	A	Lumpang	3611	ht	A	Krabi
3503	ht	NA	Chon Buri	3614	sp	NA	Bangkok
3506	ht	A	Bangkok	3617	sp	A	Nakhon Nayok
3508	alc	A	Chiang Mai	3618	alc	F	Nakhon Nayok
3509	alc	NA	Uthai Thani	3619	sp	NA	Chon Buri
3510	alc	NA	Nakhon Nayok	3627	alc	NA	Nong Khai
3516	alc	NA	Krabi	3628	ht	A	Tak
3517	alc	NA	Chiang Mai	3639	ht	A	Krabi
3523	sp	A	Kanchanaburi	3640	alc	A	Phatthalung
3525	ht	A	Nonthaburi	3647	sp	NA	Chiang Mai
3528	ht	A	Chon Buri	3651	ht	A	Saraburi
3530	dp	A	Chanthaburi	3659	sp	A	Suphan Buri
3537	alc	NA	Ratchaburi	3660	alc	A	Suphan Buri
3538	alc	A	Chiang Mai	3661	alc	NA	Mae Hong Son

[1/] KUFC = Kasetsart University Fungal Collection.
[2/] sp = soil plate method, dp = dilution plate method, alc = alcohol treatment, ht = heat treatment.
[3/] A = agriculture soil, NA = non-agricultural soil, F = forest soil.

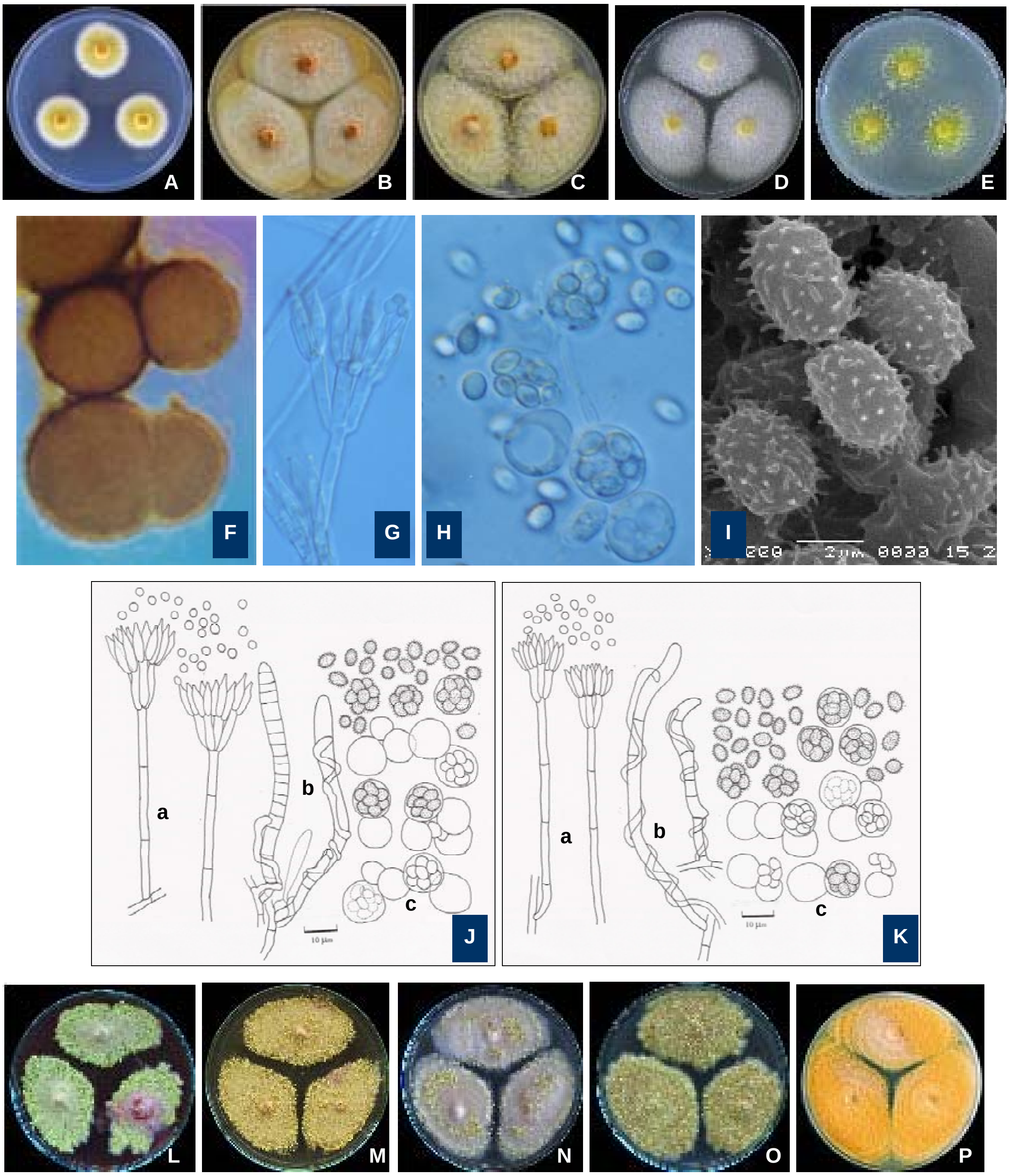

Figure 2 *Talaromyces flavus* KUFC 3530: colonies on CZA (A), CYA (B), MEA (C), CMA (D), G25N (E) incubated for 7 days at 25^{o}C; light and SEM micrographs showing ascomata (F), penicilli (G), asci and ascospores (H), ascospores (I), Camera lucida drawings of *T. flavus* KUFC 3530 (J) and KUFC 3334 (K) showing penicilli (a), ascomatal initials (b), asci and ascospores (c). Variation of colonies on MEA 14 days at 28^{o}C: *T. flavus* KUFC 3331 (L), KUFC 3406 (M), KUFC 4409 (N), KUFC 3433 (O), KUFC 3381 (P).

margin spreading broadly and submerged; exudates absent; reverse uncolored.

Colonies on G25N growing slowly, attaining a diameter of 2.0 cm in 7 days at 25^{o}C, mycelium and conidiogenesis sparse; abundant developing ascomata on the entire surface, Sulphur Yellow (R15) to Pure Yellow (R14); conidiogenesis limited; exudates absent; reverse Pale Yellow (Figure 2E).

Microscopic characters of *T. flavus* (KUFC 3530) were examined. Ascomata Sulphur Yellow (R15) to

Pure Yellow (R14), usually globose, 100–300 µm in diameter, discrete or confluent, ripening within 10 to 14 days. Covering consisting of thin interwoven hyphal networks, surrounded by loose wefts of yellow, heavily encrusted, slightly twisted, predominantly radiating hyphae, about 1-1.5 µm in diameter (Figure 2F). Ascothecial initials consisting of club-shaped ascogonia, around with thin antheridia coil tightly several times, fusing with the ascogonium at the central or higher portion of the latter cell. After fertilization, the ascogonia become septate (Figures 2J, 2K). The primordium becomes enveloped in closely wound delicate hyphae, which develop into the covering, and develops into ascogenous cell. Asci broadly ellipsoidal to subglobose, 6-7×4.5-5.5 µm (Figures 2H, 2J, 2K). Ascospores ellipsoidal, 2.5-3×1.5-2 µm, in some strains slightly larger, up to 3.7-2.2 µm, very delicately spinulose, with the spines usually irregularly disposed on the surface (Figures 2H, 2I, 2J, 2K).

Conidiophores arising from the aerial mycelium, 20-70×2-2.5 µm, yellow-green, with smooth wall. Penicilli typically biverticillate, rarely monoverticillate, and terverticillate. Metulae in small verticils of 2 to 4, yellow-green, 10-12×2-2.5 µm. Phialides 2 to 6 in the verticil, usually yellow-green, 8-10×2-3 µm, typically lanceolate. Conidia pale greenish, subglobose to ellipsoidal, 2.7-3.5×2-2.7 µm, smooth-walled (Figures 2G, 2J, 2K).

T. flavus is the most common species of *Talaromyces* from soil with a worldwide distribution. It is frequently isolated not only from soil, but also from other organic substrates. This fungus is most commonly reported from the warmer regions from different parts of the world including Turkey, Egypt, equatorial West Africa, Uganda, South Africa, India, Pakistan and tropical Australia (Asan, 2004; Domsch et al., 1993a,b). Pitt and Hocking (1997) reported *T. flavus* from food as a contaminant rather than a spoilage fungus. It has also been reported from fruit juice in Japan and Netherlands (Samson et al., 2002; Udagawa, 2000). It can survive in a heat-treated soil at 70^{o}C for 30 min, but ascospores were killed at 80^{o}C for 40 min in apple juice (Samson et al., 2002).

Antagonistic Effect of *T. flavus* Against Plant Pathogenic Fungi *in vitro*

Twenty isolates of *T. flavus* effectively inhibited mycelial growth of the 3 oomycetous plant pathogenic fungi including *Phytophthora palmivora*, *P. parasitica* and *Peronophythora litchii* on PDA, at 28^{o}C, but failed to inhibit *Pythium aphanidermatum in vitro* (Table 4, Figures 3A, 3B, 3E).

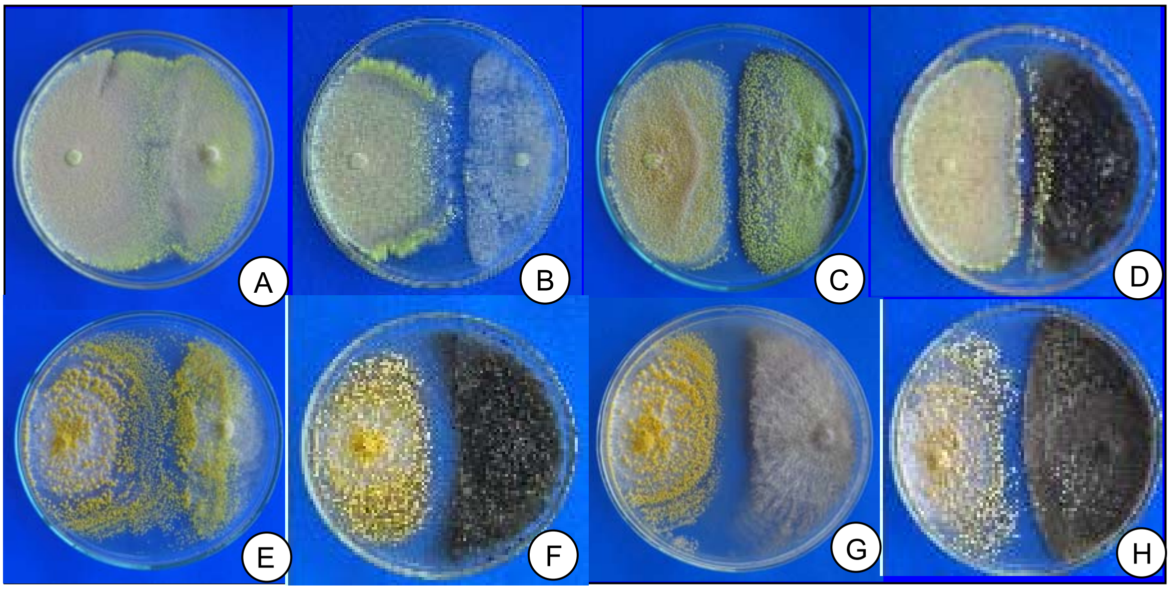

Figure 3 Antagonistic tests as dual cultures of different *Talaromyces flavus* isolates (left) and plant pathogenic fungi (right) on PDA incubated for 14 days at 28^{o}C. *T. flavus* (KUFC 3523) vs *Phytophthora palmivora* (A), *Phytophthora parasitica* (B), *Phyllosticta* sp. (C), *Colletotrichum capsici* (D). *T. flavus* (KUFC 3528) vs *Phytophthora palmivora* (E), *Colletotrichum capsici* (F), *Fusarium oxysporum* (G), *Curvularia lunata* (H).

Table 4 Percent inhibition on mycelial growth of four oomycetous and hyphomycetous plant pathogenic fungi by twenty isolates of *Talaromyces flavus* cultivated on PDA as dual culture at 28°C for 14 days.

Talaromyces flavus KUFC	Inhibition (%)							
	Phytophthora palmivora	*Phytophthora parasitica*	*Pythium aphanidermatum*	*Peronophythora litchii*	*Curvularia lunata*	*Fusarium oxysporum*	*Helminthosporium maydis*	*H. oryzae*
3334	76.92	75.00	10.27	77.50	60.00	1/	72.45	70.67
3363	75.00	74.62	19.20	76.27	54.67	67.82	70.31	1/
3381	78.05	70.59	2/	76.27	65.54	71.21	75.34	72.32
3388	78.05	75.00	9.45	75.00	60.42	72.32	70.69	70.55
3395	78.75	77.32	2/	77.50	62.00	73.33	72.51	71.89
3397	69.56	72.50	2/	75.32	65.37	1/	73.39	70.34
3400	75.20	76.65	2/	74.78	60.36	75.14	74.27	75.98
3485	72.39	71.26	7.32	75.00	61.19	75.06	76.43	76.14
3446	70.79	68.45	4.67	78.32	55.34	70.69	70.42	75.89
3450	77.27	75.00	2/	76.47	57.38	1/	78.36	74.56
3473	81.93	71.26	2/	79.51	61.45	77.14	75.56	76.78
3483	70.59	70.59	2/	75.55	62.41	75.85	76.64	1/
3501	76.47	70.59	11.89	70.58	60.37	1/	75.00	74.34
3506	70.45	67.77	4.78	68.89	56.11	68.71	74.80	72.14
3508	73.78	73.65	2/	74.34	66.91	75.11	75.67	70.45
3523	83.24	79.56	2/	80.45	2/	76.02	78.65	77.50
3525	78.22	75.00	2/	76.16	65.90	1/	79.21	76.76
3528	80.69	80.48	12.80	79.54	69.20	79.31	79.88	79.08
3530	79.01	77.50	19.24	79.50	69.39	78.35	80.31	78.32
3550	75.02	73.76	7.42	75.00	65.03	75.26	79.41	70.77

1/ Plates contamination.
2/ Plant pathogenic fungi overgrew the colony of *T. flavus*.

Twenty isolates of *T. flavus* inhibited mycelial growth of the 3 coelomycetous plant pathogenic fungi including *Colletotrichum capsici*, *C. gloeosporioides*, and *Pestalotiopsis guepinii*, but did not control *Lasiodiplodia theobromae in vitro* (Table 5, Figures 3C, 3D, 3F).

Percent inhibition of mycelial growth for the 5 hyphomycetous plant pathogenic fungi by *T. flavus* strains varied with the plant pathogens by isolate combination tested. Twenty isolates inhibited more than 70% of the radial growth of *Helminthosporium maydis* and *H. oryzae* on PDA. Two isolates of *T. flavus*, KUFC 3530 and KUFC 3528, provided 69.39 and 69.20 % inhibition of the radial growth of *Curvularia lunata*, respectively (Table 4, Figure 3H), whereas 18 other isolates of *T. flavus* produced moderate inhibition of the radial growth of this plant pathogen. Both strains of *T. flavus* showed nearly 80% inhibition of mycelial growth of *Fusarium oxysporum* (Table 4, Figure 3G). However, 20 isolates of *T. flavus* did not inhibit agonomycetous plant pathogenic fungi, *Sclerotium rolfsii* and *Rhizoctonia solani in vitro*.

Antagonistic Effect of *T. flavus* Against Plant Pathogenic Fungi in the Greenhouse

The efficacy of *T. flavus* in the greenhouse as a biological control agent against *Sclerotium rolfsii*, the causal agent of bean stem rot, indicated that the highest seedlings survival occurred at 92.59, 88.37

Table 5 Percent inhibition on mycelial growth of five coelomycetous and two agonomycetous plant pathogenic fungi by twenty isolates of *Talaromyces flavus* cultivated on PDA as dual culture at 28°C for 14 days.

Talaromyces flavus KUFC	Inhibition (%)						
	Colletotrichum capsici	*Colletotrichum gloeosporioides*	*Pestalotiopsis quepinii*	*Lasiodiplodia theobromae*	*Phyllosticta* sp.	*Sclerotium rolfsii*	*Rhizoctonia solani*
3334	70.56	65.45	55.46	10.34	79.01	4.56	6.67
3363	69.56	60.34	52.32	[1/]	62.75	[1/]	1.22
3381	70.45	65.00	50.54	[1/]	63.64	[1/]	4.45
3388	67.03	68.75	51.12	[1/]	64.80	7.9	1.99
3395	67.76	65.77	52.38	[1/]	72.73	[1/]	[1/]
3397	69.50	65.89	42.78	[1/]	68.18	[1/]	[1/]
3400	71.35	67.34	59.31	7.32	63.78	[1/]	[1/]
3485	72.78	65.00	52.44	[1/]	60.77	[1/]	[1/]
3446	73.45	66.07	54.80	[1/]	65.19	5.21	1.89
3450	74.24	66.67	55.00	[1/]	60.67	2.34	4.56
3473	76.49	69.57	50.38	6.75	57.89	[1/]	3.28
3483	77.06	66.67	55.90	7.32	67.52	[1/]	5.12
3501	75.67	67.06	60.67	7.34	72.41	[1/]	4.45
3506	67.34	55.00	58.40	[1/]	77.49	[1/]	[1/]
3508	74.00	58.71	57.47	10.45	72.84	[1/]	[1/]
3523	76.88	68.31	59.61	[1/]	85.00	1.44	7.23
3525	74.19	65.00	61.33	[1/]	73.65	4.34	3.16
3528	78.36	67.41	62.32	11.40	80.00	3.11	5.35
3530	79.45	69.90	60.82	[1/]	79.11	9.12	10.12
3550	70.22	67.34	60.34	[1/]	70.00	[1/]	[1/]

[1/] Plant pathogenic fungi overgrew the colony of *T. flavus*.

and 87.54% in the treatment with an ascospore suspension of *T. flavus* isolates KUFC 3523, 3528 and 3530, respectively, at 7 days after planting (Table 6, Figure 4). In contrast, seedlings survival was 38.44% for the control of mungbean seeds with *S. rolfsii*. Treatments with *T. flavus* KUFC 3530, 3523 and 3334 provided highly effective in increasing seedling survival at 83.33, 82.22 and 80.22 %, respectively, at 14 days after planting (Table 6).

The highest percentage of seedling survival at 30 days after planting were 45.33 and 41.34 % when mungbean seeds were treated with ascospore suspensions of *T. flavus* KUFC 3530 and KUFC 3334. All other isolates failed to control this plant pathogen at 30 days after planting (Table 6).

None of the *T. flavus* isolates gave high inhibition of *S. rolfsii in vitro*, because *S. rolfsii* thoroughly colonized *T. flavus* in the Petri dishes. However, in the greenhouse experiment, isolates KUFC 3530 and 3334 controlled *S. rolfsii*, as well or better than *Trichoderma harzianum*, which showed 34.45% seedlings survival 30 days after inoculation (Table 6). These results indicates that mechanisms other than antibiotic production may be responsible for the disease control of *S. rolfsii* in the greenhouse experiment.

T. flavus has been reported to suppress Verticillium wilt of tomato, eggplant and tomato (Fahima and Henis, 1995; Madi et al., 1997; Marois et al., 1984; Tjamos and Fravel, 1997) and parasitizes *Sclerotinia sclerotiorum* (McLaren et al., 1986; Huang et al., 2000) and *Sclerotium rolfsii* (Fravel, 1996; Madi et al., 1997). The mechanisms of biocontrol against plant pathogens include mycoparasitism (McLaren et al., 1986; Fahima et al., 1992; Madi et al., 1997), antibiotic production (Kim et al., 1990; Stosz et al., 1996) and competition

Table 6 Percent survival of mungbeen seedlings, after seeds were treated with *T. flavus* and inoculated with *Sclerotium rolfsii* at 7 and 14 days after inoculation.

Talaromyces flavus KUFC	Seedling survival (%)		
	7 days	14 days	30 days
KUFC 3334 + *S. rolfsii*	85.45	80.22	41.34
KUFC 3363 + *S. rolfsii*	85.69	74.98	0
KUFC 3381 + *S. rolfsii*	83.33	80.55	0
KUFC 3388 + *S. rolfsii*	80.24	75.10	0
KUFC 3395 + *S. rolfsii*	77.91	69.45	0
KUFC 3397 + *S. rolfsii*	87.22	78.44	0
KUFC 3400 + *S. rolfsii*	81.67	73.33	25.35
KUFC 3485 + *S. rolfsii*	81.34	77.77	0
KUFC 3446 + *S. rolfsii*	82.05	74.87	0
KUFC 3450 + *S. rolfsii*	81.67	76.11	0
KUFC 3473 + *S. rolfsii*	79.98	78.32	0
KUFC 3483 + *S. rolfsii*	78.56	70.45	11.90
KUFC 3501 + *S. rolfsii*	74.44	71.00	0
KUFC 3506 + *S. rolfsii*	86.11	80.56	0
KUFC 3508 + *S. rolfsii*	86.11	80.56	0
KUFC 3523 + *S. rolfsii*	92.59	82.22	12.43
KUFC 3525 + *S. rolfsii*	88.37	79.56	0
KUFC 3528 + *S. rolfsii*	87.54	76.42	0
KUFC 3530 + *S. rolfsii*	86.11	83.33	45.33
KUFC 3550 + *S. rolfsii*	81.15	75.66	0
Mungbean + *S. rolfsii* + *T. harzianum*[1/]	93.45	82.04	34.45
Mungbean + H_2O	97.22	94.44	94.44
Mungbean + *S. rolfsii*	38.44	30.45	0

[1/] Mungbean seeds were treated with powder formulation of *Trichoderma harzianum* (Unigreen ®).

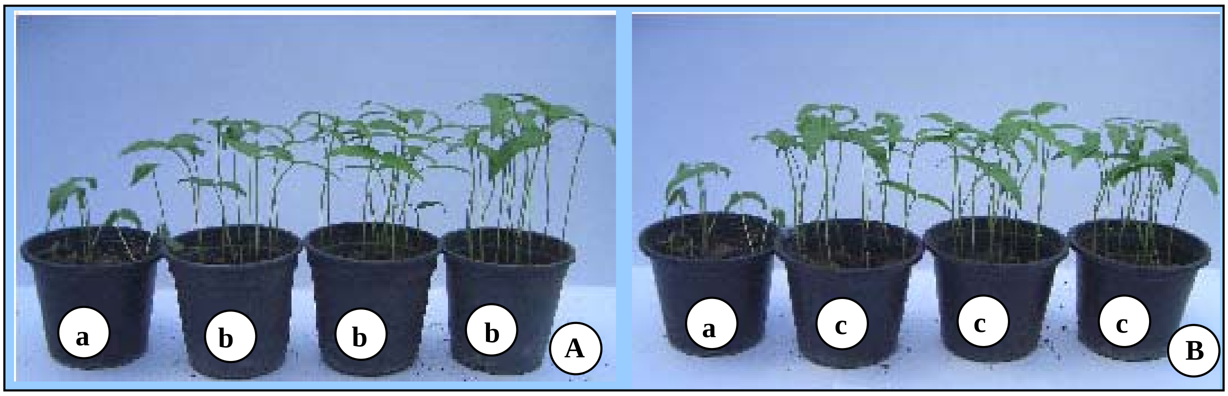

Figure 4 A. Mungbean seeds + *S. rolfsii* (control) (a), mungbean seeds + *Talaromyces flavus* KUFC 3530 + *S. roflsii* (b); B. mungbean seeds + *T. flavus* KUFC 3528 + *S. rolfsii* (c), 7 days after planting.

(Marois et al., 1982). In addition, *T. flavus* has been reported to produce several cell wall degrading enzymes responsible for antagonistic activity against phytopathogenic fungi (Madi et al., 1997). Glucose oxidase displayed important enzyme activity versus *V. dahliae* by inhibiting germination, hyphal growth, and melanization of microsclerotia (Madi et al., 1997; Stosz et al., 1998). *T. flavus* chitinase inhibited cell wall formation in *Verticillium dahliae*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani*, and inhibited spore germination and germ tube elongation of *Alternaria alternata*, *Fusarium moniliforme* and *Magnaporthe grisea* (Duo-Chuan et al., 2005; Inglis and Kawchuk, 2002).

Nagtzaam and Bollen (1997) reported colonization of roots of eggplant and potato by *T. flavus* from coated seed. They found that the ability of *T. flavus* to colonize plant roots may contribute to disease suppression by reducing the proliferation of the pathogens on the roots by direct mycoparasitism or competition. Madi et al. (1997) reported that a mutant strain of *T. flavus* exhibited high extracellular enzymes activity including chitinase, as well as mycoparasitism and in the biological control of *S. rolfsii*. Microscopic examination of the parasitic process revealed the presence of swollen segments and appressorium-like structures which have not been observed in wild-type strains of *T. flavus*.

T. flavus produces four antibiotics: vermiculine (Fuska et al., 1972), vermistatin (Fuska et al., 1979a), vermicillin (Fuska et al., 1979b) and talaron (Mizuno et al., 1974). Talaron, a pale yellow compound has been recorded as strong antifungal agent when grown in a culture medium containing 8% glucose, but no antibacterial activity was reported (Mizuno et al., 1974). Under similar culture condition, Fravel et al. (1987) reported *T. flavus* produced an extracellular metabolite with strong antimicrobial effects against fungi, bacteria and protozoa. This metabolite inhibited the radial growth and sclerotial formation of *V. dahliae*. The metabolite subsequently was identified as glucose oxidase.

Kim et al. (1990) identified the metabolite glucose oxidase, which catalyzes the oxidation of glucose to gluconate and hydrogen peroxide. Glucose oxidase in the presence of glucose killed the microsclerotia of *V. dahliae in vitro* and in sterile soil, whereas glucose oxidase, glucose, and gluconate were not inhibitory to *V. dahliae* when used individually. However, hydrogen peroxide was highly toxic to the microsclerotia of *V. dahliae* (Kim et al., 1988).

Conclusions

One hundred and twenty-two isolates of *T. flavus* have been isolated from 45 soil samples from 38 provinces of Thailand, predominantly by heat and alcohol treatment methods from non-agricultural soil. There are variations among different isolates of *T. flavus*.

The antagonistic activity tests revealed that selected 20 isolates of *T. flavus* effectively inhibited mycelial growth of *Phytophthora palmivora, P. parasitica*, *Helminthosporium maydis*, *H. oryzae*, *Fusarium oxysporum*, *Colletotrichum capsici* and *C. gloeosporioides*. However, they did not control *Pythium aphanidermatum, Lasiodiplodia theobromae, Rhizoctonia solani* and *Sclerotium rolfsii in vitro*. The greenhouse experiment indicated that 20 isolates of *T. flavus* could control *Sclerotium rolfsii,* stem rot of mungbean 7 and 14 days inoculation. However, only 6 isolates of *T. flavus* inhibited *S. rolfsii*, at 30 days after inoculation.

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