
New record of *Chaetomium* species isolated from soil under pineapple plantation in Thailand

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Pornsuriya, C., Lin, F.C., Kanokmedhakul, S. and Soyong, K. (2008). New record of *Chaetomium* species isolated from soil under pineapple plantation in Thailand. *Journal of Agricultural Technology* 4(2): 91-103.

Chaetomium species were isolated from soil in pineapple plantations in Phatthalung and Rayong provinces by soil plate and baiting techniques. Taxonomic study was based on available dichotomously keys and monograph of the genus. Five species are recorded as follows: *C. aureum*, *C. bostrychodes*, *C. cochliodes*, *C. cupreum* and *C. gracile*. Another four species are reported to be new records in Thailand as follows: *C. carinthiacum*, *C. flavigenum*, *C. perlucidum* and *C. succineum*.

Key words: *Chaetomium*, Taxonomic study

Introduction

Chaetomium is a fungus belonging to Ascomycota of the family Chaetomiaceae which established by Kunze in 1817 (von Arx *et al.*, 1986). *Chaetomium* Kunze is one of the largest genera of saprophytic ascomycetes which comprise more than 300 species worldwide (von Arx *et al.*, 1986; Soyong and Quimio, 1989; Decock and Hennebert, 1997; Udagawa *et al.*, 1997; Rodríguez *et al.*, 2002). Approximately 20 species have been recorded in Thailand (Table 1). *Chaetomium* species are well known as coprophilous, seed and soil fungi (Somrithipol, 2004; Somrithipol *et al.*, 2004), and also found in organic compost (Soyong, 1990). They degrade cellulose and other organic material and act as antagonist against plant fungal pathogens (Soyong, 2001). *C. globosum* is reported by several researchers to be a strong cellulose decomposer

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(Umikalsom, *et al.*, 1997; Umikalsom, *et al.*, 1998) and expressed a very effective antagonist of various soil microorganisms (Aggarwal, *et al.*, 2004; Dhingra, *et al.*, 2003; Soyong *et al.*, 2001). In Thailand *Chaetomium* species were screened for using as antagonist in 1989 (Soyong *et al.*, 2001). It has also been reported that some isolates of *C. globosum* produce antibiotics that can suppress damping-off of sugar beet caused by *Pythium ultimum* (Di-Pietro *et al.*, 1991). *C. cupreum* and *C. globosum* have been reported to reduce leaf spot disease of corn caused by *Curvularia lunata*, rice blast caused by *Pyricularia oryzae*, sheath blight of rice caused by *Rhizoctonia oryzae* and tomato wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* (Soyong, 1992a, 1992b).

Moreover, *Chaetomium* species are noted for their secondary metabolite content with biological activities. Several types of compounds have been investigated from *Chaetomium* spp. e.g. benzoquinone derivatives (Brewer *et al.*, 1986), a new anthraquinone-chromanone compound named chaetomanone and seven known compounds, ergosterol, ergosteryl palmitate, chrysophanol, chaetoglobosin C, alternariol monomethyl ether, echinuline and isochaetoglobosin D were found from *C. globosum* KMITL-N0802 and also reported that chaetomanone and echinulin showed activity towards *Mycobacterium tuberculosis* (Kanokmedhakul *et al.*, 2001). Three new azaphilones named rotiorinols A-C, two new stereoisomers, (-)-rotiorin and *epi*-isochromophilone II and a known compound, rubrorotiorin, were isolated from the fungus *C. cupreum* CC3003 of which compounds, rotiorinols A, rotiorinols C, (-)-rotiorin and rubrorotiorin act as antifungal activity against *Candida albicans* (Kanokmedhakul *et al.*, 2006). Four new dimeric spiro-azaphilones, cochliodones A-D, two new azaphilones, chaetoviridines E and F, a new *epi*-chaetoviridin A, and known compounds, chaetoviridin A, ergosterol, chaetochalasin A were isolated from *C. cochliodes* VTh 01 and *C. cochliodes* CTh 05. Chaetoviridines E and chaetochalasin A exhibited antimalarial activity against *Plasmodium falciparum* while cochliodones C, chaetoviridines E and F, chaetochalasin A expressed antimycobacterial activity against *M. tuberculosis*. Furthermore, *C. cochliodes* VTh 01 and *C. cochliodes* CTh 05 were reported to be antagonistic to *Fusarium oxysporum* f sp *lycopersici* causing tomato wilt (Phonkerd *et al.*, 2008), Chaetominedione is reported as a new tyrosine kinase inhibitor isolated from the algicolous marine fungus *Chaetomium* sp.(Abdel-Lateff, 2008) etc.

Chaetomium species are traditionally identified by morphological data, the type of terminal hair and lateral hairs or ascomatal hairs (straight, hooked, spiral, coiled etc.) covering the ascomata, the shape and size of asci and ascospores according to von Arx *et al.* (1986) and Seth (1970).

The objective of this research was to investigate the species of *Chaetomium* isolated from soil in pineapple plantations from Phatthalung and Rayong provinces, Thailand.

Table 1. List of *Chaetomium* species in Thailand.

Species	Reference
<i>C. ampullare</i> Chivers	Soytong, 1991
<i>C. apiculatum</i> Lodha	Udagawa, 1973
<i>C. aureum</i> Chivers	Soytong, 1991; Petcharat and Soyton, 1991
<i>C. bostrychodes</i> zopf	Soytong, 1991
<i>C. cochliodes</i> Palliser	Soytong, 1991
<i>C. cupreum</i> Ames	Soytong, 1991; Petcharat and Soyton, 1991; Somrithipol, 2004
<i>C. deceptivum</i> Malloch & Benny	Soytong, 1991
<i>C. floriforme</i> Gené & Guarro	Gené and Guarro, 1996
<i>C. fusiforme</i> Chivers	Petcharat and Soyton, 1991
<i>C. globosum</i> Kunze	Soytong, 1991; Petcharat and Soyton, 1991; Somrithipol, 2004; Somrithipol <i>et al.</i> , 2004
<i>C. gracile</i> Udagawa	Petcharat and Soyton, 1991
<i>C. hamadae</i> (Udagawa) v. Arx	Soytong, 1991
<i>C. homopilatum</i> Omvik	Soytong, 1991
<i>C. indicum</i> Corda	Somrithipol <i>et al.</i> , 2004
<i>C. longicollum</i> Krezm. & Badura	Soytong, 1991
<i>C. lucknowense</i> Rai & Tewari	Soytong, 1991; Petcharat and Soyton, 1991
<i>C. malaysiense</i> v. Arx	Soytong, 1991
<i>C. megasporum</i> Sorgel	Soytong, 1991
<i>C. seminudum</i> Ames	Soytong, 1991
<i>C. thermophilum</i> La Touche	Somrithipol, 2004
<i>C. tortile</i> Bainier	Somrithipol <i>et al.</i> , 2004
<i>C. variosporum</i> Udagawa et Horie	Udagawa, 1973
<i>C. venezuelense</i> Ames	Udagawa, 1973
<i>C. vitellinum</i> Carter	Soytong, 1991

Materials and methods

Source of isolates

Soil samples for the recovery of *Chaetomium* spp. were collected from pineapple plantations in Phatthalung and Rayong provinces, Thailand, during August to November 2007. Soil samples were kept in clean plastic bags, brought to the laboratory at King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand.

Isolation and identification

Chaetomium species were originally isolated by soil plate technique and baiting technique according to the method described by Soyong (1989).

Soil plate technique, soil samples were dried and ground to fine particles; 0.005-0.015 g of each soil sample were placed to sterilized Petri dishes and then overlaid with glucose-ammonium nitrate agar (GANA) medium (10 g glucose, 1 g NH₄NO₃, 1 g Difco bacto yeast extract, 0.5 g K₂HPO₄, 0.5 g MgSO₄·7H₂O, 20 g agar, 0.06 g rose bengal, 0.03 g streptomycin, 1,000 ml distilled water). After 2-7 d incubation at room temperature in the dark, *Chaetomium* spp. were observed under stereo microscope and isolated into pure culture by single spore isolation.

Baiting technique, each soil sample (*ca* 10 g) were placed to sterilized Petri dishes and moistened with sterile distilled water before baited with small pieces of sterilized straws, filter paper, tissue paper and pineapple leaves. After 21 d incubation at room temperature, *Chaetomium* spp. on baits were daily observed and picked their ascomata to glass slide with small amount of sterilized water before spread on water agar (WA) in a 9-cm-diameter Petri dish. The WA plates were incubated for 12 h at room temperature, then single colony was transferred onto PDA plates and isolated into pure culture. All isolates were kept in culture collection, Herbarium of Thai Mycological Association (H-TMA) at King Mongkut's Institute of Technology Ladkrabang, Thailand.

Results and discussion

Isolation and identification

Thirty isolates of *Chaetomium* were obtained in pure culture and identified into 9 species as presented in Table 1 and Fig.1-9. *C. cupreum* was the most common species which was found in soil from pineapple plantations taken from both Phatthalung and Rayong provinces. The most isolates were obtained by baiting technique, except *C. cupreum* S1 which was found by soil plate technique.

Identification of *Chaetomium* species are usually considered morphological characters (Arx *et al.*, 1986; Seth, 1970; Soyong and Quimio, 1989; Gené and Guarro, 1996; Rodríguez *et al.*, 2002) and molecular methods were used in the taxonomy of *Chaetomium* by Lee and Hanlin (1999). In the GenBank database (2008) sequences of 29 identified and of 44 unidentified *Chaetomium* species are now deposited. It is needed to do more identification

work both morphological and molecular data to confirm species in the near future.

Table 2. *Chaetomium* species isolated from soil in pineapple plantation at different locations in Thailand.

Methods	Species	Isolates	
		Phatthalung	Rayong
soil plate	<i>C. cupeum</i>	S1	-
	<i>C. aureum</i>	MB601, MB608, MB603, MB103	RY102
	<i>C. bostrychodes</i>	PR1, PR2, PR3, NB701	-
	<i>C. carinthiacum</i>	NB501	-
	<i>C. cochliodes</i>	-	RY301
	<i>C. cupreum</i>	NB201, MB303, MB301, V4B1,	RY201, RY202, RY203, RY204
	<i>C. flavigenum</i>	MB607, MB402, MB606, MB611, MB604	-
	<i>C. gracile</i>	NB401, MB605	-
baiting	<i>C. perlucidum</i>	NB202, NB501	-
	<i>C. succineum</i>	MB305, NB304	-

Five species are recorded as follows:-

Chaetomium aureum Chivers. Proc.Am.Arts Sci. 48: 87 (1942).

Young colonies usually are white by aerial mycelium. Mature colonies become red by a red pigment exudate. Ascomata are pale green, ovate in shape, 78.5-142.6 x 90.6-180.3 μm . Ascomatal hairs arcuate, septate. Asci are clavate in shape with 8 ascospores per ascus. Ascospores are elliptical, 5.0-7.7 x 8.5-12.5 μm , with two apical germ pores (Fig.1).

Isolate examined: MB607.

Chaetomium bostrychodes Zopf. Abhandl. Botan. Ver. de Prov. Brandenburg. 19: 173 (1877).

Colonies are rapidly growing, young colonies usually are white by aerial mycelium, occasionally with a purple pigment exudate. Mature colonies become green to brown with ascomata. Ascomata are olivaceous, maturing within 10-14 days, dark green to brown when old, ovate in shape, 190.2-349.8 x 272-419.8 μm . Ascomatal hairs usually spirally coiled. Asci are clavate in shape with 8 ascospores per ascus. Ascospores are lemoniform, 7.5-9.9 x 8.6-11.3 μm , with an apical germ pore (Fig.2).

Isolate examined: PR1.

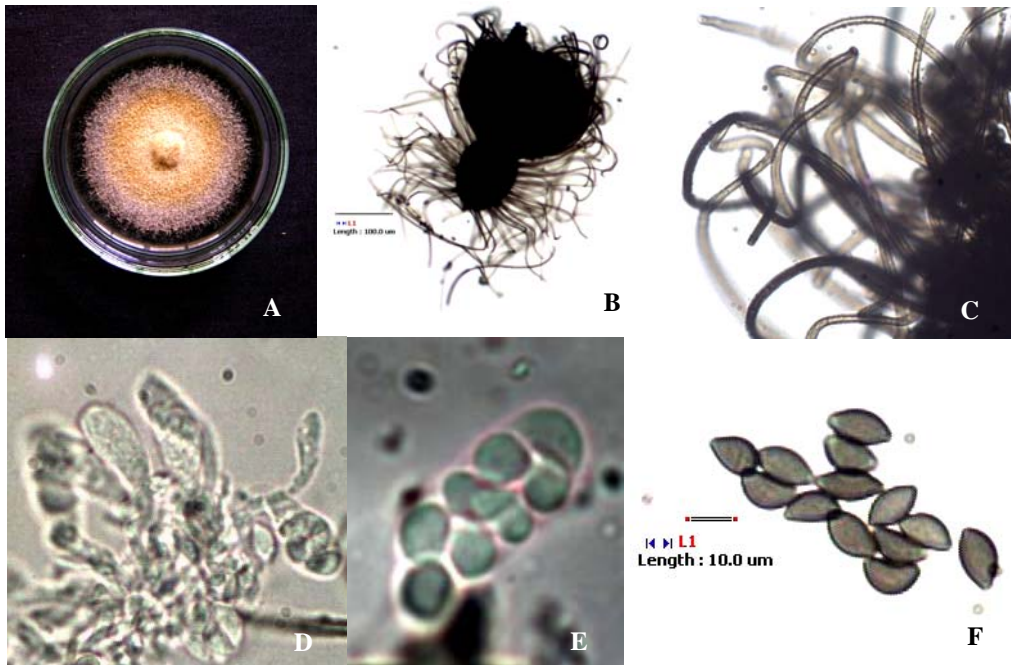


Fig.1. *Chaetomium aureum* MB601. A. 10-day-old-culture on PDA, B. ascomata, C. ascomatal hairs, D. young asci, E. 8 ascospores in an ascus, F. ascospores.

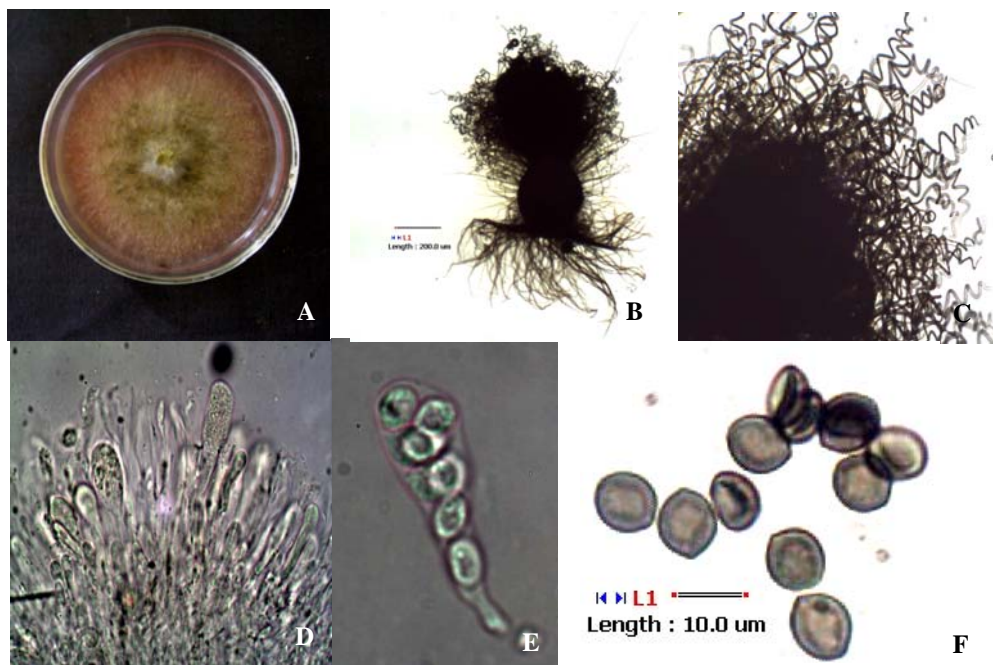


Fig.2. *Chaetomium bostrychodes* PR1. A. 10-day-old-culture on PDA, B. ascomata, C. ascomatal hairs, D. young asci, E. 8 ascospores in an ascus, F. ascospores.

Chaetomium cochliodes Palliser. North American Flora. 3 (1): 61 (1910).

Colonies are rapidly growing, young colonies usually are white by aerial mycelium, occasionally with a purple pigment exudate. Mature colonies become green to brown with ascomata. Ascomata are olivaceous, maturing within 10-14 days, dark green to brown when old, ovate in shape, 107.1-143.1x122.0-209.2 μm . Ascomatal hairs usually irregularly sinuous. Asci are clavate in shape with 8 ascospores per ascus. Ascospores are broadly ovate to lemon-shaped, 4.2-5.9x7.1-10.0 μm , with an apical germ pore (Fig.3).
Isolate examined: RY301.

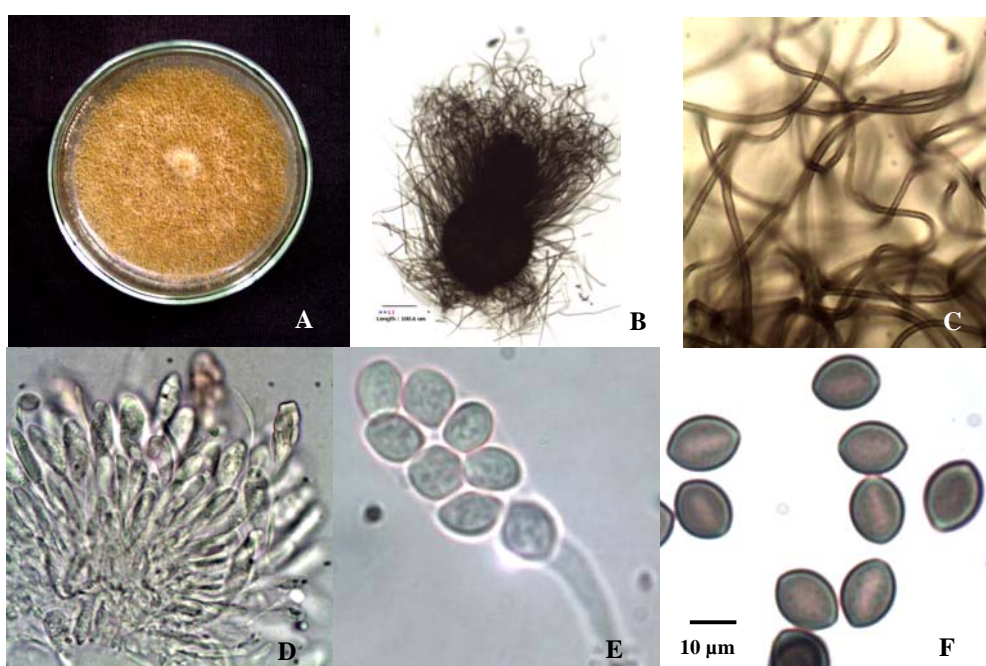


Fig.3. *Chaetomium cochliodes* RY301. A. 10-day-old-culture on PDA, B. ascomata, C. ascomatal hairs, D. young asci, E. 8 ascospores in an ascus, F. ascospores.

Chaetomium cupreum Ames. Mycologia 41(6): 642 (1950)

Colonies usually are red due to a red pigment exudate. Ascomata are red, maturing within 10-14 days, ovate in shape, 79.7-142.7 x 94.7-151.5 μm . Ascomatal hairs arcuate, apically circinate or coiled, septate. Asci are clavate in shape with 8 ascospores per ascus. Ascospores are reniform, 4.7-6.7 x 6.7-10.0 μm , with a single apical germ pore (Fig.4).
Isolate examined: RY202

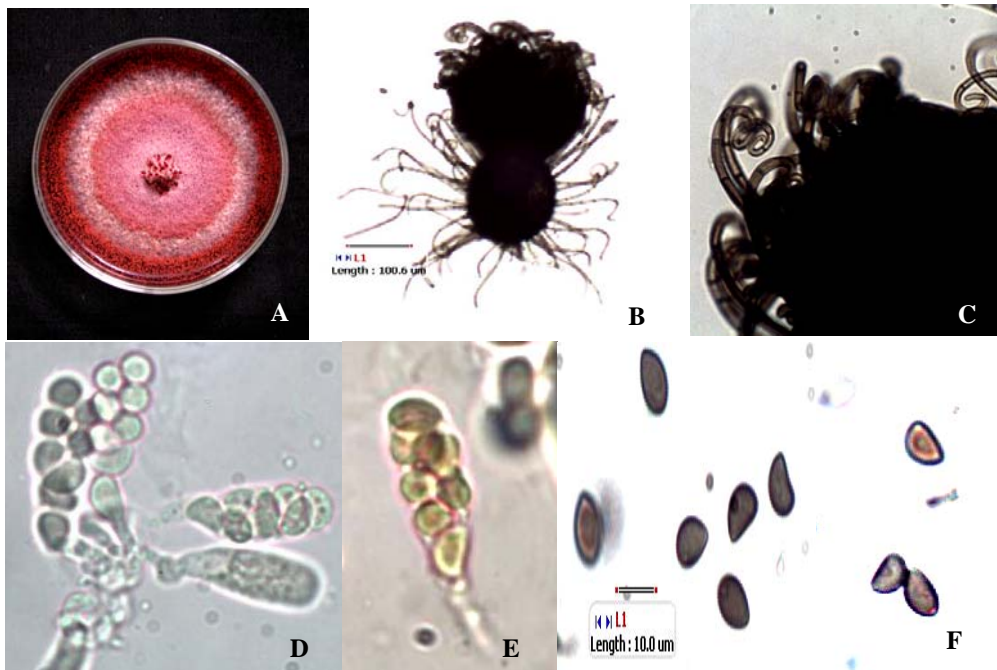


Fig.4. *Chaetomium cupreum* RY202. A. 10-day-old-culture on PDA, B. ascomata, C. ascomatal hairs, D. young asci, E. 8 ascospores in an ascus, F. ascospores.

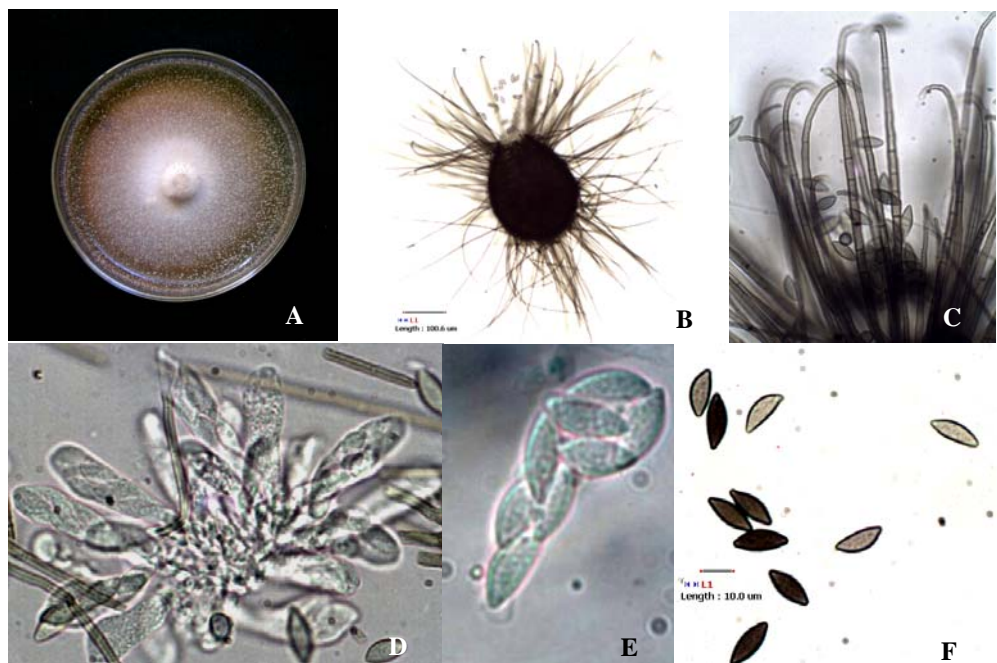


Fig.5. *Chaetomium gracile* NB401. A. 10-day-old-culture on PDA, B. ascomata, C. ascomatal hairs, D. young asci, E. 8 ascospores in an ascus, F. ascospores.

Chaetomium gracile Udagawa. J.gen.appl.Microbiol. 6: 235 (1960).

Colonies usually yellow due to yellow pigment exudates. Ascomata are olivaceous grey, maturing within 10-14 days, ovate in shape, 75.4-161.4x110.2-202.8 μm . Ascomatal hairs arcuate. Asci are clavate in shape with 8 ascospores per ascus. Ascospores are fusiform, 5.1-6.9x12.7-17.7 μm , with two apical germ pores (Fig.5).

Isolate examined: NB401.

Four species are reported to be new records in Thailand as follows:-

Chaetomium carinthiacum Sörgel. Arch. Mikrobiol 40: 393 (1961).

Colonies usually are white by aerial mycelium, without a pigment exudate. Mature colonies become green to brown with ascomata. Ascomata are olivaceous, maturing within 10-14 days, dark green when old, ovate in shape, 96.1-146.4x101.8-153.2 μm . Ascomatal hairs irregularly sinuous with roughened hairs. Asci are clavate in shape with 8 ascospores per ascus. Ascospores are elliptical, 4.3-6.2x8.4-11.6 μm , with an apical germ pore (Fig.6).

Isolate examined: NB501.

Chaetomium flavigenum van Warmelo. Mycologia. 58: 847 (1966).

Colonies usually are white by aerial mycelium, becoming red or orange due to a red pigment exudate. Mature colonies become green to brown with ascomata. Ascomata are olivaceous to brown, maturing within 10-14 days, dark grey-green when old, ovate in shape, 92.5-134.9 x 113.2-190.3 μm . Ascomatal hairs arcuate. Asci are clavate in shape with 8 ascospores per ascus. Ascospores are fusiform, 4.0-6.3 x 6.6-11.2 μm , with two apical germ pores (Fig.7).

Isolate examined: MB601.

Chaetomium perlucidum Sergejeva. Notulae Syst. Sect. Crypt. Inst. Bot. Acad. Sci. U.S.S.R. 11: 108 (1956).

Colonies usually are white or greyish by aerial mycelium, without a pigment exudate. Mature colonies dark grey to black with ascomata. Ascomata are grey, ovate in shape, 91.9-145.5x120.1-190.6 μm . Ascomatal hairs undulate and irregularly sinuous. Asci are clavate in shape with 8 ascospores per ascus. Ascospores are elliptical, 3.4-5.5x6.7-9.2 μm , with an apical germ pore (Fig.8).

Isolate examined: NB202.

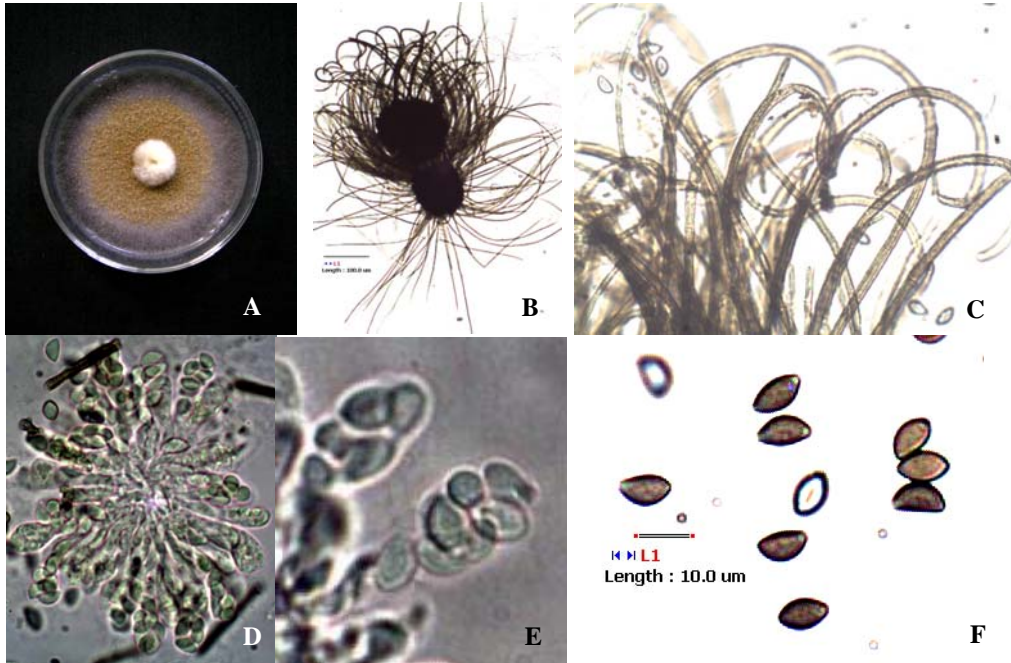


Fig.6. *Chaetomium carinthiacum* NB501. A. 10-day-old-culture on PDA, B. ascomata, C. ascomatal hairs, D. young asci, E. 8 ascospores in an ascus, F. ascospores.

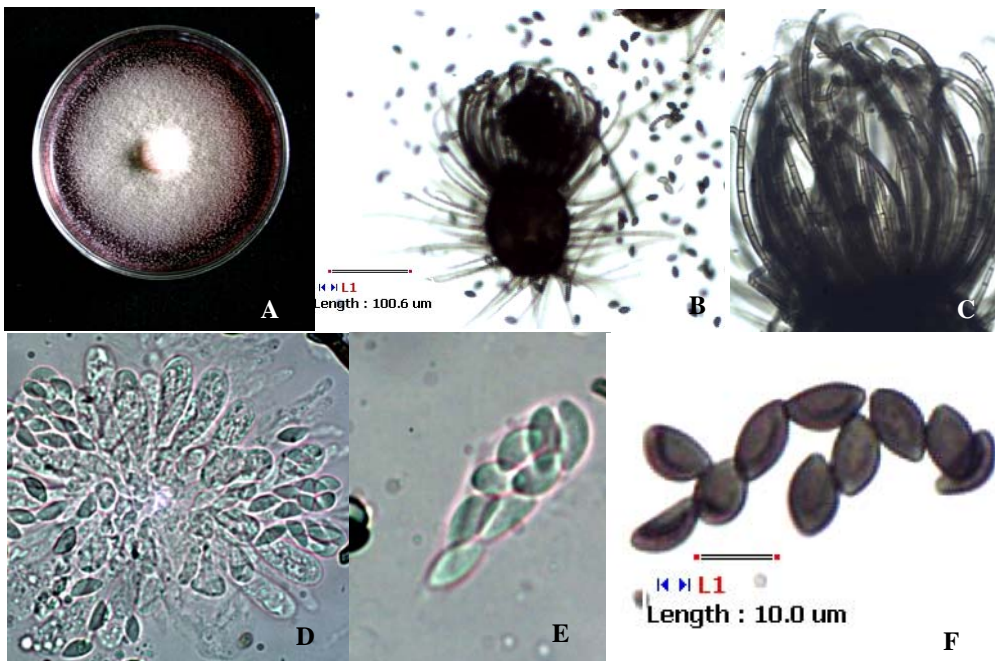


Fig.7. *Chaetomium flavigenum* MB607. A. 10-day-old-culture on PDA, B. ascomata, C. ascomatal hairs, D. young asci, E. 8 ascospores in an ascus, F. ascospores.

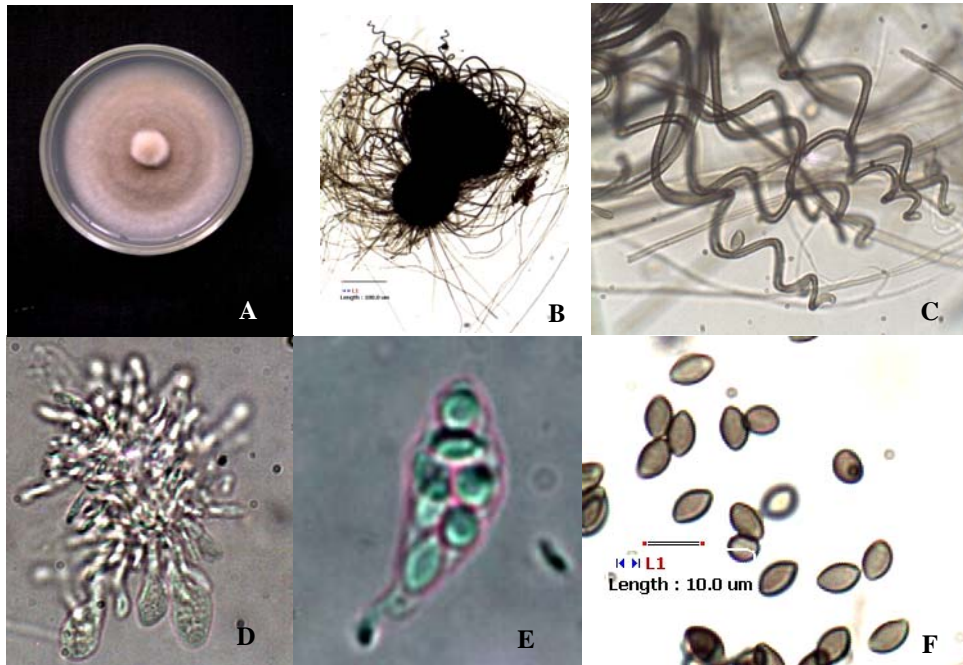


Fig.8. *Chaetomium perlucidum* NB202. A. 10-day-old-culture on PDA, B. ascomata, C. ascumatal hairs, D. young asci, E. 8 ascospores in an ascus, F. ascospores.

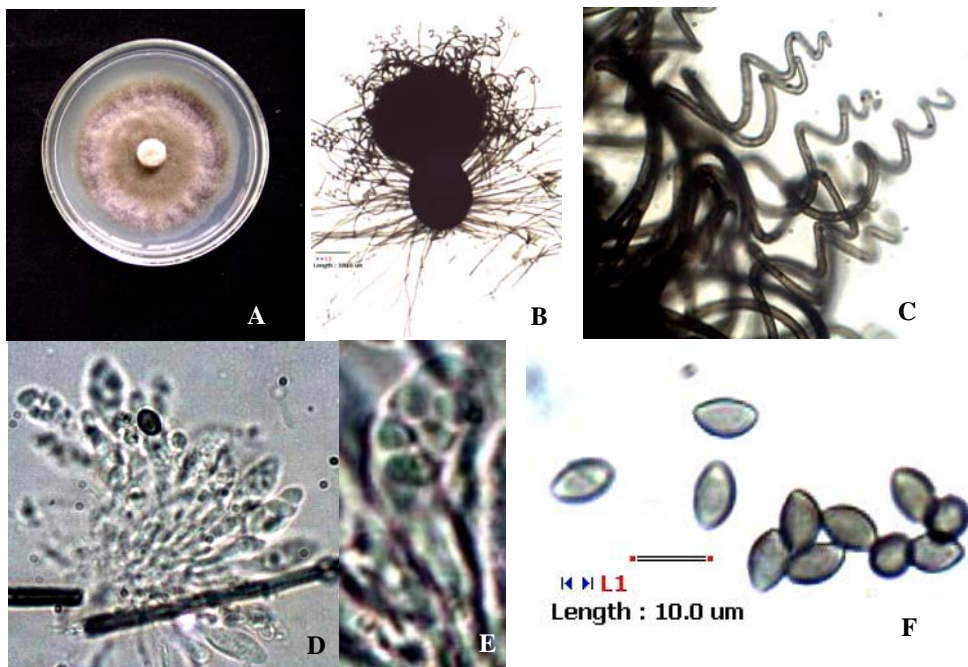


Fig.9. *Chaetomium succineum* MB304. A. 10-day-old-culture on PDA, B. ascomata, C. ascumatal hairs, D. young asci, E. 8 ascospores in an ascus, F. ascospores.

Chaetomium succineum Ames. Mycologia. 41: 445 (1949).

Colonies usually are dark green or greyish by aerial mycelium, without a pigment exudate. Mature colonies dark grey to black with ascomata. Ascomata are grey, ovate in shape, 107.1-143.1x122.0-209.2 µm. Ascomatal hairs loosely hairs. Asci are clavate in shape with 8 ascospores per ascus. Ascospores are elliptical, 4.2-5.9x7.1-10.0 µm, with an apical germ pore (Fig.9).
Isolate examined: MB304.

Acknowledgements

We would like to thank Mr. Sommart Pornsuriya and Ms. Chamaiporn Charoenporn for helping to collect soil samples. This research project is reported as a part of Ph.D.thesis.

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(Received 14 August 2008; accepted 23 October 2008)