THESIS

Talaromyces species: DIVERSITY, TAXONOMY, PHYLOGENY, ANTAGONISTIC ACTIVITY AGAINST PLANT PATHOGENIC FUNGI AND SECONDARY METABOLITES

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THESIS

Talaromyces species: DIVERSITY, TAXONOMY, PHYLOGENY, ANTAGONISTIC ACTIVITY AGAINST PLANT PATHOGENIC FUNGI AND SECONDARY METABOLITES

TIDA DETHOUP

A Thesis Submitted in Partial Fulfillment of
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Tida Dethoup 2007: *Talaromyces* species: Diversity, Taxonomy, Phylogeny, Antagonistic Activity Against Plant Pathogenic Fungi and Secondary Metabolites. Doctor of Philosophy (Plant Pathology), Major Field: Plant Pathology, Department of Plant Pathology. Thesis Advisor: Associate Professor Leka Manoch, Ph.D. 218 pages.

Forty five soil samples were collected from 38 provinces in Thailand. Different isolation methods such as the alcohol and heat treatments, soil plate and dilution plate method, and Gochenaur's glucose ammonium nitrate agar were used. Identification of the fungal isolates was based on morphological features, as colony growth and color on different agar media. Microscopic characters were examined under stereo-, light- and scanning electron microscopes. A total of 342 isolates of *Talaromyces* were obtained comprising 11 species, 1 variety and 2 unidentified species including *Talaromyces austrocalifornicus, T. bacillisporus, T. flavus, T. macrospermus, T. helicus var. helicus, T. indigoticus, T. luteus, T. rotundus, T. stipitatus, T. trachyspermus, T. wortmanii, Talaromyces* sp. 1 (KUFC 3399) and *Talaromyces* sp. 2 (KUFC 3383). *Talaromyces austrocalifornicus* and *T. indigoticus* were new records for Thailand. *Talaromyces flavus* and *T. macrosporus* were the dominant species followed by *T. stipitatus, T. trachyspermus, T. wortmannii, T. bacillisporus, T. rotundus, T. indigoticus, T. helicus* var. *major, T. austrocalifornicus, T. luteus, Talaromyces* sp. 1 (KUFC 3399) and *Talaromyces* sp. 2 (KUFC 3383).

Phylogenetic analyses were conducted using polymorphic microsatellites of 21 fungi comprising 18 species of *Talaromyces* and 3 other Trichocomaceae (KUFC 3576, 5642, 5655) from Kasetsart University Fungal Culture Collection. The results showed that species of *Talaromyces* used in this study did not show any congruence to the division either done by Stolk and Samson, 1972 or Pitt, 1979. The unidentified species, *Talaromyces* sp. 1 (KUFC 3399) was found on same clade with *T. roduntus* which occupies a basal position to the main *Talaromyces* clade and both of them belong to the Series *Lutei*.

The antagonistic activity tests revealed that 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, little inhibition was observed for *Pythium aphanidermatum, Lasiodiplodia theobromae, Rhizoctonia solani* and *Sclerotium rolfsii in vitro*. The greenhouse experimental 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* could inhibit *S. rolfsii* at 30 days after inoculation.

For secondary metabolites investigation, the oligophenalenone dimer duclauxin and two new analogues, bacillisporins D and E, were isolated from *Talaromyces bacillisporus* in addition to the previously reported bacillisporins A, B and C. Chemical study of *Talaromyces* sp. 1 (KUFC 3399) furnished the two new merodrimanes thailandolides A and B, an *O*-methylated derivative of the aromatic fragment incorporated in thailandolide B, and three known closely related 1(*3H*)-isobenzofuran derivatives, penisimplicissin, vermistatin, and hydroxydihydrovermistatin. Structures were established by spectroscopic measurements and confirmed by X-ray analyses of compounds thailandolides A and vermistatin. The unusual peptide analogue *N*-benzoylphenylalanyl-*N*-benzoylphenyl alaninate was also found.

Tida Dethoup — Leka Manoch — 3 / Apr. / 07

Student's signature — Thesis Advisor's signature

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Tida Dethoup

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Talaromyces species: DIVERSITY, TAXONOMY, PHYLOGENY, ANTAGONISTIC ACTIVITY AGAINST PLANT PATHOGENIC FUNGI AND SECONDARY METABOLITES

INTRODUCTION

Talaromyces is an ascomycete was erected by Benjamin in 1955 with *T. vermiculatus* as a type species. It belongs to the Class Ascomycetes, Order Eurotiales, Family Trichocomaceae Fischer (syn. Eurotiaceae Clem. & Shear) (Kirk *et al.*, 2001). Five different genera of anamorphic state were reported including *Penicillium*, *Paecilomyces*, *Geosmithia*, *Merimbla* and *Sagenomella*. Most anamorphic state was however, belonged to *Penicillium*, section Biverticillate– Symmetrica (Stolk and Samson, 1972; Pitt, 1979a; Heredia *et al*., 2001). *Talaromyces* produces ascomata, white, yellow to red, soft, globose to subglobose, superficial, discrete or confluent. Ascomatal coverings consisting of a network of hyphae, usually surrounded by a weft of thin hypha, straight or twisted depending on the species. Asci evanescent, mostly 8 ascospores, globose to subglobose or slightly ellipsoidal, borned in chains. Ascospores globose or ellipsoidal, smooth or showing various ornamentations (Stolk and Samson, 1972; Pitt, 1979a).

Forty six species and 6 varieties of *Talaromyces* were recorded from soil, debris, manure, agricultural and industrial wastes, dungs and foods, with a worldwide distribution (Domsch *et al*., 1993a, b; Pitt, 1979a; Pitt *et al.*, 2000; Samson, 2000; Stolk and Samson, 1972. In Thailand, 9 species and 2 varieties of *Talaromyces* were reported including *Talaromyces vermiculatus* (syn. *T. flavus*), *T. flavus* var. *flavus*, *T. flavus* var. *macrosporus*, *T. spiculisporus* (syn. *T. trachyspermus*), *T. striatus*, *T. byssochlamydoides*, *T. emersonii*, *T. trachyspermus*, *T. wortmannii*, *T. bacillisporus* and *T. rotundus*. They were isolated from forest, agricultural, mangrove soil, soil at termite mounds, dungs and decomposing starters (Chana, 1974; Cruesrisawath, 1985; Kanjanamaneesathian, 1988; Manoch, 2004; Manoch *et al*., 2004, 2005; Sudpro, 1999; Wongthong, 2001; Busarakum, 2002; Ito *et al*., 2001; Jeamjitt, 2007).

The evolutionary relationships of *Talaromyces* species are very interesting because the morphology of both teleomorphs and anamorphs of these fungi indicate a close relationship to other genera of the Trichocomaceae (Taylor *et al*., 1990; LoBuglio *et al*., 1993; Wang and Zhuang, 2007). Taylor *et al*., (1990) and Luangsa-ard *et al*., (2004) reported *Talaromyces* species (*Paecilomyces* anamorph) cluster with *Byssochlamys* and *Thermoascus* species having *Paecilomyces* anamorph, not with *Talaromyces* species having *Penicillium* anamorphs. Yaguchi *et al*., (2005) were able to demonstrate, using D1/D2 region of 28S rDNA sequence analysis, that *Geosmithia argillacea* is the anamorph of *Talaromyces eburneus*. Heredia *et al*., (2001) described a new species, *Talaromyces ocotl* with *Sagenomella* sp. anamorph, based on morphological analyses and phylogenetic inferences made from ITS and 28S rDNA sequence alignments.

Talaromyces flavus is the most common species and has been reported as an effective biological control agent against several plant pathogenic fungi including *Verticillium dahliae*, *Sclerotinia sclerotiorum* and *Sclerotium rolfsii*, the causal of verticillium wilt of eggplant, white mold of dry bean and bean stem rot respectively (Fahima and Henis, 1995; Fravel, 1996; Huang *et al*., 2000; Madi *et al*., 1997; McLaren *et al*., 1986). The mechanisms for biological control activity involved mycoparasitism (Fahima *et al*., 1992; Madi *et al*., 1997), antibiosis (Kim *et al*., 1990a, b; Stosz *et al*., 1996) and competition (Marois *et al*., 1982).

Several species of *Talaromyces* can produce bioactive compounds, such as talaroderxines A and B from *T. derxii* having activity against *Bacillus subtilis* (Suzuki *et al*.,1992), *T. trachyspermus* SANK 12191 produces trachyspic acid which inhibit tumor cell heparanase (Shiozawa *et al*., 1995) and *T convolutus* isolated from barley in Japan can produce talaroconvolutins which inhibit plant and human pathogenic fungi including *Aspergillus fumigatus*, *A. niger*, *Candida albicans* and *Cryptococcus neoformans* (Suzuki *et al*., 2000). Several new compounds were reported from other species of *Talaromyces*, such as wortmanilactones A-D from *T. wortmannii* (Dong *et al*., 2006), and three new azaphilones, luteusins A-E from *T. luteus* (Yoshida *et al*., 1996).

It is very interesting to study the genus *Talaromyces* on various topics in Thailand, especially diversity, taxonomy, phylogeny, antagonistic test against plant pathogenic fungi and the produced secondary metabolites. Because this fungus was poorly known with the only important reports contributed by Manoch *et al.* (2004) and Luangsa-ard *et al.* (2004). Their studies were limited in taxonomy and phylogeny. Therefore, more investigations on *Talaromyces* species need to be carried out in this tropical region for the discovery of new taxa, the utilization of some species as biological control agents against plant pathogenic fungi and the analysis for secondary metabolites on several species of this genus is a very challenging topic for industrial, phamaceutical and agricultural enterprises.

OBJECTIVES

1. To isolate *Talaromyces* spp. from soil from different locations in Thailand

2. To study morphological characteristics of *Talaromyces* species

3. To determine diversity and distribution of *Talaromyces* species from various soil samples

4. To maintain the pure cultures of *Talaromyces* species in a culture collection

5. To study molecular phylogeny of the isolated *Talaromyces* species

6. To study antagonistic activity tests of *Talaromyces* against 15 species of plant pathogenic fungi *in vitro* and in the greenhouse

7. To investigate the secondary metabolites of the two species of *Talaromyces*; *T. bacillisporus* and *Talaromyces* sp. 1 (KUFC 3399)

LITERATURE REVIEWS

1. Diversity and taxonomic study of *Talaromyces*

1.1 Diversity and distribution of *Talaromyces* in Thailand

Chana (1974) isolated microfungi from paddy and garden soil from 10 provinces in Central, Thailand including Bangkok, Kanchanaburi, Nonthaburi, Samut Songkhram, Prachin Buri, Sing Buri, Ratchaburi, Nakhon Pathom, Nakhon Nayok and Suphan Buri. Alcohol treatment, Glucose Ammonium Nitrate Agar (GAN) and streptomycin were used. *Talaromyces vermiculatus* was found from all soil samples. *T. spiculisporus* was isolated from most soil samples excepted soil from Nakhon Nayok and Kanchanaburi. Whereas, *T. striatus* was reported from garden soil in Samut Songkhram and paddy soil from Sing Buri and Suphan Buri respectively.

Cruesrisawath (1985) studied thermophilic and thermotolerant fungi from soil and debris in Thailand, using modification of Warcup's soil plate method and Yeast Starch Agar (YSA). *Talaromyces dupontii* with *Paecilomyces* - anamorph was found from decomposting starters. The optimum temperature of this fungi is ranging from 45-50^{o}C, but it can grow at 30^{o}C.

Kanjanamaneesathian (1988) reported the diversity of thermophilic and thermotolerant fungi from various substrates in Thailand. Soil plate method, YSA and Yeast Glucose Agar (YGA) with Streptomycin were used. *Talaromyces byssochlamydoides* and *T. emersonii* were recorded from agricultural soil from Prachuap Khiri khan and Chachoengsao respectively.

Six isolates of *Talaromyces* species were isolated from forest soil, Amphur Thong Pa Phum, Kanchanaburi; soil at Kasetsart University, Bangkok; forest soil and strawberry garden soil from Doi Intanonth, Chiang Mai. Alcohol and heat treatment with "GAN" were used (Manoch and Chana ,1996).

Manoch *et al.*, (1997) studied on Ascomycetes and Deuteromycetes from forest, agricultural soil and debris from various locations in Thailand, using alcohol and heat treatment and GAN. They could isolated *Talaromyces bacillisporus* and *Talaromyces* spp. from soil. Manoch *et al.*, (2000) reported 7 isolates of *Talaromyces* spp. from different forest types at Khao Yai National Park, Huay Kha Khang Wildlife Sanctuary, Uthai Thani. Alcohol and heat treatment techniques were employed for isolation.

Sudpro (1999) reported the diversity of soil and plant pathogenic fungi in agricultural soil from Sakhon Nakorn, using the soil plate method and "GAN". Two isolates of *Talaromyces* spp. were recorded from corn and cassava field soils in June and September, respectively.

Wongthong (2001) isolated fungi from mangrove forest at Ranong Coastal Research Station, Ranong Province, using dilution plate method on Glucose-Yeast Extract Agar with 14 ppt of salt. The soil samples were collected in December (winter), April (summer) and July (rainy) during 1999 – 2000. *Talaromyces flavus* was recorded from mangrove soil in December and July. Four isolates of *Talaromyces* spp. were reported from mangrove soil in all season.

Busarakum (2002) reported 3 isolates of *Talaromyces* spp. from rhizosphere soils of *Lusidia discolor*, *Calanthe rosea* and *Spathoglottis plicata* at Queen Sirikit Botanic Garden, Chiang Mai, using alcohol and heat treatment techniques with "GAN".

Manoch *et al.*, (2004) isolated soil fungi from termite mounds in Thailand. Alcohol and heat treatment methods and "GAN" were used. Scanning electron microscope (SEM) photomicrographs showed the ascospores ornamentation of *Talaromyces bacillisporus* from termite mounds in Sakon Nakhon and Trat, whereas, *T. flavus* var. *flavus* was observed from termite mounds in Nakhon Pathom. Sixteen isolates of *Talaromyces* spp. were found from termite mounds from Krabi and Nakron Nayok.

Ito *et al.*, (2001) reported microfungi from mangrove soil in Thailand at Ranong and Phang Nga. *Talaromyces byssochlamydoides* was isolated from rhizosphere soil of *Avicennia alba*, *Rhizophora mucronata*, *Rhizophora apiculata* and *Sonneratin alba*. *Talaromyces flavus* was found from rhizosphere soil of *Ceriops tage* and *Bruguiera sexangula*, whereas *T. wortmannii* was recorded from rhizosphere soil of *Avicennia alba*.

Jeamjitt (2007) studied coprophilous fungi from domestic and animals, using heat treatment and "GAN". *Talaromyces bacillisporus* and *T. rotundus* were recored from rat and buffalo dung in Bangkok respectively.

Manoch *et al.*, (2004) studied teleomorph of *Aspergillus* and *Penicillium* from soil at termite mounds using alcohol and heat treatment and "GAN". *Talaromyces flavus* var. *macrospermus* and *T. trachyspermus* were found from termite mound at Sakhon Nakhon, whilst, *T. wortmannii* were recorded from termite mound in Ratchaburi.

1.2 Diversity and taxonomic study of *Talaromyces*

Satanimi (1971) isolated fungi from soil at Mt. Pelion, Greece using alcohol treatment technique and "GAN". Six isolates of *Talaromyces luteus* were found. Hudson (1973) isolated *Talaromyces thermophilus* from air at Cambridge, England by using 2 % malt extract agar.

Intensive researches on diversity of *Talaromyces* species have been conducted in Japan. New species of *Talaromyces* were found from various substrates including *T. flavus*, *T. helicus* (Udagawa, 1963), *T. wortmannii* (Tokumasu, 1974), *T. trachyspermus* (Horie *et al.*, 1977), *T. derxii*- heterothallic species (Takada and Udagawa, 1988), *T. helicus* var. *boninensis* (Yaguchi *et al.*, 1992), *T. subinflatus* (Yaguchi *et al.*, 1993a), *T. convolutus*, *T. emodensis*, *T. tardifaciens* (Udagawa, 1993) *T. wortmannii* var. *sublevisporus*, *T. trachyspermus* var. *assiutensis*, *T. muroii*

(Yaguchi *et al.*,1994a, b), *T. hachijoensis* (Yuguchi *et al.*, 1996), *T. retardatus* (Udagawa *et al.*, 1993), *T. spectabilis* (Udagawa and Suzuki, 1994) and *T. euchlorocarpius* (Yaguchi *et al.*, 1999).

In the United States, Tansey (1971) reported *Talaromyces emersonii* and *T. thermophilus* from self-heated wood chips. Huang and Schmitt (1975) isolated 7 species of this genus including *T. flavus* var. *flavus, T. helicus, T. luteus, T. trachyspermus, T. udagawae, T. ucrainicus* and *T. wortmannii* form soils of Southern Ohio. In addition, Yuguchi *et al.*, (1993a) described a new species, *T. austrocalifornicus* from soil at Southern California, USA.

Rosenberg (1975) studied the effect of temperature and pH on growth of thermophilic and thermotolerant properties of *Talaromyces emersonii* and *T. thermophilus*. Optimum temperatures and pH of both species are 35-50°C, pH 3.4 – 5.4 and 45-50 °C, pH 7.2 – 8.1, respectively.

Jesenska *et al.*, (1992) reported 6 species of *Talaromyces* from soil at Slovak Republics, including *Talaromyces avellaneus, T. bacillisporus, T. emersonii, T. flavus* var. *flavus, T. trachyspermus* and *T. wortmannii*.

Tzean *et al.*, (1992) isolated *Talaromyces unicus* from soil, in Taiwan. The soil samples was suspended in 60°C aqueous solution for 20 min and placed on dichloram rose bengal chloramphenical agar plate. In addition Yaguchi *et al.*, (1994b) reported *T. eburneus* with *Geosmithia* anamorph from soil in Taipei, Taiwan.

Domsch *et al.*, (1993a, b) studied microfungi from soil and reported five species of *Talaromyces* including *T. emersonii, T. flavus, T. trachyspermus, T. helicus* and *T. wortmannii*. They were found worldwide from various substates such as soil, fertilizer and dungs.

Some species of *Talaromyces* were obtained from Arsenic-polluted soil and able to produce a volatile As-compound from As(III), such as *T. thermophilus*, an

thermopilic fungi was isolated from citrus waste decomposing in Japan (Hiroki and Yoshiwara, 1993; Heerden *et al*., 2002).

Udagawa *et al*, (1996) isolated ascomycetous microfungi from 58 house dust samples from detached house and apartment in Kobe City, by using dilution plate method and potato dextrose agar with chloramphenical. The most frequent species were *Talaromyces flavus, T. trachyspermus, T. macrospermus T. wortmannii and T. helicus*.

Sage *et al.*, (1997) reported 4 species *Talaromyces* including *T. flavus*, *T. helicus*, *T. stipitatus* and *T trachyspermus* from polluted soils, Oued Sebou, Morocco. Soil plate and malt extract agar (MEA) mixed with chloramphenical were used. *Talaromyces flavus* was more often found at heavily polluted sites than at lightly polluted ones.

Heredia *et al.*, (2001) reported *Talaromyces rotundus* and *T. ocotl,* new species with *Saganomella* anamorph from soil planted with *Pinus hartwegii* and *Pinus patula*, using heat and alcohol treatment. For heat treatment, 2-3 g of soil was filled in aluminum foil in hot air oven at 100 ^{o}C for 1hour. Alcohol treatment was by soil sample treated with 60% ethanol, 2% phenol, "GAN" mixed cyclosporin A, streptomycin sulfate and chlortetracycline in a petridish.

Asan (2004) studied *Aspergillus*, *Penicillium* and related species from various substrates in Turkey. Fourteen species of *Talaromyces* were reported from pepper powder, wheat seed, greenhouse and agriculture soil, drug tablets, dust, leather and surgical strings including *T. bacillisporus, T. byssochlamydoides, T. emersonii, T. flavus, T. helicus, T. intermedius, T. luteus, T. leycettanus, T. ohiensis, T. purpureus, T. rotundus, T. stipitatus, T. udagawae* and *T. wortmannii.*

Cavalcanti *et al*., (2002) reported the diversity of microfungi from mangrove in Itamaraca islands, Pernambuco state, Brazil, using a dilution technique and Sabouraud dextrose agar mixed with chloramphenical. *Talaromyces bacillisporus*,

T. trachysperus and *T. flavus* were found. *T. bacillisporus* was reported as a new recorded for Brazil.

Talaromyces flavus and *T. macrosporus* are heat-resistant and reported fairly frequently isolated from heat processed food and pastuerized juice. Ascospores may be soil-born and thus contaminating the fruit (Pitt and Hocking, 1997; Samson *et al*., 2002).

1.3 Study on morphology of *Talaromyces*

Stolk and Samson (1972) studied and revision monograph of *Talaromyces* morphology for identifying and key of 16 species and 2 varieties including *T. bacillisporus, T. byssochlamydoides, T. emersonii, T. flavus* var. *flavus, T. flavus* var. *macrosporus, T. helicus* var. *helicus, T. helicus* var. *major, T. intermedius, T. leycettanus, T. luteus, T. purpurea, T. rotundus, T. stipitatus, T. thermophilus, T. trachyspermus, T. udagawae, T. ucrainicus* and *T. wortmannii*. The main characteristics of *Talaromyces* for identification including **1)** ascomatal initial developing; **2)** ascomatal covering; **3)** shape, size and ornamentation of ascospores under scanning electron microscope and **4)** anamorph (imperfect state).

1) Characteristics of ascomatal initial or young cleistothecia (ascomata) of genus *Talaromyces* shows variable including 1) developing as short branches or as intercalary portions of hyphae which swell considerably, become strongly gnarled and branched profusely (e.g. *T. trachyspermus*) (Figure 1A); 2) consist of a pair of gemetangium (e.g. *T. flavus*) (Figure 1B); 3) consist of swollen cells resembling chlamydospores (e.g. *T. emersonii*) (Figure 1C); 4 coiled hyphae (e.g. *T. leycettanus*) (Figure 1D); 5) swollen irregular septate hyphae producing coiled branches (e.g. *T. wortmannii*) (Figure 2A) and 7) consisting one-celled and chlamydospore-like and produce ascogenous hyphae directly (Figures 2B-C) then the initials become septate and begin to produce branch hyphae for further development.

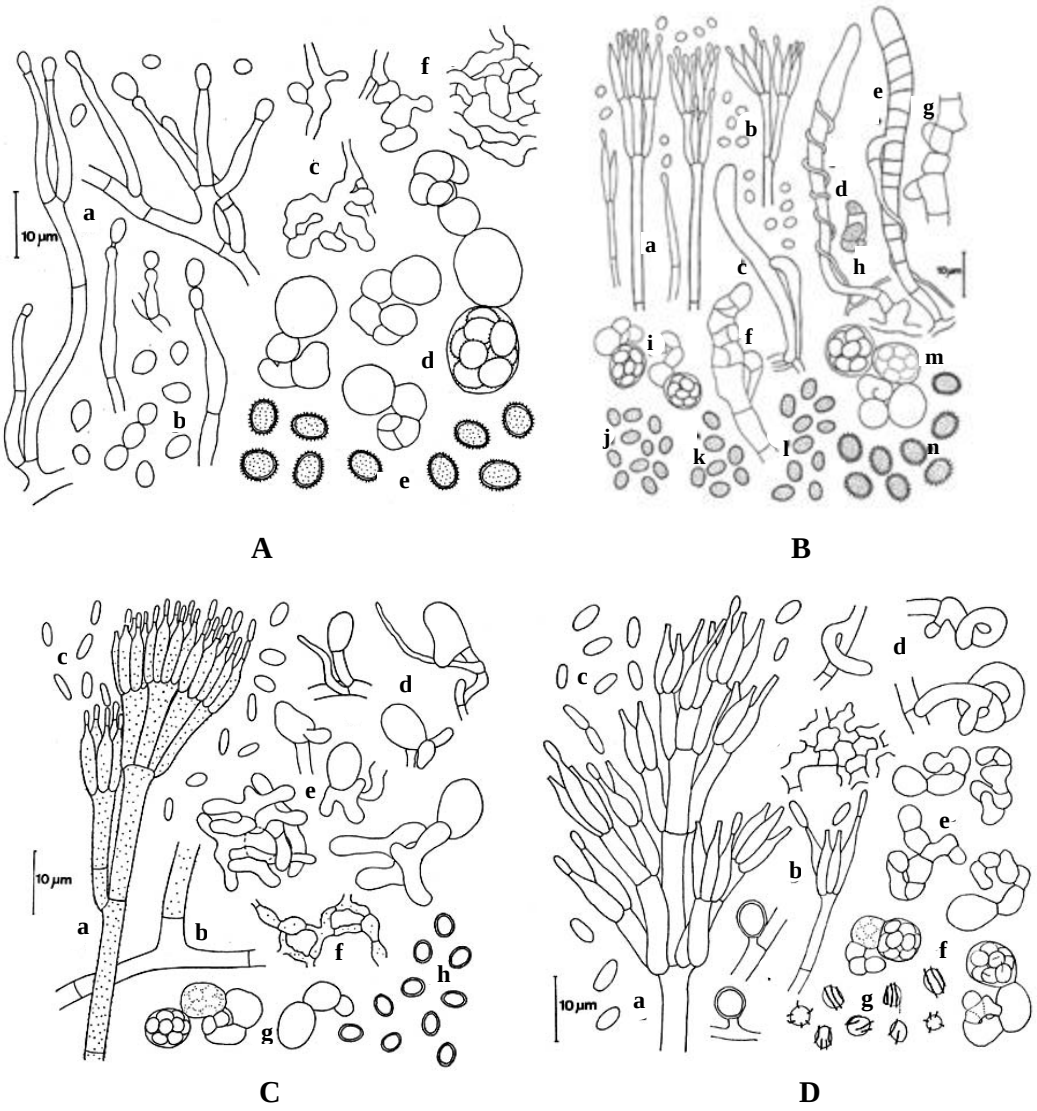


Figure 1 Morphological characteristics of four *Talaromyces* species

A) ***T. intermedius***, a. conidiogenous structures; b. conidia; c. ascomatal initials; d. chains of asci; e. ascospores; f. ascomatal covering on surface view

B) ***T. flavus***, a. conidiogenous structures; b. conidia; c-h. development of ascogonia and antheridia; i-m. chains of asci; j-n. ascospores

C) ***T. emersonii,*** a. conidiogenous structure; b. foot-cell; c. conidia; d. ascomatal initials; e. primordium consisting of profusely branching hyphae; f. fragment of ascomatal covering; g. asci in chains; h. ascospores

D) ***T. leycettanus***, a. complicated conidiogenous structure; b. monoverticillate penicillus; c. conidia; d. ascomatal initials, e-f. chains of asci; g. ascospores; h. fragment of ascomatal covering; i. chlamydospores

Source : Stolk and Samson, (1972)

Figure 2 Morphological characteristics of three *Talaromyces* species
A) ***T. wortmannii***, a. conidiogenous structures; b. conidia; c. ascomatal initials; d. chains of asci; e. ascospores
B) ***T. trachyspermus***, a. conidiogenous structures; b. conidia; c. ascomatal initials; d. chains of asci; e. ascospores; Type collection USDA 5798; f. ascospores; Herbarium specimen 7b. g. conidiogenous structures with conidia; h. ascospores.
C) ***T. bacillisporus***, a. conidiogenous structures; b. conidia; c. swollen cells from which the initials will develop; d. ascomatal initials, producing branches; e. developing asci; f. chain of asci; g. ascospore

Source : Stolk and Samson, (1972)

2) Ascomatal coverings of its species consist of a network of hyphae, differing markedly in their density, 1) covering with scanty and inconspicuous hyphae (e.g. *T. byssochlamydoides*); 2) distinct and loose-textured (e.g. *T. flavus*) and 3) thick-walled and closely knit, becoming pseudoparenchymatous in age (e.g. *T. thermophilus*).

3) Morphology of ascospores which various patterns (Figure 3) including globose, subglobose or ellipsoidal, their walls may be smooth, spinulose or provided with ridges. For a few species, very little variation in the sizes of the ascospores produced in different strains of one *Talaromyces* species.

4) Anamorph-state of *Talaromyces* show much variable. Five genera were reported including *Penicillium*, *Paecilomyces*, *Geosmithia*, *Merimble* and *Sagenomella* (Table 1). In 1972, Stolk and Samson reported anamorph state of *Talaromyces* in 2 genera, *Penicillium* and *Paecilomyces*. *Paecilomyces* is anamorph of *T. leycettanus* and *T. byssochlamydoides*, whereas other *Talaromyces* species have *Penicillium*-anamorph. The genus *Paecilomyces* differs from *Penicillium* by the shape of the phialides, consisting of a cylindrical or swollen basal portion, tapering into a long distinct neck. The conidial structures are usually divergent, verticillate or irregularly arranged.

In the course of a revisionary study of the genus *Penicillium*, Pitt (1979a) recognized that certain species are closely related, but differed significantly in microscopic characters and coloration from other *Penicillium* species. Thus he erected the genus *Geosmithia* gen. nov. comprising 6 species: *G. emersonii* and *G. swiftii* were replaced for *P. emersonii* (teleomorph- *Talaromyces emersonii) and P. bacillisporum* (teleomorph- *Talaromyces bacillisporum*) respectively. *Geosmithia* is distinguished from *Penicillium* primarily by the formation of conidia borne as cylinders from cylindroidal, rough-walled phialides, which commonly lack the distinctive narrowed collula (necks) of *Penicillium* and *Paecilomyces*. In addition, conidia of *Geomithia* viewed on mass are not green: *G. namyslowskii,* an apparent exception, produces grey

conidia with a pale green cast, a color quite distinct from the blue-grey or green-grey characteristic of *Penicillium* (Pitt, 1979b).

Pitt (1979c) reported *Merimble* gen. nov. differs from *Penicillium* by the absence of green in colony colors, and from *Paecilomyces* by the formation of phialides with short straight collula. It differs from both these genera and from *Geosmithia* by the characteristic formation of long, wide, spathulate conidiophores similar to those of *Aspergillus* and *Raperia* (Subramanian and Rajendran, 1975). However, the absence of foot cells and the successive formation of phialides by *Merimbla* provide clear distinctions from both species (Pitt, 1979c).

Heredia *et al.*, (2001) reported *Sagenomella* sp. as an anamorph of *Talaromyces ocotl*. *Sagenomella* is resembled to *Acremonium*, but differs producing a short conidiophore erect from mycelium, simple phialides or a short lateral conidiophore bearing 2-4 whorls of basitonously branched phialides. Phialides acerose, usually tapering apically, but sometimes cylindrical, slightly swollen at the base.

Stolk and Samson, (1972) proposed *Talaromyces* differs from the other perfect penicillate genus *Eupenicillium* by its soft ascomata. *Eupenicillium* is characterized by sclerotioid ascomata that are very hard to gritty when young. *Talaromyces* can easily be distinguished from the related genera in the Eurotiaceae: *Byssochlamys* Westling, *Hamigera* Stolk & Samson and *Thermoascus* Miehe by its catenate asci.

In the Family *Gymnoascaceae*, four genera produce ascomata approximating those of *Talaromyces* including *Arachniotus*, *Narasimhella*, *Amauroascus* and *Arachnotheco*. *Talaromyces* can easily be separated from these genera by its conidial state. In these four gymnoascaceous genera, the imperfect state is lacking or represented by arthro-or aleurioconidia (Stolk and Samson, 1972).

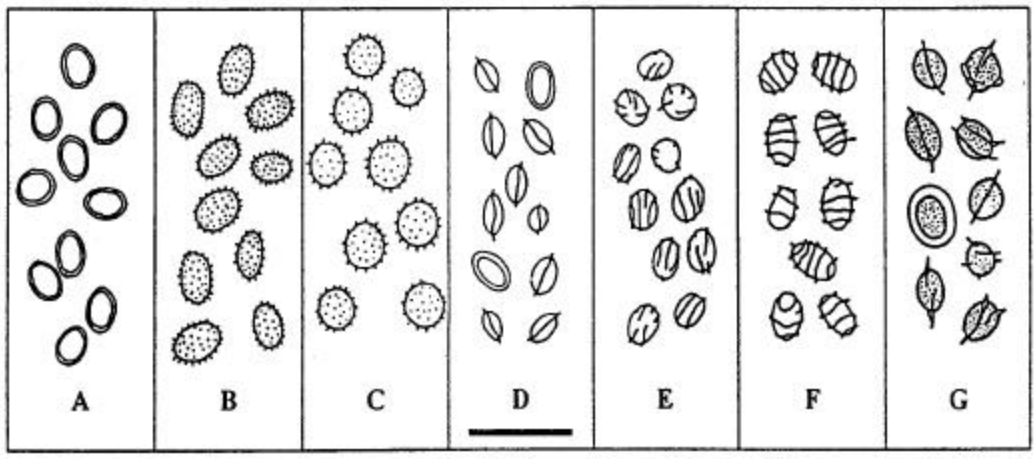


Figure 3 A synoptic illustrations of the ascospores ornamentation of *Talaromyces* species: *T. emersonii* (A), *T. flavus* (B), *T. bacillisporus* (C), *T. stipitatus* (D), *T. thermophilus* (E), *T. udagawae* (F) and *T. unicus* (G). Bar = 10 um.

Source : Stolk and Samson, 1972; Tzean *et al.*, 1992

Table 1 Teleomorph and anamorph connections of *Talaromyces* species

***Talaromyces* (Teleomorph)**	**Anamorph**	**References**
Talaromyces assiutensis	*Penicillium assiutense*	Samson and Abdel-Fattah, 1978
Talaromyces austrocalifornicus	*Penicillium austrocalifornicum*	Yaguchi *et al*.,1993a
Talaromyces bacillisporus	*Penicillium bacillisporum*	Stolk and Samson, 1972
	Geosmithia swiftii Pitt	Pitt, 1979b
Talaromyces barcinensis	*Penicillium barcinense*	Yaguchi *et al*., 1993b
Talaromyces brevicompactus	*Penicillium brevicompacta*	Kong, 1999
Talaromyces byssochlamydoides	*Paecilomyces byssochlamydoides*	Stolk and Samson, 1972
Talaromyces convolutus	*Penicillium convolutum*	Udagawa, 1993
Talaromyces derxii	*Penicillium derxii*	Takada and Udagawa, 1988
Talaromyces dupontii	*Penicillium dupontii*	Stolk and Samson, 1972
Talaromyces eburneus	*Geosmithia eburnea*	Yaguchi *et al*, 1994a
Talaromyces emersonii	*Penicillium emersonii*	Stolk and Samson, 1972
Talaromyces emodensis	*Penicillium emodense*	Udagawa, 1993
Talaromyces euchlorocarpius	*Penicillium euchlorocarpium*	Yaguchi *et al*., 1999
Talaromyces flavus	*Penicillium vermiculatum*	Stolk and Samson, 1972
Talaromyces galapagensis	*Penicillium galapagense*	Pitt, 1979a
Talaromyces gossypii	*Penicillium gossypii*	Pitt, 1979a
Talaromyces hachijoensis	-	Yaguchi *et al*., 1996

Table 1 (Continued)

Talaromyces	**Anamorph**	**References**
Talaromyces helicus var. *boninensis*	*Penicillium spirillum*	Yaguchi *et al*., 1992
Talaromyces helicus var. *helicus*	*Penicillium helicum*	Stolk and Samson, 1972
Talaromyces helicus var. *major*	*Penicillium helicum*	Stolk and Samson, 1972
Talaromyces indigoticus	*Penicillium indigoticum*	Takada and Udagawa, 1993
Talaromyces intermedius	*Penicillium intermedium*	Stolk and Samson, 1972
Talaromyces lagunensis	*Penicillium lagunense*	Udagawa *et al.*, 1994
Talaromyces leycettanus	*Paecilomyces leycettanus*	Stolk and Samson, 1972
Talaromyces luteus	*Penicillium luteum*	Stolk and Samson, 1972
	Penicillium udagawae	Pitt, 1979a
Talaromyces macrosporus	*Penicillium macrosporum*	Frisvad *et al*., 1990
Talaromyces mimosinus	*Penicillium mimosinum*	Pitt, 1979a
Talaromyces muroii	-	Yaguchi *et al*., 1994a
Talaromyces ocotl	*Sagenomella* sp.	Heredia *et al*., 2001
Talaromyces ohiensis	*Penicillium ohiense*	Pitt, 1979a
Talaromyces panasenkoi	*Penicillium panasenkoi*	Pitt, 1979a
Talaromyces purpureus	*Penicillium purpureum*	Stolk and Samson, 1972
Talaromyces retardatus	*Penicillium retardtum*	Udagawa *et al.*, 1993
Talaromyces rotundus	*Penicillium rotundum*	Stolk and Samson, 1972
	Penicillium sphaerum	Pitt, 1979a
Talaromyces spectabilis	*Paecilomyces spectabilis*	Udagawa and Suzuki, 1994
Talaromyces stipitatus	*Penicillium stipitatum*	Stolk and Samson, 1972
	Penicillium emmonsii	Pitt, 1979a
Talaromyces striatus	*Penicillium lineatum*	Pitt, 1979a
Talaromyces subinflatus	*Penicillium subinflatum*	Yaguchi *et al*., 1993a
Talaromyces tardifaciens	*Penicillium tardifaciens*	Udagawa, 1993
Talaromyces thermophilus	*Penicillium dupontii*	Stolk and Samson, 1972
Talaromyces trachyspermus	*Penicillium lehmanii*	Stolk and Samson, 1972
Talaromyces trachyspermus var. *assiutensis*	*Penicillium lehmanii*	Yaguchi *et al*., 1994b
Talaromyces ucrainicus	*Penicillium ucrainicum*	Stolk and Samson, 1972
Talaromyces udagawae	*Penicillium udagawae*	Stolk and Samson, 1972
Talaromyces unicus	*Penicillium unicum*	Tzean *et al.*, 1992
Talaromyces wortmannii	*Penicillium wortmannii*	Stolk and Samson, 1972
Talaromyces wortmannii var. *sublevisporus*	*Penicillium kloeckeri*	Yaguchi *et al*., 1994b

1.4 Identification of *Talaromyces*

Hawksworth *et al.*, (1995) classified *Talaromyces* as below;

Kingdom Eumycota
Phylum Eumycotina
Class Ascomycetes
Order Eurotiales
Family Trichocomaceae
Genus *Talaromyces*

Raper and Thom (1949) studied morphology of the genus *Penicillium*. They found many species of *Penicillium* including *P. wortmanni*, *P. bacillisporus*, *P. vermiculatum*, *P. stipitatum* and *P. luteum* could produce soft ascomata and asci in chains with 8 ascospores.

In 1955 C.R. Benjamin erected the genus *Talaromyces* with Type species; *T. vermiculatus* (syn. *T. flavus* (Klocker) Stolk & Samson (Stolk and Samson, 1972). For generic description "Ascomata globose to subglobose, soft, superficial, discrete or confluent, of indeterminate growth. Ascomatal covering varying from scanty to dense, consisting of a network of hyphae, which may range from very loosed-textured to closely knit, usually surround by a weft of thin, usually encrusted radiating hyphae, straight or twisted depending on the species. Ascomatal initials of various shape. Asci evanescent, 4-, 6- or 8-spored, globose to subglobose or slightly ellipsoildal, borne in chains. Ascospores globose or ellipsoidal, smooth or showing various ornamentations, yellow, rarely become reddish" (Stolk and Samson, 1972; Pitt, 1979a).

Stolk and Samson (1972) divided *Talaromyces* to 4 sections including section *Talaromyces*, *Thermophila*, *Purpurea* and *Emersonii* (Table 2) showed their different anamorph states as follows;

1. Section *Talaromyces* - conidial state belonging to the *Penicillium* Biverticillate- symmetrical type
2. Section *Emersonii* - conidial state *Paecilomyces* or belonging to the *Penicillium cylindrosporum*-series
3. Section *Thermophila* - conidial state belonging to *Penicillium* asymmetrica-Divaricata, near the *P. janthinellum*-series
4. Section *Purpurea* - conidial state belonging to the *Penicillium restrictum*-series

Section *Talaromyces*

Cleistothecia yellow, occasionally white, creamish, pinkish or reddish. Ascospores yellow, in some strains producing abundant red pigment. Conidial state: *Penicillium* biverticillate–symmetrica type. Phialide usually lanceolate, in a few species showing a wider base. Species belonging to this section show their best development on malt and oatmeal agar. On Czapek's agar (CZA), they develop better than the species belonging to the other sections. The species belonging to this section are mesophillic, with optimum temperatures about 25°C and maximum temperatures not exceeding 40°C. Ascomatal initials are of various types. Paired gemetangia occur in 3 species of this section: *T. flavus*, *T. helicus* and *T. stipitatus*. In the other species initials develop as branches or as intercalary portions of hyphae. Asci are produced in short coiled chains, they are usually 8-spored. Ascospores are generally ellipsoidal and their walls showing various ornamentation.

Section *Talaromyces* comprising 10 species, 2 varieties: *Talaromyces rotundus, T. luteus, T. udagawae, T. stipitatus, T. ucrainicus, T. flavus, T. macrosporus, T. helicus* var. *helicus, T. helicus* var. *major, T. wortmannii, T. trachyspermus* and *T. intermedius*

Section *Emersonii*

Ascomatal coverings scanty to distinct. Ascospores yellow. Imperfect state: *Penicillium cylindrosporum - series* or *Paecilomyces*; thermophilic or thermotolerant. Optimum temperatures of the 4 species are relatively high, ranging from 35°C to 45°C depending on the species. All species grow well at 40°C. Ascomatal initials show different structures. Asci occur in short, curved or coiled chains, usually 8-spored. Ascospores ellipsoidal or globose, smooth wall or showing various ornamentation.

Section *Emersonii* comprising 4 species: *Talaromyces byssochlamydoides*, *T. leycettanus*, *T. emersonii* and *T. bacillisporus*.

Section *Thermophila*

The ascomata have well-developed, thick, parenchymatous walls. Asci are not produced in helicoidal chains as occurring in most species of *Talaromyces*, but in rather straight and branched chains. In addition to the conidial state of the only species is classified in the Asymmetrica–Divaricata near the *Penicillium janthinellum-series*. Ascomata grow well on sterilized oat grains at 45°C.

Section *Thermophila* comprising only one species: *Talaromyces thermophilus*

Section *Purpurea*

Covering of ascomata distinct, consisting of a thin, loose-textured network of hyphae, yellow. Ascospores yellow, becoming reddish from diffusing red pigment. Anamorph state is *Penicillium restrictum – series*.

Section *Purpurea* comprising only one species: *Talaromyces purpureus*

Table 2 Morphological characteristics of different species of *Talaromyces* in four sections and three series (Modified from Stolk and Samson, 1972; Pitt, 1979a)

1. Section *Talaromyces*

Series Flavi	***Series Lutei***	***Series Trachyspermus***
1. *Talaromyces flavus*	1. *Talaromyces luteus*	1. *Talaromyces trachyspermus*
2. *Talaromyces macrosporus*	2. *Talaromyces wortmannii*	2. *Talaromyces gossypii*
3. *Talaromyces helicus*	var. *wortmannii*	3. *Talaromyces ohiensis*
var. *helicus*	var. *sublevisporus*	4. *Talaromyces galapagensis*
var. *major*	3. *Talaromyces rotundus*	5. *Talaromyces mimosinus*
var. *boninensis*	4. *Talaromyces austrocalifornicus*	6. *Talaromyces barcinensis*
4. *Talaromyces stipitatus*	5. *Talaromyces retardatus*	7. *Talaromyces assiutensis*
5. *Talaromyces striatus*	6.*Talaromyces euchlorocarpius*	
6. *Talaromyces panasenkoi*	7. *Talaromyces udagawae*	
7. *Talaromyces muroii*	8. *Talaromyces ucrainicus*	
8. *Talaromyces unicus*	9. *Talaromyces lagunensis*	
9. *Talaromyces intermedius*		

2. Section *Emersonii*	**3. Section *Thermophila***	**4. Section *Purpurea***
1. *Talaromyces bacillisporus*	1. *Talaromyces thermophilus*	1. *Talaromyces purpureus*
2. *Talaromyces leycettanus*		
3. *Talaromyces emersonii*		
4. *Talaromyces byssochlamydoides*		
5. *Talaromyces eburneus*		
6. *Talaromyces spectabilis*		

2. The molecular study of *Talaromyces*

2.1 Microsatellites or simple sequence repeats (SSRs)

Microsatellites or simple sequence repeats (SSRs) are composed of tandemly repeated, simple DNA sequence motif of as many as six nucleotides in length. These loci are commonly found throughout both prokaryotic and eukaryotic genomes and typically are highly polymorphic within species and populations. In addition, these codominant genetic markers are relatively easy to score and have high reproducibility and specificity. As such, microsatellites have become one of the most popular classes of molecular markers and are commonly employed to investigate the population genetics of a diverse range of organisms (Bruford and Wayne, 1993; Goldstein and Schlotterer, 1999; Dettman and Taylor, 2004).

For fungi, SSRs have been also used for phylogenetic analysis and these analyses have examined evolutionary relationships from the kingdom to species level and genetic polymorphism (Chen *et al.*, 2002; Nascimento *et al.*, 2004; Fisher *et al.*, 2004a, b.

White mold and stem rot caused by *Sclerotinia sclerotiorum* is a serious disease in agricultural and native plants at Canada. Sirjusingh and Kohn (2001) was able to show using microsatellites–based analysis the diversity of this fungus. It was also shown successfully amplified the closely related *S. trifoliorum* and *S. minor.*

Paracoccidioides brasiliensis, is a cause of paracoccidioidomycosis (PCM) and widespread in Central and South America, from Mexico to Argentina. Its natural habitat in soil or in plants in areas where PCM endemic. Microsatellite markers were used to elucidate the phylogenetic relationships of the environmental strains and the types of human disease they cause. The result showed no correlation between the clinical form of human PCM and SSR patterns (Fisher *et al.*, 2004a, b; Nascimento *et al.*, 2004) used multilocus microsatellite typing to examine the emergence of *Penicillium marneffei* in human HIV-positive populations.

2.2 Phylogenetic study of *Talaromyces*

Ribosomal DNA of 29 species of *Talaromyces* and related genera were examined in their phylogenetic relationships. The variability in the nuclear rDNA repeat unit was studied by the restriction fragments of total DNA that hybridized to the rDNA repeat unit of *Neurospora crassa* (pMF2). The result showed *Talaromyces* species with *Paecilomyces* anamorphic states cluster with *Byssochlamys* and *Thermoascus* species having *Paecilomyces* anamorphic states and not with *Talaromyces* species having *Penicillium* anamorphs. Whereas, the strictly anamorphic *Penicillium* species are not mixed in with the holomorphic species, but are clustered in a group that it well separated from most of the *Talaromyces* species (Taylor *et al.*, 1990).

LoBuglio *et al.*, (1993) was able to reveal the relationship between *Talaromyces* with *Penicillium* anamorphs and strictly mitotic species of *Penicillium* subgenus *Biverticillium* using ribosomal DNA (rDNA) nucleotide characters. They demonstrated that some mitotic *Penicillium* species have a closer phylogenetic relationship to meiotic species than they do to other strictly mitotic *Penicillium* species.

Berbee *et al.*, (1995) concluded that *Penicillium* is not monophyletic based on analysis of the variable ITS region and 5.8, 18S regions of the ribosomal DNA. *Penicillium* species that have a *Eupenicillium* teleomorph grouped with *Aspergillus* whereas *Penicillium* species with *Talaromyces* teleomorphs grouped with *Paecilomyces*. This study, however, only looked at three of *Penicillium* spp. and *Aspergillus* spp. with two *Paecilomyces* species.

Talaromyces ocotl with *Sagenomella* sp.-anamorph described as new species, based on morphological analyses and phylogenetic inferences made from ITS and 28S rDNA sequence alignments. *Talaromyces ocotl* and *T. rotundus* were isolated soil from *Pinus hartwegii* and *Pinus patula* forests at the Volcanic Cordillera in Mexico (Heredia *et al.*, 2001).

Yaguchi *et al.*, (2005) were able to demonstrate, using D1/D2 region of 28 S rDNA sequence analysis, that *Geosmithia argillacea* is the anamorph of *Talaromyces eburneus* as a heat resistant fungus. They are often reported spoilage agents in fruit juices, food and other heat processed fruit based products (Samson *et al*., 2002; Udagawa, 2000).

3. Biological Control of plant pathogenic fungi by *Talaromyces*

Talaromyces flavus (Klöcker) Stolk & Samson (conidial state: *Penicillium vermiculatum* Dangeard) was reported to be antagonistic to many plant pathogenic fungi, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Verticillium albo-atrum*, and *V. dahliae*. A variety of mechanisms have been postulated for biocontrol of pathogens by *T. flavus* including hyperparasitism, competition for nutrients, and antibiosis (Boosalis, 1956; Duo-Chuan *et al.*, 2005; McLaren *et al*., 1986; Kim *et al.*, 1988; Stosz *et al*., 1998).

Boosalis (1956) observed that *T. flavus* invades hyphae of *Rhizoctonia solani* directly by producing penetration pegs. These pegs developing from either a mycelium coiling around the host hyphae or form a hyphae in direct contact with the host. Later, McLaren *et al.*, (1986) demonstrated hyperparasitism of sclerotinia wilt caused by *Sclerotinia sclerotiorum* by *T. flavus*, using light and scanning electron microscopy. The hyphae of *T. flavus* could grow toward and coiled around the host hyphal cells. The coiling effect intensified as the hyphae of *T. flavus* branched repeatedly on the host surface. Tips of hyphal branches often invaded the host by direct penetration of the cell wall without formation of appressoria. Moreover infection of host cells by *T. flavus* resulted in granulation of the cytoplasm and collapse of the cell walls.

Production of the enzymes glucose oxidase has been shown to be involved in the biological control of Verticillium wilt by *T. flavus* (Ayer and Racok, 1990a). Stosz *et al.*, (1998) reported the location of glucose oxidase in *T. flavus* by immunocytochemistry using glucose oxidase-specific polyclonal antibody. The results

showed that glucose oxidase was found in both intracellular and extracellular, both mature and young hyphal cells contained this enzyme, but decreased as the cells aged. Enzyme stability studies confirmed that the glucose oxidase of *T. flavus* is an extremely stable enzyme, retaining 13% of its original activity after 2 weeks at 25°C and retaining activity for several days at temperatures up to 50°C.

Duo-Chuan *et al.*, (2005) purified two chitinases from the culture filtrate of *T. flavus*. By SDS-PAGE, the molecular weight of the two enzymes was estimated to be 41 and 32 kDa respectively. The two chitinases can degrade cell wall of *Verticillium dahliae, Sclerotinia sclerotiorum* and *Rhizoctonia solani*, and inhibited spore germination and germ tube elongation of *Alternaria alternata, Fusarium moniliforme*, and *Magnaporthe grisea*.

Hydrogen peroxide is one of the products of a reaction catalyzed by glucose oxidase in the presence of glucose. Kim *et al.*, (1988) purified and characterized the glucose oxidase from *T. flavus* culture filtrates and determined that this enzyme inhibited germination of *V. dahliae* microsclerotia *in vitro* in the presence of glucose. *In vitro* exposure to hydrogen peroxide or to glucose oxidase in the presence of glucose is lethal to the microsclerotia of *V. dahliae*. (Kim *et al.*, 1988; Stosz *et al.*, 1996).

Nagtzaam and Bollen (1997) reported *Talaromyces flavus* has potential as biocontrol agent against of *Verticillium dahliae*. The fungus could colonize potato and eggplant roots. In pot experiments with field soils, the fungus could growth from potato seed tube or eggplant seeds coated to developing roots, including the root tips. The population of *T. flavus* decreased log-linearly with distance from the seed and was higher with potatoes than with eggplants.

Tjamos and Fraval (1997) reported the distribution and establishment of *T. flavus* in the soil and on roots of solanaceous crops. Ascospores of *T. flavus* were applied to eggplant, tomato and potato as either a drench or in spherical granular (alginate prill). The percentage colonization of roots was greater than that of root tips.

When populations of *T. flavus* were expressed as colony forming units g-1 fresh weight, recovery of *T. flavus* was greater in root tips and superficially associate with the roots. Populations were lower in roots, rhizosphere and non-rhizosphere soils, respectively. Populations of *T. flavus* were greater when it was applied as a drench rather than in alginate prill.

Jun *et al.*, (1999) studied on the antagonism of *T. flavus* against pathogenic fungi infecting cotton in China, including *Verticillium dahliae*, *Fusarium oxysporum* f. sp. *vasinfectum*, *Rhizoctonia solani* and *Colletotrichum gossypii in vitro* on CZA and wheat grain media. Results showed that *T flavus* inhibited the radial growth of *V. dahiae*, *F. oxysporum* f. sp. *vasinfectum*, *R. solani* and *C. gossypii*, and the conidial germination of *Verticillium dahliae*, *Fusarium oxysporum* f. sp. *vasinfectum*, but did not inhibit *R. solani* and *C. gossypii*. The inhibitory activity of *T. flavus* was affected by glucose concentration in Czapek medium, with the highest activity recorded when glucose was at 0.5-4.0%.

In Canada, Inglis and Kawchuk (2002) tested the ability of *Talaromyces flavus* to degrade cell walls of *Pythium ultimum* (Oomycete), *Fusarium equiseti* (Ascomycete) and *Rhizoctonia solani* (Basidiomycete) *in vitro*. The results showed that *T. flavus* could degrade cell walls of *P ultimum* and *F equiseti*. Production of carboxymethyl cellulase, ß- glucanases, chitinases and chitosanases were reported.

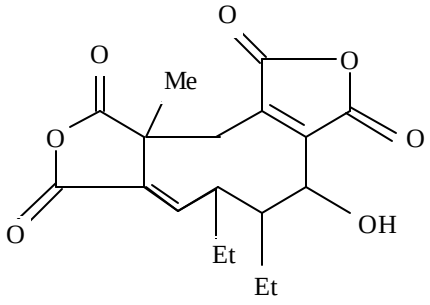
4. Chemistry of the Genus *Talaromyces* C.R. Benjamin

Fungi are know to produce a vast number of bioactive compounds. After the discovery of penicillin G from a *Penicillium* species about 80 years ago that led to many researches were motivated to search and screening large collection of fungi especially antibiotic property for novel drug (Larsen *et al.*, 2005). Until 1995, the top twenty selling prescription medicines worldwide were of fungal origin (Langley, 1997; Peberdy, 1999; Singh and Aneja, 1999; Skehan *et al.*, 1990). *Talaromyces* is an interesting fungus for this purpose.

In recent years, seventeen species of the genus *Talaromyces* including *T. assiutensis, T. austrocalifornicus, T. bacillisporus, T. convolutes, T. derxii, T. emodensis, T. flavus, T. luteus, T. macrosporus, T. mimosinus, T. ohiensis, T. panasenkoi, T. stipitatus, T. tardifaciens, T. trachyspermus, T. udagawae* and *T. wortmannii* have been investigated for their secondary metabolites and its activity (Ayer and Racok, 1990 a,b; Dong *et al.*, 2006; Frisvad *et al.*, 1990; Ishii *et al.*, 1995; Nozawa *et al.*,1995; Phillips *et al.*, 1987; Shiozawa *et al.*, 1995; Suzuki *et al.*, 1992, 1999, 2000; Yamazaki and Okuyama, 1980; Yoshida *et al.*, 1996).

4.1 *Talaromyces assiutensis*

Frisvad *et al.*, (1990) investigated the profiles of secondary metabolites of *Talaromyces* and found that *T. assiutensis* can produce glauconic acid (**1**).



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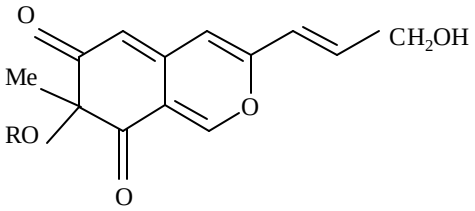
4.2 *Talaromyces austrocalifornicus*

Suzuki *et al.*, (1999) reported the isolation of (-)- mitorubrinal (**2**) and (-)- mitorubrinic acid (**3**) from yellow pigments on ascomata of *T. austrocalifornicus*.



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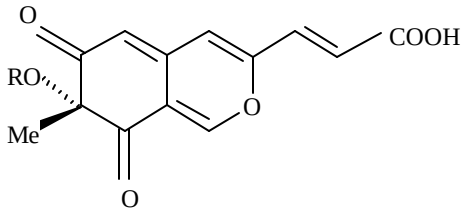
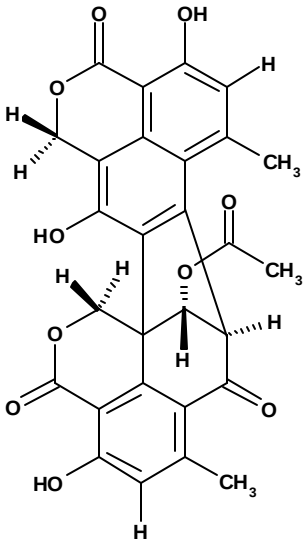


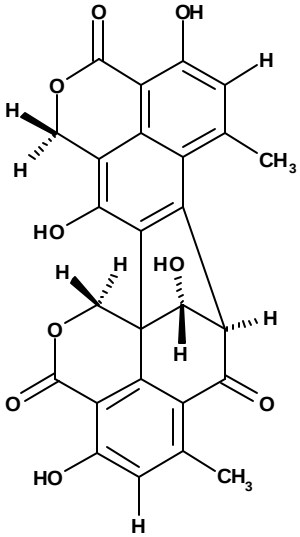
3

4.3 *Talaromyces bacillisporus*

Oxaphenalenone dimers and xanthone carboxylic acid were isolated from *T. bacillisporus* as bacillosporins A (**4**), B (**5**), C (**6**), pinselin and pinselic acid. Compound **4** exhibited antibacterial activity against *Bacillus subtilis* and *Sarcina lutea* (Yamazaki and Okuyama, 1980).



4



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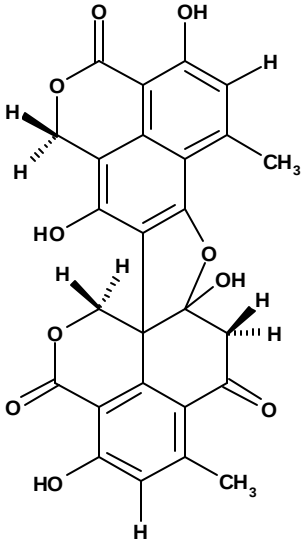
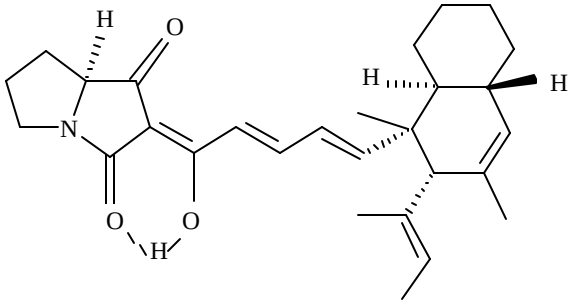


6

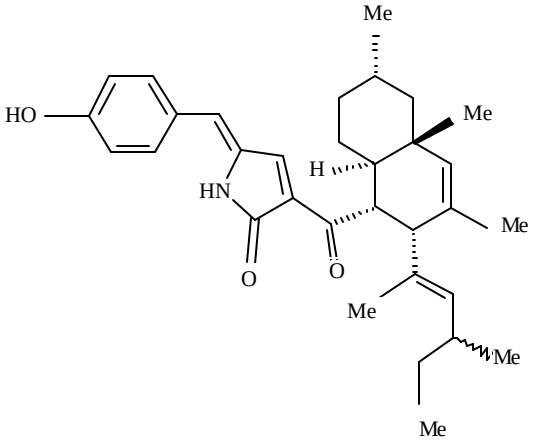
Furthermore, Ishii *et al.*, (1995) isolated talarotoxin (**7**) from *T. bacillisporus* that showed cytotoxic activity inhibited the proliferation of both mouse myeloma X63.Ag8.6.5.3 cells and BALB/3T3 mouse fibroblasts.



7

4.4 *Talaromyces convolutus*

The fungus contained the secondary metabolites: talaroconvolutins A (**8**), B (**9**), C (**10**) and D (**11**). Compounds **8** and **9** were reported to inhibit he growth of *Aspergillus fumigatus*, *A. niger* and *Candida albicans* (Suzuki *et al.*, 2000).



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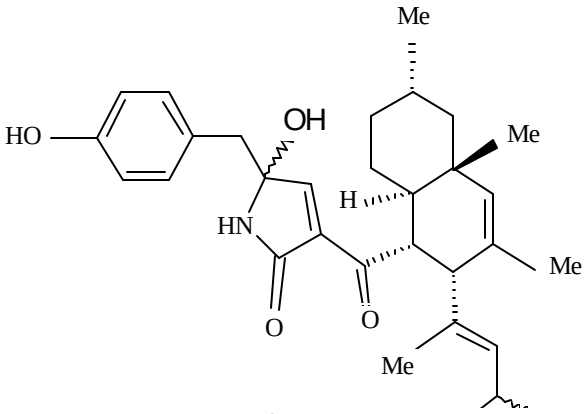

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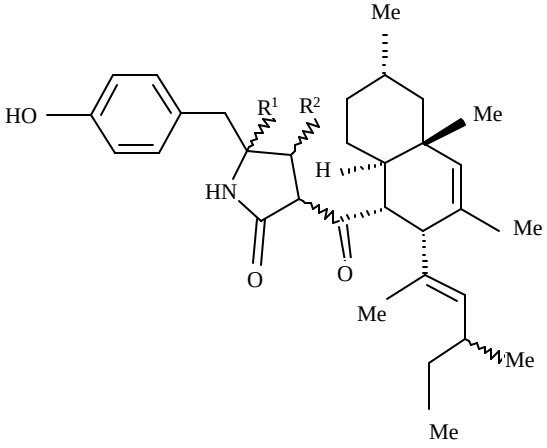
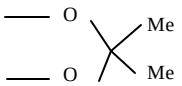


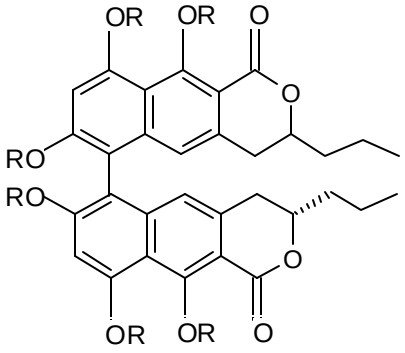
10 R^1 = OH, R^2 = OEt

11 R^1, R^2 = 

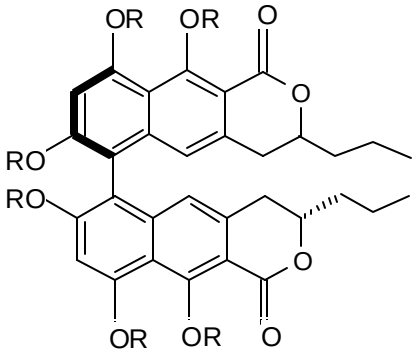
Moreover, Suzuki *et al.*, (1999) have reported the isolation of (-)-mitorubrinal (**2**) and (-)- mitorubrinic acid (**3**) from yellow pigment on ascomata of *T. convolutus*.

4.5 *Talaromyces derxii*

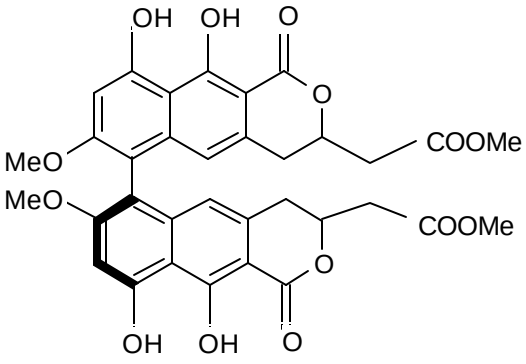
Secondary metabolites isolated from *T. derxii* including; talaroderxines A (**12**) and B (**13**), viriditoxin (**14**), vioxanthin (**15**), semiviriditoxin (**16**), hexamethyl ether (**17**) and vioxanthin (**18**) have strong antibacterial activity against *Bacillus subtilis* (Suzuki *et al.*,1992)



12 R = H
17 R = Me



13 R = Ac



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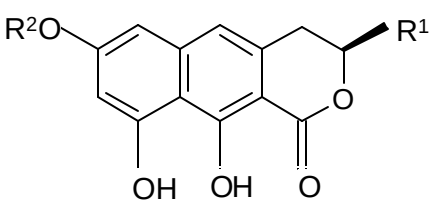
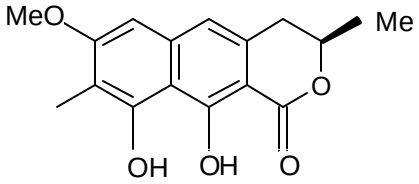


15 R^1 = Me, R^2 = H

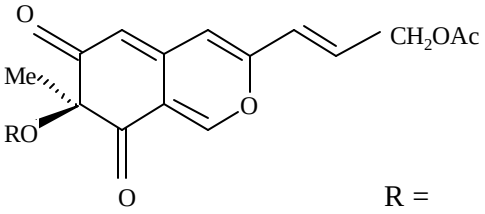
16 R^1 = CH_2COOMe, R^2 = Me



18

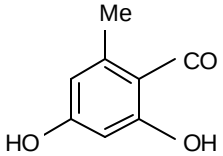
4.6 *Talaromyces emodensis*

Suzuki *et al.*, (1999) isolated (+)- mitorubrinol acetate (**19**) from yellow pigment on ascomata of *T. emodensis.*



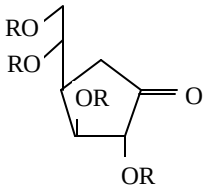
19

R =

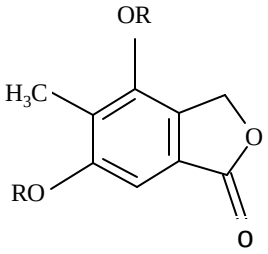


4.7 *Talaromyces flavus*

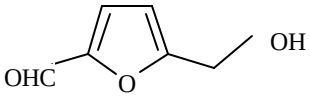
A number of secondary metabolites have been isolated from *T. flavus* including D-glucono-1,4-lactone (**20**), acetylation of D-glucono-1,4-lactone (**21**), 4,6 –dihydroxy-5-methylphthalide (**22**), methylation of 4,6 –dihydroxy-5-methylphthalide (**23**), 5-hydroxymethylfurfural (**24**), 7-hydroxy-2,5-dimethylchromone (**25**), methylation of 7-hydroxy-2,5-dimethylchromone (**26**), methyl 4-carboxy-5-hydroxyphthaladehydate (**27**), (**28**), 3-hydroxymethyl-6,8-dimethoxycoumarin (**29**), altenusin (**30**), dehydroaltenusin (**31**), dehydroaltenusin diacetate (**32**), desmethyldehydroaltenusin (**33**), acetylation of desmethyldehydroaltenusin (**34**), talaroflavone (**35**), acetylation of talaroflavone (**36**), deoxytalaroflavone (**37**),



20 R = H
21 R = $COCH_3$



22 R = H
23 R = CH_3



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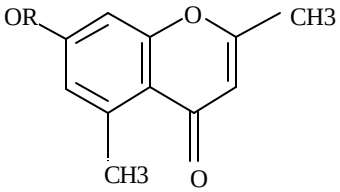
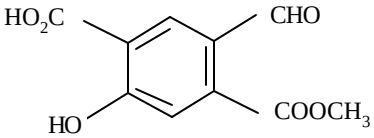


25 R = H
26 R = CH_3



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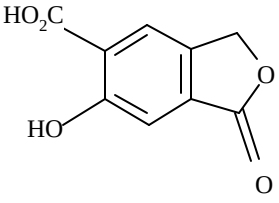
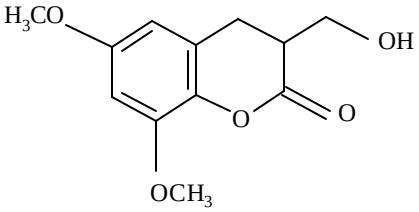


28



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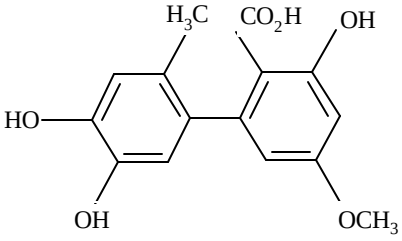

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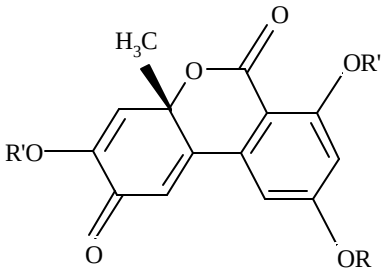
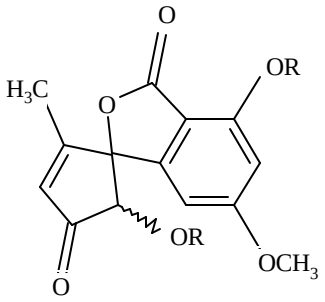
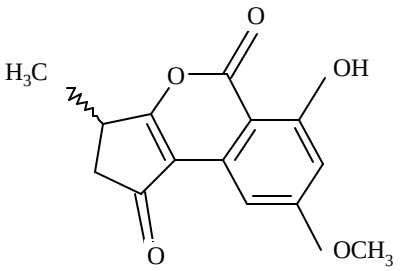


31 R = CH_3, R' = H
32 R = CH_3, R' = $COCH_3$
33 R = H, R' = H
34 R, R' = $COCH_3$

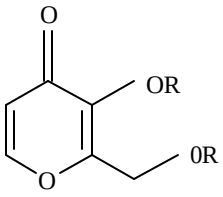


35 R = H
36 R = $COCH_3$

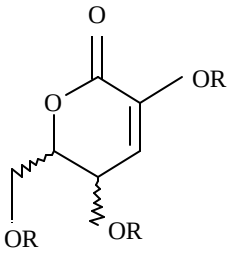


37

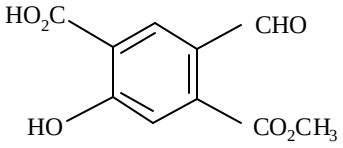
hydroxymethylmaltol (**38**), acetylation of hydroxymethylmaltol (**39**), 6-hydroxymethyl-2H-pyran-2-one (**40**), acetylation of 6-hydroxymethyl-2H-pyran-2-one (**41**), methyl 4carboxy-5-hydroxyphthalaldehydate (**42**), vermiculine (**43**) and wortmannin (**44**) (Ayer and Racok, 1990 a,b; Frisvad *et al.*, 1990).



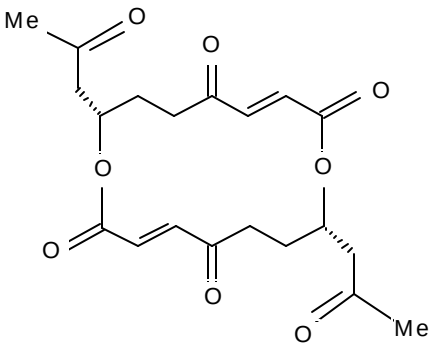
38 R = H
39 R = $COCH_3$



40 R = H
41 R = $COCH_3$



42



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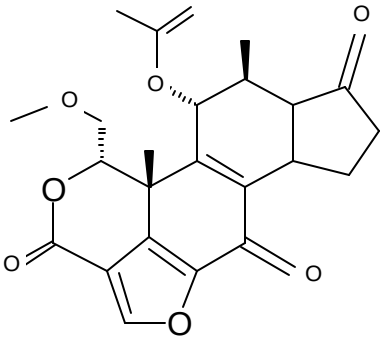
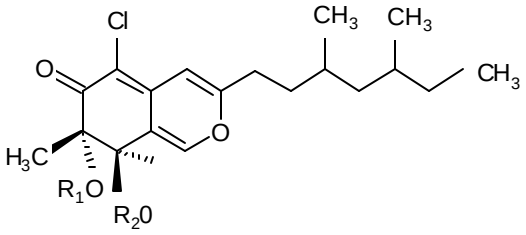


44

4.8 *Talaromyces luteus*

Yoshida *et al.*, (1996) have isolated three new azaphilones, luteusins A (**45**), B (**46**), C (**47**), D (**48**) and E (**49**) from *T. luteus*.



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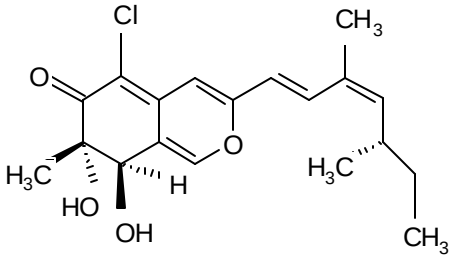

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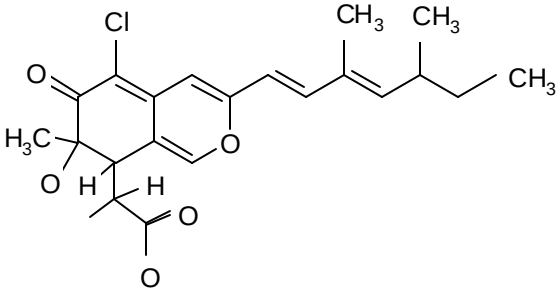

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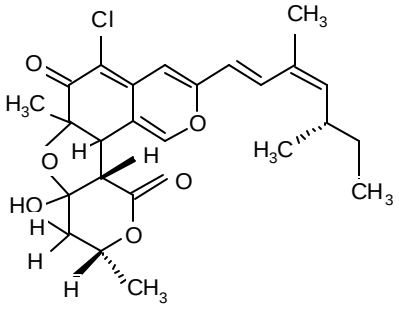
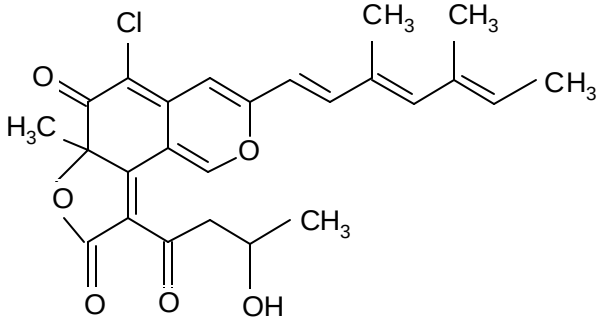


48



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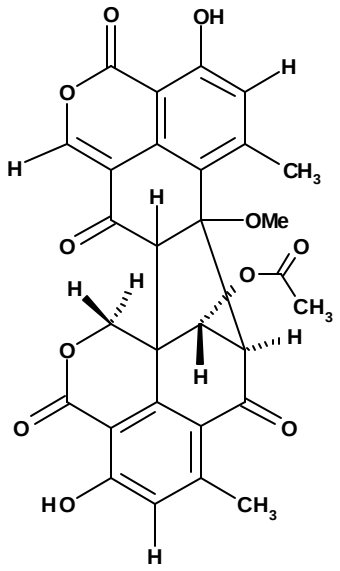
4.9 *Talaromyces macrosporus*

Frisvad *et al.*, (1990) reported the isolation of duclauxin (**50**) from this fungus.



50

4.10 *Talaromyces mimosinus*

Frisvad *et al.*, (1999) have reported the isolation of (-)- mitorubrinal (**2**) and (-)- mitorubrinic acid (**3**) from yellow pigments on ascomata of this fungus.

4.11 *Talaromyces ohiensis*

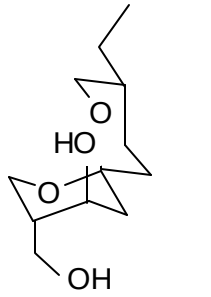
Frisvad *et al.*, (1990) reported glauconic acid (**1**) and vermiculine (**43**) from this fungus.

4.12 *Talaromyces panasenkoi*

Frisvad *et al.*, (1990) reported glauconic acid (**1**) from this fungus.

4.13 *Talaromyces stipitatus*

Phillips *et al.*, (1987) reported four new spiroketal talaromycins derived, talaromycins C (**51**), D (**52**), E (**53**) and F (**54**) from *T. stipitatus* as well as the known talaromycins A, B from *Talaromyces flavus*. Later, Frisvad *et al.*, (1990) reported more compounds from this fungus including duclauxin (**50**), catenarin (**55**), emodin (**56**), stipitatic acid (**57**) and erythroglaucin (**58**).



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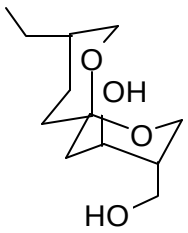

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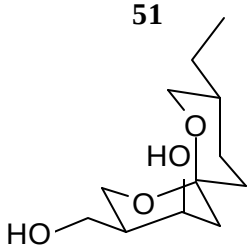

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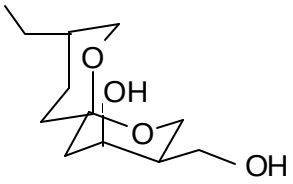

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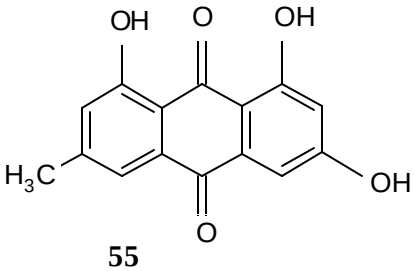
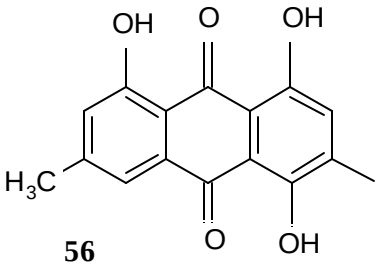


55



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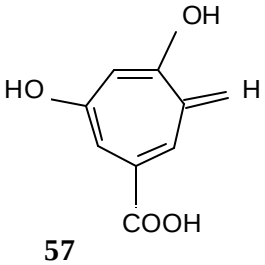

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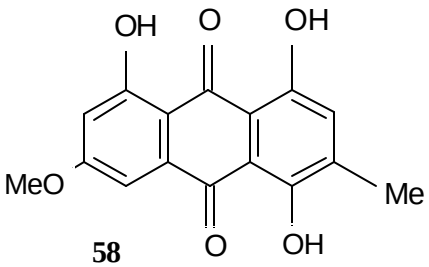
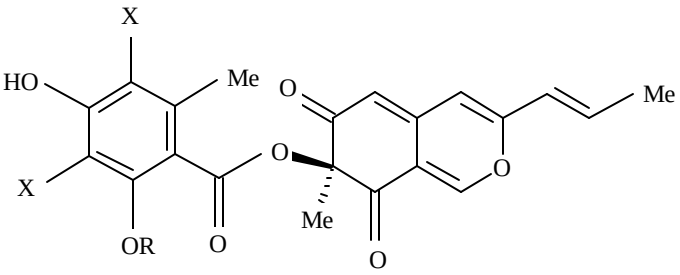


58

4.14 *Talaromyces tardifaciens*

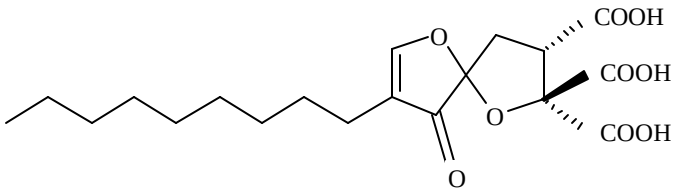
The monomethyl-(+)–mitorubrin (**59**), (+)–mitorubrin (**60**) and falconensin H (**61**) have been isolated from this fungus (Nozawa *et al.*,1995).



59 R = Me, X = H
60 R = H, X = H
61 R = Me, X = Cl

4.15 *Talaromyces trachyspermus*

Shiozawa *et al.*, (1995) reported a new metabolites, trachyspic acid (**62**) from *T. trachyspermus* that inhibits tumor cell heparanase.



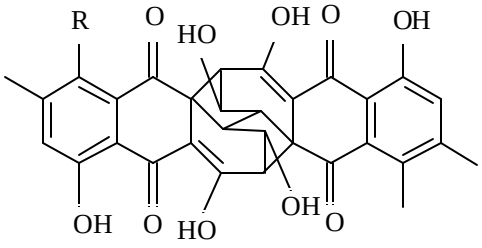
62

4.16 *Talaromyces udagawae*

Frisvad *et al.*, (1990) reported the isolation of (-)- mitorubrinal (**2**) and (-)- mitorubrinic acid (**3**) from this fungus.

4.17 *Talaromyces wortmannii*

Frisvad *et al.*, (1990) isolated mitorubrin (**2**), mitorubrinol acetate (**19**), rugulosin (**63**) and skyrin (**64**) from this fungus. Later, Dong *et al.*, (2006) reported four new compounds including wortmanilactones A (**65**), B (**66**), C (**67**) and D (**68**) from *T. wortmannii*. All compounds were screened for cytotoxic activities against a panel of five human cell lines (HCT-5, HCT-115, A549, MDA-MB-231, and K562). The IC50 values of the compounds range from 28.7 to 130.5 µM.



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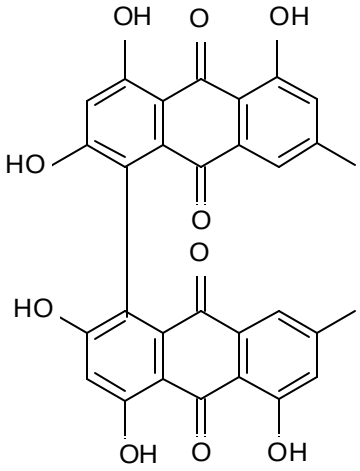

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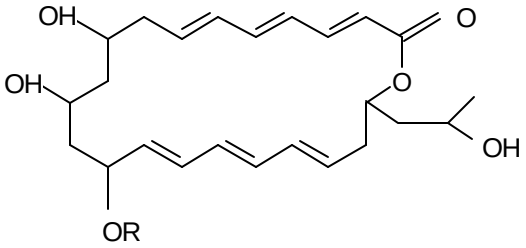
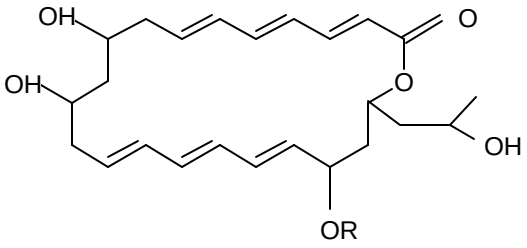


65 R = H
66 R = CH_3



67 R = H
68 R = CH_3

MATERIALS AND METHODS

Materials

1. Isolation and identification of *Talaromyces*

1.1 Materials for collected soil samples

1.1.1 shovel

1.1.2 permanent marker

1.1.3 plastic bags

1.1.4 rubber band

1.1.5 camera

1.1.6 note book

1.2 Laboratory Materials

1.2.1 permanent marker

1.2.2 forceps

1.2.3 fine needle s

1.2.4 Petri dishes

1.2.5 test tube s

1.2.5 beakers

1.2.6 agar media

1.2.7 electric scale

1.2.8 hot air oven

1.2.9 autoclave

1.2.10 alcohol lamp

1.2.11 65%, 70% and 95% ethyl alcohol

1.2.12 slide s and coverslips

1.2.13 distilled water

1.2.14 lactophenol

1.2.15 stereo microscope (SZ-PT Olympus)

1.2.16 light microscope (BH-2 Olympus)

1.2.17 camera lucida

1.2.18 Scanning Electron Microscope (JEOL JSM 6400)

1.2.19 thermometer

1.2.20 oil emersion

2. Preservation

2.1 sterilized soil

2.2 sterilized filter paper Whatman No.1

2.3 liquid paraffin

2.4 aluminum foil

2.5 paper bags

2.6 plastic bags

2.7 vials, size 1 dram.

2.8 Petri dishes

2.9 forceps

2.10 dessicator, electronic dry cabinet (WEIFO)

3. Media (Appendix)

3.1 Glucose Ammonium Nitrate Agar (GAN)

3.2 Cornmeal Agar (CMA)

3.3 25% Glycerol Nitrate Agar (G25N)

3.4 Potato Dextrose Agar (PDA)

3.5 Malt Extract Agar (MEA)

3.6 Czapek's Agar (CZA)

3.7 Czapek Yeast Autolysate Agar (CYA)

3.8 Oatmeal Agar (OMA)

3.9 Water agar

4. Phylogeny study of *Talaromyces*

4.1 Centrifuge

4.2 Hot water bath

4.3 Spatulas

4.4 Pipettes 1,000, 200, 100, 20, 2 µl

4.5 Pipette tips

4.6 Mortar and Pestle

4.7 Liquid nitrogen

4.8 Micro centrifuge tubes

5. Glasshouse Material

5.1 pots 10 cm

5.2 trays

5.3 sterilized soil

5.4 mungbean seeds

6. Isolation and purification of the secondary metabolite from *Talaromyces*

6.1 cultivating medium

6.2 Petri dishes

6.3 cork borer

6.4 Erlenmeyer flask 250, 500,1,000 and 2,000 ml

6.5 fitrate pump

6.6 paper filtrate Whatman No. 1

6.7 rotary evaporator

6.8 column chromatography

6.9 TLC aluminium sheets 20 x 20 cm silica gel 60 F_{254}, Merck

6.10 silica gel 60 F_{254} (0.063–0.200 mm), Merck for column chromatography

6.11 silica gel 60 F_{254} (0.063–0.200 mm), Merck for thin layer chromatography

6.12 20 x 20 cm glass plates

6.13 sea sand

6.14 cotton

6.15 ethyl acetate (EtOAc)

6.16 chloroform ($CHCl_3$)

6.17 acetone ($(CH_3)_2CO$)

6.18 petroleum ether (Petrol)

6.19 methanol (CH_3OH)

6.20 formic acid (HCOOH)

6.21 distilled water

6.22 microcapillary pipettes, calibrated size 10 µl

6.23 vials, 4 dram

6.24 volumetric flask

6.25 hot plate

6.26 UV detector

6.27 ultrasonic machines

6.28 aluminum foil

6.29 tank chamber

7. Stucture elucidation of the compounds

7.1 Proton Nuclear Magnetic Resonance (^{1}H NMR)

7.2 Carbon-13 Nuclear Magnetic Resonance (^{13}C NMR)

7.3 Correlation Spectroscopy (COSY)

7.4 Heteronuclear Single Quantum Coherence (HSQC)

7.5 Heteronuclear Multiple Bond Correlation (HMBC)

7.6 High Resolution Mass Spectrometry (HRMS)

Methods

1. Isolation and identification of *Talaromyces*

1.1 Soil samples collection

Forty-five soil samples were collected from agricultural fields, unagricultural fields, forest and along the roadside (Table 3, Figure 4). Labelled with locations, dates, and names of the collecter and brought to the laboratory for isolated this fungus.

2. Isolation of *Talaromyces*

2.1 Soil plate method (A modification of Warcup, 1950)

A small amount of soil (0.005-0.015 g) was placed onto a sterile Petri dish. About 10 ml of warm GAN containing rose bengal and streptomycin was added and the Petri dish was gently rotated to disperse the soil particles before the agar solidified. The plates were, then placed in covered boxes for incubation in darkess at room temperature. Hyphal tips were transferred onto PDA and maintained as pure cultures for identification.

2.2 The dilution plate method (Barron 1968)

A 10 g of soil samples was added to 100 ml of sterile distilled water. Suspensions were vigorously shaken until thoroughly mixed and 10 ml the suspension was mixed with 90 ml of sterile distilled water in a flask. Ten ml samples were then transferred through a succession of 90 ml sterile distilled water blanks until the desired dilution was reached. One-ml aliquots of the selected dilution (usually 10^{-2}, 10^{-3}, 10^{-4}) were pipetted into Petri dishes for each selected dilution. The same procedures described in the previous method were followed.

2.3 Alcohol treatment method (A modification of Warcup and Baker, 1963)

0.03 g of soil samples was placed in 65% ethanol for 10-20 min. The liquid was decanted, bits of the treated soil were dispensed into several sterile Petri dishes, and the plates were immediately poured with GAN. The same procedures described in the previous method were follwed.

2.4 Heat treatment method (A modified of Warcup and Baker, 1963)

One g of soil samples was placed in a sterile test tube in a water bath at 60-80°C for 20-30 min. Excess water was drained off and soil particle were placed into Petri dishes. The same procedures (2.1-2.3) described previously were followed.

2.5 Single ascospore isolation (Intana, 2003)

PDA plus 100 ppm streptomycin was poured in a Petri dish. A sterile glass rod was used to spread 0.5 ml ascospores suspension (10^3 ascospores / ml) on a solidified agar media and incubated for 24 h at room temperature. Ascospore germination was examined under a light or compound microscopes and a piece of agar containing a single ascospore was transferred to slant PDA.

3 Identification of *Talaromyces* species (Stolk and Samson, 1972; Ramirez, 1982; Manoch *et al.*, 2004)

3.1 Macroscopic examination

Morphological characteristics of colonies were determined as growth pattern, color, texture on different media, such as CZA, MEA, CYA, CMA, OMA and G25N agar for 7 to 14 days, at 25°C, 28°C and 37°C (Pitt, 1979a). Diameters of

colonies were measured in milimetres, most effectively by transmitted light and from the reverse side.

Colony characteristics were examined under a stereoscopic microscope and naked eyes. The microscope was used for assessing texture of colonies and the appearance of penicilli and conidial chains. For judgement of conidial and colony colours, Rayner's "A Mycological Colour Chart" (Rayner, 1970) has been employed.

3.2 Microscopic examination

Microscopic characteristics were examined on a slide preparation using sterile distilled water and lactophenol as mounting media and examined under a light microscope (Olympus BH-2 with Normaski Interference Contrast). Camera lucida drawings were employed. Photomicrographs of fungal structure were taken under stereo, light and scanning electron microscopes.

Study on ornamentation of ascospore was conducted using Scanning Electron Microscopy. Matured ascomata and ascospores of *Talaromyces* from dry culture agar media were transferred with a fine needle and placed onto double-strick scotch tape on aluminium stubs. The specimens were coated with gold for 5-7 min. and examined in a JEOL JSM 6400 scanning electron microscope (Manoch *et al.*, 2004).

Identification was based on morphological characteristics examination under a stereo, light and scanning electron microscopes. *Talaromyces* were identified following the researches done in previous reports (Stolk and Samson, 1972; Pitt, 1979a; Takada and Udagawa, 1988; Yaguchi *et al.*, 1992; Yaguchi *et al.*, 1993a, b; Udagawa, 1993; Yaguchi *et al.*, 1994a, b; Yuguchi *et al.*, 1996; Udagawa *et al.*, 1993; Udagawa and Suzuki, 1994).

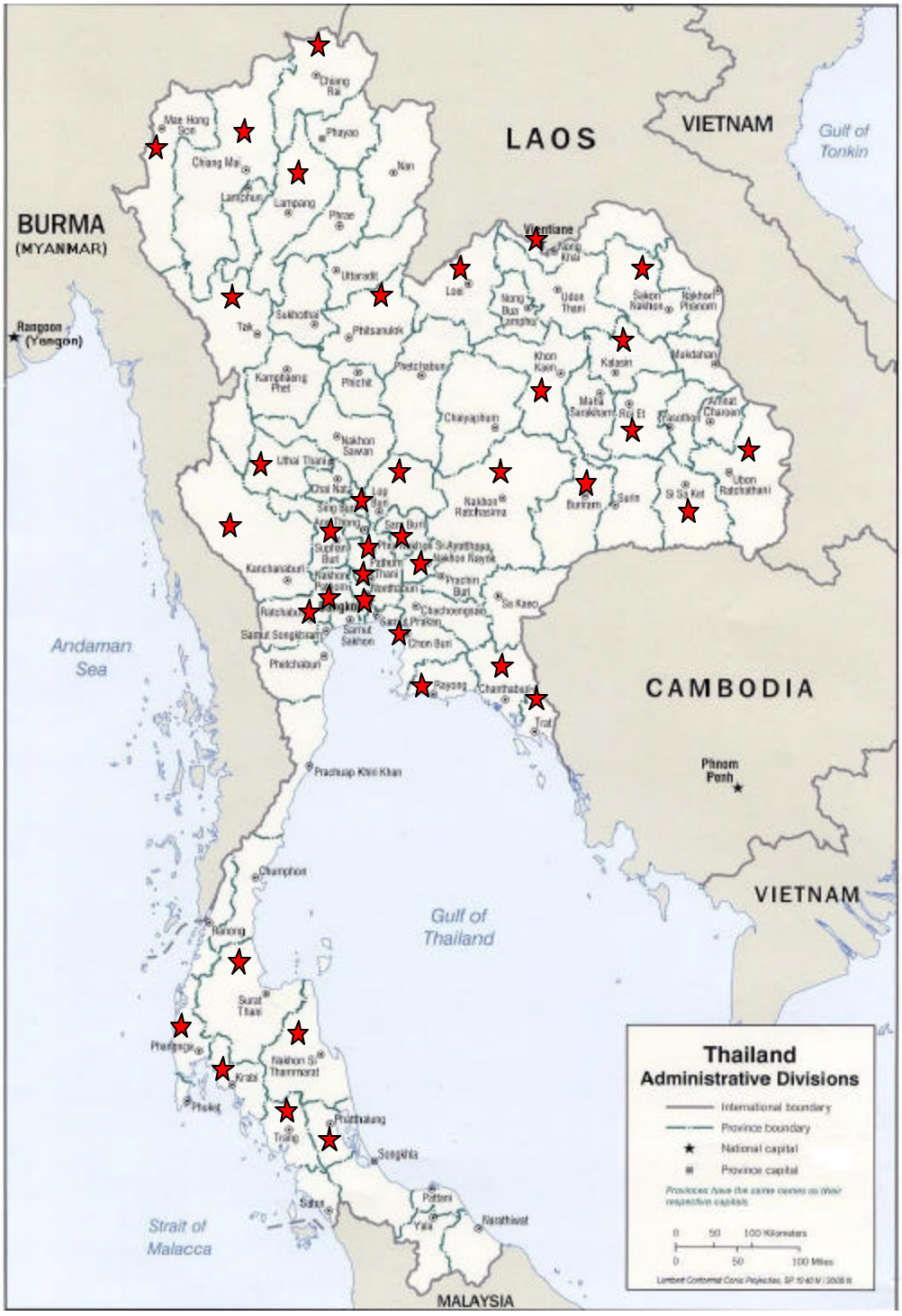


Figure 4 Map of Thailand indicating the collection sites of the soil samples from 38 provinces

Table 3 Forty-five soil samples were collected from various locations in Thailand

Part and Province	Location	Collecting date
North		
Chiang Mai	Agricultural soil, Queen Sirikit Botanic Garden	11 June 2003
	Agricultural soil, Amphur Mae Tang	17 July 2004
	Nonagricultural soil, Amphur Mae Sa	29 January 2002
	Forest soil, Mok Fa water fall	17 December 2004
Chiang Rai	Agricultural soil, Horticulture Research Institute	16 January 2004
Lumpang	Agricultural soil, Amphur Maung	15 December 2004
Mae Hong Son	Forest soil, Amphur Maung	3 December 2003
Tak	Nonagricultural soil, Amphur Maung	5 November 2003
North-East		
Buri Rum	Nonagricultural soil, Amphur Chareamprakient	15 January 2002
Pitsanulok	Forest soil, Pu Kin Rang Kha	1 January 2002
Kalasin	Nonagricultural soil, Amphur Maung	16 April 2003
Khon Kaen	Nonagricultural soil, Amphur Chumpae	2 July 2003
Loei	Forest soil, Pu Kra Doug	17 November 2001
Ubon Ratchathani	Agricultural soil, Amphur Maung	29 November 2001
Nakhon Ratchasima	Agricultural soil, Amphur Pak Thong Chai	25 October 2002
	Agricultural soil, Amphur Dan Kun Tod	19 August 2003
Nong Khai	Forest soil, Amphur Maung	11 September 2004
Roi Et	Nonagricultural soil, Amphur Maung	6 October 2003
Sakon Nakhon	Forest soil, Amphur Pupan	16 September 2001
	Nonagricultural soil, Amphur Kum Ta Kra	21 January 2000
Si Sa Ket	Nonagricultural soil, Amphur Kantharak	27 October 2001
Central		
Ang Thong	Agricultural soil, Amphur Maung	29 November 2004
Phra Nakhon Si Ayutthaya	Agricultural soil, Amphur Wangnoi	18 October 2003
Bangkok	Agricultural soil, Kasetsart Univ., Bang Khan	11 July 2003
	Nonagricultural soil, Kasetsart Univ., Bang Khan	5 June 2004
Kanchanaburi	Agricultural soil, Tong Pa Poom	30 June 2002
Lop Buri	Nonagricultural soil, Amphur Maung	11 May 2003
Uthai Thani	Nonagricultural soil, Amphur Maung	19 May 2004
Nakhon Pathom	Agricultural soil, Amphur Kumpangsan	4 February 2002
Nonthaburi	Agricultural soil, Amphur Maung	9 May 2003
Ratchaburi	Nonagricultural soil, Amphur Nong Po	10 October 2001
Saraburi	Nonagricultural soil, Amphur Maung	19 May 2004
Sing Buri	Nonagricultural soil, Amphur Maung	27 April 2004
Suphan Buri	Agricultural soil, Amphur Dam Bang Nang Buon	5 January 2002
East		
Chanthaburi	Agricultural soil, Amphur Ta Mai	24 November 2004
Chon Buri	Agricultural soil, Tambol Bangsarai, Amphur Sattahip	2 January 2002
Rayong	Nonagricultural soil, Amphur Ban Phe	15 August 2003
Trat	Forest soil, Ko Koh	9 August 2003
South		
Krabi	Nonagricultural soil, Ko Lanta	10 August 2003
Nakhon Si Thammarat	Nonagricultural soil, Walairak Univ., Amphur Maung	14 March 2004
Phatthalung	Agricultural soil, Amphur Bang Kaew	25 March 2005
Phang Nga	Nonagricultural soil, Amphur Maung	22 July 2003
Surat Thani	Nonagricultural soil, Amphur Maung	8 December 2003
Trang	Nonagricultural soil, Amphur Maung	10 December 2003

4 Preservation

Pure cultures were maintained on slant PDA covered with liquid paraffin, filter paper and sterilized soil at the culture collection, Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok.

4.1 PDA slant method (Smith and Onions, 1994)

Pure cultures of *Talaromyces* spp. were maintained on PDA slants at 28°C. Subculturing was carried out every 6 months.

4.2 Liquid paraffin method (Smith and Onions, 1994)

Pure cultures were maintained on PDA agar slant in a small vial (1 drams). Liquid paraffin was placed in a vial and autoclaved three times. Covering the pure culture on agar with sterile liquid paraffin about 2/3 of a vial and stored at 28 °C in order to prevent dehydration and slow down metabolic activity and growth through reduced oxygen tension.

4.3 Filter paper method (Fong *et al.*, 2000)

Fifteen pieces (0.5 x 0.5 cm^2) of sterile filter paper Whatman No. 1 were placed on PDA in sterile Petri dish. The mycelia were transferred on PDA and incubated for 7-14 days depend on the species. The filter papers with fungal mycelium were transferred to new sterile Petri dish by using sterile forcep and placed in a dessicator or electric dessicator (35°C) for 7-10 days. Dried filter papers covered mycelium mass and ascomata were kept in an aluminum foil, labeled and placed in a box for storage at -20 °C.

4.4 Soil Culture (Smith and Onions, 1994)

Loamy soil was placed in a vial about 2/3 full and autoclaved twice at 121°C for 15 min. One ml of spore suspension in sterile water was added The soil cultures were left to grow at room temperature and then left to dry while stored in a refrigerator at 4-10°C.

5. The molecular study of *Talaromyces* species

5.1 Cultivation of *Talaromyces* strains for DNA extraction using CTAB method

Twenty-one fungi were selected for this studied (Table 4). The preparative for DNA extraction of fungal mycelium, the fungi were grown in 250 ml flask containing 100 ml of PDB and inoculated with two mycelial plugs taken from stock culture of each fungal grown on PDA. Culture flasks were incubated at 28-30°C on rotary shakers at 150 rpm for 2-3 days. The mycelia were harvested from broth through filter paper (Whatman No. 1) by vacuum filtration.

5.2 Methods for DNA preparation

5.2.1 DNA extraction from fungal material

- Preheat extraction CTAB (2% w/v CTAB, 20mM EDTA, 100 mM Tris-HCl, pH 8.0, 1.4 M NaCl) buffer at 65°C.

- Grind 1-2 g of mycelial by adding liquid nitrogen in a mortar and pestle until the tissues are powdery.

- Add 500 µl of warm extraction buffer to the powdered tissue. Mix and incubate in waterbath at 65°C for 10 min.

- Add 500 µl Chloroform-Isoamyl alcohol (24:1). Mix and spin at 12,000-14,000 rpm for 5 min.

- Transfer aqueous phase into a clean microtube and add 0.8 vol. of isopropanol.

- Mix carefully and spin at 5,000 rpm for 2-3 min. (repeat if top phase is not clear) and discard aqueous phase.

- Add 600-700 µl cold absolute ethanol.

- Centrifuge, remove ethanol and wash with 70% ethanol. Air dried the pellet and resuspend in Rnase. Store DNA samples at 4°C or -20°C for longer periods.

Table 4 Taxa and selected isolates used for studying molecular phylogeny

Taxon	**Strains**
Genus *Talaromyces*	
Section *Talaromyces*	
Series *Flavi*	
T. flavus	KUFC 3381
T. helicus var. major	KUFC 3598
T. indigoticus	KUFC 3366
T. macrosporus	KUFC 3381
T. stipitatus	KUFC 3594
Series *Lutei*	
T. austrocalifornicus	KUFC 3401
T. luteus	KUFC 3331
T. rotundus	KUFC 3359, KUFC 3446
T. wortmannii	KUFC 3333
Talaromyces sp. 1	KUFC 3399, KUFC 3631
Talaromyces spp.	KUFC 3352, KUFC 3370, KUFC 3470
Series *Trachyspermus*	
T. trachyspermus	KUFC 3355
Talaromyces sp. 2	KUFC 3383
Section Emersonii	
T. bacillisporus	KUFC 3350
Genus *Byssochlamys*	
B. fulva	KUFC 2849
Class Trichocomaceae	
Acremonium anamorph	KUFC 3645
Penicillium anamorph	KUFC 3580

5.3 PCR conditions for amplification of microsettalites genes

The SSR regions were amplified by the polymerase chain reaction (PCR) using the respective primer combination (Table 5). DNA amplification was performed in final volume of 50 µl containing 1X buffer (10mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$), 0.2 mM each deoxynucleoside triphosphate, 50 pmole of each primer, 2.5 U/µl of *Taq* DNA polymerase, and 50 ng of genomic DNA from each fungi. The DNA was amplified by thermocycler (Perkin-Elmer 9600/ Applied Biosystem) and the following parameters: 5 min of denaturation at 94 ^{o}C, followed by 30 cycles of amplication with denaturation at 94 ^{o}C for 45 sec, annealing at melting temperature of each primer pairs for 45 sec as shown in table 5, DNA elongation at 72 ^{o}C for 30 sec and a final elongation for 7 min at 72 ^{o}C. A negative control lacking template DNA was included for each set of reactions. Ten microliters from each PCR reaction were electrophoresed on 3 % agarose gel electrophoresis in 0.5X Tris-acetate buffer. DNA in gels were stained with ethidium bromide (0.5 ug per ml) and viewed under UV- transilluminator.

Table 5 List of primer sequences of 10 microsatellite loci

Primer	Primer sequence (5'-3')	Tm (^{0}C)
MG 13	5' CAC-GTG-TCA-AGT-CAT-AAT-AAA-TAG 3'	56.3
MG 14	5' AAT-CTG-CTG-CCA-ATA-GTC-AT 3'	56.3
PM1F	5' CCT-GTT-TGT-CTT-TTG-TGC-TG 3'	52.7
PM1R	5' GTA-CGG-GCT-AGC-TGT-CAG-TG 3'	52.7
PM2F	5' TTA-CTC-GAT-ACG-GCA-GTT-GG 3'	52.7
PM2R	5' TGT-TAC-GAT-AAC-CGC-GTC-TG 3'	52.7
PM7F	5' TCC-CTC-ACA-TGC-TAA-TGA-TG 3'	52.7
PM7R	5' ACG-ACT-CGG-AGG-AAT-TGA-GA 3'	52.7
PM12F	5' GCC-CAC-ACT-GAC-ACA-CTA-TG 3'	52.7
PM12R	5' ATA-TCT-TGG-TGC-CAC-CTG-AC 3'	52.7

Source: Fisher *et al.* (2004a)

5.4 Data analysis

For each bands of DNA in gels were assigned a number that fragments of the same size from different strains have the same number. Numbered fragments were treated as characters with two states, present (=1) or absent (=0), and their distribution was tabulated for all the fungi studied. For molecular phylogeny analysis: The data was aligned by TreeCon Software for the neighbor-joining analysis (Nei and Li, 1979). A bootstrap analysis was conducted with 100 replications.

6. Test of antagonism against plant pathogenic fungi by *Talaromyces*

Talaromyces spp. were subcultured on PDA for 14-21 days or until ascospores became mature. Ascospore suspension was prepared by Petri dish flooding the culture surface with sterile water and then ascomata of *Talaromyces* spp. were scraped from medium surface by sterile spatula. The concentration was determined with haematocytometer before adjusted with sterile water to 10^6 ascospore / ml for antagonistic test in this study (Intana , 2003).

6.1 *In vitro* growth inhibition and overgrowing of mycelia of plant pathogen

Twenty isolates of *T. flavus*, each isolate of *T. bacillisporus* and *Talaromyces* sp. KUFC 3399 were selected to test for antagonistic activity against 15 species of plant pathogenic fungi (Tables 6-7). The young mycelium from the colony margin of *Talaromyces* spp. and the specific plant pathogenic fungus were cut with sterile cork borer (0.8 cm diam) and placed as a dual culture on PDA, 7 cm apart. All Petri dishes were incubated at room temperature (28 ^{o}C) for 14 days. The inhibition levels were calculated by using the formula: G_1-G_2 / G_1 x 100 where G_1 = colony radius of plant pathogenic fungi in the control and G_2 = colony radius of plant pathogenic fungi in the dual culture test (Intana, 2003). Each treatment was performed with three replicates.

6.2 Antagonistic tests of *Talaromyces flavus* in greenhouse

A modification of Madi *et al.*, (1997) method for antagonistic tests of *T. flavus* in the greenhouse was conducted. Dried mungbeen seeds (*Vigna radiata* (L.)R. Wilczek) were surface disinfested with 0.525% sodium hyperclorite for 5 min, rinsed 3 times with sterile water and immersed in ascospore suspension (10^6 ml) of *T. flavus* strains for 24 hr. 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 of *Sclerotium rolfsii* were placed 0.5-1 cm apart from each bean seed. Seeds and sclerotia were covered with 150 g of soil, and the pots were incubated in the greenhouse at temperature ranging from 28 to 30°C. Disease symptoms were recorded at 7 and 14 days after planting. There were three treatments in the experiment: mungbean + *S. rolfsii* + *Trichoderma harzianum* (Unigreen®), 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 6 Isolates of *Talaromyces flavus*, *T. bacillsporus* and *Talaromyces* sp. 1 (KUFC 3399) from various locations, used for antagonistic test against plant pathogenic fungi

T. flavus strains KUFC	Location	Methods
3334	Forest soil, Mae Hong Son	Heat treatment
3363	Watermelon field soil, Chon Buri	Alcohol treatment
3381	Chili field soil, Kasetsart Univ., Bangkok	Heat treatment
3388	Mango field soil, Chiang Mai	Soil plate technique
3395	Paddy soil, Suphan Buri	Soil plate technique
3397	Cucumber field soil, Nonthaburi	Heat treatment
3400	Corn field soil, Chiang Mai	Alcohol treatment
3485	Mungbean field soil, Nakhon Ratchasima	Dilution plate technique
3446	Paddy soil, Chiang Mai	Alcohol treatment
3450	Longan field soil, Chiang Rai	Heat treatment
3473	Nonagricultural soil, Krabi	Alcohol treatment
3483	Tomato field soil, Nonthaburi	Heat treatment
3501	Cucumber field soil, Chiang Mai	Alcohol treatment
3506	Paddy soil, Bangkok	Heat treatment
3508	Cabbage field soil, Chiang Mai	Soil plate technique
3523	Cucumber field soil, Kanchanaburi	Soil plate technique
3525	Kale field soil, Nonthaburi	Heat treatment
3528	Mungbean field soil, Chon Buri	Heat treatment
3530	Durian field soil, Chanthaburi	Dilution plate technique
3550	Forest soil, Sakon Nakhon	Soil plate technique
T. bacillisporus	Nonagricultural soil, Kasetsart Univ., Bangkok	Heat treatment
Talaromyces sp. KUFC 3399	Forest soil, Trat	Heat treatment

Table 7 Fifteen species of plant pathogenic fungi from various diseased plants used for testing of antagonistic activity of *Talaromyces* spp.

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

7 Analytical secondary metabolites of *Talaromyces bacillisporus* and *Talaromyces* sp.1 (KUFC 3399)

7.1 General Experimental

- Merck Si gel 60 (0.2-0.5 mm; 70-230 mesh) was used for column chromatography
- Analytical and preparative TLC was performed on silica gel-60 (GF_{254}; Merck), 0.25 thickness. The plates were activated at 110^{o}C in the oven for 1 hour. All TLC plates were visualized under UV 254 nm or developed with iodine vapor.
- Melting points were recorded on a Bock Monoscope and are uncorrected.
- Rotations were determined on a Polax-2L instrument.
- ^{1}H and ^{13}C NMR spectra were recorded at ambient temperature in DMSO on a Bruker DRX instrument operating at 500 and 125 MHz respectively, ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR spectra were measured on a Bruker CxP spectrometer. The solvents used were deuterated chloroform (Merck) or hexadeuterated dimethylsulfoxide (Merck).
- EI mass spectra were measures on a Hitachi Perkin-Elmer RMV-GM instrument. For HR mass spectra were measured on CONCEPT II, 2 sectors mass spectrometer. The accelerating voltage was 8 KV.
- X-ray diffraction studies were performed with a Stoe IPOS image plant equipped with Mo Kα radiation. The structure was solved using SHELX 597 and refined with SHELXL 97. A perspective view of the molecule was obtained with ORTEP.
- The solvents used were commercial grade of Vidrolab 2 which were distilled prior to use and analytical reagent grade of brand Merck and Lab-Scan.
- All solvents were evaporated either by reduced pressure using " Buchi evaporator" or nitrogen gas.
- The weight was measured on the balance Mettler AE 200.

7.2 Isolation and Purification of the Secondary Metabolites from *Talaromyces bacillisporus*

7.2.1 Fungus material

Talaromyces bacillisporus C.R. Benjamin was isolated from a soil sample collected on the campus of Kasetsart University, Bangkok, Thailand in July 2003 and identified by Assoc. Prof. Dr. Leka Manoch on the basis of the description in Pitt (1979a) and with standard tests. A sample with accession number KPFC 3350 has been deposited in the Department of Plant Pathology, Faculty of Agriculture, Kasetsart University.

7.2.2 Extraction and isolation of the constituents

7.2.2.1 Preparation of the crude extract

Twenty-five 1,000 mL Erlenmeyer flasks each containing 200 g of rice and 100 mL of H_2O were autoclaved at 121°C for 15 min., inoculated with three mycelium plugs of *Talaromyces bacillisporus* KPFC 3350 culture and incubated at 28 °C for 30 days. To each flask containing the moldy rice was added 400 ml of EtOAc, after which the contents were left to macerate for 3 days and then filtered using filter paper (Whatman No. 1). Evaporation of the combined filtrates to a volume of 1,000 ml at reduced pressure followed by addition of anhydrous sodium solephate, filtration and evaporation of the filtrate at reduced pressure furnished 105 g of dark brown crude EtOAc extract which was extract with $CHCl_3$ (3 x 500 ml). The $CHCl_3$ extracts were combined and concentrated at reduced pressure to afford 85 g of a brown viscous mass.

7.2.2.2 Fractionation of the crude extract

The crude $CHCl_3$ extract was applied to a silica gel column (200 g), and eluted with petrol-$CHCl_3$, $CHCl_3$ and $CHCl_3$-acetone, 300-500 ml / fractions being collected as follows:

Fractions	Eluents
1-142	$CHCl_3$-petrol (1:1)
143- 218	$CHCl_3$-petrol (7 : 3)
219- 286	$CHCl_3$-petrol (9 : 1)
287- 315	$CHCl_3$-acetone (9 : 1)
316- 343	$CHCl_3$-acetone (4 : 1)
344- 365	$CHCl_3$-acetone (7 : 3)

The fractions were analyzed by analytical TLC and combined, according to their composition, as follows:

Fractions	Isolated compounds
1-6 (1,038.9 mg)	not purified
7-19 (1,638.2 mg)	not purified
20-22 (447.2 mg)	not purified
23-28 (600 mg)	**bacillisporin A** (**4**, 300 mg)
	duclauxin (**50**, 20 mg)
29-111 (2,000 mg)	**bacillisporin A** (**4**, 1,250 mg)
	bacillisporin D (**69**, 9 mg)
	duclauxin (**50**, 27 mg)
112-122 (276.6 mg)	not purified
123-132 (244.5 mg)	not purified
133-142 (224.4 mg)	not purified
143-160 (2,000 mg)	**bacillisporin E** (**70**, 22 mg)
	bacillisporin C (**6**, 600 mg)
161-174 (795 mg)	not purified
175-185 (424.6 mg)	not purified
186-190 (165.3 mg)	not purified
191-201 (317.1 mg)	not purified
202-210 (404.4 mg)	not purified
211-215 (280 mg)	**bacillisporin B** (**4**, 200 mg)
216-225 (570.8 mg)	not purified

Fractions	Isolated compounds
226-256 (656.8 mg)	not purified
257-267 (795 mg)	not purified
268-271 (20.8 mg)	not purified
272-279 (66.2 mg)	not purified
280-286 (47.4 mg)	not purified
287-293 (623.2 mg)	not purified
294-306 (299.4 mg)	not purified
307-315 (177.6 mg)	not purified
316-343 (604.4 mg)	not purified
344-365 (400.1 mg)	not purified

7.2.2.3 Isolation and Purification of the compounds

Fractions 23-28 (600 mg) were combined and recrystallized from $CHCl_3$-petrol to give bacillisporin A (**4**) as a pale yellow solid (300 mg).

TLC of the mother liquor (silica gel, $CHCl_3$-acetone-HCO_2H, 95:5:1) gave 35 mg of duclauxin (**50**) as a yellow solid.

Fractions 29-111 (2 g) were combined, applied to a silica gel column (50 g) and eluted with $CHCl_3$-petrol and $CHCl_3$-acetone, 100 ml subfractions being collected as follows:

Fractions	Eluents
1-22	$CHCl_3$-petrol (7 : 3)
23-64	$CHCl_3$-petrol (9 : 1)
65- 98	$CHCl_3$-acetone (9 : 1)

Subfractions 3-5 were combined (800 mg) and recrystallized from $CHCl_3$-petrol to give more bacillisporin A (**4**, 250 mg).

Purification of the mother liquor by TLC (silica gel, $CHCl_3$-acetone-HCO_2H, 95:5:1) gave 27 mg of duclauxin (**50**) as a yellow solid.

Subfractions 43-64 (30 mg) were combined and purification by TLC (silica gel, $CHCl_3$-acetone-HCO_2H, 85:15:1) to give 9 mg of bacillisporin D (**69**) as a yellow solid.

Fractions 143-160 (2 g) were combined and recrystallized from $CHCl_3$-acetone to give 480 mg of bacillisporin E (**70**). The mother liquor was chromatographed over silica gel (20 g) and eluted with $CHCl_3$-petrol and $CHCl_3$-acetone as follows using 100 mL/ subfractions.

Fractions	**Eluents**
1-61	$CHCl_3$-petrol (7 : 3)
62-72	$CHCl_3$-petrol (9 : 1)
73-87	$CHCl_3$-acetone (9 : 1)
88-100	$CHCl_3$-acetone (4 : 1)

Subfraction 16-21 (200 mg) were combined and purified by TLC (silica gel, $CHCl_3$-acetone-HCO_2H, 85: 15: 1) to give 22 mg of bacillisporin E (**70**).

Combination of fractions 161-180 (1.5 g) and recrystallization from $CHCl_3$-acetone afforded 600 mg of bacillisporin C (**6**). Combination of fractions 211-215 (180 mg) and recrystalization from $CHCl_3$-acetone afforded 200 mg of bacillisporin B (**5**).

7.3 Isolation and purification of the secondary metabolites from *Talaromyces* sp. 1 (KUFC 3399)

7.3.1 Fungal material

The fungus was isolated from a soil sample collected at Trat Province, Easthern Thailand in August 2003. The strain was deposited at the Department of Plant Pathology, Faculty of Agriculture, Kasetsart University with the accession number KPFC 3399.

7.3.2 Method of culture and extraction of the constituents

7.3.2.1 Preparation of the crude extract

Fifty 1000 ml Erlenmeyer flasks containing 200 g of rice and 100 ml of water, autoclaved at 121ºC for 15 min, were inoculated with ten mycelium plugs from the *Talaromyces* sp. 1 (KUFC 3399) culture and incubated at 28ºC for 30 days. Each flask with the moulded rice was added 400 ml of ethyl acetate and the content was left to macerate for 3 days. The content of the flasks was filtered by filter paper and the filtrate was evaporated under reduced pressure to give 3 litres of the solution and then anhydrous sodium solephate was added and filtered. The ethyl acetate solution was evaporated under reduced pressure to give 79.2 g of dark brown viscous mass of a crude ethyl acetate extract which was extracted by $CHCl_3$ (3x500 ml). The chloroform extracts were combined and evaporated under reduced pressure to give a brown viscous mass of crude chloroform extract (51.4 g).

7.3.2.2 Fractionation of the crude extract

The crude $CHCl_3$ extract was chromatographed over silica gel column (200 g), and eluted with Petrol-$CHCl_3$, $CHCl_3$ and $CHCl_3$-acetone, 300-500 ml / fractions being collected as follows;

Fractions	Eluents
1- 202	$CHCl_3$-petrol (1 : 1)
201-265	$CHCl_3$-petrol (7 : 3)
266- 285	$CHCl_3$-petrol (9 : 1)
286- 316	$CHCl_3$-acetone (9 : 1)
317- 343	$CHCl_3$-acetone (4 : 1)
344- 368	$CHCl_3$-acetone (7 : 3)

All collected fractions were analyzed by analytical TLC and combined, according to their composition, as follows;

Fractions	Isolated compounds
1-4 (52 mg)	not purified
5-15 (8 mg)	not purified
16-24(3 mg)	not purified
25-27 (2 mg)	not purified
28-37 (443 mg)	not purified
38-46 (273 mg)	not purified
47-64 (273 mg)	not purified
65-76 (273 mg)	***N*-benzoylphenylalanyl-*N*-benzoylphenylalaninate** (**77**, 22 mg)
77-90 (123 mg)	**vermistatin** (**75**, 59 mg)
91-92 (145 mg)	**vermistatin** (**75**, 20 mg) **thailandolide B** (**72**, 11.3 mg)
93-95 (56 mg)	**vermistatin** (**75**, 15 mg)
96 (28.5 mg)	not purified
97-107 (244 mg)	**thailandolide A** (**71**, 200 mg)

Fractions	Isolated compounds
108-110 (58.6 mg)	not purified
111-130 (570 mg)	**penisimplicissin** (**74**, 15.4 mg)
31-144 (275.3 mg)	not purified
145- 149 (121 mg)	**2-glyceryl palmitate** (**78**, 11.7 mg)
150-162 (387 mg)	not purified
163-179 (336 mg)	not purified
180 -202 (517 mg)	not purified
203 -220 (756 mg)	not purified
221-280 (1,190 mg)	**hydroxydihydrovermistatin** (**76**, 37 mg)
281-291(1,270 mg)	not purified
292-297 (542 mg)	not purified
298-303 (302 mg)	not purified
304-320 (498 mg)	not purified
321-339 (521 mg)	not purified
340-355 (569 mg)	not purified
356-368 (671 mg)	not purified

7.3.4 Isolation and Purification of the compounds

Fractions 65-75 were combined (345 mg) and recrystallized from a mixture of $CHCl_3$ and petrol to give white solid of ***N*-benzoylphenylalanyl-*N*-benzoylphenylalaninate** (**77**, 200 mg).

Fractions 77-90 and 93-95 were combined (179 mg) and recrystallized from a mixture of $CHCl_3$ and petrol to give white solid of **thailandolide B** (**72**, 59 mg).

Fractions 91 and 92 were combined (45 mg) and purified with TLC (silica gel, $CHCl_3$-Me_2O-HCO_2H; 95:5:1) to give **thailandolide B** (**72**, 11.3 mg) and **vermistatin** (**75**, 20 mg).

Fractions 97-107 were combined (244 mg) followed by recrystallized from a mixture of $CHCl_3$ and petrol to give white solid of **thailandolide A** (**71**, 200 mg).

Fractions 111-130 were combined (570 mg) and recrystallized from $CHCl_3$ and petrol gave yellow solid (45 mg), which was further purified by TLC (silica gel, $CHCl_3$-Me_2O-HCO_2H; 95:5:1) to give **penisimplicissin** (**74**, 15.4 mg).

Fractions 145-159 were combined (121 mg) and purified by TLC (silica gel, $CHCl_3$-Me_2O-EtOAc: HCO_2H; 85:10:5:1) to give **2-glyceryl palmitate** (**78**, 11.7 mg).

Fractions 221-280 were combined (1.19 g) and applied on a silica gel column (10 g) and eluted with $CHCl_3$-petrol, 100 ml / subfractions were collected as follows:

Fractions	**Eluents**
1- 50	$CHCl_3$-petrol (1 : 1)
51- 74	$CHCl_3$-petrol (7 : 3)
75- 80	$CHCl_3$-acetone (9 : 1)

Subfractions 28-32 (127 mg) were combined and purified by TLC (silica gel, $CHCl_3$-Me_2O-HCO_2H; 4:1:0.1) to give ***O*-methylated derivative** (**73**, 37 mg).

Fractions 286-313 were combined (2.2 g) and applied on a silica gel column (12 g) and eluted with $CHCl_3$-petrol, 100 ml sfrs were collected as follows: Sfrs 1-25 ($CHCl_3$-petrol, 7:3), 26-50 ($CHCl_3$-petrol, 9:1). Sfrs 16-24 were combined (220 mg) and purified by TLC (silica gel, $CHCl_3$-Me_2O-HCO_2H; 4:1:0.1) to give **hydroxydihydrovermistatin** (**76**, 46 mg).

7.4 Structure elucidation of the compounds

The structure of the compounds were established by spectroscopic methods (^{1}H, ^{13}C NMR, COSY, HSQC, HMBC, HRMS) as well as comparison of their NMR data with those in the literatures.

8. Place

The experiments of taxonomic study and antagonistic test were conducted at Mycology Labolatory, Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok. For scanning electron photomicrographs were examined at Scientific Equipment Centre, Biomolecular Sciences, Kasetsart University. The isolation, purification and structure elucidation of the secondary metabolites were conducted at Instituto de Ciencias Biomedicas de Abel Salazar (ICBAS), Universidade do Porto, Portugal.

9. Duration

The study was carried out during October 2002 to October 2006.

RESULTS AND DISCUSSION

1. Diversity and distribution of *Talaromyces*

Three hundred and forty-two isolates of the genus *Talaromyces* comprising 11 species, 2 unidentified species and 1 variety were found from 45 soil samples collected from 38 provinces in Thailand. *Talaromyces flavus* and *T. macrosporus* were the dominant species followed by *T. stipitatus*, *T. trachyspermus*, *T. wortmannii*, *T. bacillisporus*, *T. rotundus*, *T. indigoticus*, *T. helicus* var. *major*, *T. austrocalifornicus*, *T. luteus*, *Talaromyces* sp. 1 (KUFC 3399) respectively, whereas only one isolate of *Talaromyces* sp. 2 (KUFC 3383) was found from agricultural soil, Chon Buri (Table 8, Figure 5).

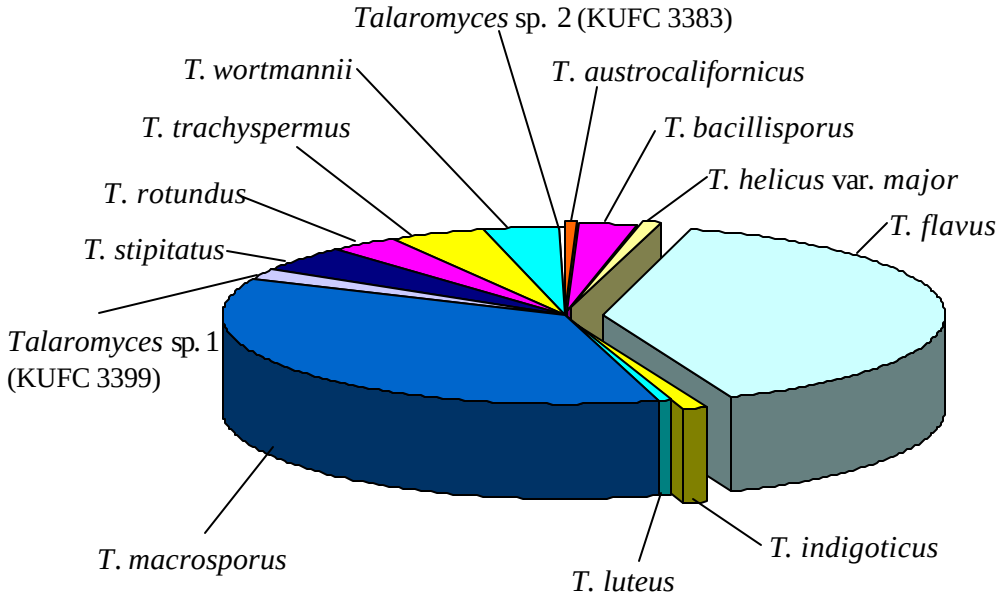


Figure 5 Occurrence of *Talaromyces* species from various soil samples at different locations

Talaromyces flavus and *T. macrosporus* were found from 45 soil samples in 38 provinces (Tables 8-9). Most isolates of both species were derived from heat treatment and alcohol treatment methods followed by the soil plate and dilution plate methods

(Table 9). Similar result was recorded by Udagawa *et al.*, (1996) when they isolated microfungi from house dust in Kobe using heat treatment method, a number of *T. flavus* were isolated. It has been also recorded as a heat resistant fungus in food products (Udagawa, 2000). *Talaromyces flavus* and *T. macrosporus* were commonly found in nonagricultural soil followed by agricultural soil and forest soil (Table 9). 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.

Two isolates of *Talaromyces luteus* and *T. helicus* var. *major* were found from hot spring soil at Mae Hong Son and agricultural soil at Chanthaburi respectively. In addition, two isolates of *T. austrocalifornicus* were isolated from forest soil at Mae Hong Son and nonagricultural soil, Chiang Mai whereas 4 isolates of *T. indigoticus* were found from Sakon Nakhon, Chiang Mai, Nakhon Pathom and Chon Buri (Table 9). For Unidentified species, *Talaromyces* sp.1 (KUFC 3399) was isolated from forest soil at Trat and nonagricultural soil, Chiang Mai, whereas only one isolate of *Talaromyces* sp. 2 (KUFC 3383) was found from forest soil, Trat (Table 9).

Nine species and two varieties of *Talaromyces* were previously recorded from Thailand including *T. flavus* var. *flavus*, *T. flavus* var. *macrosporus*, *T. bacillisporus*, *T. byssochlamydoides*, *T. emersonii*, *T. rotundus*, *T. striatus*, *T. stipitatus*, *T. trachyspermus* and *T. wortmannii*. They were isolated from forest, agricultural, mangrove soil, soil at termite mounds, dungs and decomposing starters from eighteen provinces (Chana, 1974; Cruesrisawath, 1985; Kanjanamaneesathian, 1988; Manoch, 2004; Manoch *et al.*, 2004, 2005; Sudpro, 1999; Wongthong, 2001; Busarakum, 2002; Ito *et al.*, 2001; Jeamjitt, 2007). In this study, *Talaromyces austrocalifornicus* and *T. indigoticus* are new record for Thailand.

Table 8 Frequency and number of isolates of *Talaromyces* species from various soil samples at different locations

Fungal species	KUFC	Total isolates
Talaromyces austrocalifornicus	3351, 3401	2
Talaromyces bacillisporus	3350, 3378, 3393, 3404, 3417, 3493, 3580, 3590, 3633, 3652	10
Talaromyces helicus var. *major*	3593, 3595, 3598	3
Talaromyces flavus	3332, 3334, 3335, 3336, 3337, 3338, 3340, 3341, 3344, 3358, 3360, 3361, 3362, 3368, 3369, 3370, 3375, 3376, 3381, 3382, 3388, 3389, 3390, 3391, 3392, 3394, 3395, 3400, 3402, 3403, 3405, 3406, 3407, 3408, 3409, 3411, 3412, 3414, 3415, 3416, 3418, 3419, 3420, 3424, 3426, 3427, 3429, 3430, 3431, 3432, 3433, 3434, 3435, 3436, 3441, 3442, 3445, 3446, 3455, 3456, 3457, 3458, 3459, 3466, 3467, 3473, 3474, 3477, 3478, 3479, 3480, 3481, 3482, 3491, 3492, 3502, 3503, 3507, 3508, 3509, 3510, 3516, 3517, 3521, 3530, 3533, 3536, 3537, 3538, 3539, 3542, 3543, 3544, 3549, 3550, 3554, 3555, 3556, 3557, 3558, 3559, 3565, 3571, 3576, 3585, 3586, 3587, 3588, 3600, 3601, 3603, 3604, 3605, 3614, 3617, 3618, 3619, 3627, 3628, 3639, 3640, 3646, 3647, 3651, 3652, 3659, 3660, 3661, 3662, 3663, 3664, 3665, 3666, 3667, 3668, 3669, 3670, 3671, 3672	139
Talaromyces indigoticus	3366, 3562, 3592, 3611	4
Talaromyces luteus	3331, 3364	2
Talaromyces macrosporus	3339, 3342, 3343, 3345, 3346, 3347, 3348, 3349, 3356, 3363, 3365, 3367, 3371, 3373, 3374, 3377, 3384, 3385, 3386, 3387, 3396, 3397, 3398, 3437, 3438, 3439, 3440, 3443, 3444, 3448, 3450, 3451, 3452, 3453, 3454, 3460, 3461, 3462, 3463, 3465, 3468, 3469, 3470, 3471, 3476, 3483, 3484, 3485, 3486, 3494, 3497, 3498, 3499, 3500, 3501, 3504, 3505, 3506, 3511, 3512, 3513, 3518, 3520, 3522, 3523, 3524, 3525, 3526, 3527, 3528, 3529, 3531, 3532, 3534, 3535, 3540, 3541, 3545, 3546, 3547, 3548, 3552, 3563, 3564, 3566, 3567, 3568, 3569, 3570, 3572, 3573, 3674, 3575, 3577, 3578, 3579, 3581, 3582, 3583, 3584, 3589, 3591, 3597, 3599, 3602, 3606, 3607, 3608, 3610, 3612, 3613, 3623, 3624, 3625, 3626, 3629, 3630, 3631, 3632, 3638, 3642, 3643, 3644, 3645, 3653, 3654, 3655	127
Talaromyces rotundus	3359, 3379, 3410, 3447, 3475, 3609, 3620	7
Talaromyces stipitatus	3357, 3422, 3449, 3464, 3472, 3487, 3514, 3515, 3551, 3553, 3560, 3561, 3594, 3636, 3641, 3648, 3658	17
Talaromyces trachyspermus	3352, 3353, 3355, 3372, 3380, 3421, 3423, 3425, 3428, 3495, 3596, 3621, 3635, 3649, 3657	15
Talaromyces wortmannii	3333, 3354, 3488, 3489, 3490, 3519, 3615, 3616, 3622, 3634, 3637, 3650, 3656	13
Talaromyces sp. 1	3399, 3413	2
Talaromyces sp. 2	3383	1
	Totel	342

Table 9 Distribution of *Talaromyces* spp. from various locations using different isolation methods

KUFC[1/]	Isolation method[2/]	Source[3/]	Location	KUFC[1/]	Isolation method[2/]	Source[3/]	Location
Talaromyces austrocalifornicus				3381	ht	A	Bangkok
3351	ht	F	Mae Hong Son	3382	ht	A	Suphan Buri
3401	ht	NA	Chiang Mai	3388	ht	F	Chiang Mai
Talaromyces bacillisporus				3389	ht	NA	Phatthalung
3350	ht	NA	Bangkok	3390	ht	NA	Surat Thani
3378	ht	NA	Kalasin	3391	ht	A	Suphan Buri
3393	ht	NA	Chiang Mai	3392	ht	A	Nonthaburi
3404	alc	F	Loei	3394	alc	NA	Nakhon Ratchasima
3417	alc	NA	Chiang Mai	3395	alc	NA	Chiang Mai
3493	alc	A	Chiang Mai	3397	ht	A	Nonthaburi
3580	sp	F	Mae Hong Son	3402	ht	NA	Chiang Mai
3590	alc	NA	Sakon Nakhon	3403	ht	NA	Chiang Mai
3633	ht	A	Chiang Rai	3405	alc	NA	Chiang Mai
3652	ht	A	Chiang Rai	3406	alc	F	Mae Hong Son
Talaromyces helicus* var. *major				3407	alc	F	Mae Hong Son
3593	alc	A	Chanthaburi	3408	ht	NA	Nakhon Ratchasima
3595	sp	A	Chanthaburi	3409	ht	NA	Chiang Mai
3598	alc	A	Chanthaburi	3411	ht	NA	Nakhon Si Thammarat
Talaromyces flavus				3412	ht	NA	Tak
3332	alc	NA	Bangkok	3414	alc	NA	Krabi
3334	alc	F	Mae Hong Son	3415	ht	NA	Krabi
3335	alc	NA	Krabi	3416	ht	NA	Nakhon Ratchasima
3336	alc	A	Nakhon Ratchasima	3418	ht	NA	Krabi
3337	alc	A	Chonburi	3419	ht	NA	Nakhon Ratchasima
3338	sp	A	Chonburi	3420	ht	NA	Rayong
3340	alc	A	Lop Buri	3424	ht	NA	Nakhon Si Thammarat
3341	ht	A	Chiang Rai	3426	sp	NA	Phang Nga
3344	ht	A	Chiang Rai	3427	alc	F	Chiang Mai
3358	alc	NA	Bangkok	3429	alc	A	Bangkok
3360	alc	NA	Bangkok	3430	ht	NA	Bangkok
3361	sp	NA	Bangkok	3431	ht	NA	Roi Et
3362	alc	A	Lop Buri	3432	ht	F	Chiang Mai
3368	ht	A	Lumpang	3433	ht	F	Mae Hong Son
3369	ht	A	Chiang Rai	3434	ht	NA	Chiang Mai
3370	alc	F	Pitsanulok	3435	ht	F	Mae Hong Son
3375	sp	A	Chiang Mai	3436	ht	NA	Kalasin
3376	alc	A	Lop Buri	3441	ht	NA	Chiang Mai

[1/] KUFC = Kasetsart University Fungal Collection

[2/] sp = soil plate method, dp = dilution plate method, alc = alcohol treatment, ht = heat treatment

[3/] A = agricultural soil, NA = nonagricultural soil, F = forest soil

Table 9 (Continued)

KUFC[1/]	Isolation method[2/]	Source[3/]	Location
	Talaromyces flavus		
3442	ht	NA	Chiang Mai
3445	ht	A	Nonthaburi
3446	ht	F	Mae Hong Son
3455	ht	NA	Ratchaburi
3456	sp	A	Chathaburi
3457	sp	A	Chathaburi
3458	ht	NA	Chiang Mai
3459	ht	F	Chiang Mai
3466	alc	NA	Chiang Mai
3467	alc	NA	Saraburi
3473	sp	NA	Ang Thong
3474	alc	A	Lop Buri
3477	alc	NA	Nakhon Ratchasima
3478	alc	NA	Chiang Mai
3479	alc	F	Pitsanulok
3480	alc	F	Trat
3481	alc	NA	Tak
3482	alc	NA	Nakhon Si Thammarat
3485	sp	A	Nakhon Ratchasima
3492	alc	A	Lop Buri
3502	ht	A	Chiang Rai
3503	ht	NA	Phang Nga
3507	alc	NA	Phatthalung
3508	alc	NA	Ang Thong
3509	sp	A	Chon Buri
3510	alc	A	Lop Buri
3516	ht	NA	Khon Kaen
3517	ht	NA	Khon Kaen
3521	ht	F	Trat
3530	ht	F	Chiang Rai
3533	sp	NA	Sing Buri
3536	alc	A	Lop Buri
3537	ht	A	ChiangRai
3538	ht	F	Trat
3539	alc	NA	Bangkok
3542	alc	NA	Bangkok
3543	ht	F	Chiang Mai
3544	alc	NA	Buri Rum
3549	alc	NA	Krabi
3550	ht	NA	Sing Buri
3554	ht	A	Suphan Buri
3555	ht	F	Trat
3556	ht	F	Nong Khai
3557	alc	NA	Nakhon Ratchasima
3558	alc	A	Kanchanaburi
3559	ht	A	Kanchanaburi
3570	sp	A	Chonburi
3571	ht	F	Sakon Nakhon
3576	sp	NA	Phang Nga
3585	alc	NA	Chiang Mai
3586	ht	NA	Nakhon Ratchasima
3587	ht	A	Lop Buri
3588	ht	A	Phra Nakhon Si Ayutthaya
3600	ht	NA	Uthai Thani
3601	alc	NA	Nakhon Ratchasima
3603	ht	NA	Surat Thani
3604	ht	NA	Sing Buri
3605	ht	NA	Sing Buri
3614	ht	F	Mae Hong Son
3617	ht	F	Mae Hong Son
3618	ht	F	Trat
3619	ht	A	Chanthaburi
3627	sp	NA	Phang Nga
3628	alc	F	Chiang Mai
3639	alc	NA	Roi Et
3640	ht	NA	Roi Et
3646	ht	NA	Si Sa Ket
3647	ht	NA	Udon Ratchathani
3651	sp	NA	Phang Nga
3652	ht	A	Chiang Rai
3659	sp	F	Loei
3660	alc	NA	Trang
3661	ht	A	Chiang Rai
3662	ht	F	Mae Hong Son
3663	ht	F	Mae Hong Son
3664	ht	F	Sakon Nakhon
3665	alc	NA	Chiang Mai
3666	ht	A	Nakhon Pathom
3667	ht	A	Chon Buri
3668	ht	F	Trat
3669	ht	NA	Chiang Rai

[1/] KUFC = Kasetsart University Fungal Collection

[2/] sp = soil plate method, dp = dilution plate method, alc = alcohol treatment, ht = heat treatment

[3/] A = agricultural soil, NA = nonagricultural soil, F = forest soil

Table 9 (Continued)

KUFC[1/]	Isolation method[2/]	Source[3/]	Location
	Talaromyces flavus		
3670	ht	F	Sakon Nakhon
3671	ht	F	Sakon Nakhon
3672	ht	F	Sakon Nakhon
	Talaromyces luteus		
3331	ht	F	Mae Hong Son
3364	ht	F	Mae Hong Son
	Talaromyces macrosporus		
3339	ht	A	Chiang Rai
3342	ht	F	Sakon Nakhon
3343	ht	F	Sakon Nakhon
3345	ht	F	Khon Kaen
3346	ht	NA	Chiang Mai
3347	ht	NA	Chiang Mai
3348	alc	F	Nong Khai
3349	alc	NA	Chiang Mai
3356	alc	A	Nakhon Pathom
3363	sp	F	Chiang Mai
3365	alc	F	Mae Hong Son
3367	ht	A	Chon Buri
3371	ht	NA	Nakhon SiThammarat
3373	alc	NA	Phang N ga
3374	alc	NA	Krabi
3377	sp	NA	Krabi
3384	alc	NA	Krabi
3385	alc	NA	Surat Thani
3386	alc	NA	Surat Thani
3387	alc	A	Ang Thong
3396	alc	NA	Phatthalung
3397	alc	NA	Bangkok
3398	alc	NA	Bangkok
3437	sp	NA	Uthai Thani
3438	alc	A	Lop Buri
3439	ht	A	Chiang Rai
3440	ht	NA	Kalasin
3443	alc	NA	Si Sa Ket
3444	alc	NA	Nakhon Ratchasima
3448	sp	NA	Saraburi
3450	alc	A	Suphan Buri
3451	ht	A	Suphan Buri
3452	ht	F	Trat
3453	ht	NA	Phatthalung
3454	ht	A	Chiang Rai
3460	sp	A	Chiang Mai
3461	alc	A	Lop Buri
3462	alc	A	Lop Buri
3463	ht	F	Chiang Mai
3465	alc	A	Chonburi
3467	alc	A	Chonburi
3469	ht	A	Chiang Rai
3470	ht	A	Chiang Rai
3476	alc	NA	Chiang Mai
3483	ht	NA	Sing Buri
3484	ht	A	Suphan Buri
3485	ht	F	Trat
3486	ht	F	Trat
3494	alc	NA	Ratchaburi
3497	alc	NA	Ratchaburi
3498	ht	NA	Nakhon Ratchasima
3499	ht	NA	Nakhon Si Thammarat
3500	ht	NA	Nakhon Si Thammarat
3501	sp	NA	Phangnga
3504	alc	F	Chiang Mai
3505	ht	NA	Chiang Mai
3506	ht	A	Nakhon Ratchasima
3511	ht	F	Chiang Mai
3512	ht	F	Chiang Mai
3513	alc	A	Suphan Buri
3518	ht	NA	Buri Ram
3520	ht	NA	Buri Ram
3522	ht	NA	Kalasin
3523	ht	A	Lumpang
3524	ht	A	Lumpang
3525	ht	F	Trat
3526	ht	F	Trat
3527	sp	NA	Phang Nga
3528	alc	F	Chiang Mai
3529	alc	A	Nakhon Ratchasima
3531	ht	NA	Sakon Nakhon
3532	ht	F	Pitsanulok

1/ KUFC = Kasetsart University Fungal Collection

2/ sp = soil plate method, dp = dilution plate method, alc = alcohol treatment, ht = heat treatment

3/ A = agricultural soil, NA = nonagricultural soil, F = forest soil

Table 9 (Continued)

KUFC[1/]	Isolation method[2/]	Source[3/]	Location	KUFC[1/]	Isolation method[2/]	Source[3/]	Location
	Talaromyces macrosporus				***Talaromyces macrosporus***		
3534	ht	NA	Sakon Nakhon	3624	alc	NA	Chiang Mai
3535	sp	NA	Phang Nga	3625	sp	NA	Chiang Mai
3540	sp	NA	Roi Et	3626	alc	NA	Buri Rum
3541	sp	NA	Rayong	3629	ht	F	Loei
3545	alc	NA	Chiang Mai	3630	ht	A	Chiang Rai
3546	ht	F	Mae Hong Son	3631	ht	A	Chiang Rai
3547	ht	F	Mae Hong Son	3632	ht	A	Nonthaburi
3548	ht	F	Loei	3638	sp	NA	Chiang Mai
3552	alc	NA	Chiang Mai	3642	sp	F	Trat
3563	alc	A	Ayutthaya	3643	alc	A	Lop Buri
3564	alc	A	Ayutthaya	3644	ht	A	Chiang Rai
3566	alc	F	Chiang Mai	3645	alc	F	Mae Hong Son
3567	alc	NA	Chiang Mai	3653	alc	F	Mae Hong Son
3568	ht	F	Mae Hong Son	3654	ht	NA	Krabi
3569	ht	NA	Chiang Mai	3655	ht	NA	Si Sa Ket
3570	ht	F	Mae Hong Son		***Talaromyces rotundus***		
3572	ht	NA	Kalasin	3359	alc	NA	Chiang Mai
3573	ht	NA	Chiang Mai	3379	ht	NA	Sing Buri
3574	alc	NA	Chiang Mai	3410	ht	A	Suphan Buri
3575	alc	A	Ubol Ratchathani	3447	ht	F	Trat
3577	alc	A	Ang Thong	3475	alc	NA	Bangkok
3578	sp	NA	Chonburi	3609	alc	F	Mae Hong Son
3579	alc	A	Lop Buri	3620	ht	F	Mae Hong Son
3581	ht	A	Chiang Rai		***Talaromyces stipitatus***		
3582	ht	A	Chiang Rai	3357	ht	F	Sakon Nakhon
3583	alc	F	Nong Khai	3422	sp	NA	Krabi
3584	alc	NA	Trang	3449	alc	NA	Chiang Mai
3589	sp	NA	Trang	3464	ht	F	Mae Hong Son
3591	alc	A	Lop Buri	3472	ht	NA	Nakhon Ratchasima
3597	alc	NA	Roi Et	3487	ht	NA	Bangkok
3599	alc	NA	Buri Rum	3514	ht	NA	Nakhon Si Thammarat
3602	alc	A	Ubol Ratchathani	3515	alc	A	Bangkok
3606	alc	A	Nonthaburi	3551	ht	NA	Chiang Mai
3607	alc	F	Nong Khai	3553	ht	NA	Chiang Mai
3608	sp	NA	Sing Buri	3560	ht	NA	Bangkok
3610	alc	A	Lop Buri	3561	ht	A	Chiang Rai
3612	ht	A	Chiang Rai	3594	ht	A	Chon Buri
3613	ht	A	Chiang Rai	3636	ht	F	Sakon Nakhon
3623	alc	A	Chiang Mai	3641	ht	NA	Bangkok

[1/] KUFC = Kasetsart University Fungal Collection

[2/] sp = soil plate method, dp = dilution plate method, alc = alcohol treatment, ht = heat treatment

[3/] A = agricultural soil, NA = nonagricultural soil, F = forest soil

Table 9 (Continued)

KUFC[1/]	Isolation method[2/]	Source[3/]	Location
Talaromyces stipitatus			
3648	sp	NA	Phangnga
3658	alc	NA	Chiang Mai
Talaromyces trachyspermus			
3352	ht	NA	Bangkok
3353	ht	NA	Bangkok
3355	ht	NA	Chiang Mai
3372	sp	NA	Phangnga
3380	sp	NA	Phangnga
3421	sp	F	Mae Hong Son
3423	alc	NA	Chiang Mai
3425	ht	F	Loei
3428	ht	F	Sakon Nakhon
3495	ht	NA	Saraburi
3596	alc	NA	Chiang Mai
3621	alc	NA	Bangkok
3635	alc	NA	Buri Rum
3649	alc	NA	Chiang Mai
3657	alc	F	Chiang Rai
Talaromyces wortmannii			
3333	ht	F	Mae Hong Son
3354	ht	NA	Krabi
3488	ht	NA	Chiang Mai
3489	ht	NA	Uthai Thani
3490	alc	A	Chon Buri
3634	ht	NA	Chon Buri
3615	alc	NA	Bangkok
3616	sp	NA	Krabi
3622	sp	NA	Chiang Mai
3634	alc	A	Chiang Rai
3637	alc	A	Bangkok
3650	ht	NA	Sing Buri
3656	ht	A	Suphan Buri
***Talaromyces* sp. 1**			
3399	alc	A	Trat
3413	ht	NA	Chiang Mai
***Talaromyces* sp. 2**			
3383	ht	F	Trat

1/ KUFC = Kasetsart University Fungal Collection

2/ sp = soil plate method, dp = dilution plate method, alc = alcohol treatment, ht = heat treatment

3/ A = agricultural soil, NA = nonagricultural soil, F = forest soil

2. Morphological study of *Talaromyces* spp.

1. *Talaromyces austrocalifornicus* Yaguchi et Udagawa (Figures 6-10)

Strains examined: KUFC 3351 forest soil, Mae Hong Son; KUFC 3401 nonagricultural soil, Chiang Mai

Reference: Yaguchi *et al.*, 1993b

Stat. Anam. ***Penicillium austrocalifornicum*** Yaguchi et Udagawa

Colonies on CZA growing slowly, reaching a diameter of 10-12 mm within 7 days at 25°C, umbonate, slightly sulcate, consisting of a thin basal felt, Light Yellow or Pure Yellow (R 14); producing developing ascomata on the felt; conidiogenesis sparse, inconspicuous; exudates small, yellow; reverse Sienna (R 8) or Bay (R 6) (Figures 6 A, a). Colonies on CZA at 28°C, attaining a diameter of 15 mm and 20-22 mm within 7 and 14 days respectively, velvety to funiculose, slightly sulcate, consisting of a compact basal felt, producing immature ascomata on the agar surface, Pure Yellow (R 14); reverse Straw at the margin (R 46), Scarlet (R 5) at the centre (Figures 7-8 A, a).

Colonies on CYA growing restrictedly, reaching a diameter of 8-10 mm within 7 days at 25°C, umbonate, floccose, consisting of a compact basal felt, Sulphur Yellow (R 15) to Pure Yellow (R 14); producing young ascomata in a layer on the felt, conidiogenesis limited; exudates absent; reverse Umber (R 9) to Chestnut (R 40) (Figures 6 B, b). Colonies on CYA at 28°C, reaching 17 and 20 mm in diameter within 7 and 14 days respectively, funiculose, consisting of a thick basal felt, Pure Yellow (R 14); producing abundant ascomata over the entire surface; exudates absent; reverse Salmon (R 41) to Umber (R 8) (Figures 7-8 B, b).

Colonies on MEA growing moderately, attaining a diameter of 20 mm within 7 days at 25°C, plane, more or less funiculose, with central area raised up to 3-4 mm deep floccose, consisting of a compact mycelial felt, Pure Yellow (Rayner 14); producing young ascomata intermixed with yellow mycelial hyphae; conidiogenesis inconspicuous and sparse; margins entire; exudates as pale yellow drops, abundantly

produced in central area; odor musty; reverse Cinnamon (R 62) or Sepia (R 63) (Figures 6 C, c). Colonies on MEA at 28°C, reaching 22 mm and 35-37 mm in diameter within 7 and 14 days respectively, plane, fasciculate, consisting of a compact basal felt, Pure Yellow (R 14); developing abundant ascomata which form a continuous layer over the entire surface; conidiogenesis absent; reverse Amber (R 47) to Umber (R 9) (Figures 7-8 C, c).

Colonies on CMA growing moderately, attaining a diameter of 20 mm within 7 days at 25°C; plane, consisting of a very thin mycelial felt, vegetative mycelium submerged and forming sparse growth of aerial hyphae, Light Yellow or Pure Yellow (R 14); ascomata moderately produced on the agar surface; conidiogenesis sparse; exudates scattered, as small clear drops; reverse uncolored (Figures 6 D, d). Colonies on CMA at 28°C, reaching 22 mm and 35-40 mm in diameter within 7 and 14 days respectively, fasciculate, consisting of a thin basal felt, with surface appearing granular due to the production of abundant ascomata, Pure Yellow (R 14); conidiogenesis absent; reverse Straw (R 46) (Figures 7-8 D, d).

Colonies on OMA growing moderately, attaining a diameter of 22-24 mm within 7 days at 25°C, plane, consisting of a thin mycelial felt where abundant yellow ascomata developed, showing an increased yellowish coloration, Light Yellow or Pure Yellow (R 14); conidiogenesis inconspicuous and sparse; exudates absent; reverse uncolored (Figures 6 E, e). Colonies on OMA at 28°C, reaching 25 mm and 40-45 mm in diameter within 7 and 14 days respectively, fasciculate, colonies characters and colored as on CMA; reverse Straw (R 46) to Luteous (R 12) (Figures 7-8 E, e).

Colonies on G25N agar growing extremely slowly within 7 days at 25°C and 28°C (Figures 6-7 F, f), attaining a diameter of 9-12 mm within 14 days at 28°C, consisting of a compact mycelial felt, producing abundant penicilli over the entire surface, Pale Greenish Grey (R 123); ascomata absent; exudates small, Pale Orange (R 7); margins entire; reverse uncolored (Figures 8 F, f).

At 37°C, growth is extremely restricted.

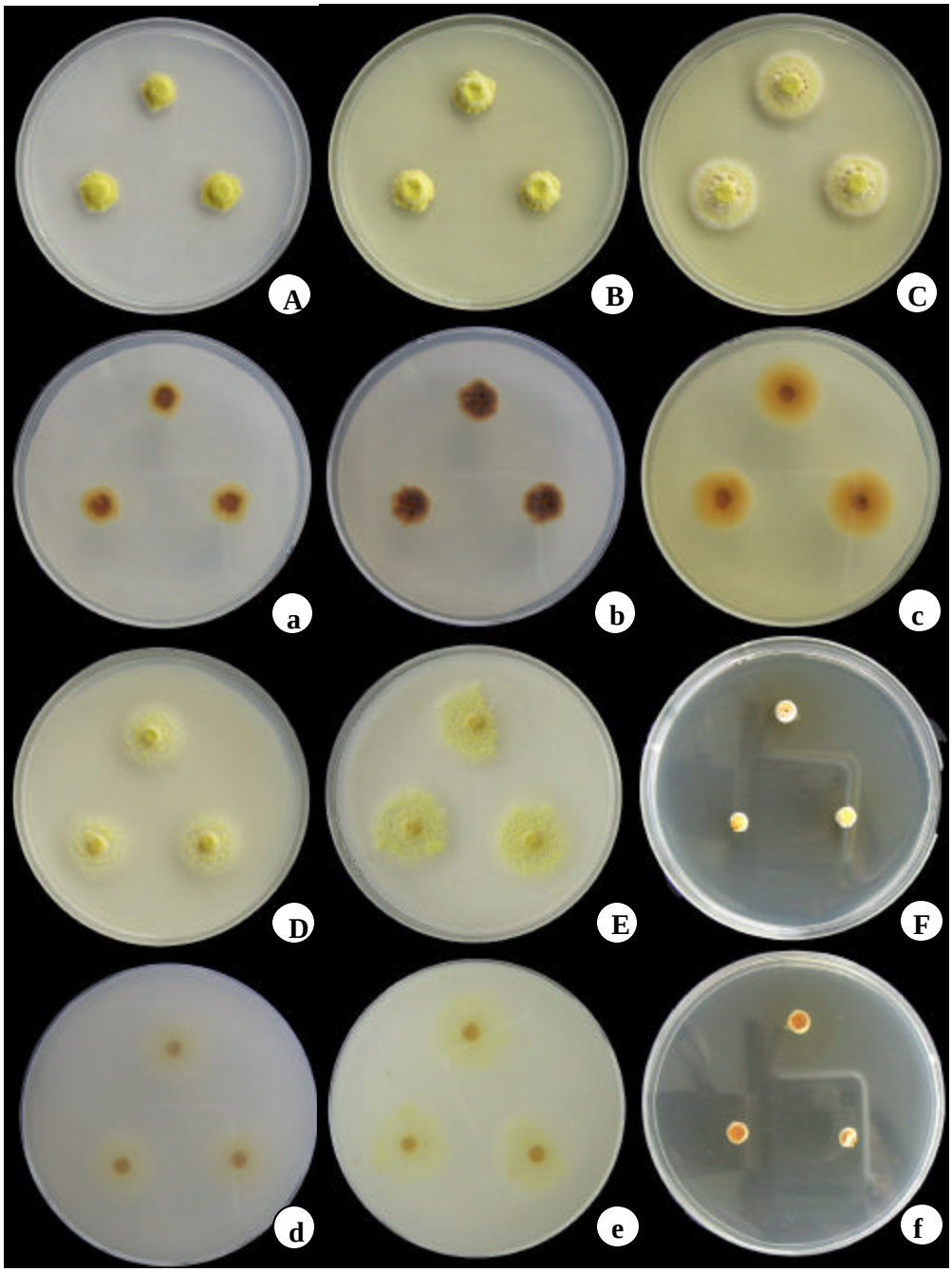


Figure 6 *Talaromyces austrocalifornicus* KUFC 3401. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

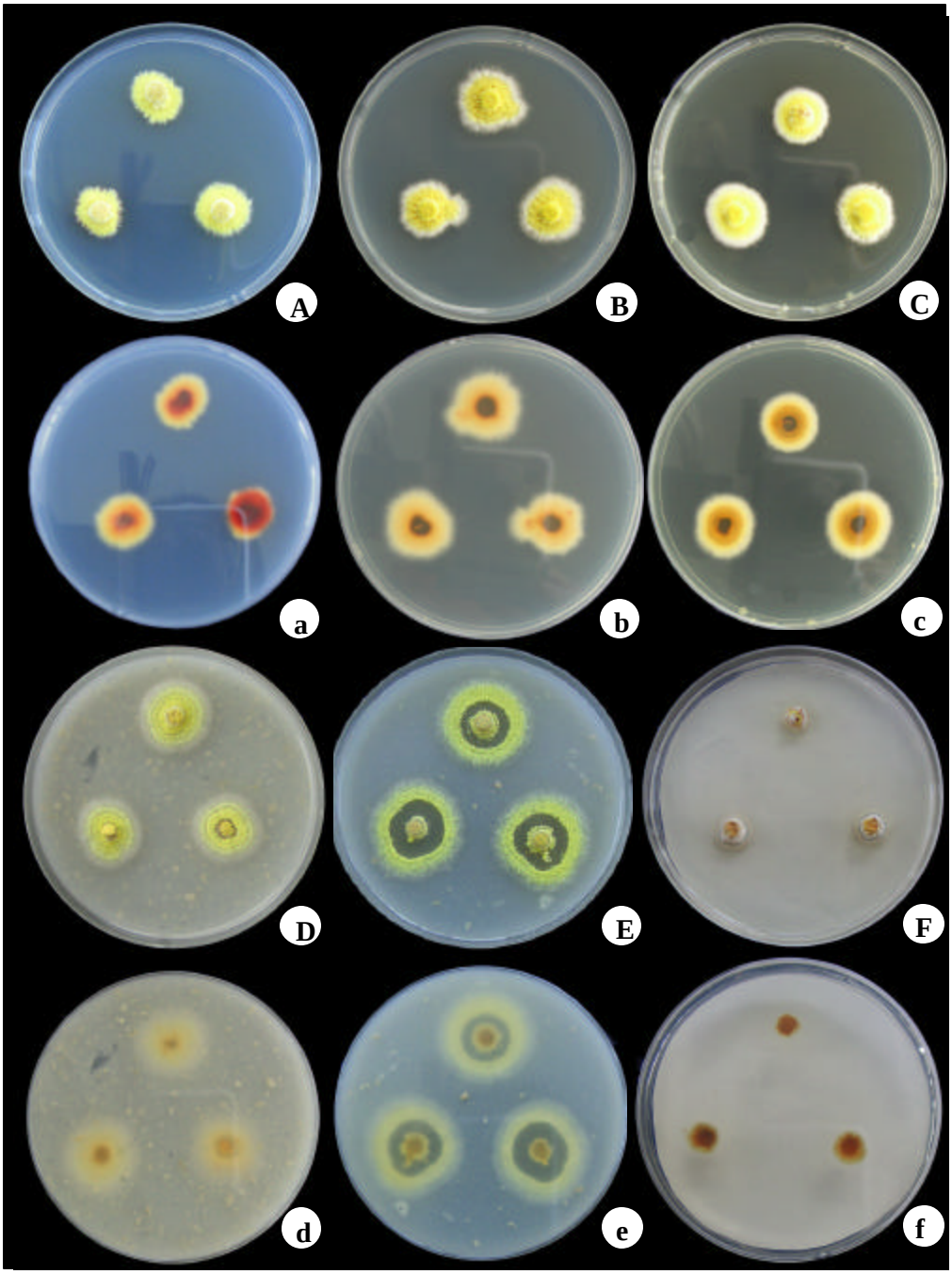


Figure 7 *Talaromyces austrocalifornicus* KUFC 3401. Obverse and reverse views of colonies on different media, incubated for 7 days at 28°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

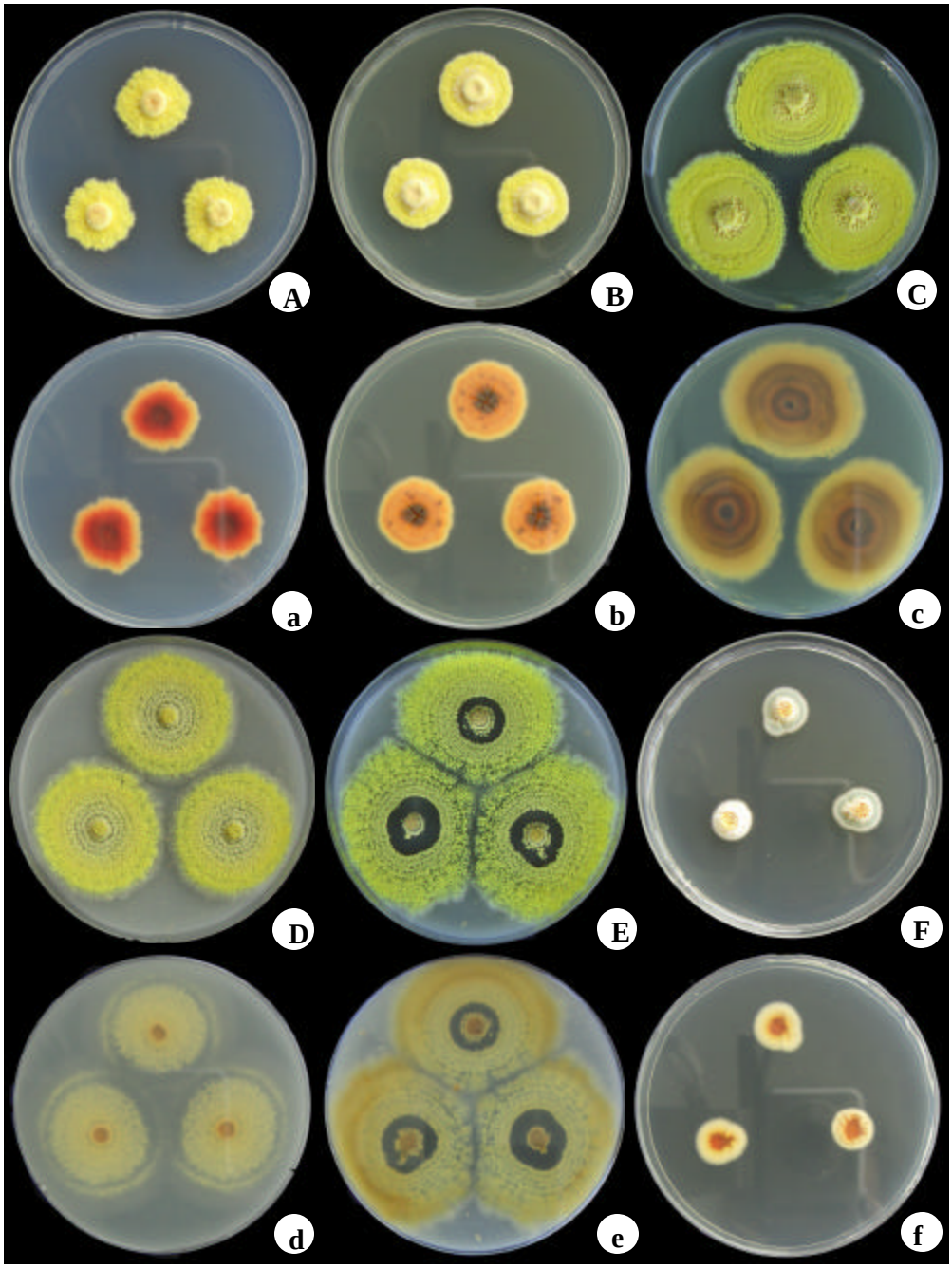


Figure 8 *Talaromyces austrocalifornicus* KUFC 3401. Obverse and reverse views of colonies on different media, incubated for 14 days at 28°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

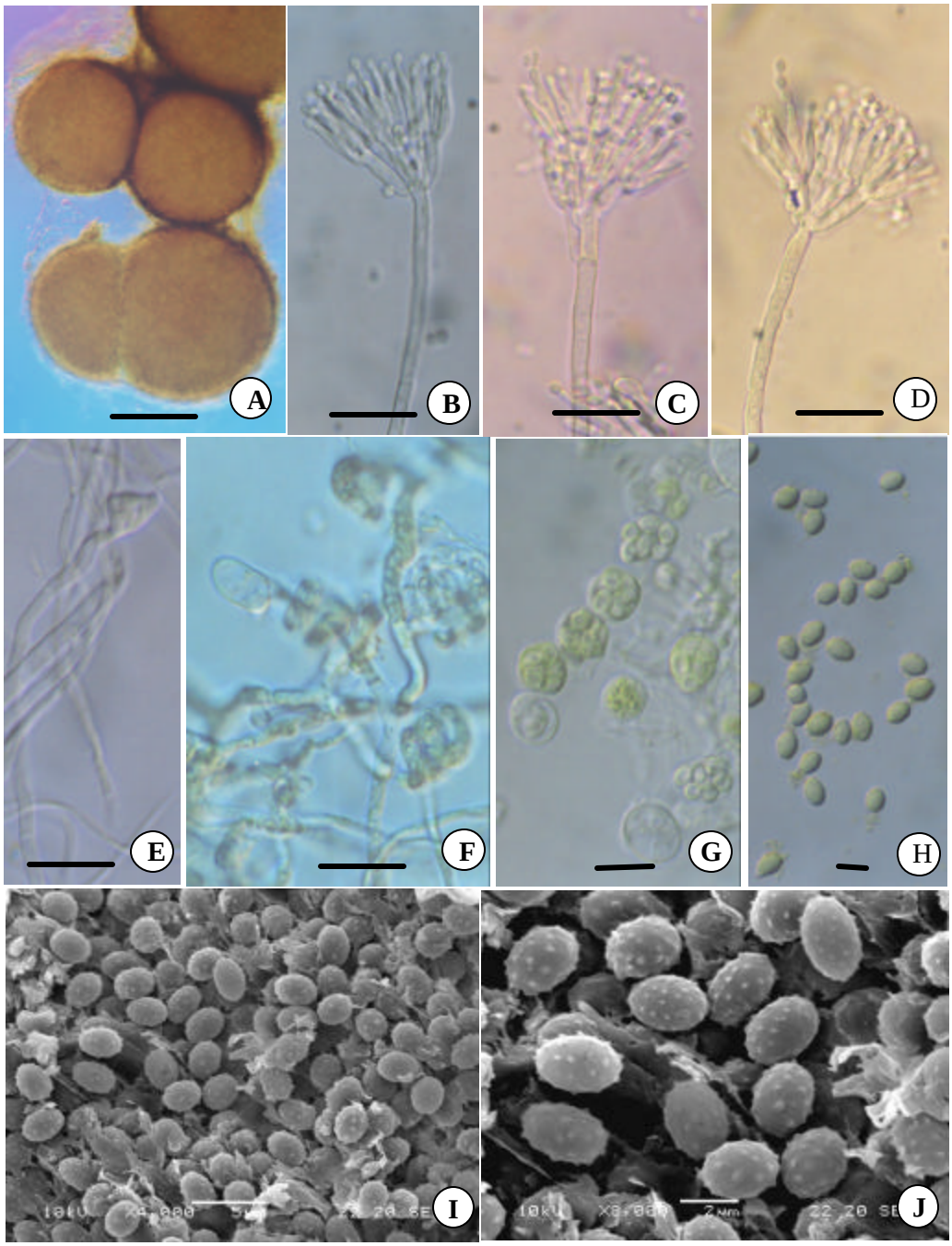


Figure 9 *Talaromyces austrocalifornicus* KUFC 3401

A. ascomata; B-D. penicilli; E-F. ascomatal initials; G-H. asci and ascospores; I-J. ascospores (SEM)

(Bars: A = 100 µm; B-G = 10 µm; H = 3 µm; I = 5 µm; J = 2 µm)

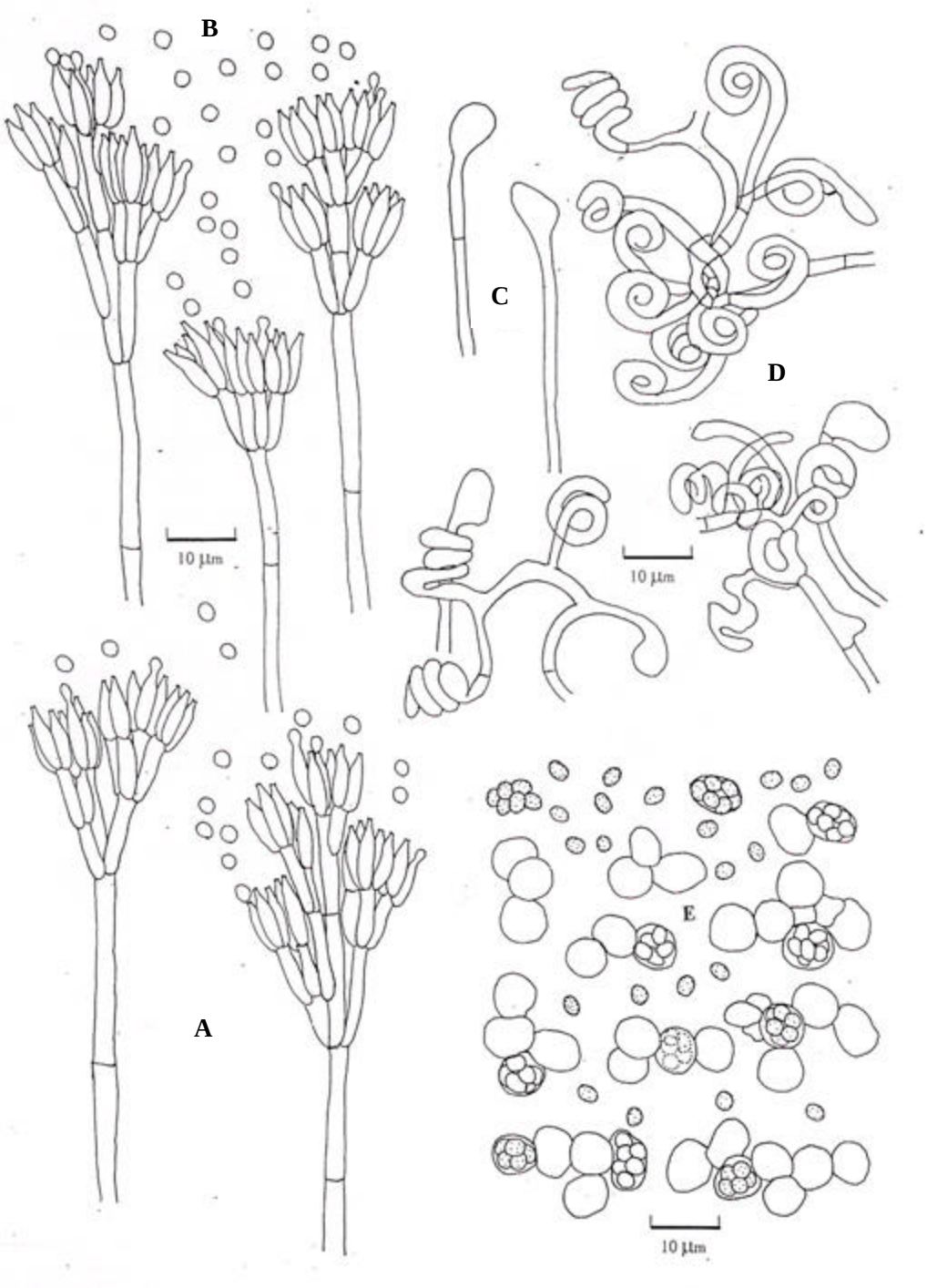


Figure 10 *Talaromyces austrocalifornicus* KUFC 3401

Camera lucida drawings of A. Penicilli; B. conidia; C. ascogonia; D. ascomatal initials; E. asci and ascospores

Ascomata discrete or confluent, non-ostiolate, soft, ripening within 14 to 21 days (Figures 8 A-E), globose to subglobose, (150-) 200-300 (-400) µm in diameter, ascomatal wall composed of a loose network of branched, septate, radiate, yellow, interwoven hyphae (Figure 9 A). Ascomatal initials pattern comprising the formation of long-stipitate ascogonium which swollen at apex and soon enveloped in incurved or twisted branches of aerial hypha (anteridia) (Figures 9 E-F, 10 C-D). Asci borne in chains, 8-spored, globose to ovoidal, 6-7.5 x 4.5-5.5 µm, evanescent (Figures 9 G, 10 E). Ascospores pale yellow, broadly ellipsoidal, small, 2.2-3.3 x 2.2-2.5 µm, finely spinulose with sparse spines (Figures 9 H-J, 10 E).

Conidiophores arising from the basal mycelium or aerial hyphae; stipes hyaline, smooth, 75-100(-120) x 2.7-3(-3.5) µm. Penicilli typically biverticillate, sometime terverticillate or quaterverticillate; rami 10-25 µm long. Metulae mostly appressed verticils of 4-6, 8-10 x 2-3 µm. Phialides acerose, 4-6 in the verticil, (9-) 10-12 x 2-3 µm (Figures 9 B-D, 10 A). Conidia hyaline, subglobose to globose, 2-3 µm, smooth-walled (Figures 9 B-D, 10 B).

Talaromyces austrocalifornicus was first reported as new species from soil at the University of Southern California, Los Angelies, California, U.S.A. (Yaguchi *et al*., 1993b). It is related to *T. convolutes* which has been reported as new species from soil in Nepal in the same year (Udagawa, 1993). The growth rate on common media, colony morphology, well-developing penicilli, ascospores size and morphology and especially ascomatal initial pattern are in similar. They seemed to be the same species. However, both species differ in ascomata size and conidiophore lenght. *T. austrocalifornicus* belonged to the section *Talaromyces*, series *Lutei* in its growth-rate on MEA, colony morphology, ascomata size and color and well-developing penicilli. Suzuki *et al.*, (1999) have reported the isolation of (-)- mitorubrinal and (-)-mitorubrinic acid from yellow pigment on ascomata of this species.

2. ***Talaromyces bacillisporus*** C.R. Benjamin (Figures 11-13)

Strains examined: KUFC 3350 nonagricultural soil, Kasetsart Univ., Bangkok; KUFC 3417 forest soil, Mok Fa water fall, Chiang Mai; KUFC 3580 forest soil, Mae Hong Son

Reference: Stolk and Samson, 1972

Stat. Anam. ***Geosmithia swiftii*** Pitt (1979)

Penicillium bacillisporum Swift (1932)

Colonies on CZA growing moderately, attaining a diameter of 20 mm within 7 days at 25°C, plane, velvety, slightly radiate furrow, consisting of a compact basal felt, Creamish to Straw (R 46); ascomata absent; conidiogenesis profuse; exudates and soluble pigment absent; reverse Dark Mouse Grey (R 119), but Straw (R 46) at the margin (Figures 11 A, a).

Colonies on CYA growing rapidly, attaining a diameter of 40 mm within 7 days at 25°C, velvety, zonate, sulcate, consisting of a compact basal felt, producing numerous young ascomata on the mycelium felt, Creamish to Pale Sulphur Yellow (R 15); conidiogenesis sparse and inconspicuous; exudates absent; reverse Orange (R 7), Umber (R 9) to Hazel (R 88) (Figures 11 B, b).

Colonies on MEA growing rapidly, attaining a diameter of 40-43 mm within 7 days at 25°C, plane, velvety, consisting of a thin mycelial felt, producing abundant conidiogenesis over the entire surface, Pale Mouse Grey (R 117); ascomata absent; margins entire and lower; exudates absent; reverse Dark Green (R 21) at centre but margins uncolor (Figures 11 C, c).

Colonies on CMA growing moderately, attaining a diameter of 35 mm within 7 days at 25°C, plane, consisting of a compact tough Saffron (R 10) mycelial felt, showing Salmon (R 41) to Saffron (R 10) shade, occasionally furrowed in radial pattern, white to Pale Luteous (R 11) sectors occur, ascomata absent; exudates absent; reverse Orange (R 7), Umber (R 9) to Hazel (R 88) (Figure 11 D, d).

Colonies on OMA growing rather rapidly, attaining a diameter of 45 mm within 7 days at 25°C, plane, consisting of a compact mycelial felt in which abundant developing ascomata, covered by a floccose to funiculose aerial mycelium bearing penicilli, showing creamish to Pale Luteous (R 11) color; conidiogenesis abundant; exudates absent; reverse Dark Green (R 21) to Dark Bluish Green (R 24) (Figures 11 E, e).

Colonies on G25N agar growing slowly; attaining a diameter of 15 mm within 7 days at 25°C, producing abundant penicilli, Glaucus Blue Green (R 94); ascomata absent; exudates absent; reverse Straw (R 46) (Figures 11 F, f).

Colonies on all media at 37°C, 7 days, growing rapidly, attaining a diameter of 20-45 mm, commonly similar in appearance to colonies on CYA, plane, furrow, consisting of a compact basal felt, Vinaceous Buff (R 86) to Hazel (R 88); ascamata absent; conidiogenesis abundant; reverse Sienna (R 8) to Umber (R 9).

Ascomata creamish to pale yellow, usually globose, 50-200 µm in diameter, discrete or confluent, ripening within 2 weeks. Covering consisting of a thin network of loosely interwoven hyphae, surrounded by a weft of radiating, white to creamish (Figure 12 A). Ascomatal initials developing directly from vegetative hyphae, consisting of swollen, somewhat twisted hyphal elements, which become irregularly septate and produce coiling branches thus developing a compact mass of ascogenous hyphae (Figures 12 D, 13 C). Asci 8-spored, globose to ovoidal, 12-14 x 9.5-13 µm (Figures 12 E, 13 D). Ascospores globose, 4.5-5.2 µm in diameter, thick-walled, spinulose with spines up to 0.5 µm in length (Figures 12 F-H, 13 E).

Conidiophores arising from aerial hyphae, short, 25-75 x 1.5-2.5 µm, Penicilli typically monoverticillate or occasionally biverticillate. Phialides smooth, consisting of a cylindrical base, tapering abruptly to a short conidium-bearing tip, about 2 to 6 in the verticil (Figures 12B-C, 13A). Conidia hyaline, cylindrical, 3.5 x 1.5-2 µm, smooth-walled (Figures 12 B-C, 13 B).

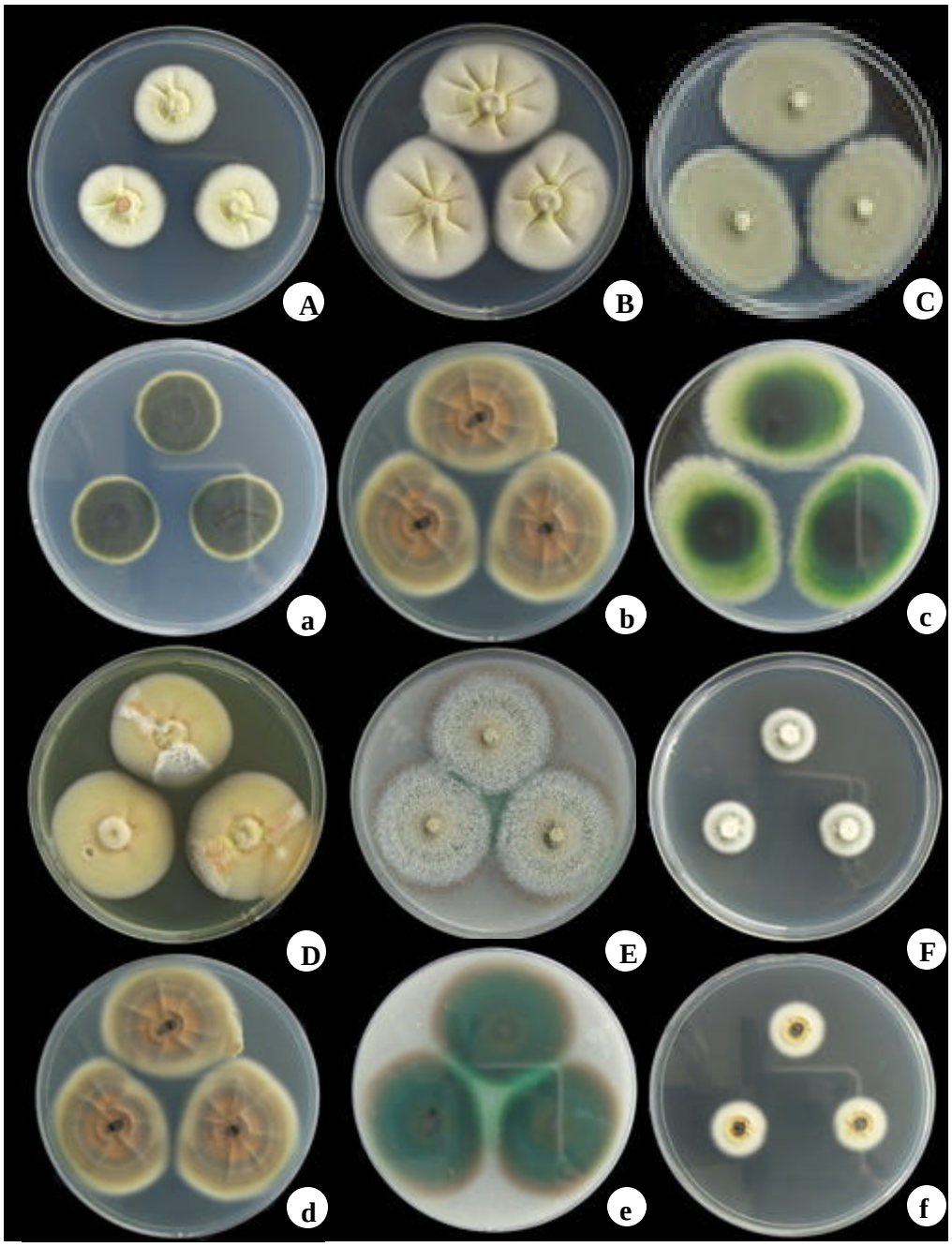


Figure 11 *Talaromyces bacillisporus* KUFC 3350. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

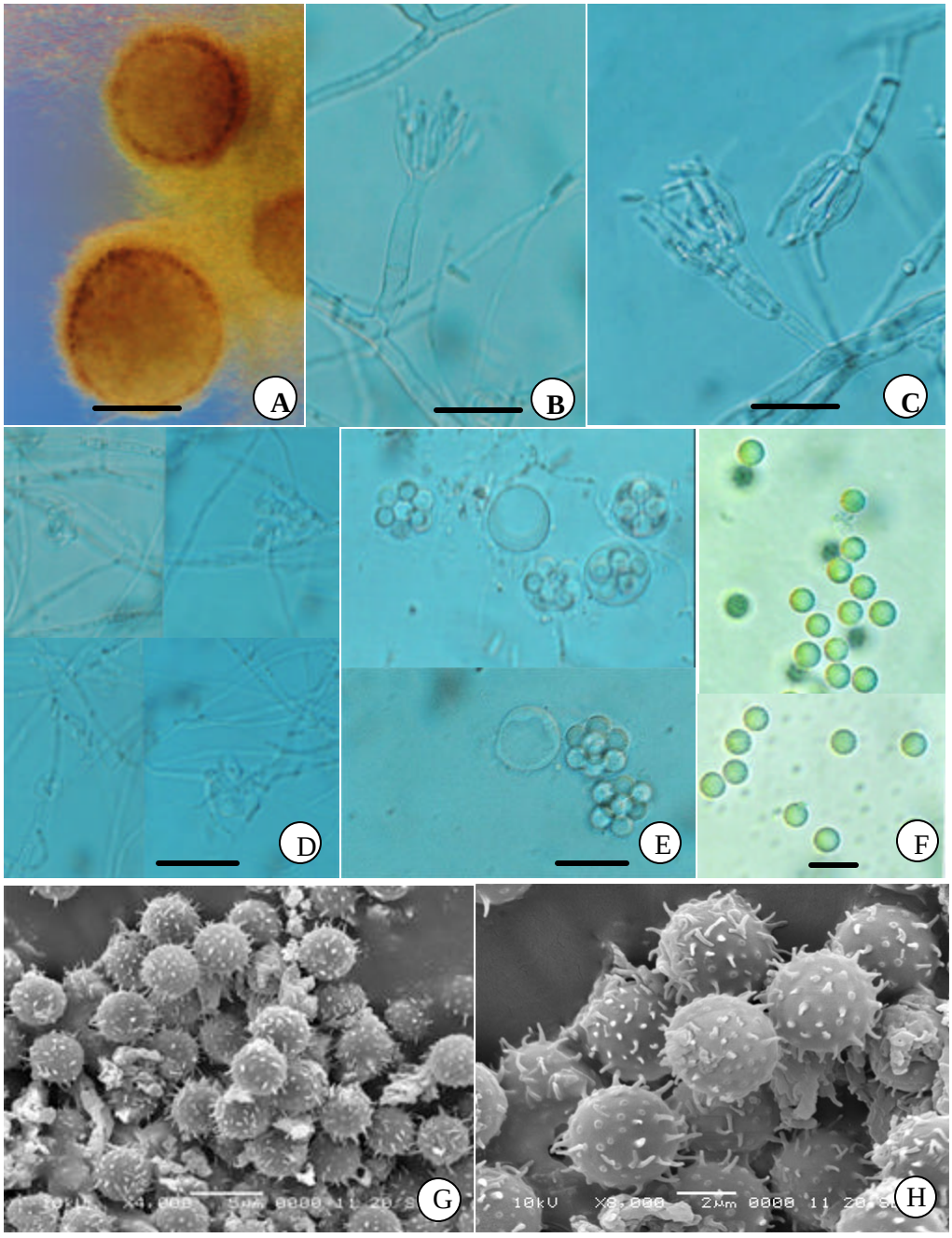


Figure 12 *Talaromyces bacillisporus* KUFC 3350

A. ascomata; B-C. penicilli and conidia; D. ascomatal initials; E-F. asci and ascospores; G-H. ascospores (SEM)

(Bars: A = 100 µm; B-E = 10 µm; F-G = 5 µm; H = 2 µm)

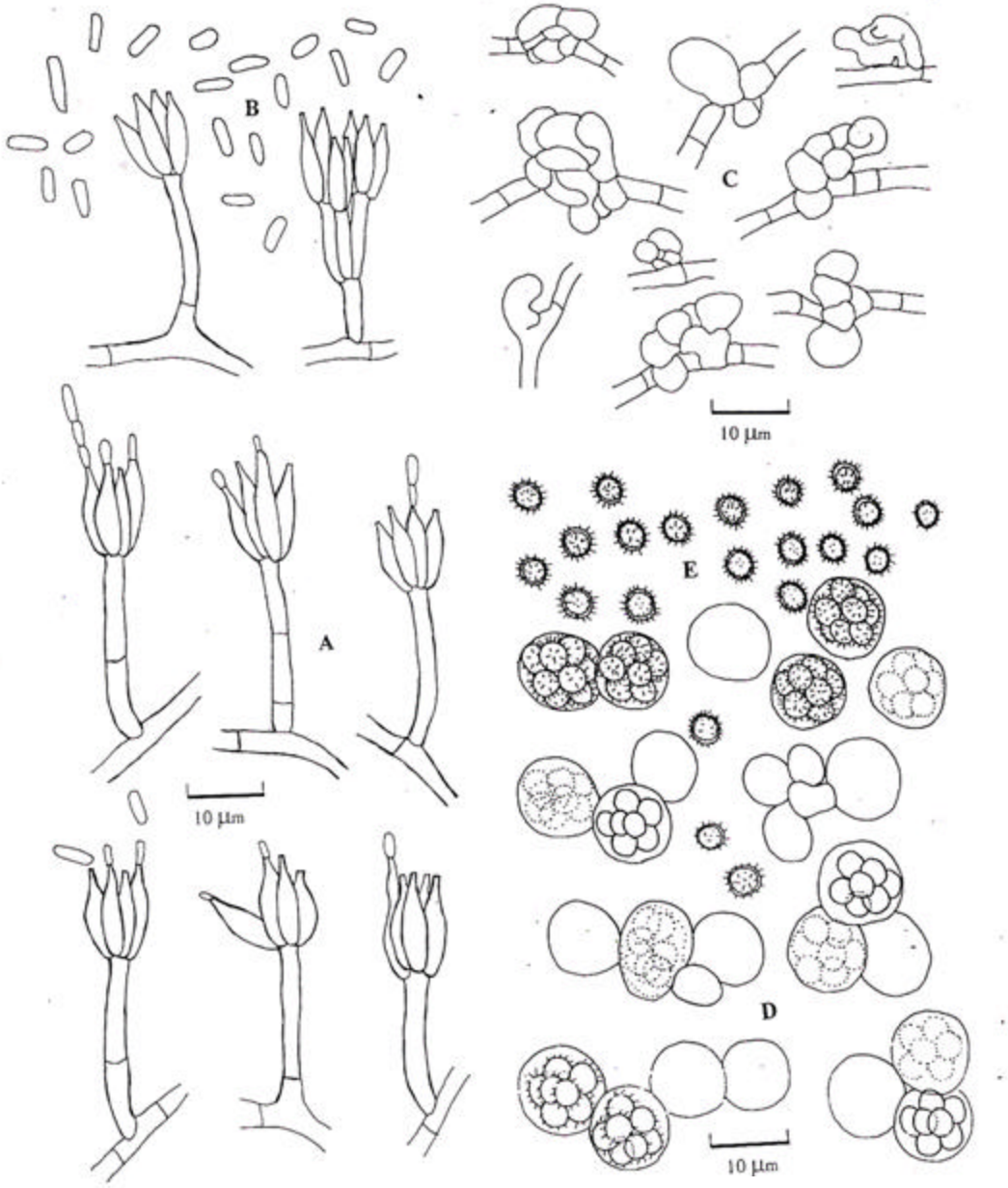


Figure 13 *Talaromyces bacillisporus* KUFC 3350

Camera lucida drawings of A. penicilli; B. conidia; C. ascomatal initials; D. asci and ascospores

Talaromyces bacillisporus can easily be distinguished from other species by the green color shade of colony reverse, conspicuous globose and spinulose ascospores. This species has been regarded as a very rare species, however it reported from soils and pasteurized fruit juice from many countries (Stolk and Samson, 1972; Pitt and Hocking, 1997; Asan, 2004). In Thailand, *T. bacillisporus* was isolated from agricultural, forest soils, termite mounds and rat dung (Manoch *et al*., 2004, Jeamjitt, 2007). Yamazaki and Okuyama (1980) reported oxaphenalenone dimers and xanthone carboxylic acid from *T. bacillisporus* as bacillosporins A-C and pinselin and found that bacillosporins A can exhibit antibacterial activity against *Bacillus subtilis* and *Sarcina lutea*. In addition, Ishii *et al.*, (1995) was also reported talarotoxin from this fungus. This compound showed cytotoxic activity which inhibited the proliferation of both mouse myeloma X63.Ag8.6.5.3 cells and BALB/3T3 mouse fibroblasts. In this study, two new compounds, bacillisporins D and E have been isolated from *T. bacillisporus* (KUFC 3350).

3. ***Talaromyces flavus*** (Klöcker) Stolk & Samson (Figures 14-19)

Strains examined: KUFC 3332 nonagricultural soil, Bangkok; KUFC 3334 forest soil, Mae Hong Son; KUFC 3335 nonagricultural soil, Krabi; KUFC 3340 agricultural soil, Lop Buri; KUFC 3344 agricultural soil, Chiang Rai; KUFC 3360 nonagricultural soil, Bangkok; KUFC 3363 agricultural soil, Chon Buri; KUFC 3381 agricultural soil, Kasetsart Univ., Bangkok; KUFC 3395 agricultural soil, Suphan Buri; KUFC 3397 agricultural soil, Nonthaburi; KUFC 3400 agricultural soil, Chiang Mai; KUFC 3408 agricultural soil, Nakhon Ratchasima; KUFC 3420 nonagricultural soil, Rayong; KUFC 3455 nonagricultural soil, Ratchaburi; KUFC 3485 agricultural soil, Nakhon Ratchasima; KUFC 3446 agricultural soil, Chiang Mai; KUFC 3473 nonagricultural soil, Krabi; KUFC 3501 agricultural soil, Chiang Mai; KUFC 3510 agricultural soil, Chiang Mai; KUFC 3523 agricultural soil, Kanchanaburi; KUFC 3525 agricultural soil, Nonthaburi; KUFC 3528 agricultural soil, Chon Buri; KUFC 3530 agricultural soil, Chanthaburi; KUFC 3549 nonagricultural soil, Buri Rum; KUFC 3550 forest soil, Sakon Nakhon; KUFC 3554 agricultural soil, Suphan Buri; KUFC 3556 forest soil, Trat

References: Stolk and Samson, 1972; Pitt, 1979a; Domsch *et al.*, 1993a, b

Stat. Anam. ***Penicillium vermiculatum*** Dangeard

Morphological characteristics of *T. flavus* isolate KUFC 3530: colonies on CZA growing slowly, attaining a diameter of 25-28 mm in 7 days at 25°C, plane, more or less funiculose, consisting of a thick basal felt with Pure Yellow (R 14) aerial mycelium, producing numerous developing ascomata on the felt, Light Yellow or Pure Yellow (R 14); conidiogenesis sparse, inconspicuous; exudates small, pale orange; reverse Red (R 2) but white at the margin (Figures 14 A, a). Colonies on CZA at 28°C, reaching a diameter of 40 mm within 7 days, plane, funiculose, consisting of a thick basal felt, producing abundant immature ascomata, Pure Yellow (R 14); conidiogenesis sparse, inconspicuous; exudates small, pale orange; reverse Saffron (R 10) to Apricot (R 42) (Figures 15 A, a).

Colonies on CYA spreading broadly, reaching 55 mm diameter within 7 days at 25°C, velvety, plane, zonate, consisting of a thin basal felt, producing developing ascomata in a layer on the felt; conidiogenesis sparse, Sulphur Yellow (R 15) to Pure Yellow (R 14); exudates pale orange at the centre; reverse ranging from Luteous (R 12), Ochreous (R 44) to Scarlet (R 5) (Figures 14 B, b). Colonie on CYA at 28°C, attaining a diameter 60 mm within 7 days, velvety later becoming more fasciculate, zonate, producing limited ascomata in the central area, Pale Sulphur Yellow (R 15); conidiogenesis lacking; exudated scattered; reverse ranging Straw (R 46), Scarlet (R 5) to Rust (R 39) (Figures 15 B, b).

Colonies on MEA growing rapidly, attaining a diameter 70 mm within 7 days at 25°C, thin, funiculose, producing numerous young ascomata over the entire surface, usually forming a conspicuous thick layer, showing Sulphur Yellow (R 15) to Pure Yellow (R 14), but some strains, such as KUFC 3332, KUFC 3344 growing moderately, 40-45 mm within 14 days and produced paler yellow shades; conidial sparse and intermixed with aerial hyphae; exudates limited; reverse conspicuous brown shading, ranging from Peach (R 4) to Umber (R 9) (Figures 14 C, c). Colonies on MEA at 28°C, reaching 50-55 mm diameter within 7 days, funiculose, producing abundant ascomata on the agar surface, showing an increased yellowish coloration; conidiogenesis moderate; exudates absent; reverse Peach (R 4) to Flesh (R 37) (Figures 15 C, c).

Colonies on CMA growing rapidly, attaining a diameter 40-50 mm within 7 days at 25°C, plane, funiculose, consisting of a thin basal felt, producing limited ascomata on the felt, loosely covered by somewhat funiculose hyphae, White to Yellowish White; conidiogenesis moderate; exudates absent; reverse uncolored (Figures 14 D, d). Colonies on CMA at 28°C, reaching 50-55 cm in diameter within 7 days, plane, funiculose, producing abundant ascomata on the agar surface, Light Yellow or Pure Yellow (R 14); conidiogenesis moderate; exudates absent; reverse Straw (R 14) (Figures 15 D, d).

Colonies on OMA spreading broadly, reaching 45-50 cm diameter within 7 days at 25°C, velvety to more or less funiculose, consisting of a compact funiculose basal felt, ascomata slowly developing on the felt, White; conidiogenesis limited; margins spread broadly and submerged; exudates absent; reverse uncolored (Figures 14 E, e). Colonies on OMA spreading broadly, reaching 50–55 cm diameter within 7 days at 25°C, velvety to more or less funiculose, consisting of a thin basal felt, ascomata slowly developing on the felt, Sulphur Yellow (R 15); conidiogenesis lacking; margins spread broadly and submerged; exudates absent; reverse uncolored (Figures 15 E, e).

Colonies on G25N agar growing slowly; attaining a diameter of 20 mm within 7 days at 25°C, mycelium and conidiogenesis sparse; abundant developing ascomata on the entire surface, Sulphur Yellow (R 15) to Pure Yellow (R 14); conidiogenesis limited; exudates absent; reverse Pale Yellow (Figures 14-15 F, f).

Microscopic characteristics of *T. flavus* (KUFC 3530) were examined under light and scanning electron microscopes. Ascomata sulphur yellow to yellow, usually globose, 200-450 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, slightly twisted, predominantly radiating hyphae. Ascomatal initials consisting of club-shaped ascogonia, around with thin antheridia coil tightly several times. After fertilization, the ascogonia become septate (Figures 18 A-B). The primordium becomes enveloped in closely wound delicate hyphae, which develop into the covering and develop to ascogenous cell. Asci broadly ellipsoidal to subglobose, 6-7 x 4.5-5.5 µm (Figures 18 A-B). Ascospores ellipsoidal, 2.5-3 x 1.5-2 µm, in some strains slightly larger, up to 3.7 x 2.2 µm, very delicately spinulose, with the spines usually irregularly disposed on the surface (Figures 18 A-B, 19 A-D).

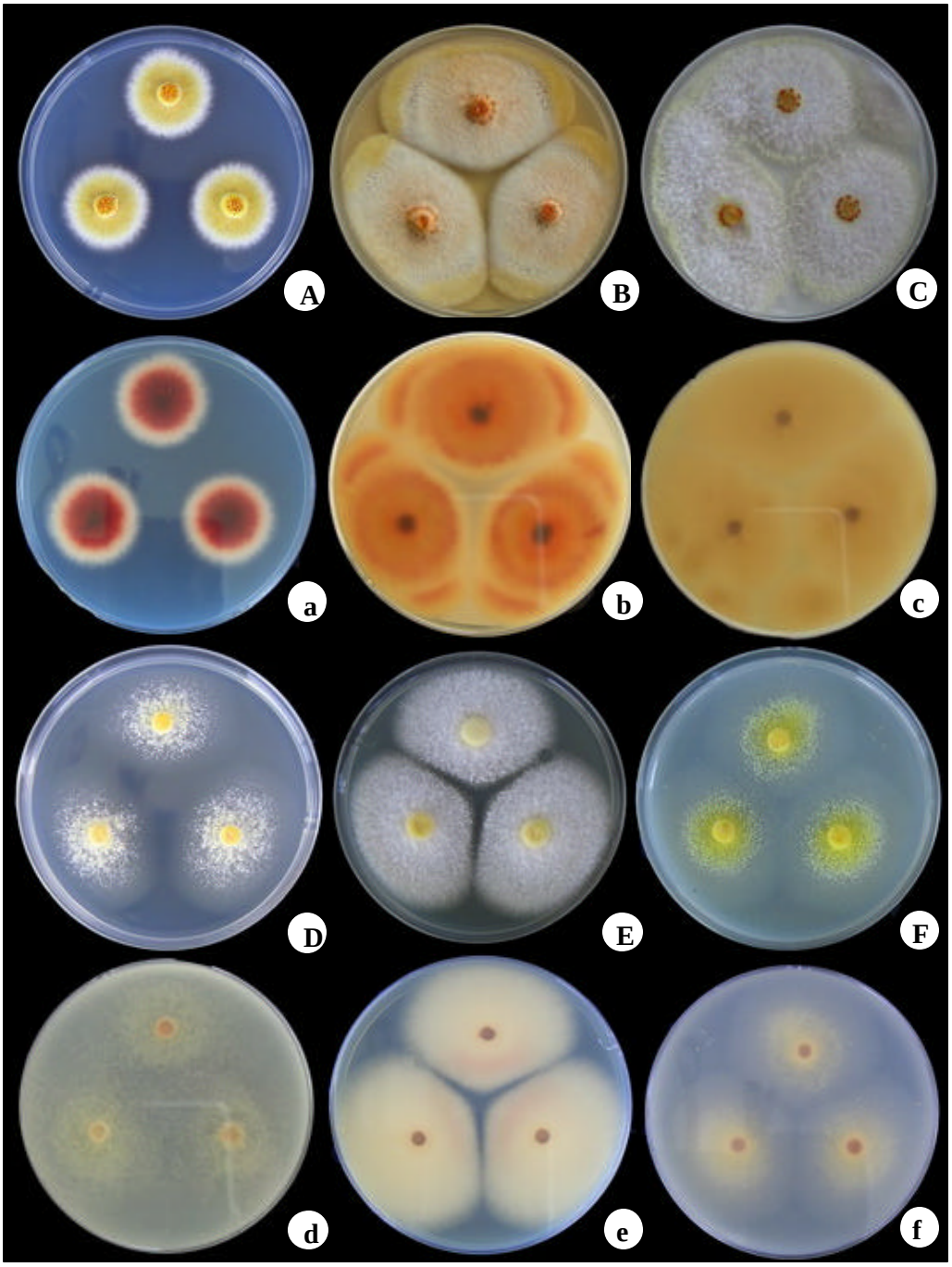


Figure 14 *Talaromyces flavus* KUFC 3530. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

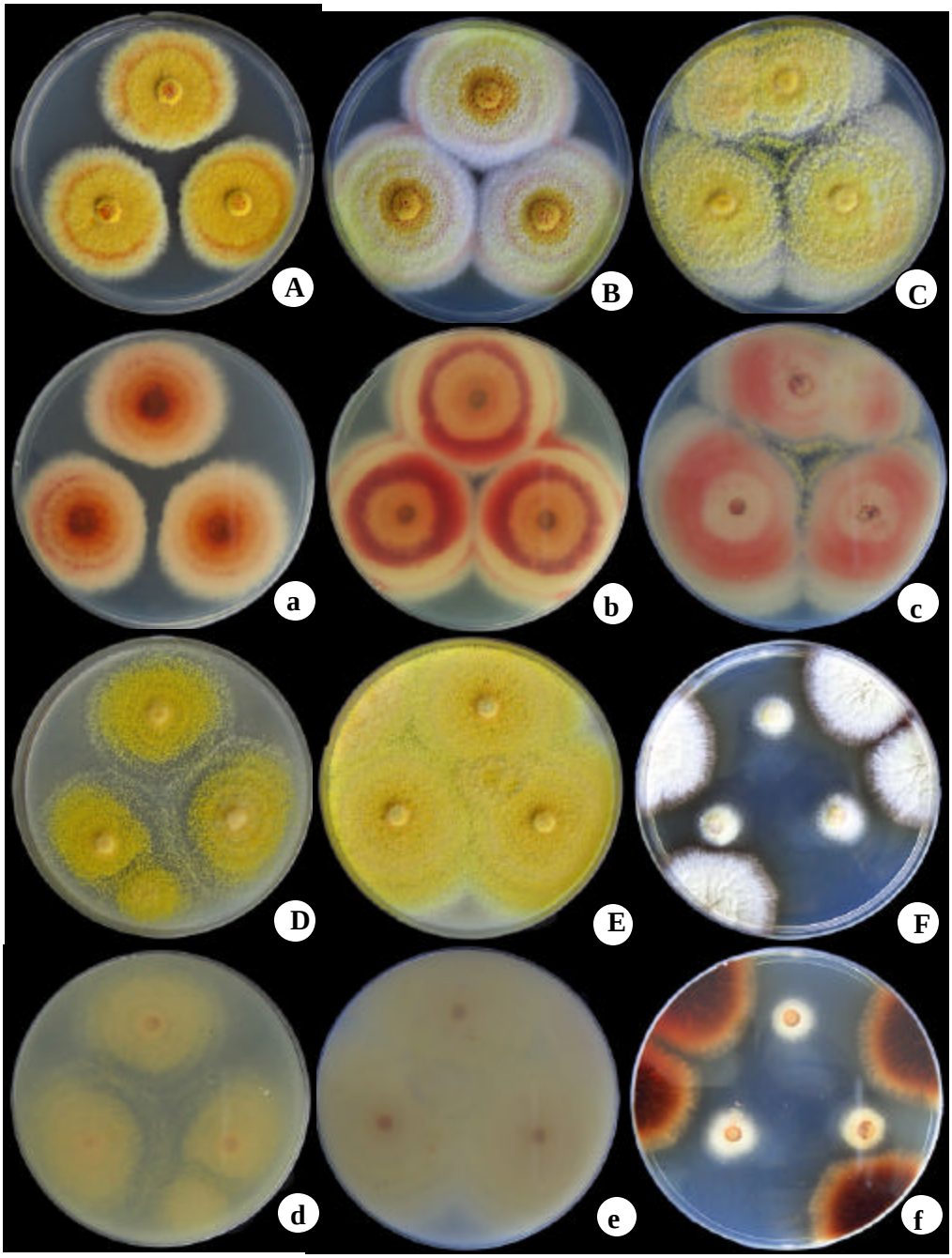


Figure 15 *Talaromyces flavus* KUFC 3530. Obverse and reverse views of colonies on different media, incubated for 7 days at 28°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

Conidiophores arising from the aerial mycelium, 20-70 x 2-2.5 µm, yellow-green, smooth-walled. Penicilli typically biverticillate, rarely monoverticillate and terverticillate (Figures 17 A-E, 18 A-B). Metulae in small verticils of 2 to 4, yellow-green, 10-12 x 2-2.5 µm. Phialides 2 to 6 in the verticil, 8-10 x 2-3 µm, typically lanceolate. Conidia pale greenish, subglobose to ellipsoidal 2.7-3.5 x 2-2.7 µm, smooth-walled (Figures 17 A-D, 18 A-B).

Two varieties of this fungus were recorded, in differs of colony appearance and size of ascospore including *T. flavus* var. *flavus* (ascospore size, 2.2-3.5 x 3-5 µm) and *T. flavus* var. *macrosporus* (ascospore size, 3.5-5.2 x 5-6.5 (-7) µm) (Stlok and Samson, 1972). On the basis of differences in secondary metabolites production. Frisvad *et al.*, (1990) raised the variety to species status. In this study, both of species were isolated from all soil samples and have been reported from many substrates in Thailand in previous report. In addition, *T. macrosporus* have been recorded as a cause of pasteurised orange juice and food (Pitt and Hocking, 1997).

Stolk and Samson (1972) stated that *Talaromyces 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 ascospores. We also found the variation among different *T. flavus* isolates (Figure 16). Differences in the shape, size and ornamentation of ascospores were also noted.

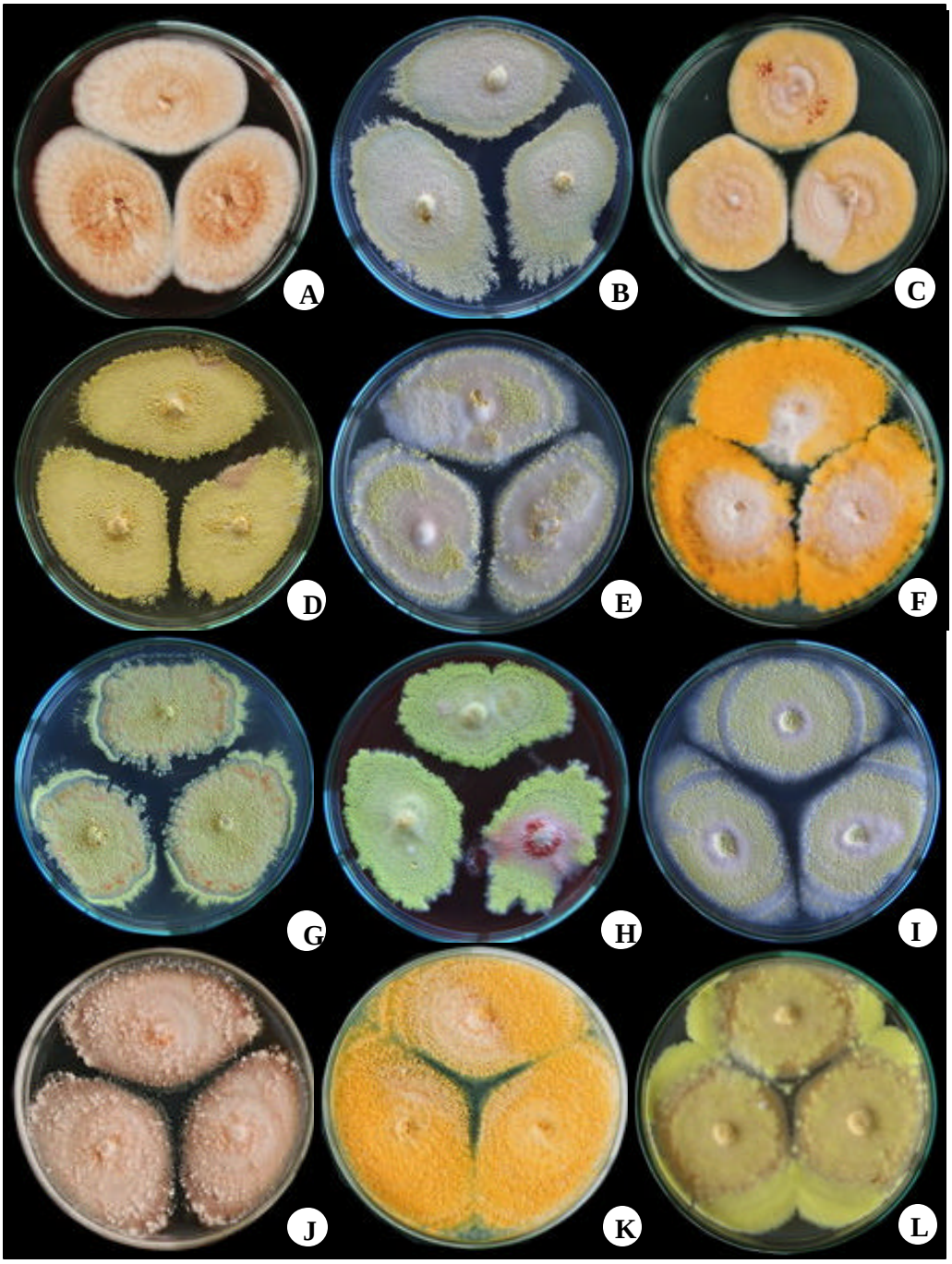


Figure 16 Variations of colony pattern of *T. flavus* on MEA at 28°C for 14 days

A. KUFC 3332, B. KUFC 3335, C. KUFC 3408, D. KUFC 3344
E. KUFC 3556, F. KUFC 3340, G. KUFC 3455, H. KUFC 3420
I. KUFC 3360, J. KUFC 3510, K. KUFC 3549, L. KUFC 3554

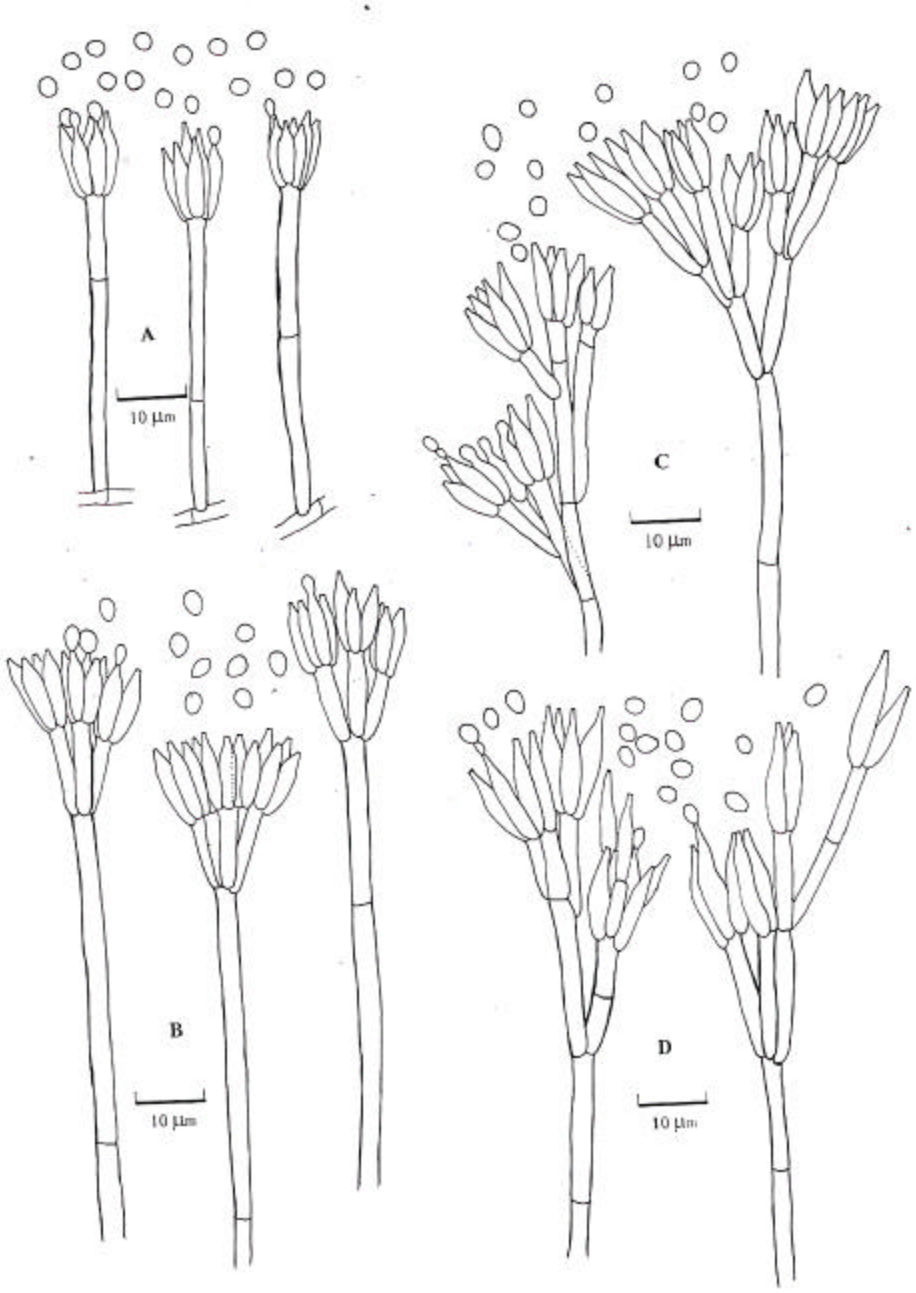


Figure 17 Variations of penicili of *Talaromyces flavus*

A. KUFC 3334 B. KUFC 3340

C. KUFC 3501 D. KUFC 3506

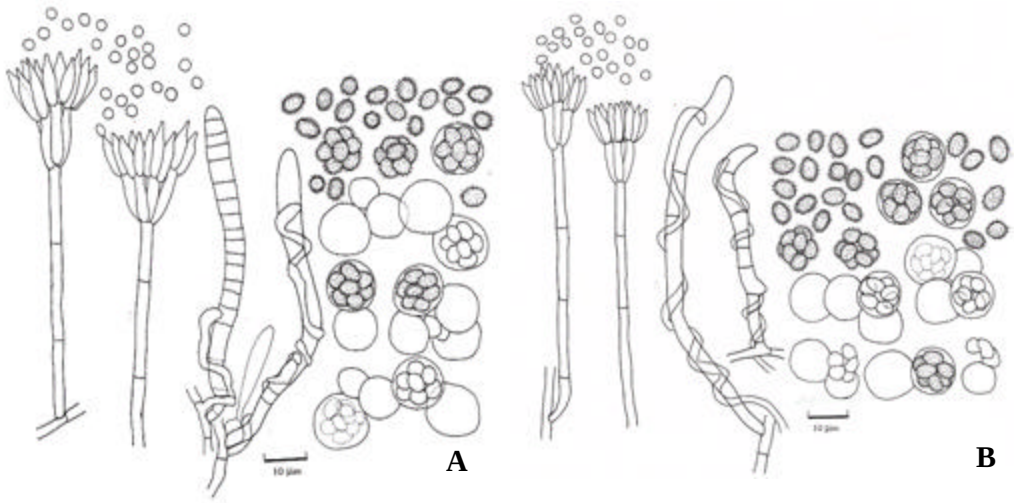


Figure 18 Camera lucida drawings of *Talaromyces flavus* showing penicilli, conidia, ascomatal initials, asci and ascospores
A. KUFC 3523 B. KUFC 3528

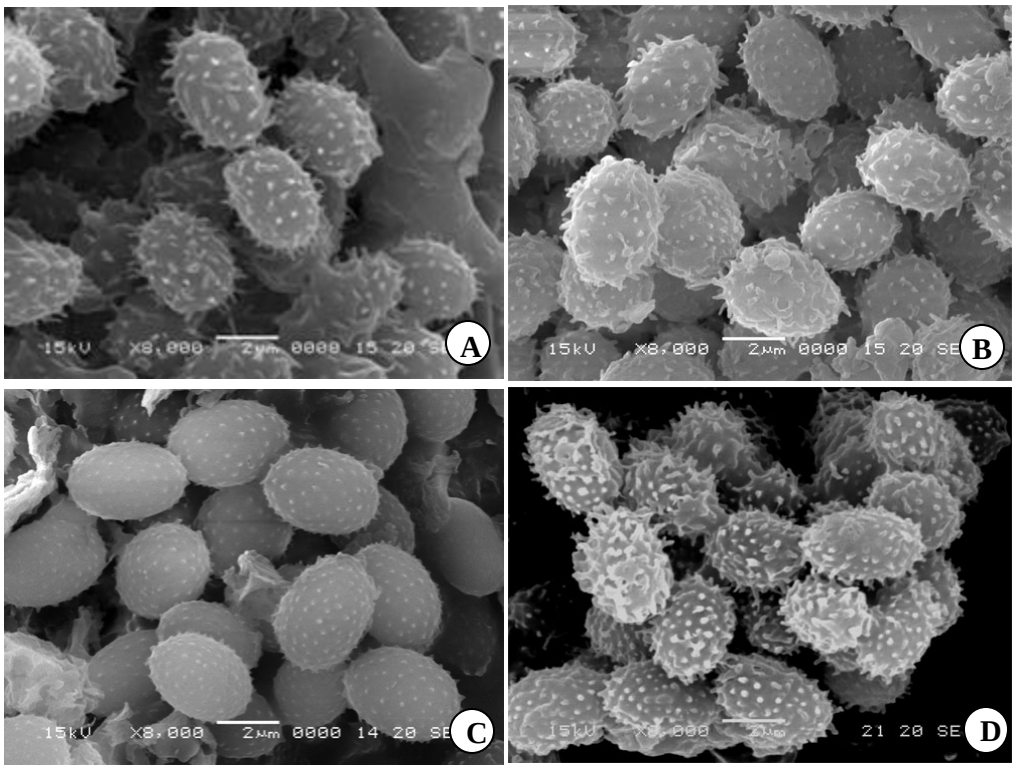


Figure 19 SEM photomicrographs showing variations in shape, size and ornamentation of ascospores among different isolates of *T. favus*
A. KUFC 3523; B. KUFC 3528; C. KUFC 3530; D. KUFC 3340

4. ***Talaromyces helicus*** C.R. Benjamin **var. *major*** Stolk & Samson (Figures 20-22)

Strains: KUFC 3593, KUFC 3598 agricultural soil, Chanthaburi

Reference: Stolk and Samson, 1972

Stat. Anam. *Penicillium helicum* Raper & Fennell

Colonies on CZA growing slowly, attaining a diameter of 15 mm within 7 days at 25°C, plane, consisting of a very thin basal mycelial felt, Straw (R 46); ascomata and conidiogenesis absent; exudates absent; reverse uncolored to Pale Luteous (R 11) (Figures 20 A, a).

Colonies on CYA growing rather rapidly, attaining a diameter of 22-24 mm within 7 days at 25°C, velvety, floccose at the central area, consisting of a compact basal felt, producing only white mycelium; ascomata and conidiogenesis lacking; exudates absent; reverse Straw (R 46) to Pale Luteous (R 11) (Figures 20 B, b).

Colonies on MEA spreding broadly, reaching a diameter of 65-70 mm within 7 days at 25°C, plane, more or less funiculose, consisting of a compact mycelial felt, producing abundant ascomata over the entire surface, Sulphur Yellow (R. 15) to Pure Yellow (R 14); conidiogenesis inconspicuous and sparse, margins entire; exudates abundant at central area, as pale yellow drops; reverse Luteous (R 11) (Figures 20 C, c).

Colonies on CMA growing rapidly, attaining a diameter of 50 mm within 7 days at 25°C, plane, thin, consisting of a spreading, submerged vegetative mycelium, with limited development of funiculose aerial hyphae, producing yellow ascomata in central area, Straw (R 46) or Pure Yellow (R 14); conidiogenesis inconspicuous and sparse; margins entire; exudates absent; reverse uncolored (Figures 20 D, d).

Colonies on OMA growing rapidly, attaining a diameter of 60-70 mm within 7 days at 25°C, plane, consisting of a thin mycelial felt in which abundant

developing ascomata, Sulphur Yellow (R 15); conidiogenesis inconspicuous and sparse; exudates absent; reverse uncolored (Figures 20 E, e).

Colonies on G25N agar growing extremely slowly; attaining a diameter of 5-6 mm within 7 days at 25°C, producing only sparse white mycelium (Figures 20 F, f).

Ascomata discrete or often confluent, ripening within 10 to 14 days, globose to subglobose, (150-) 170-210 µm in diameter (Figure 21A), soft, surrounded by loose wefts of yellow hyphae; ascomatal wall soft, consisting of thin, branched, interwoven hyphae. Ascomatal initials consisting of club-shaped ascogonia around which thin antheridia coil tightly several times in the basal pattern, growing out to coil terminally in a conspicuous helical pattern (Figures 21 D, 22 C). Asci in chains, 8-spored, broadly subglobose to globose, 6.5-10 x 6-8 µm (Figures 21 E, 22 D). Ascospores hyaline, broadly ellipsoidal, smooth-walled, 3.3-4 x 2.3-2.6 µm (KUFC 3623), whereas ascospores size of KUFC 3628 rather bigger, 3.6-4.7 x 2.7-3 (Figures 21 F-I, 22 E-F).

Conidiophores arising from basal mycelium or as short branches from aerial hyphae, hyaline, 30-50 x 2-2.5 µm, smooth-walled. Penicilli typically biverticillate or monoverticillate. Metulae in small verticils of 2 to 4, hyaline, 10-12 x 2.5-2.7 µm. Phialides 2 to 6 in the verticil, hyaline, 10-12 (-15) x 2-3 µm (Figures 21 B-C, 22 A-B). Conidia globose to subglobose, 3-4 µm or 3.5-4.5 µm in diameter, smooth-walled (Figures 21 B-C, 22 A-B).

The main characteristic of *Talaromyces helicus* var. *major* is the production of poorly developed penicilli and smooth-walled ascospores. This fungus was reported from soil in Sweden, Switzerland, Argentina and Australia (Domsch *et al.*, 1993a, b). *Talaromyces helicus* isolated from co-contaminated sludge of the east channel, Argentina could detoxificately copper and biphenyl in environmental (Ormero *et al.*, 2006).

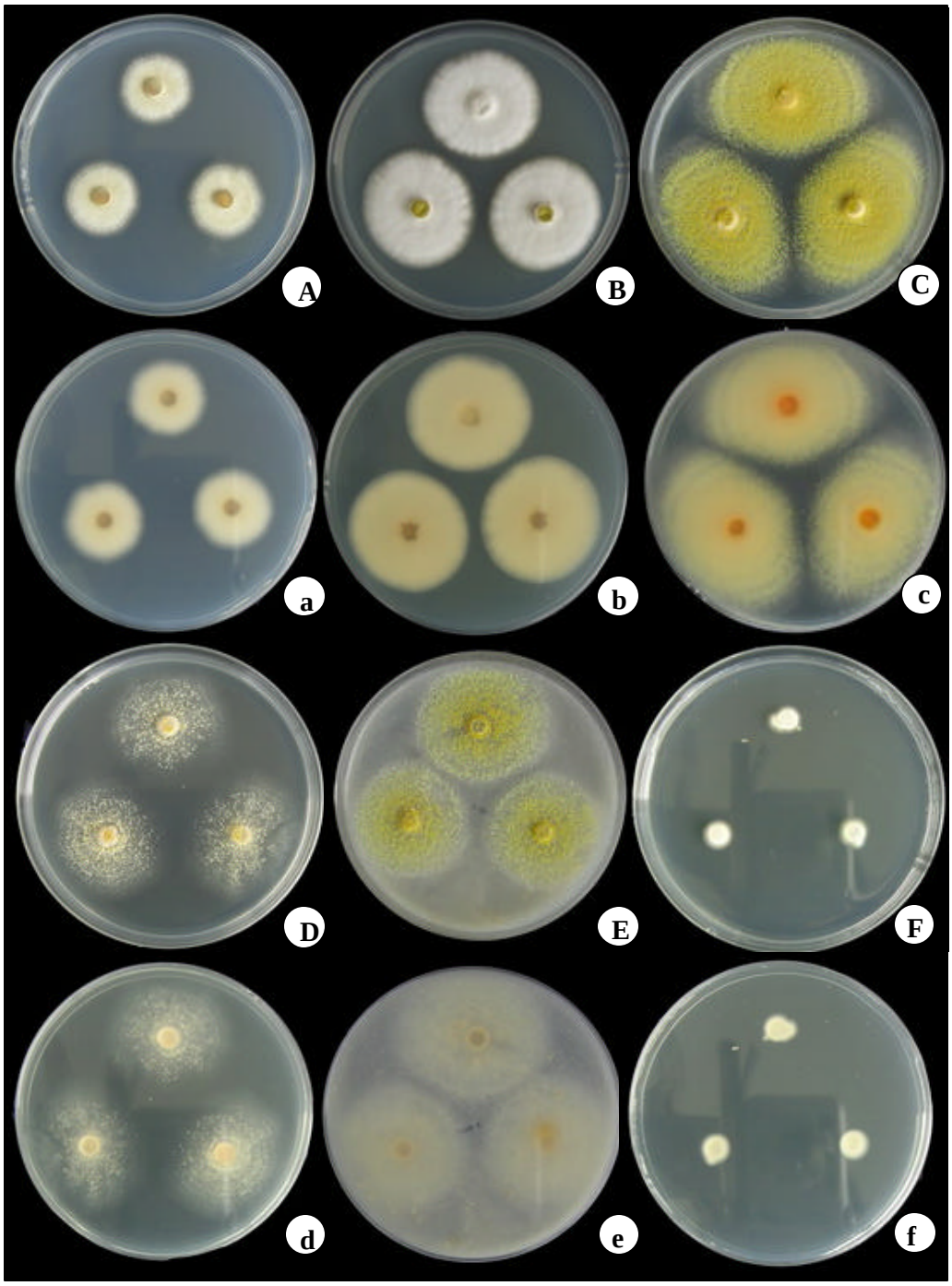


Figure 20 *Talaromyces helicus* var. *major* KUFC 3598. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

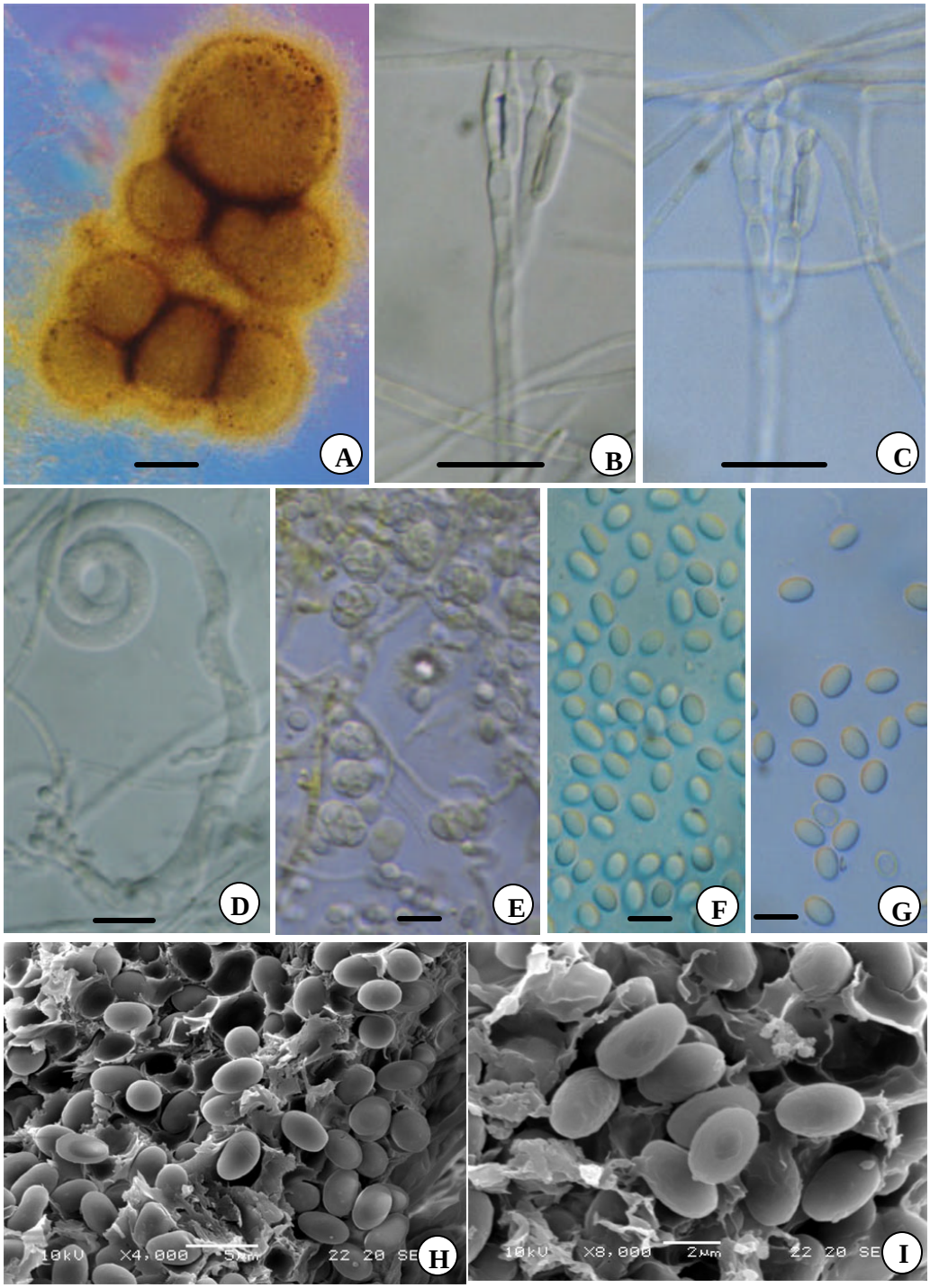


Figure 21 *Talaromyces helicus* var. *major* KUFC 3598
A. ascomata; B-C. penicilli; D. ascomatal initial; E. asci and ascospores; F. ascospores KUFC 3593; G. KUFC 3598; H-I. ascospores (SEM) (Bars: A = 100 μm; B-D = 10 μm; E-H = 5 μm; I = 2 μm)

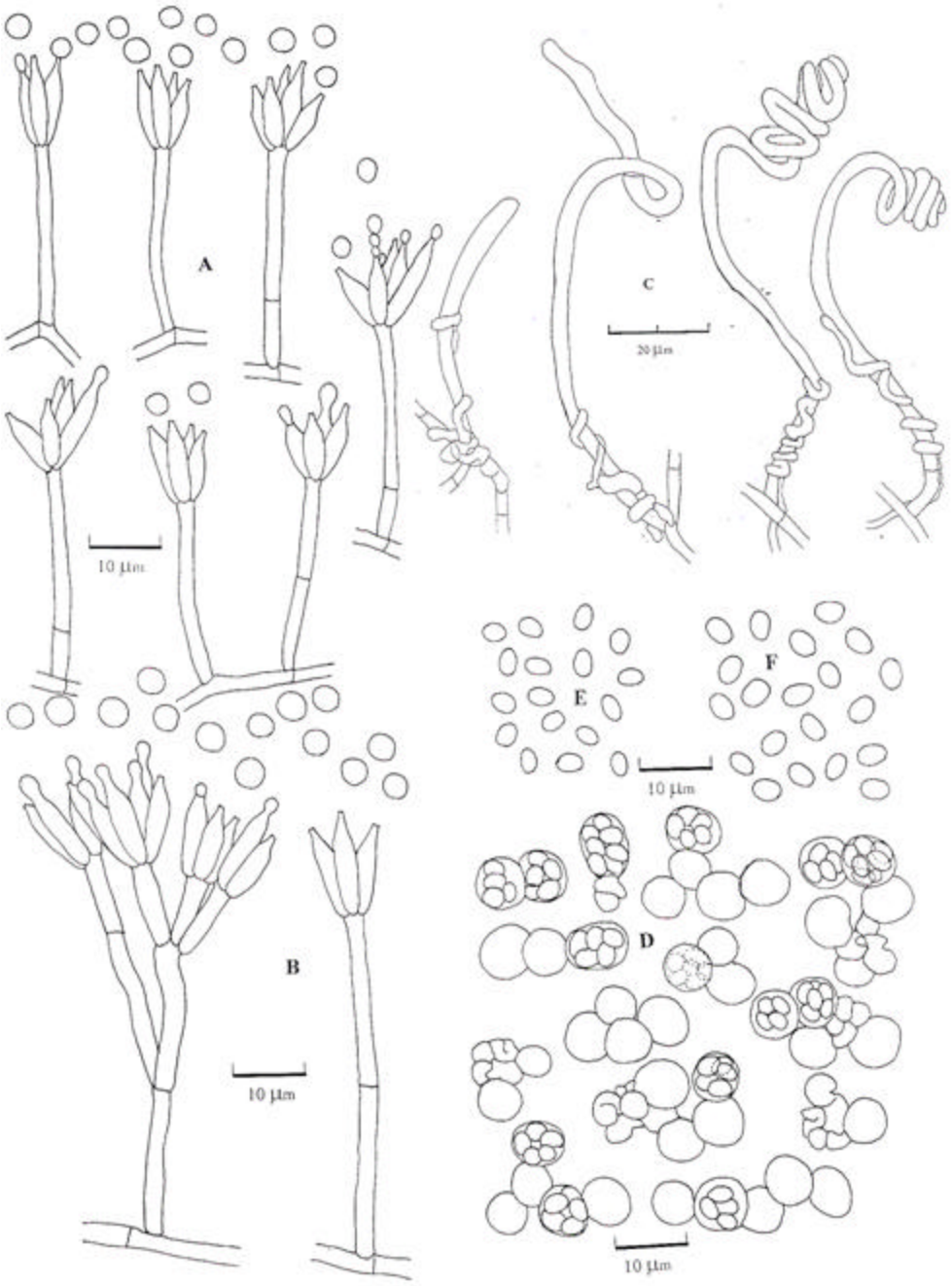


Figure 22 *Talaromyces helicus* var. *major*

Camera lucida drawings of A. penicilli KUFC 3593; B. KUFC 3598 C. ascomatal initials; D. ascus and ascospores; E. ascospores KUFC 3593, F. KUFC 3598

5. ***Talaromyces luteus*** (Sacc.) Stolk & Samson (Figures 23-25)

Strains examined: KUFC 3331, KUFC 3364 soil at hot spring, Mae Hong Son

References: Stolk and Samson, 1972; Pitt, 1979a

Stat. Anam. ***Penicillium udagawae*** Zukal

Colonies on CZA growing moderately, attaining a diameter of 20 mm within 7 days at 25°C, plane, velvety to funiculose, sulcate, consisting of a compact basal felt, producing only Pure Yellow (R 14) mycelium on the felt; conidiogenesis sparse, inconspicuous; exudates absent; reverse Pale Luteous (R 11). Colonies on CZA at 28°C, reaching a diameter of 25 mm and 40 mm within 7 and 14 days respectively, plane, velvety to fasciculate, sulcate, consisting of a compact basal felt, producing abundant young ascomata over the entire surface, Pure Yellow (R 14); conidiogenesis sparse, inconspicuous; exudates absent; reverse Straw (R 46) (Figures 23 A, a).

Colonies on CYA growing moderately, attaining a diameter of 20-22 mm within 7 days at 25°C, floccose to funiculose, slightly sulcate, commonly umbonate at the central area, consisting of a compact basal felt, producing developing ascomata in a layer on the felt, Pure Yellow (R 14); conidiogenesis sparse and inconspicuous; exudates absent; reverse Cinnamon (R 62). Colonies on CYA at 28°C, reaching a diameter of 25 mm and 45 within 7 and 14 days respectively, funiculose, slightly zonate, consisting of a compact basal felt, producing young ascomata in the central area, Pure Yellow (R 14) to Orange (R 7); conidiogenesis sparse and inconspicuous; exudates absent; reverse Pale Luteous (R 11) to Orange (R 7) (Figures 23 B, b).

Colonies on MEA growing moderately, attaining a diameter of 20-22 mm within 7 days at 25°C, plane, velvety, more or less funiculose, producing abundant ascomata over the entire surface; conidiogenesis limited, Pure Yellow (R 14); margins broad, entire; exudates scattered, as small clear drops; reverse Straw (R 46). Colonies on MEA at 28°C, reaching a diameter of 25 mm and 40 within 7 and 14 days respectively, plane, consisting of a thick interwoven basal felt, in which numerous

ascomata are embedded near the agar surface, Luteous (R12) to Orange (R 7) in the central area, surrounded by a thin, submerged margin; conidiogenesis sparse and inconspicuous; exudates clear to orange drops; reverse Pale Luteous (R 11) (Figures 23 C, c).

Colonies on CMA growing slowly, attaining a diameter of 17-18 mm within 7 days at 25°C, plane, consisting of a thin mycelial felt, plane, producing moderately ascomata on the agar surface, Pure Yellow (R 14); conidiogenesis limited; exudates absent; reverse uncolored. Colonies on CMA at 28°C, reaching a diameter of 20 mm and 35 mm within 7 and 14 days respectively, colony as mentioned above, but ascomata are more abundantly overgrown by aerial mycelium, Pure Yellow (R 14); conidiogenesis absent; exudates absent; reverse uncolored.

Colonies on OMA growing slowly, attaining a diameter of 15-18 mm within 7 days at 25°C, plane, consisting of a thin mycelial felt in which abundant yellow ascomata soon develop intermix with conidiogenesis, showing an increased yellowish coloration, Pure Yellow (R 14); conidiogenesis limited; exudates absent; reverse uncolored. Colonies on OMA at 28°C, reaching a diameter of 20 mm and 35 mm within 7 and 14 days respectively, vegetative mycelium submerged or forming a sparse growth of aerial hyphae, producing abundant ascomata near the agar surface, Pure Yellow (R 14); conidiogenesis limited; exudates absent; reverse uncolored.

Colonies on G25N agar growing extremely slowly; attaining a diameter of 10 mm within 14 days at 28°C, producing only sparse aerial growth.

At 37°C, growth is extremely restricted.

Ascomata yellow to orange, subglobose or globose, about 200-300 µm in diameter, usually confluent, occasionally discrete, ripening within 2 weeks. Covering consisting of thin networks of loosely interwoven hyphae (Figure 24 A). Ascomatal initials start with the terminal or intercalary swelling and branching of hyphae, occasionally forming a small plexus of swollen cells, from which long, single, coiling

aseptate hyphae develop, producing several loose coils in a helical pattern (Figures 24 E, 25 C). Asci 8-spored, broadly ellipsoidal to subglobose, 7.3-11 x 6-9 µm (Figures 24 F, 25 D). Ascospores ellipsoidal, 4-4.7 x 2.7-3.3 µm, ornamented with 3 to 5 regularly transverse, nearly parallel ridges, 1-1.5 µm wide (Figures 24 G-I, 25 D).

Conidiophores arising both from submerged and aerial hyphae, short, 30-50 x 3-3.5 µm. Penicilli typically irregularly arranged, sometimes terverticillate or monoverticillate. Metulae in small verticils of 2 to 3, 7-10 x 2.7-3 µm. Phialides ampulliform to acerose, about 2 to 4 in the verticil (Figures 24 B-D, 25 A). Conidia hyaline, subglobose to globose, 3-5 x 1.5-2 µm, smooth-walled, borne in tangled, disordered chains (Figures 24 B-D, 25 B).

Yoshida *et al.*, (1996) isolated three new azaphilones, luteusins A, B, C, D and E form this fungus.

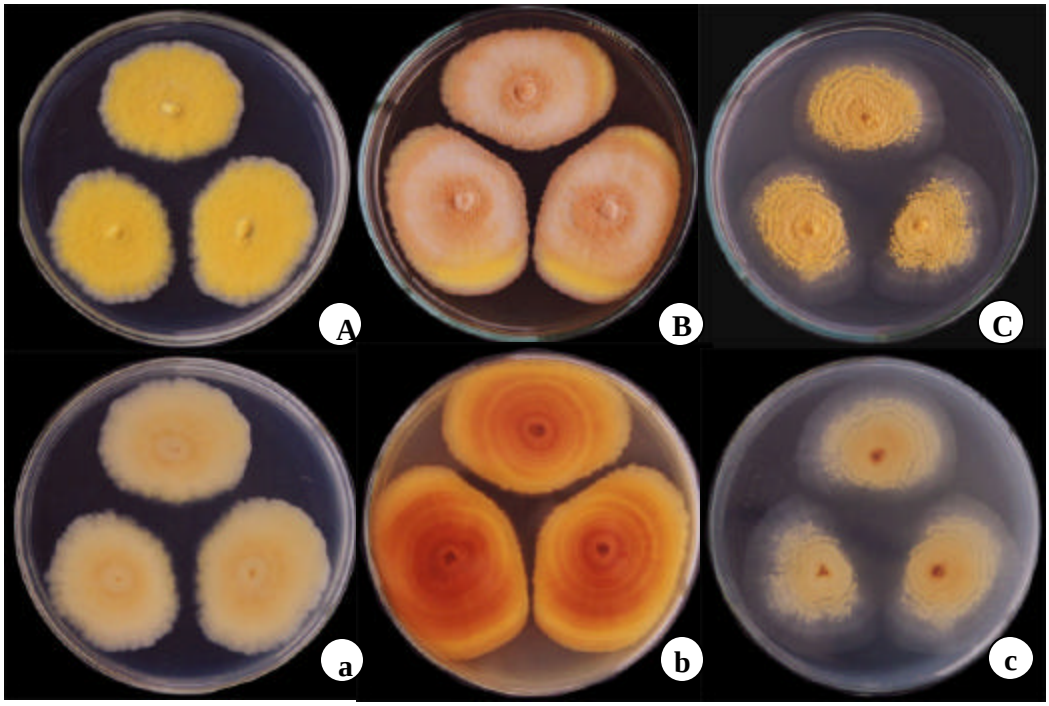


Figure 23 *Talaromyces luteus* KUFC 3364. Obverse and reverse views of colonies on different media, incubated for 7 days at 28°C; CZA (A, a), CYA (B, b) , MEA (C, c)

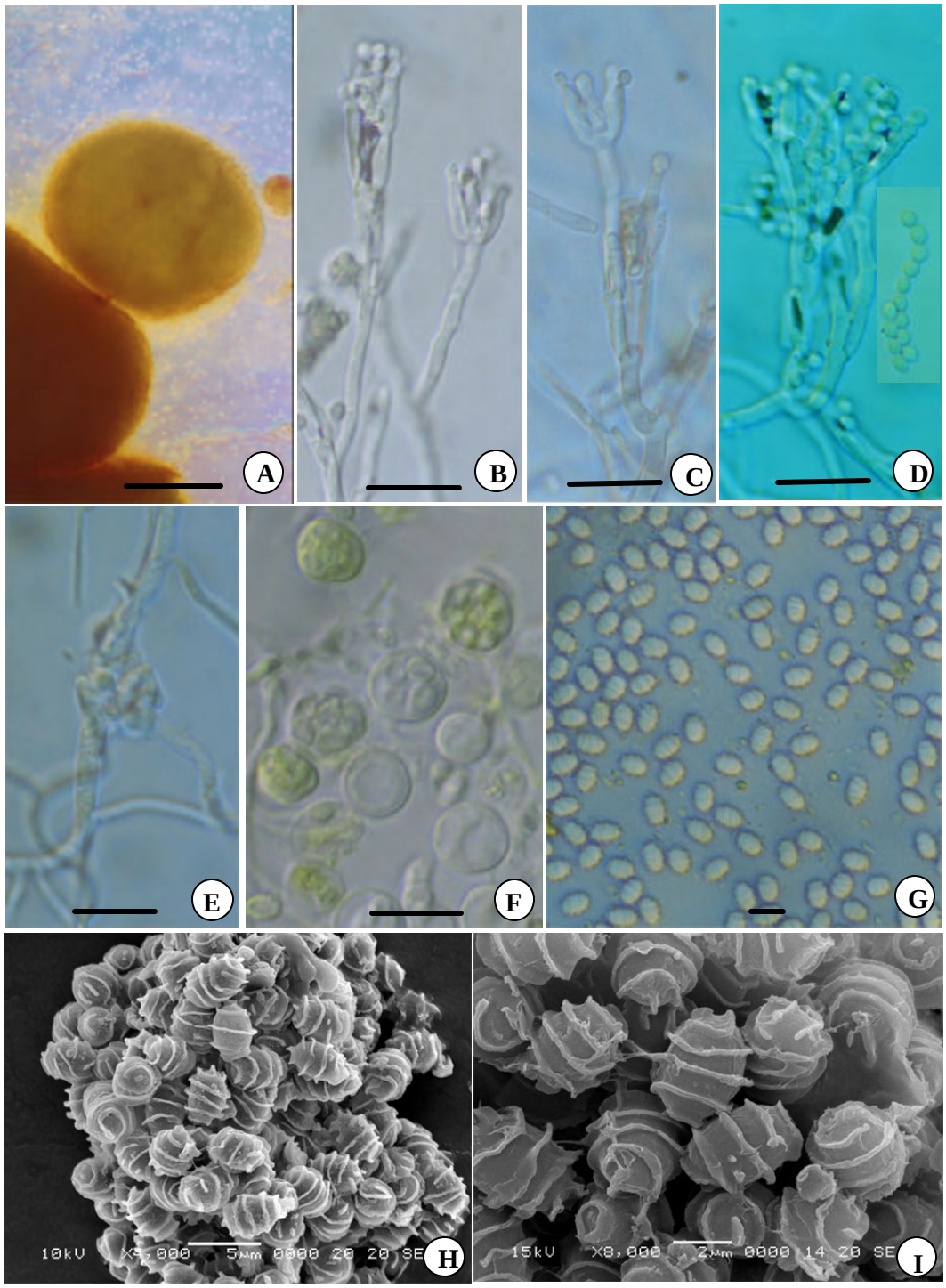


Figure 24 *Talaromyces luteus* KUFC 3364

A. ascomata; B-D. penicilli; E. ascomatal initial; F-G. asci and ascospores; H-I. ascospores (SEM)

(Bars: A = 100 μm; B-F = 10 μm; G-H = 5 μm; I = 2 μm)

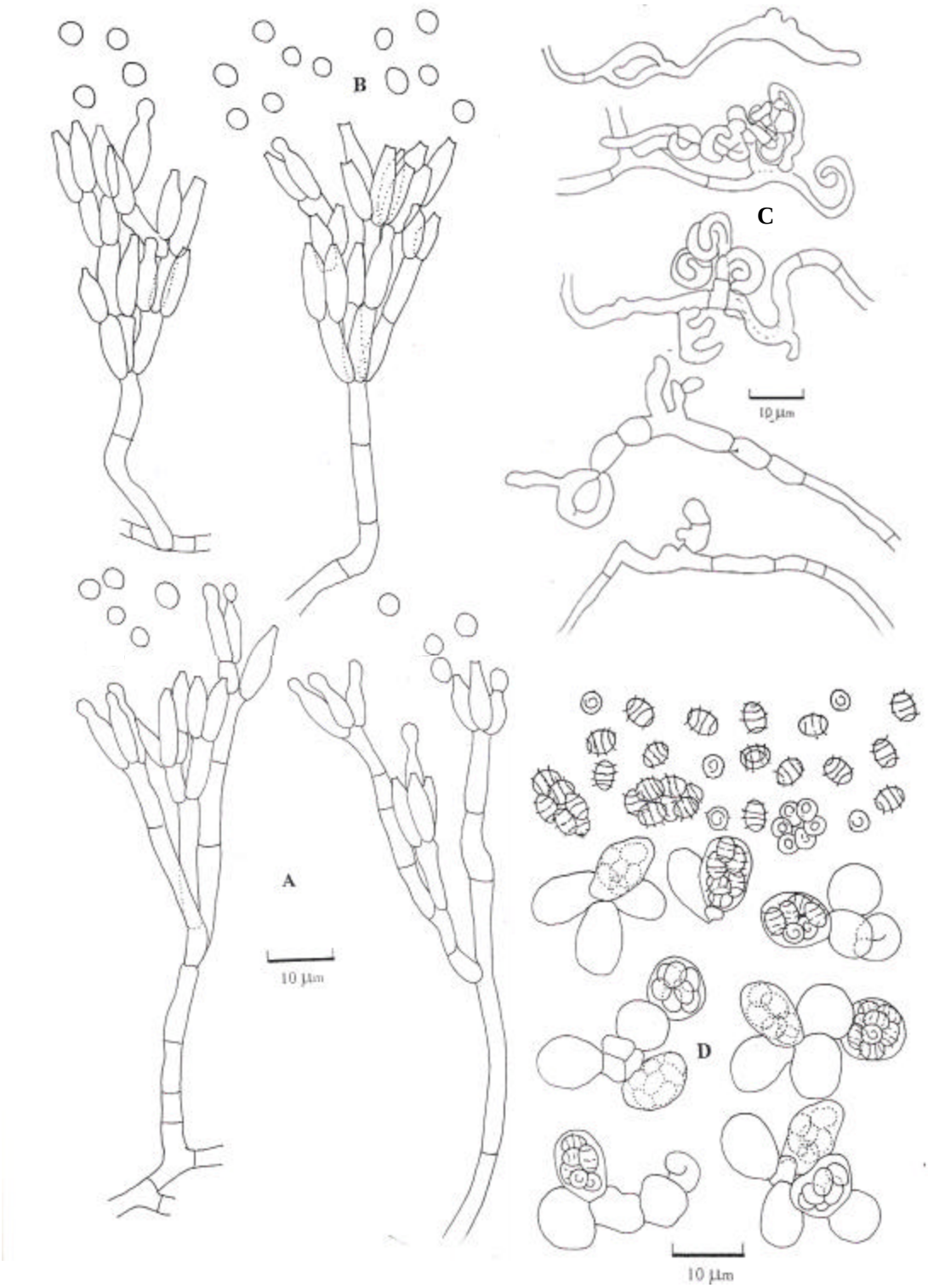


Figure 25 *Talaromyces luteus* KUFC 3364

Camera lucida drawings of A. penicilli; B. conidia;
C. ascomatal initials; D. ascus and ascospores

6. ***Talaromyces macrosporus*** (Stolk & Samson) Frisvad (Figures 26-30)

Strains examined: KUFC 3339 agricultural soil, Chiang Rai; KUFC 3363 forest soil, Chiang Mai; KUFC 3367 agricultural soil, Chon Buri , KUFC 3450 agricultural soil, Suphan Buri; KUFC 3506 agricultural soil, Nakhon Ratchasima; KUFC 3568 forest soil, Mae Hong Son; KUFC 3623 agricultural soil, Chiang Mai; KUFC 3638 forest soil, Trat; KUFC 3589 nonagricultural soil, Trang

References: Stolk and Samson, 1972; Pitt, 1979a; Frisvad *et al.*, 1990

Stat. Anam. ***Penicillium macrosporum*** Stolk and Sanson

Colonies on CZA growing moderately, attaining a diameter of 20-22 mm within 7 days at 25°C, plane, consisting of a compact basal felt, producing abundant young ascomata in a layer on the felt, Scarlet (R 5) at the central area, but elsewhere usually Pure Yellow (R 14); conidiogenesis moderately; exudates clear to reddish; soluble pigment Scarlet (R 4) to Rust (R 39); reverse Scarlet (R 4) to Blood (R 3) (Figures 26 A, a). Colonies on CZA at 28°C, reaching a diameter of 20-22 mm within 7 days, plane, fasciculate, consisting of a compact basal felt, producing numerous developing ascomata on the entire surface, Sulphur Yellow (R 15); conidiogenesis limited; exudates absent; soluble pigment Apricot (R 42); reverse Amber (R 9) (Figures 27 A, a).

Colonies on CYA growing rather rapidly, attaining a diameter of 25-28 mm within 7 days at 25°C, fasciculate, plane, showing the same cultural characteristics as on CZA, Scarlet (R 5) at the central area, Pure Yellow (R 14) at the margin; soluble pigment Scarlet (R 4) to Rust (R 39); reverse Pale Bay (R 3) (Figures 26 B, b). Colonies on CYA at 28°C, reaching a diameter of 40 mm within 7 days, plane, velvety, lightly radically sulcate, consisting of a compact basal felt, producing abundant developing ascomata over the entire surface, Sulphur Yellow (R 15); conidiogenesis scattered; exudates absent; soluble pigment Apricot (R 42); reverse Scarlet (R 4) to Rust (R 39) (Figures 27 B, b).

Colonies on MEA growing moderately, attaining a diameter of 22-25 mm within 7 days at 25°C, velvety, floccose to funiculose, commonly umbonate at the

central area, consisting of a compact basal felt, producing abundant young ascomata in a layer on the felt, intermixed with yellow aerial hyphae, Sulphur Yellow (R 15) to Pure Yellow (R 14); conidiogenesis sparse and inconspicuous; exudates abundant, Scarlet (R 5) to Rust (R 39); reverse Scarlet (R 4) to Blood Colour (R 3) (Figures 26 C, c). Colonies on MEA at 28°C, attaining a diameter of 35 mm within 7 days, plane, velvety, consisting of a compact basal felt, developing abundant ascomata which form a continuous layer, Sulphur Yellow (R 15); conidiogenesis limited; exudates absent; soluble pigment Apricot (R 42); reverse Scarlet (R 4) to Coral (R 38) (Figures 27C, c).

Colonies on CMA growing rapidly, attaining a diameter of 40-42 mm within 7 days at 25°C, plane, consisting of a very thin mycelial felt, vegetative mycelium submerged or forming a sparse growth of aerial hyphae, plane, producing moderately ascomata on the agar surface, Pure Yellow (R 14); conidiogenesis profuse; exudates scattered, as small clear drops; reverse uncolored (Figures 26 D, d). Colonies on CMA at 28°C, reaching a diameter of 45 mm within 7 days, plane, thin, with vegetative mycelium submerged, producing abundant ascomata in a thin layer, Pure Yellow (R 14); conidiogenesis sparse; exudates scattered; reverse uncolored (Figures 27 D, d).

Colonies on OMA growing rapidly, attaining a diameter of 40-42 mm within 7 days at 25°C, plane, consisting of thin mycelial felt in which abundant yellow ascomata soon develop intermix with conidiogenesis, showing an increased yellowish coloration, Pure Yellow (R 14); conidiogenesis inconspicuous and sparse; exudates absent; reverse uncolored (Figures 26 E, e). Colonies on OMA at 28°C, attaining a diameter of 45 mm within 7 days, plane, thin, with vegetative mycelium submerged, granular in appearance due to the numerous production of ascomata in a layer, Pure Yellow (R 14); conidiogenesis sparse; exudates scattered; reverse uncolored (Figures 27 D, d).

Colonies on G25N agar growing slowly, attaining a diameter of 15-20 mm and 22 mm within 7 days at 25°C and 28°C respectively, funiculose, with Pure Yellow (R 14) vegetative mycelium; ascomata limited; conidiogenesis moderately showing Pale Greenish Grey (R 123) color; reverse Umber (R 9) (Figures 26-27 F, f).

Ascomata yellow, subglobose to globose, 300-550 µm in diameter, confluent, occasionally discrete, ripening within 2 to 3 weeks. Covering consisting of well-developed networks of interwoven hyphae surrounded by weft of short, twisted, branched hyphae (Figure 29 A). Ascomatal initials conspicuous, consisting of large, cylindrical cells (ascogonia) encircled by fine hyphae (antheridia) (Figure 30 C). Asci evanescent, borne in chains, globose to ellipsoidal, 11.5-13 x 9-11.5 µm (Figures 29 D, 30 D). Ascospores yellow, occasionally reddish, ellipsoidal to broadly ellipsoid, 5.5 x 7.5 µm, thick-walled, conspicuous spinose (Figures 29 B-G, 30 D).

Conidiophores borne from aerial hyphae and ropes of hyphae, smooth, sometimes encrusted, 40-70 x 2-2.5 µm. Penicilli typically biverticillate, rarely monoverticillate and terverticillate. Metulae in small verticils of 2 to 4, 10-12 x 2-2.5 µm. Phialides 2 to 6 in the verticil, 8-10 x 2-3 µm, typically lanceolate (Figure 30 A). Conidia pale greenish, subglobose to ellipsoidal, 2.7-3.5 x 2–2.7 µm, smooth-walled (Figure 30 B).

This fungus was earlier classification as *T. flavus* var. *macrosporus*, but later described as a separate species (Frisvad *et al.*, 1990). It is an extremely variable species same as *T. flavus*. Different strains may show vary in colour and texture of colonies, in the amount of red pigment produced, in the number of penicilli and ascomata, and in size and color of ascospores (Figures 28, 29 B-G).

Talaromyces macrosporus is heat resistance fungus and used as a model system to study heat resistance and heat activation of ascospores (Van Der Spuy *et al.*, 1975; Beuchat 1986, 1988a, b; Dijksterhuis *et al.*, 2002). In addition, it was reported cause spoilage outbreaks in food and drink products after pasteurization treatments (Pitt and Hocking, 1997; Samson *et al.*, 2002). Many study have been performed heat isolation of this fungus, heat shock is necessary to germinate the ascospores (Conner and Beuchat 1987a, b; Beuchat, 1988a; King and Whitehand, 1990; Dijksterhuis and Teunissen, 2004).

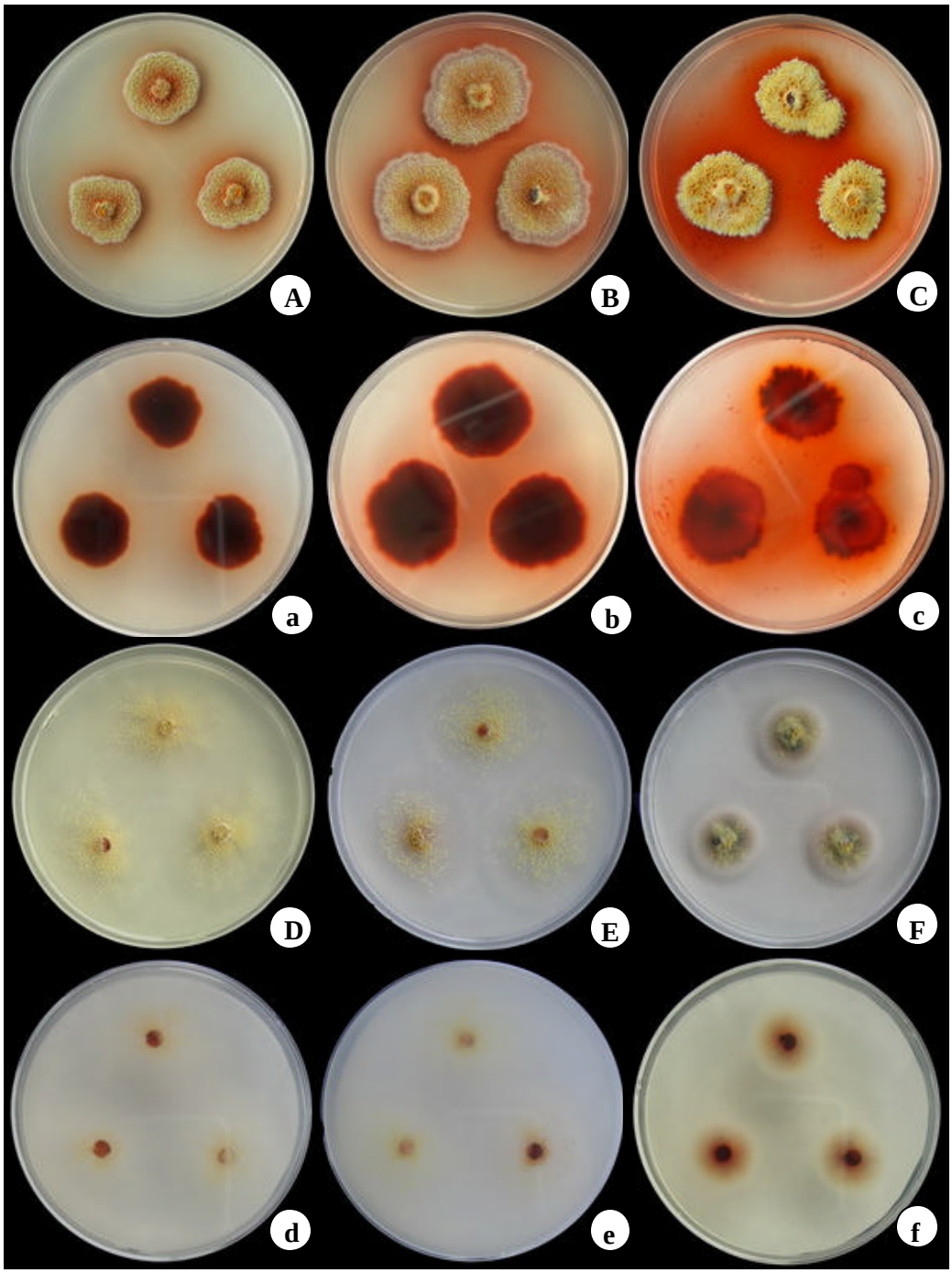


Figure 26 *Talaromyces macrosporus* KUFC 3381. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

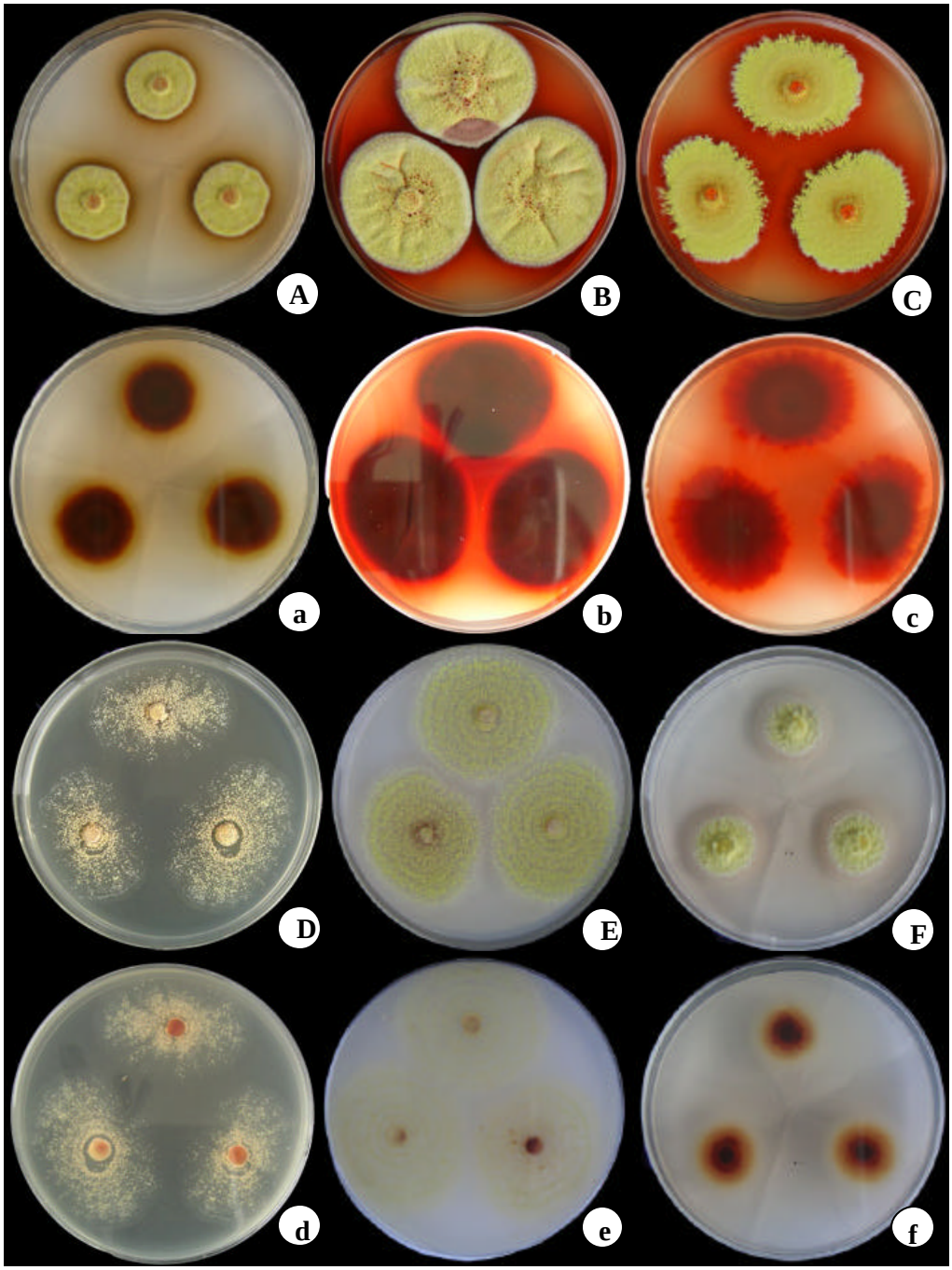


Figure 27 *Talaromyces macrosporus* KUFC 3363. Obverse and reverse views of colonies on different media, incubated for 7 days at 28°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

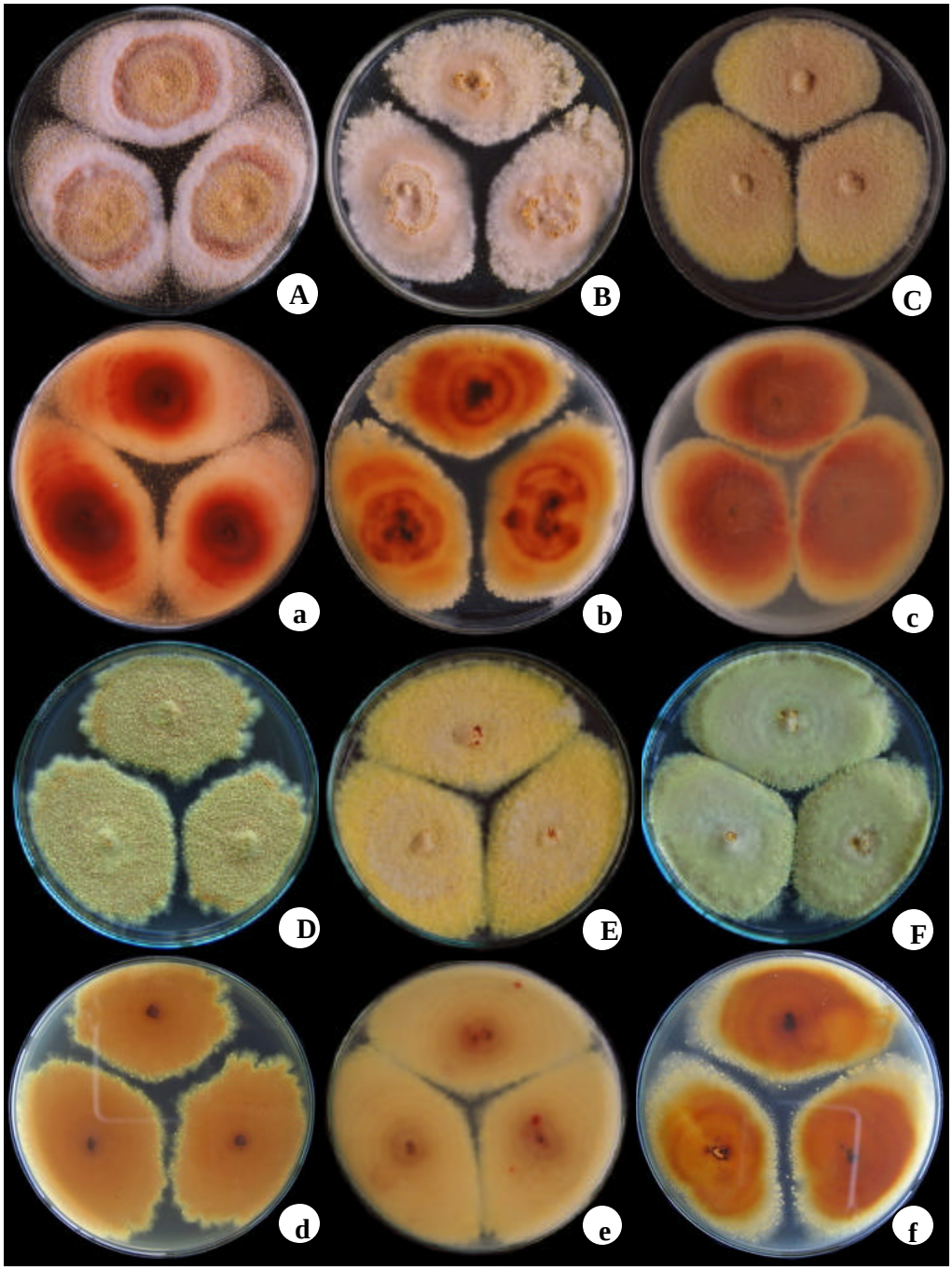


Figure 28 Variations of colony pattern showing obverse and reverse views of various isolates of *T. macosporus* on MEA at 28°C, 14 days

KUFC 3367 (A, a), KUFC 3450 (B, b), KUFC 3506 (C, c)
KUFC 3568 (D, d), KUFC 3623 (E, e), KUFC 3638 (F, f)

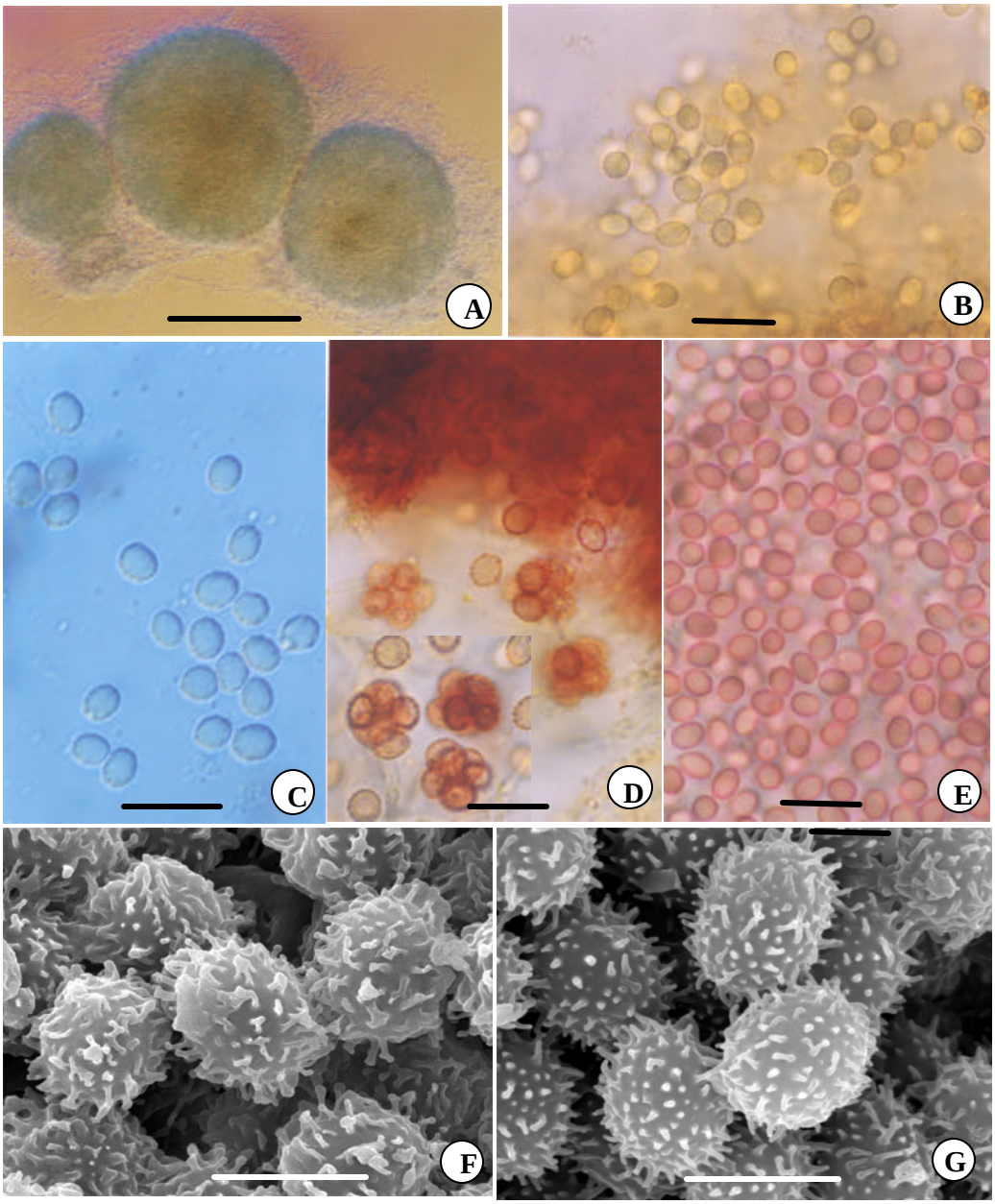


Figure 29 *Talaromyces macrosporus* KUFC 3363

A. ascomata; B-E. asci and ascospores; F-G. ascospores (SEM)

(Bars: A = 100 µm; B-E = 10 µm; F-G = 5 µm)

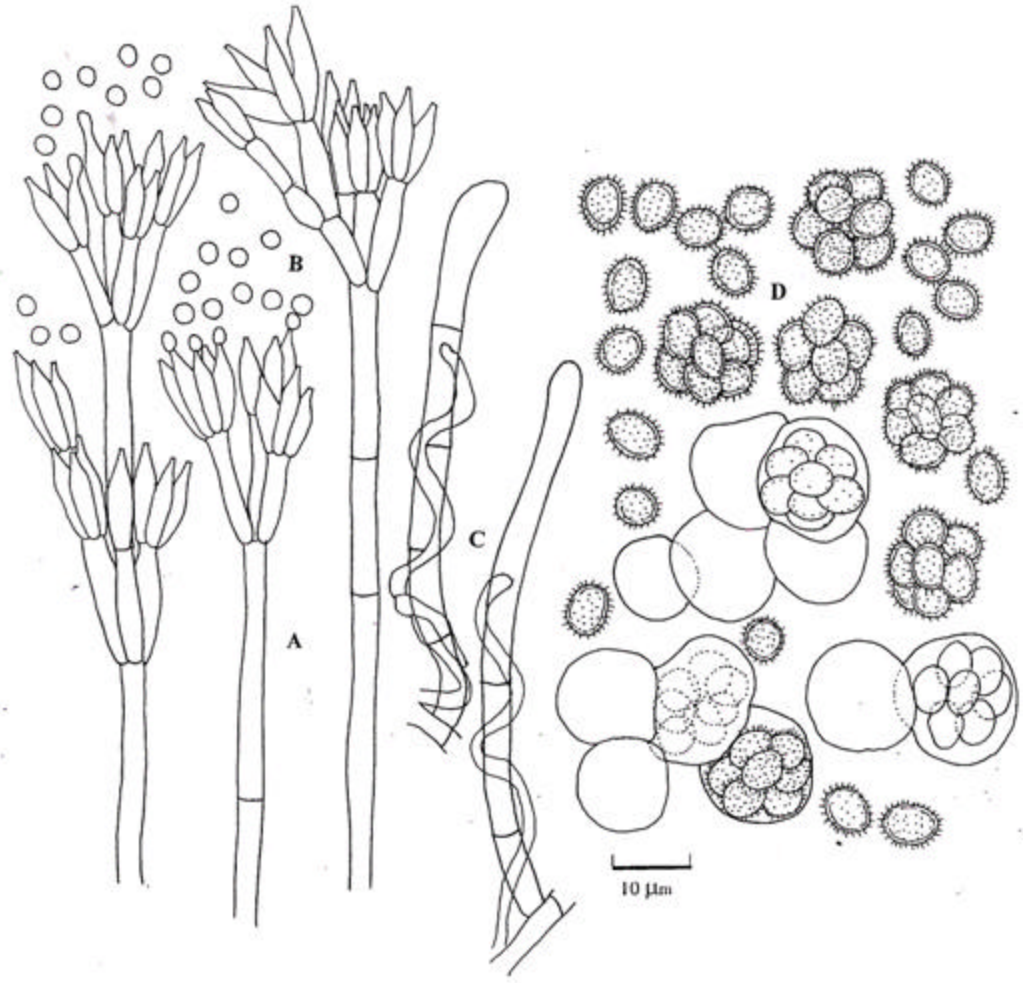


Figure 30 *Talaromyces macrosporus* KUFC 3363
Camera lucida drawings of A. penicilli; B. conidia
C. ascomatal initials; D. asci and ascospores

7. ***Talaromyces indigoticus*** Takada et Udagawa (Figures 31-35)

Strain examined: KUFC 3366 forest soil, Sakon Nakhon

Reference: Takada and Udagawa, 1993

Stat. Anam. ***Penicillium indigoticum*** Takada et Udagawa

Colonies on CZA growing moderately, attaining a diameter of 25-26 mm within 7 days at 25°C, floccose, consisting of a thin basal felt with White aerial hyphae; ascomata and conidiogenesis absent; exudates absent; reverse Scarlet (R 5) to Rust (R 39) at the central area, Straw (R 46) at the margin (Figures 31 A, a). Colonies on CZA at 28°C, reaching a diameter of 35-37 mm within 7 days, floccose, producing only white to pinkish shade mycelium; ascomata and conidiogenesis absent; reverse Peach (R 4) to Scarlet (R 5) at the centre, Straw (R 46) at the margin (Figures 32 A, a).

Colonies on CYA growing rather rapidly, attaining a diameter of 35-37 mm within 7 days at 25°C, plane, floccose, consisting of a thin basal felt with white mycelial hyphae; ascomata and conidiogenesis absent, white; exudates clear, small; odor musty; reverse Peach (R 4) to Scarlet (R 5) (Figures 31 B, b). Colonies on CYA at 28°C, reaching a diameter of 55 mm within 7 days, floccose, slightly zonate at the central area, producing white mycelium but central area showing Smoke Grey (R 105) color where young ascomata develop; conidiogenesis absent; reverse Peach (R 4) to Scarlet (R 5) (Figures 32 B, b).

Colonies on MEA growing moderately, attaining a diameter of 25-27 mm within 7 days at 25°C, floccose, plane, very thin, producing limit developing ascomata in the central area, white; conidiogenesis limited; reverse Pale Luteous (R 11) (Figures 31 C, c). Colonies on MEA at 28°C, reaching a diameter of 35 mm within 7 days, plane, consisting of a thin basal mycelial felt, white; raised to umbonate at the margin; ascomata and conidiogenesis absent; reverse Straw (R 46) (Figures 32 C, c).

Colonies on OMA growing rapidly, attaining a diameter of 35-36 mm within 7 days at 25°C, plane, thin, consisting of a spreading of submerged vegetative

mycelium, producing yellow ascomata in limited numbers in central area; exudates absent; odor musty; reverse uncolored (Figures 31 D, d). Colonies on OMA at 28°C spreading broadly, reaching a diameter of 60-70 mm within 7 days, consisting of a thin basal felt, with vegetative mycelium submerged, producing limit ascomata in a thin layer, showing Sulphur Yellow (R 15) shade; exudates moderate, small; odor musty; reverse uncolored (Figures 32 D, d).

Colonies on G25N agar growing extremely slowly; attaining a diameter of 10 mm and 20 mm within 7 days at 25°C and 28°C respectively, floccose, producing only white mycelium (Figures 31-32 E, e).

Colonies on all media at 37°C, 14 days growing rapidly, attaining a diameter of 20-45 mm, commonly similar in appearance to colonies on CYA, plane, floccose, consisting of a compact basal felt, producing young ascomata in central area, White to Sienna (R 8); conidiogenesis absent; reverse Sienna (R 8) to Umber (R 9) (Figures 33 A-F, a-f).

Ascomata superficial, discrete or confluent, ripening within 21-30 days, non-ostiolate, globose to subglobose, 250-480 µm in diameter, soft, ascomatal wall consisting of densely interwoven hyphae (Figure 34 A). Ascomatal initials composed of short cylindrical ascogonium, around with thick antheridia coil tightly several times (Figures 34 D-E, 35 C). Asci in short chains, 8-spored, ovoidal or subglobose, 7.5-8.5 x 6-7 µm (Figures 34 F, 35 D). Ascospores at first hyaline to pale yellow, soon becoming blue, finally indigo-blue, ellipsoidal, 3.5-4.5 x 2.4-2.85 µm, spinose (Figures 34 F-I, 35 D).

Conidiophores arising from aerial hyphae, stipes short, 12-15 (-20) x 2-3 µm. Penicilli biverticillate, sometimes monoverticillate (Figures 34 B-C, 35 A). Metulae in small verticils of 2-4, 7-11 x 2- 2.5 µm. Phialides 3-6 per metula, lanceolate, 8.57-10.71 x 2-3 µm. Conidia hyaline, ovoidal to ellipsoidal, 2-3 x 2-2.5 µm, smooth-walled (Figures 34 B-C, 35 B).

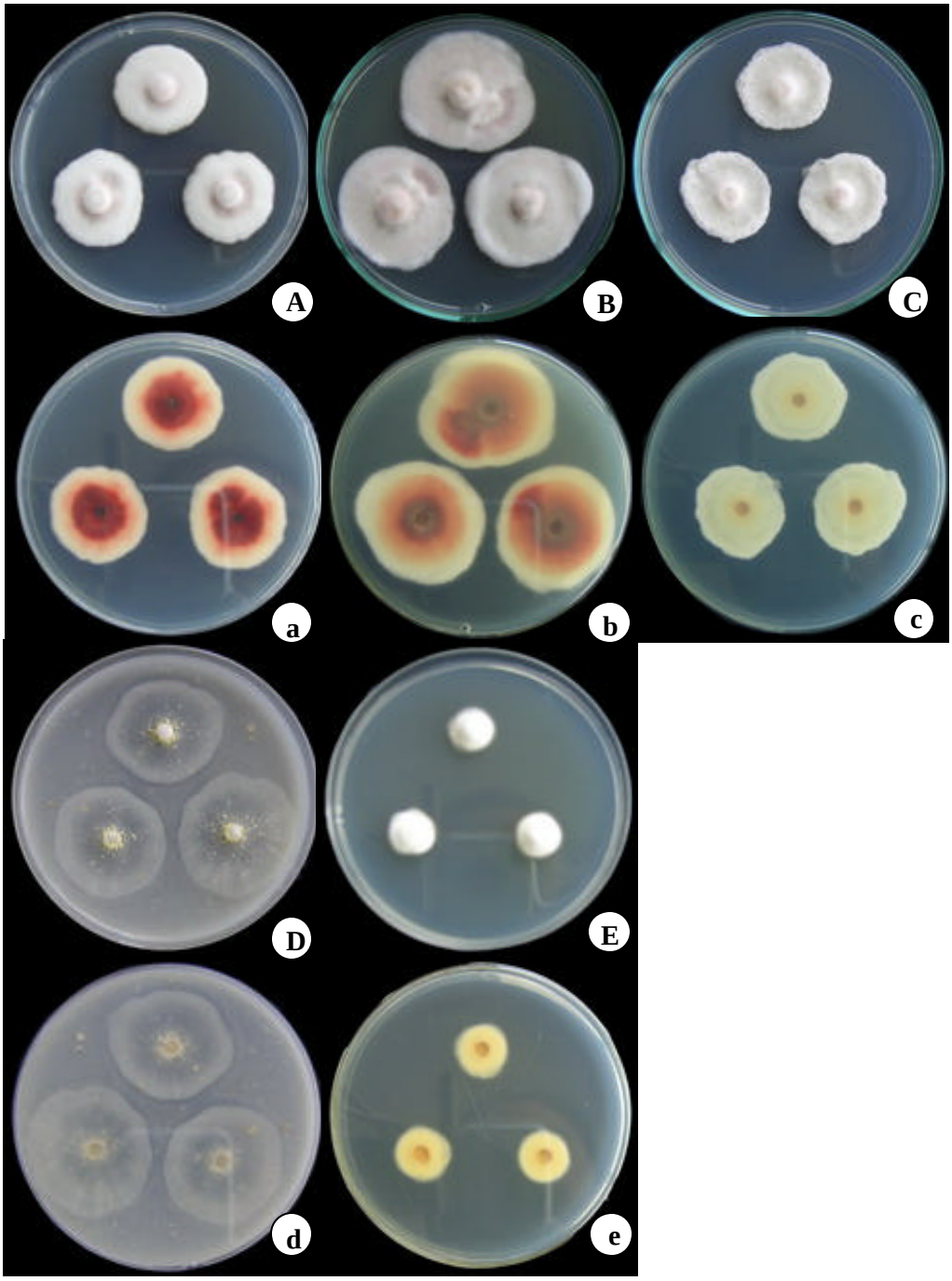


Figure 31 *Talaromyces indigoticus* KUFC 3366. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c), OMA (D, d), G25N (E, e)

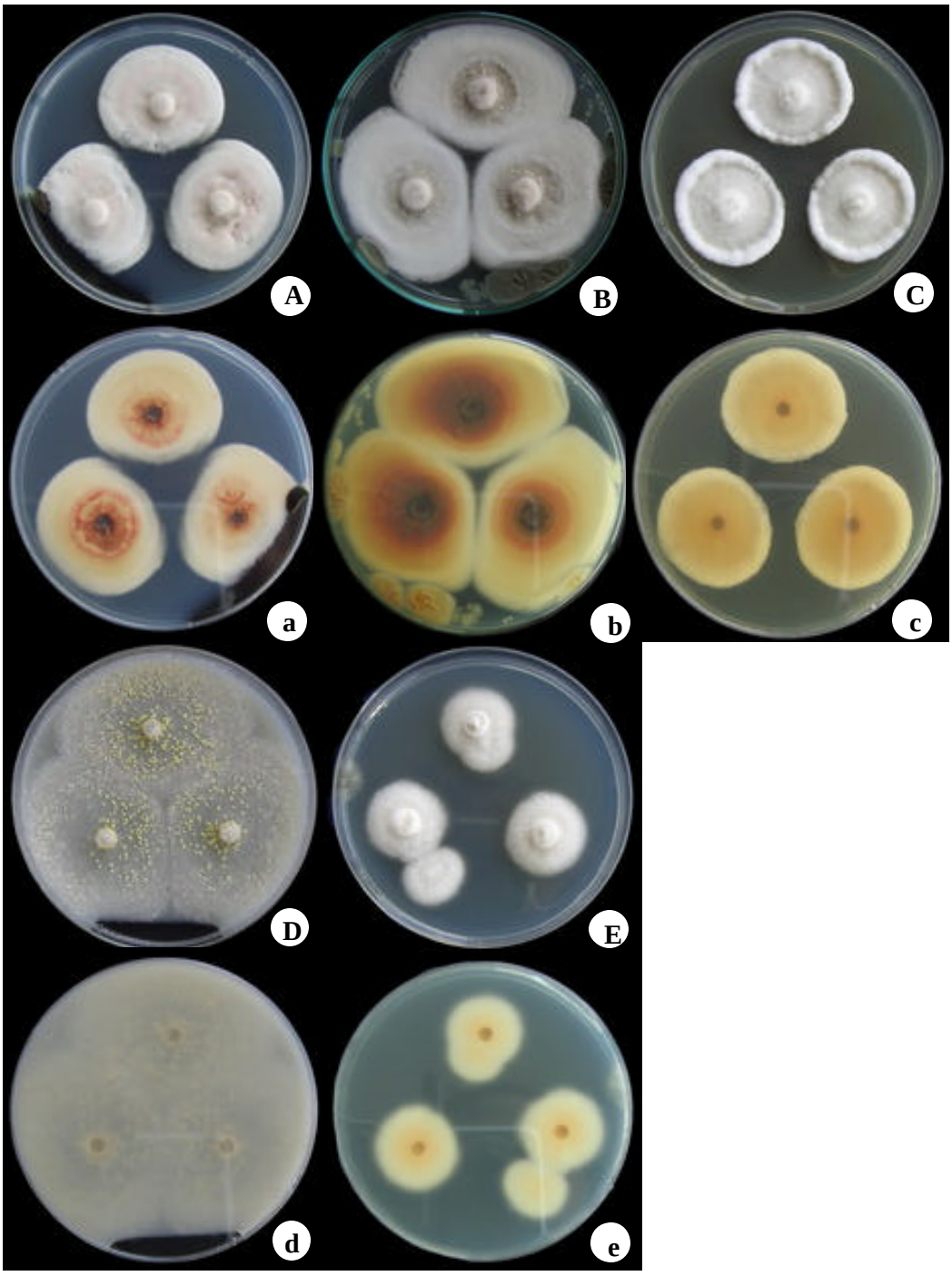


Figure 32 *Talaromyces indigoticus* KUFC 3366. Obverse and reverse views of colonies on different media, incubated for 7 days at 28°C; CZA (A, a), CYA (B, b), MEA (C, c), OMA (D, d), G25N (E, e)

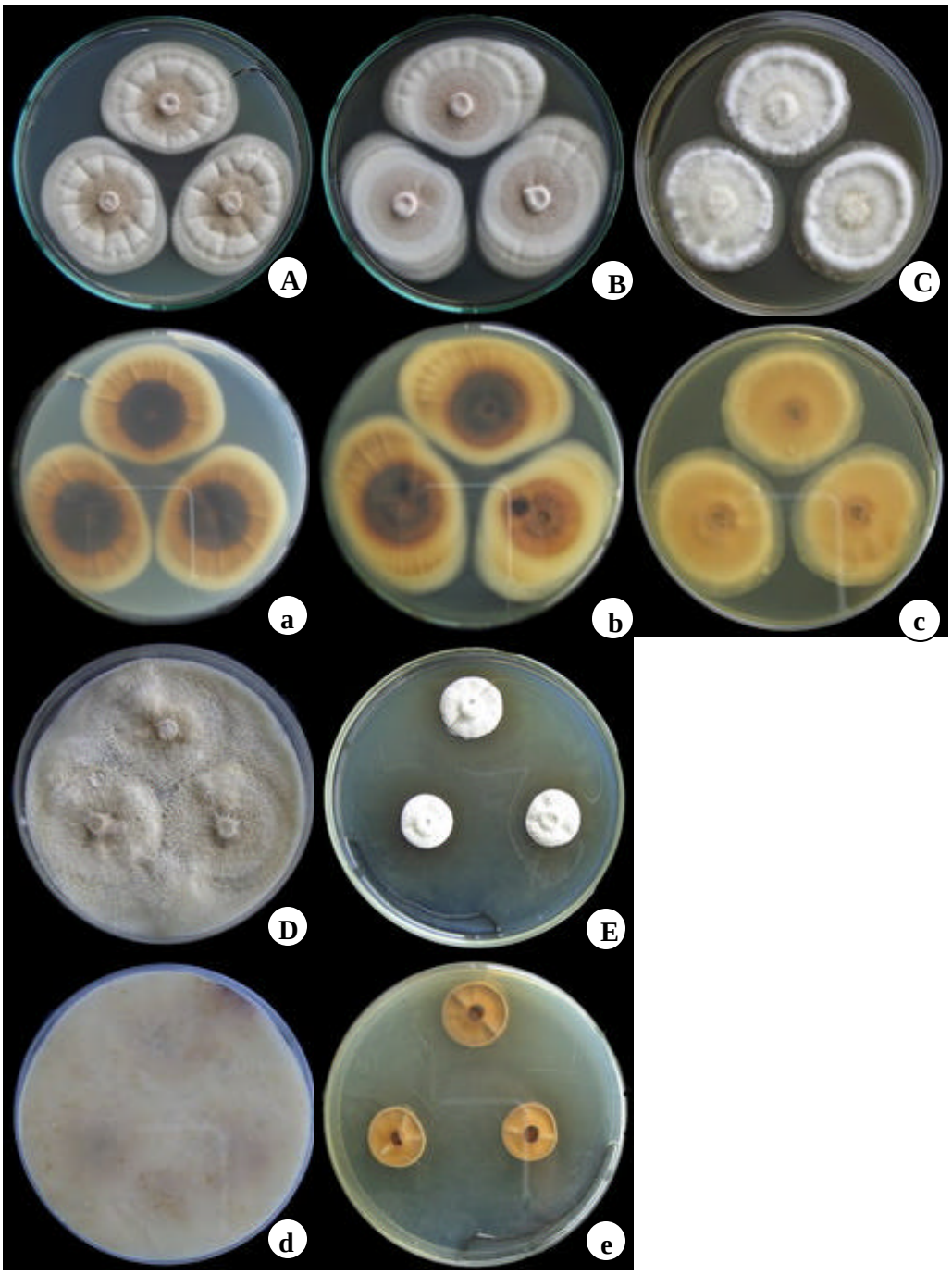


Figure 33 *Talaromyces indigoticus* KUFC 3366. Obverse and reverse views of colonies on different media, incubated for 14 days at 37°C; CZA (A, a), CYA (B, b), MEA (C, c), OMA (D, d), G25N (E, e)

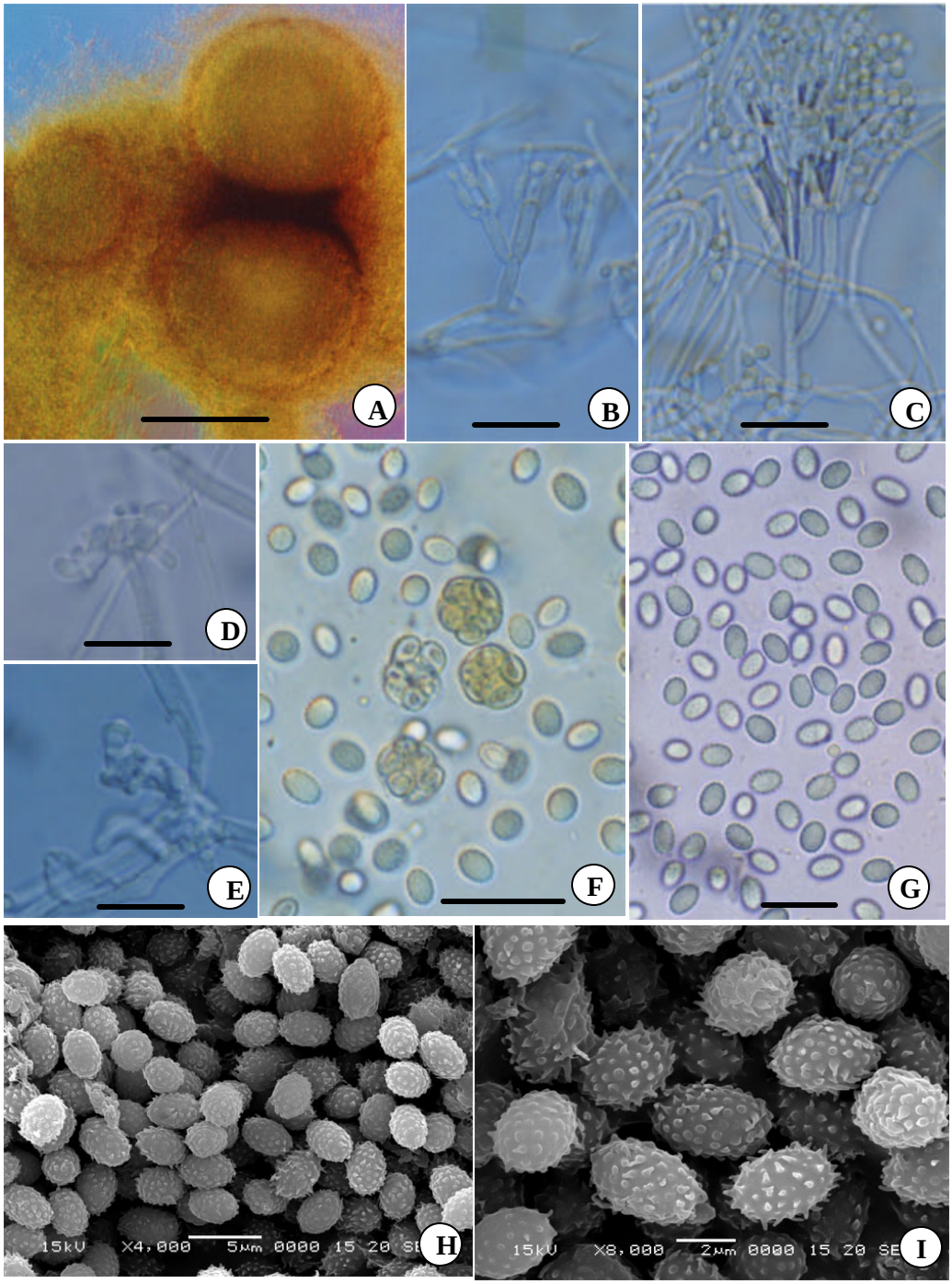


Figure 34 *Talaromyces indigoticus* KUFC 3366

A. ascomata; B-C. penicilli; D-E. ascomatal initials; F-G. asci and ascospores; H-I. ascospores (SEM)

(Bars: A = 200 µm; B-F = 10 µm; G-H = 5 µm; I = 2 µm)

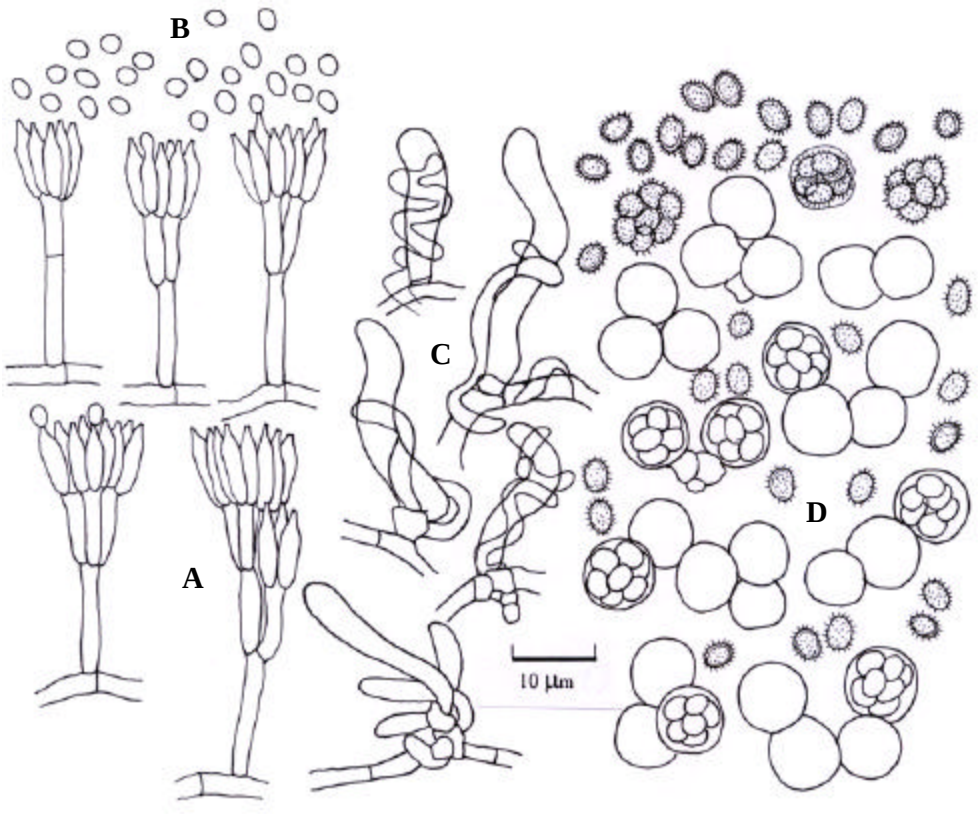


Figure 35 *Talaromyces indigoticus* KUFC 3366

Camera lucida drawings of A. penicilli; B. ascomatial initials C. ascogonium; D. Asci and ascospores

8. ***Talaromyces rotundus*** C.R. Benjamin (Figures 36 - 38)

Strains examined: KUFC 3359 agricultural soil, Chiang Mai; KUFC 3410 agricultural soil, Suphan Buri

References: Stolk and Samson, 1972; Pitt, 1979a

Stat. Anam. ***Penicillium rotundum*** Raper and Fennell

Colonies on CZA growing slowly, attaining a diameter of 15-18 mm within 7 days at 25°C, velvety, plane but centrally wrinkled and sulcate, consisting of a thin basal mycelial felt, Pure Yellow (R 14); ascomata and conidiogenesis absent; exudates and soluble pigment absent; margins entire and white; reverse brown shades ranging Sienna (R 8) to Umber (R 9) (Figures 36 A, a).

Colonies on CYA growing rather rapidly, attaining a diameter of 25-28 mm within 7 days at 25°C, velvety, radially sulcate or wrinkled, floccose to funiculose, consisting of a tough mycelial felt, Pale Luteous (R 11); ascomata and conidiogenesis absent; margins entire; exudates limited; reverse Sienna (R 8) to Umber (R 9) (Figures 36 B, b).

Colonies on MEA growing rapidly, attaining a diameter of 28-30 mm within 7 days at 25°C, plane, funiculose, consisting of a thin basal mycelial felt, Saffron (R 10); ascomata absent; conidiogenesis inconspicuous and sparse; margins white and broad; exudates absent; reverse Ochreous (R 44) to Amber (R 9) (Figures 36 C, c).

Colonies on CMA spread broadly, attaining a diameter of 28-30 mm within 7 days at 25°C, plane, consisting of a very thin mycelial felt, vegetative mycelium submerged, plane, Pale Luteous (R 11); conidiogenesis moderately; margins broad, submerged and translucent; exudates scattered, as small clear drops; reverse uncolored (Figures 36 D, d).

Colonies on OMA growing rather rapidly, attaining a diameter of 30-35 mm within 7 days at 25°C, plane, consisting of a very thin mycelial felt in which

granular due to densely packed ascomata, Pale Luteous (R 11); conidiogenesis inconspicuous and sparse; margins broad and submerged; exudates absent; reverse uncolored (Figures 36 E, e).

Colonies on G25N agar growing extremely slowly; attaining a diameter of 10 mm within 7 days at 25°C, producing only sparse aerial growth (Figures 36 F, f).

At 37°C, growth is extremely restricted.

Ascomata soft, pale orange, globose to subglobose, 400-530 µm in diameter, discrete or confluent, ripening within 2-3 weeks. Covering consisting of a thin network of loosely interwoven hyphae, yellow to orange (Figure 37 A). Ascomatal initials develop within short branches or as intercalary portions of hyphae, become strongly gnarled and branch profusely (Figures 37 C-E, 38 E). Asci 8-spored, subglobose to globose, 10-11 x 9.5-10 µm (Figures 37 B, 38 F-G). Ascospores globose, (3.5-) 4-5.5 (-6) µm in diameter, thick-walled, spinulose (Figures 37 F-I, 38 F-G).

Conidial state lacking or produced very limited, best development on CZA + 20% sucrose. Conidiophores arising as short branches from aerial mycelium, (20-) 26-35 (-40) µm long, 2-2.5 µm wide, smooth-walled. Metulae in verticils of 2 to 3, measuring 10-12 x 2-2.5 µm. Philides about 4 to 6 in the verticil, lanceolate, 10-12.5 (-14.5) x 2.2-2.5 µm (Figures 38 A, C). Conidia oblate, 3-3.5 x 1.5-2 µm, smooth, hyaline (Figures 38 B, D).

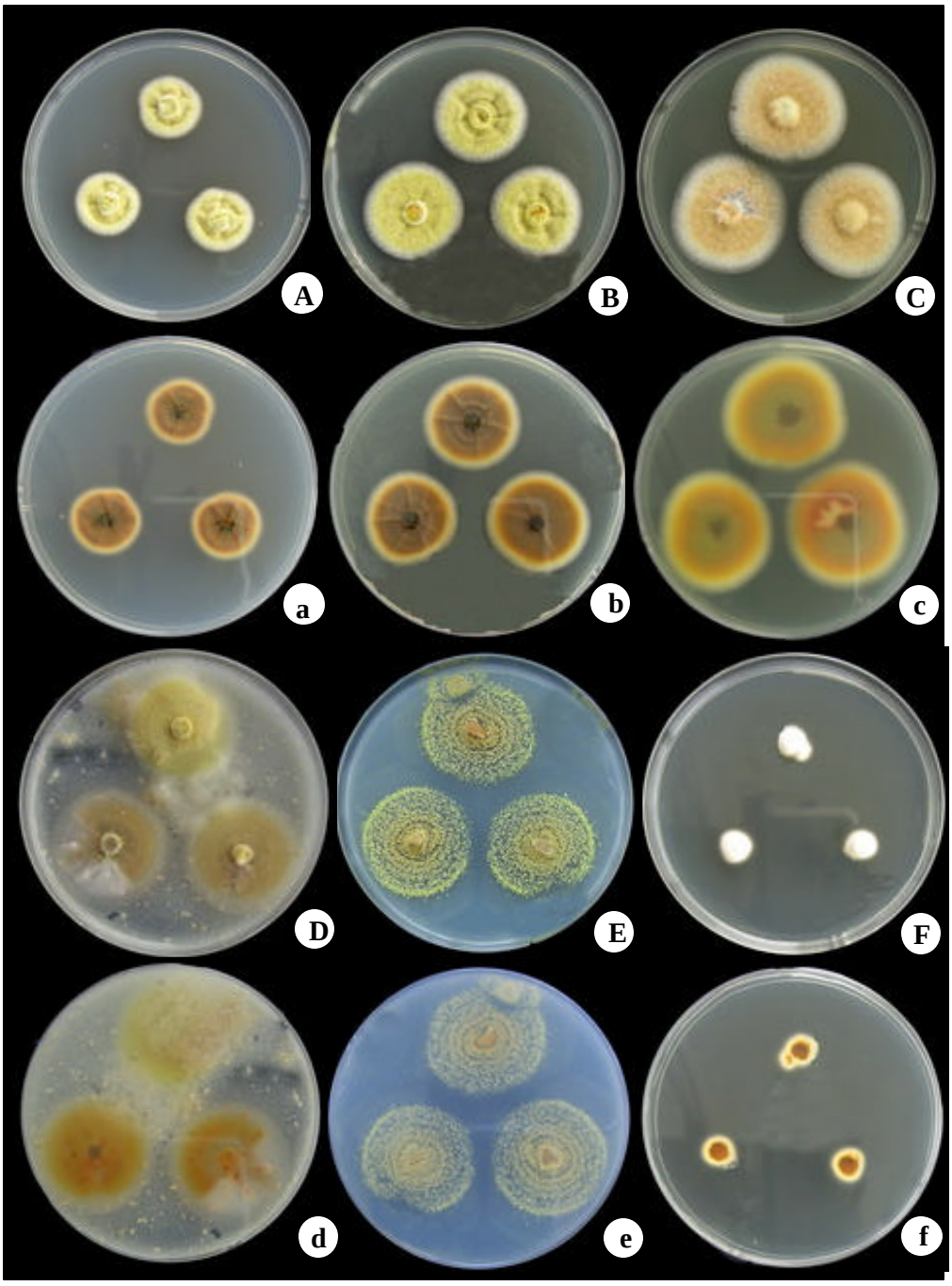


Figure 36 *Talaromyces rotundus* KUFC 3359. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

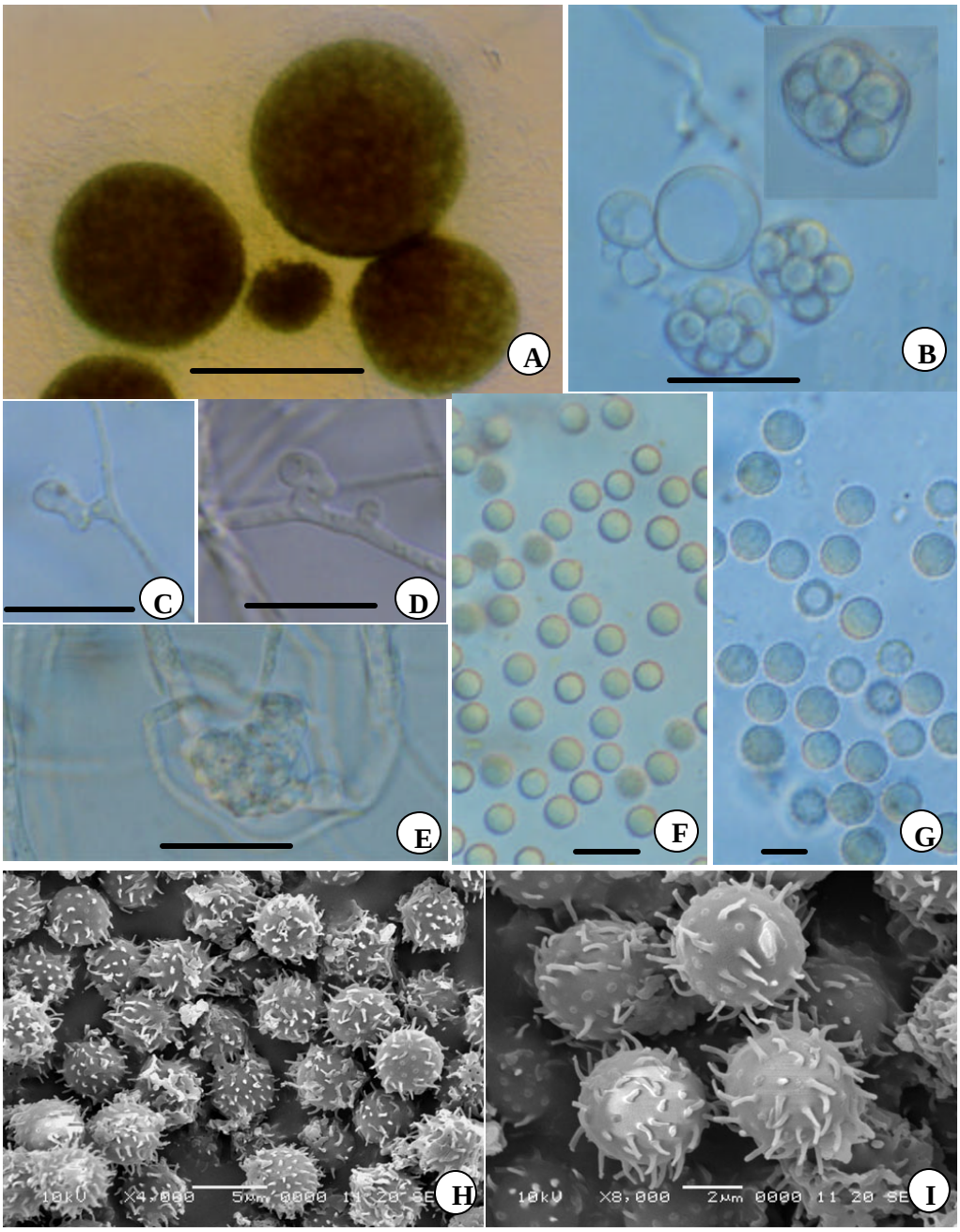


Figure 37 *Talaromyces rotundus* KUFC 3359

A. ascomata; B. Asci and ascospores; C-E. ascomatal initials;

F. ascospores KUFC 3359; G. ascospores KUFC 3410; H-I. ascospores (SEM)

(Bars: A = 100 µm; B-E = 10 µm; F-H = 5 µm; I = 2 µm)

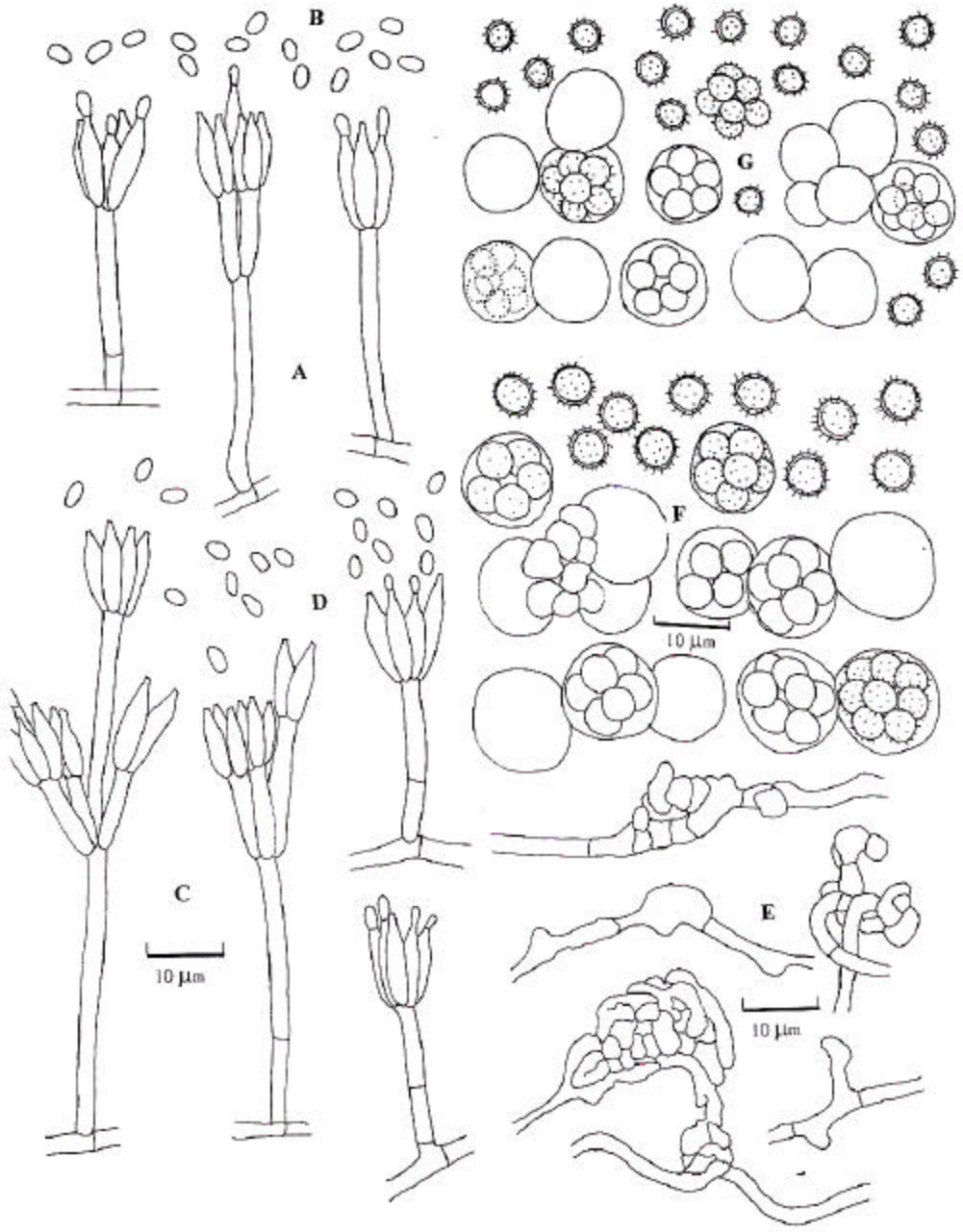


Figure 38 *Talaromyces rotundus*

Camera lucida drawings of A. penicilli; B. conidia KUFC 3359; C. penicilli, D. conidia KUFC 3410; E. ascomatal initials; F. asci and ascospores KUFC 3410; G. Asci and ascospores KUFC 3359

9. ***Talaromyces stipitatus*** C.R. Benjamin (Figures 39-43)

Strain examined: KUFC 3357 forest soil, Sakon Nakhon, KUFC 3422 nonagricultural soil, Krabi

References: Stolk and Samson, 1972; Pitt, 1979a

Stat. Anam. ***Penicillium stipitatum*** Thom

Colonies on CZA growing rapidly, attaining a diameter of 45-50 mm within 7 days at 25°C, plane, consisting of a thin basal felt with white aerial mycelial; ascomata and conidiogenesia absent; exudates and soluble pigment absent; reverse Straw (R 46) (Figures 39 A, a). Colonies on CZA at 28°C, attaining a diameter of 50 mm and 65-70 mm within 7 and 14 days repectively, funiculose, consisting mainly of a comparatively thin layer of ascomata, produced near the agar surface, showing conspicuous Pure Yellow (R 14) shade, becoming Pale Amber (R 47) to Ochreous (R 44) in age; conidiogenesis sparse; reverse ranging Pure Yellow (R 14), Luteous (R 12) to Umber (R 9) (Figures 40 A, a, D, d).

Colonies on CYA growing rather rapidly, attaining a diameter of 55-60 mm within 7 days at 25°C, floccose to funiculose, consisting of a thin basal felt, producing only Pure Yellow (R 14) mycelium; ascomata absent; conidiogenesis sparse at the margin; exudates absent; reverse brown shades ranging Pale Luteous (R 11), Luteous (R 12), Amber (R 47) to Ochreous (R 44) (Figures 39 B, b). Colonies on CYA at 28°C, attaining a diameter of 50 mm and 55 mm within 7 and 14 days respectively, floccose or funiculose, consisting of a thin basal felt in which abundant young ascomata embedded near the agar surface, conspicuous Pure Yellow (R 14), becoming Luteous (R 12) in age; conidiogenesis sparse; reverse ranging Pure Yellow (R 14) to Luteous (R 12) (Figures 40 B, b, E, e).

Colonies on MEA growing fairly rapidly, attaining a diameter of 60-70 mm within 7 days, 25°C, more or less funiculose, consisting of a thin layer of numerous young ascomata produced near the agar surface, intermixed with yellow aerial hyphae, Pale Luteous (R 11); conidiogenesis sparse and inconspicuous; reverse Straw (R 46) (Figures 39 C, c). Colonies on MEA at 28°C, attaining a diameter of 70

mm and 75 mm within 7 and 14 days respectively, funiculose, slightly zonate, showing the same cultural characteristics and color as on CZA, consisting of a thin mycelial felt in which granular due to densely packed ascomata, Pale Luteous (R 11), becoming pinkish shade in age; reverse Luteous (R 12) (Figures 40 C, c, F, f).

Colonies on CMA growing rapidly, attaining a diameter of 60-62 mm within 7 days at 25°C, plane, thin, with vegetative mycelium submerged, Straw (R 46); ascomata absent; conidiogenesis inconspicuous and sparse; margins entire; exudates absent; reverse uncolored (Figures 39 D, d). Colonies on CMA at 28°C, attaining a diameter of 70-80 mm within 14 days at 25°C, plane, consisting of a thin mycelial felt, Straw (R 46); ascomata and conidiogenesis absent; margins entire; exudates absent; reverse uncolored.

Colonies on OMA growing rapidly, attaining a diameter of 70-75 mm within 7 days at 25°C, more or less funiculose, plane, consisting of a thin mycelial felt in which numerous developing ascomata over the entrie surfuce, Straw (R 46) or Pure Yellow (R 14); conidiogenesis inconspicuous and sparse; exudates absent; reverse uncolored (Figures 39 E, e). Colonies on OMA at 28°C, attaining a diameter of 70-75 mm within 7 days, plane, consisting of thin mycelial felt in which numerous Pure Yellow (R 14) ascomata develop; conidiogenesis inconspicuous and sparse; exudates absent; reverse uncolored.

Colonies on G25N agar growing moderately, attaining a diameter of 20-22 mm within 7 days at 25°C, producing only sparse white to Pale Yellow (R 14) mycelium (Figures 39 F, f).

Colonies on all media within 7 days at 37°C, well developed except on G25N agar, mostly consisting of a thin mycelial felt, white to Pale Yellow (R 14); slowly developed of ascomata; conidiogenesis limited; reverse uncolored to brown shades.

Ascomata subglobose or globose, at first yellow, then pale luteous or luteous, becoming pink in age, (150-) 200-250 µm in diameter, discrete or confluent, ripening within 10 to 14 days. Ascomatal walls composed of a thin network of loosely interwoven hyphae (Figure 41 A). Ascomatal initials resemble at first those of *T. flavus*, consisting of thick, club-shaped ascogonia, around which thin antheridia tightly coil at the basal parts. After fertilization, at the ascogonia apices, they produce a few gnarled branches which continue to branch profusely (Figures 41 E, 42 E, F). Asci 8-spored, broadly subglobose to globose, 6-7.3 x 6-7 µm (Figures 41 F, 43 A). Ascospores pale yellow, flattened ellipsoidal, with a single equatorial ridge, 3.3-4 x 2.5-3 µm, smooth-walled to very finely roughened (under SEM) (Figures 41 F-I, 43 B).

Conidiophores short, arising from aerial hyphae, 14-30 (-35) µm x 2.33-3.33 µm. Penicilli typically monoverticillate, 2-4 in the verticil, phialides 10-13.5 (-16.67) x 3–3.5 µm, occasionally also solitary phialides, 22.5–28.67 x 4-4.5 µm (Figures 41 B-D, 42 A, C). Conidia subglobose to globose, 3.3-4.3 (-4.5) µm, smooth-walled (Figures 41 C-D, 42 B, D).

Talaromyces stipitatus can easily be distinguished from the other species of *Talaromyces* by the unique pattern of the ascospored and ascomatal initials. Because of the presence of paired gametangia it shows relationships with *T. flavus* and *T. helicus*. This species reported as rarely species, but from a variety of sources (Pitt, 1979 a).

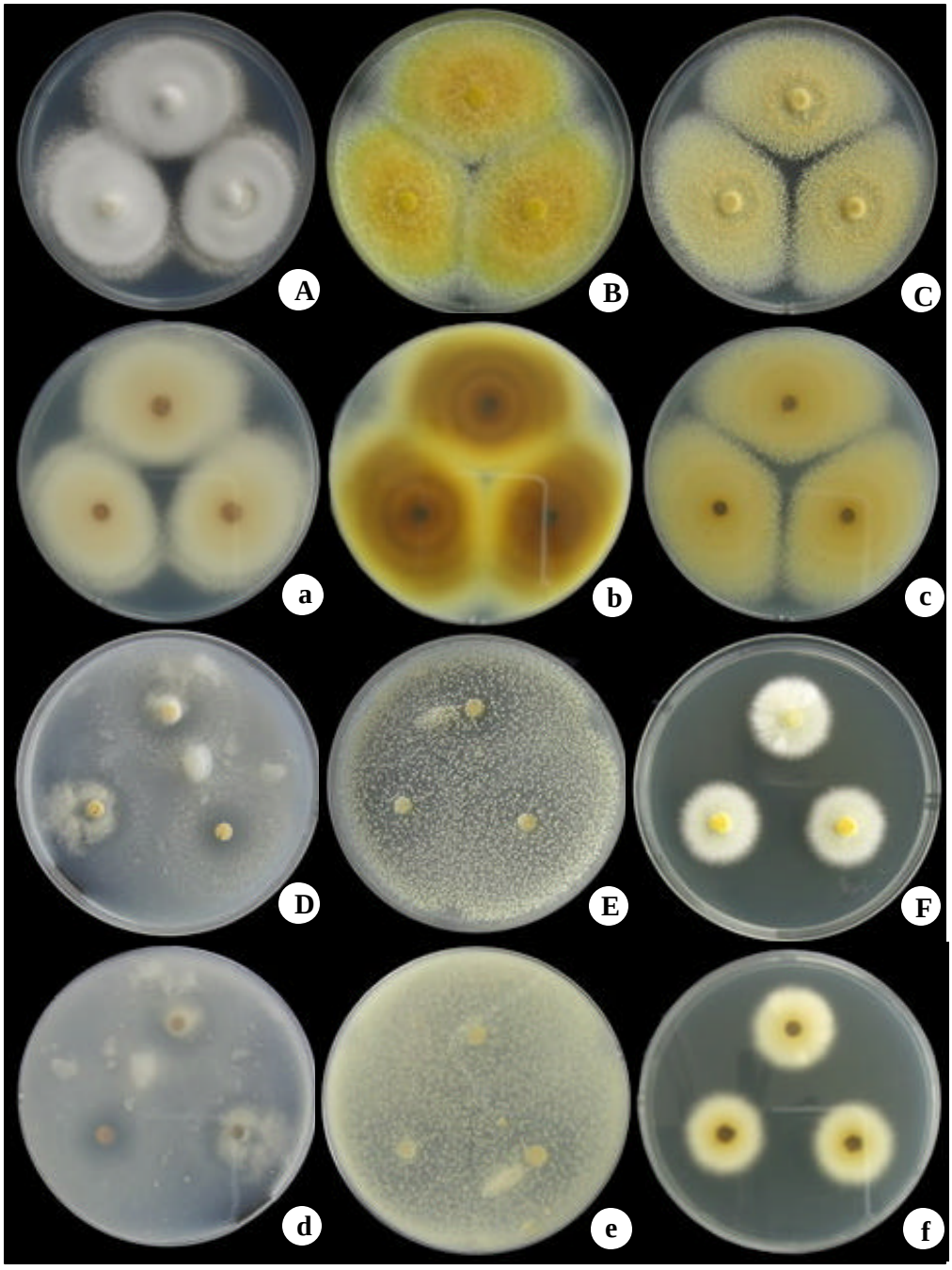


Figure 39 *Talaromyces stipitatus* KUFC 3357. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

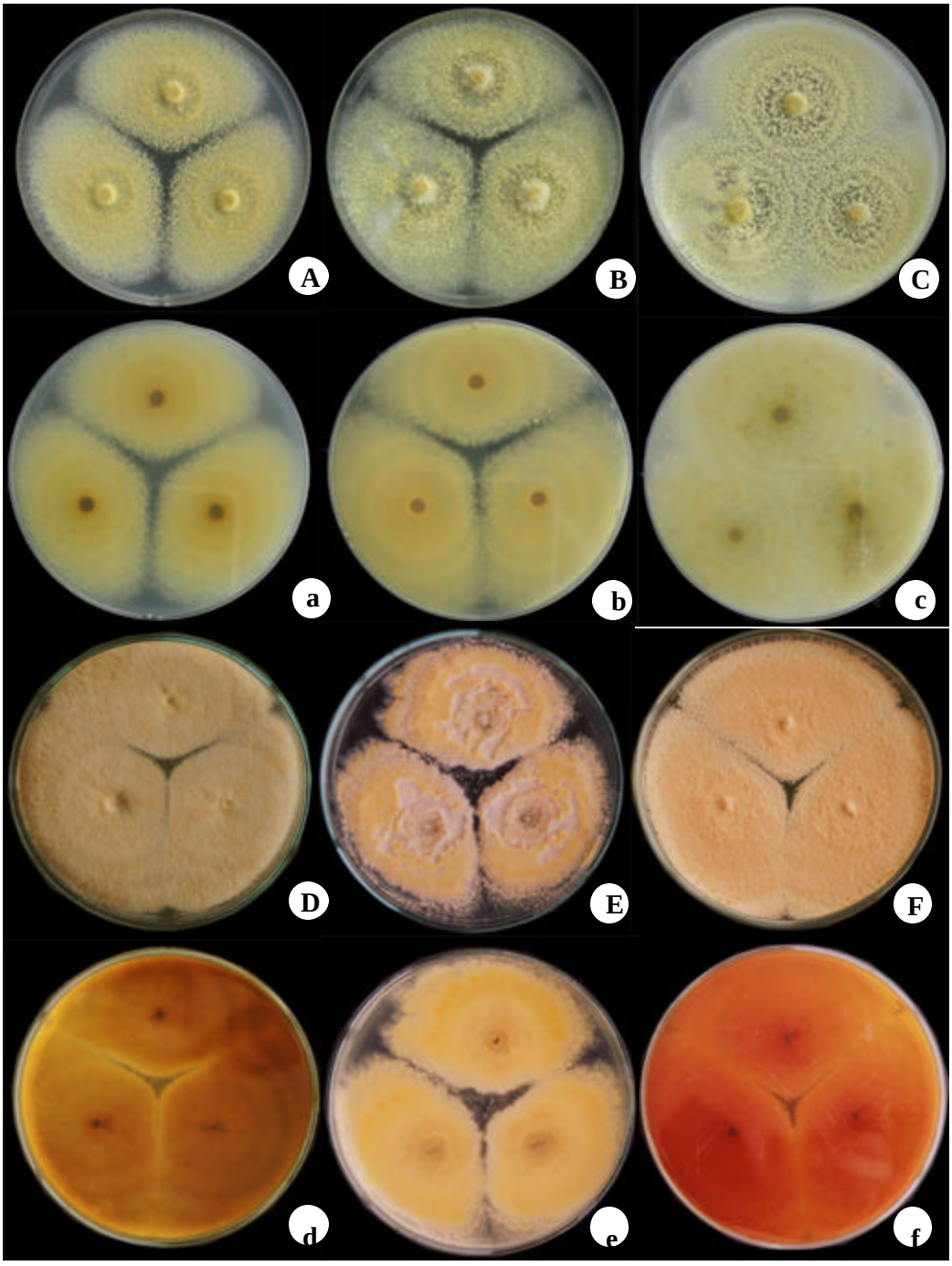


Figure 40 *Talaromyces stipitatus* KUFC 3357. Obverse and reverse sides of colonies on different media, incubated for 7 days at 28°C: CZA (A, a), CYA (B, b), MEA (C, c), incubated for 14 days at 28°C: CZA (D, d), CYA (E, e), MEA (F, f)

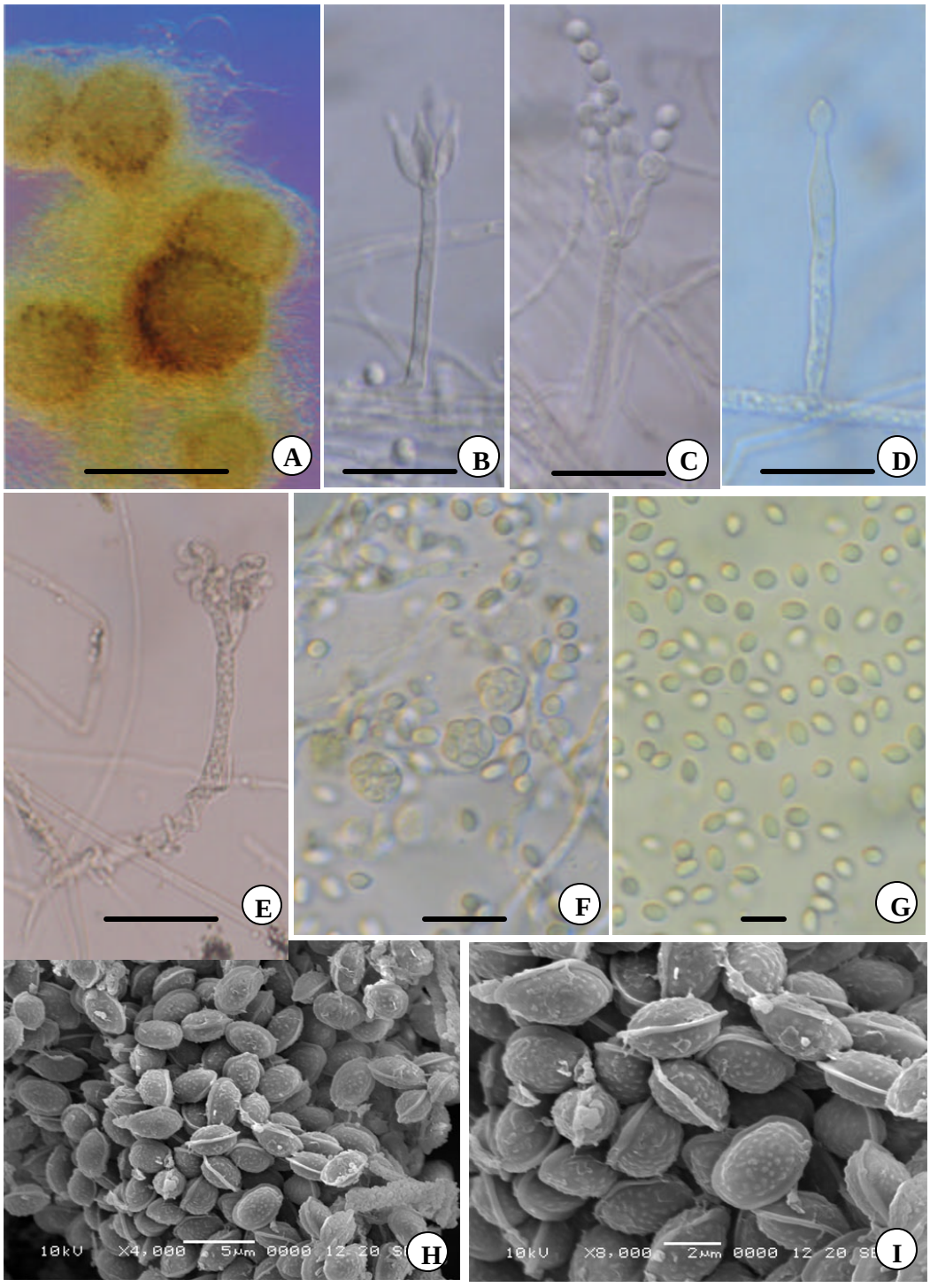


Figure 41 *Talaromyces stipitatus* KUFC 3357

A. ascomata; B-D. penicilli; E. ascomatal initial; F-G. asci and ascospores; H-I. ascospores (SEM)

(Bars: A = 100 µm; B-E = 20 µm; F = 10 µm; G-H = 5 µm; I = 2 µm)

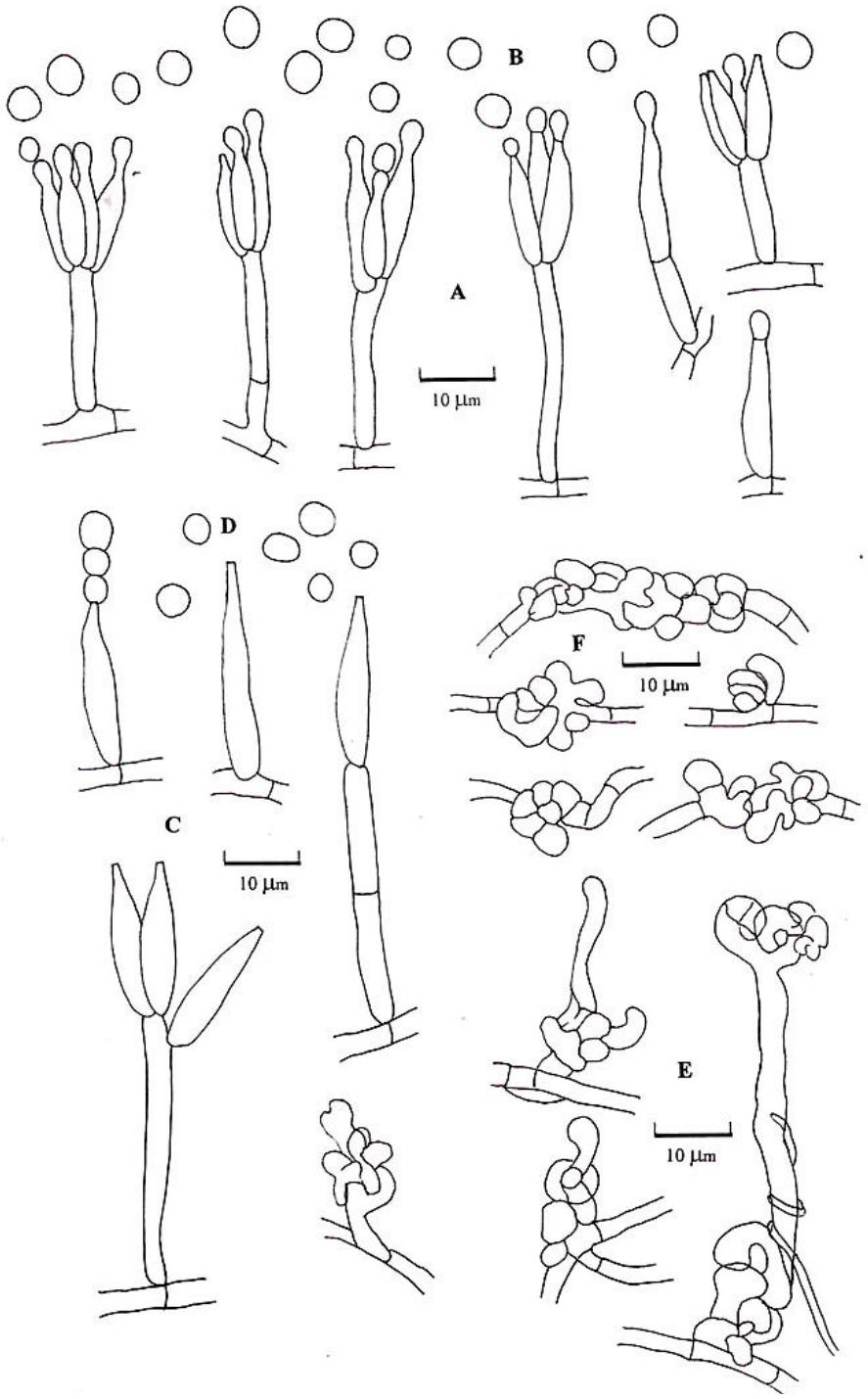


Figure 42 *Talaromyces stipitatus*

Camera lucida drawings of A-B. penicilli and conidia KUFC 3357; C-B. penicilli and conidia KUFC 3422; E. ascomatal initials KUFC 3357; F. KUFC ascomatal initials 3422

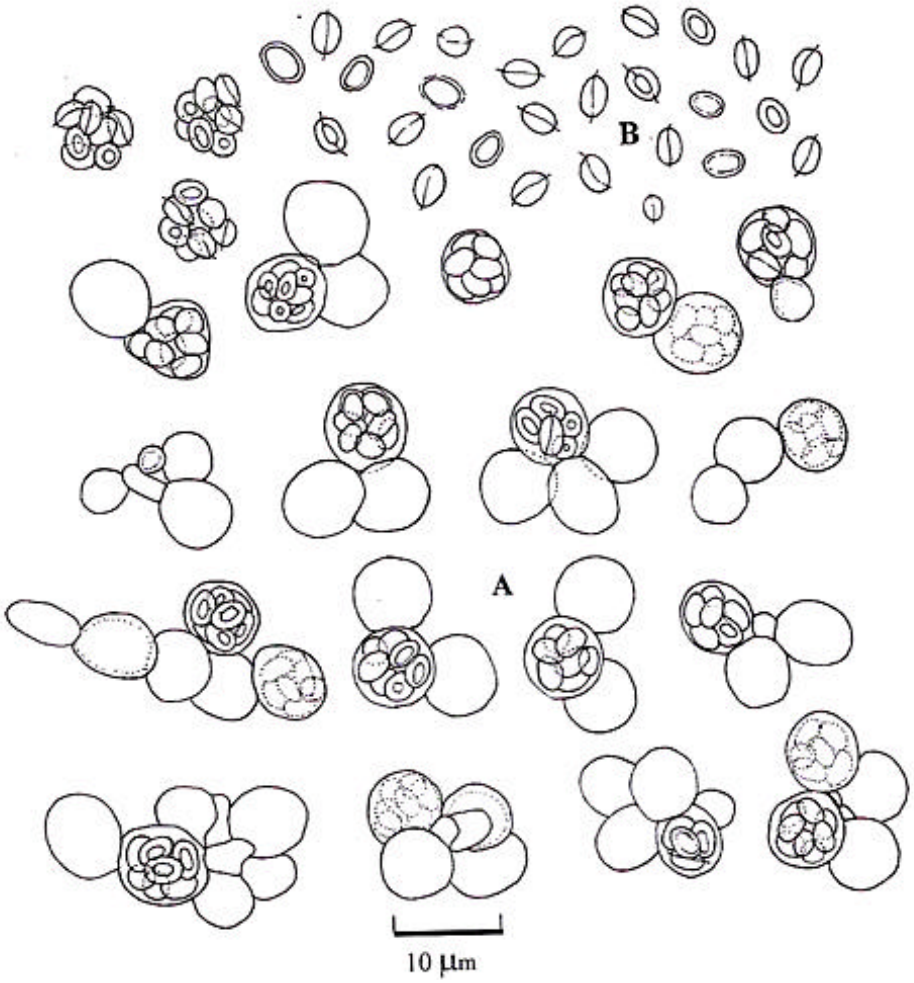


Figure 43 *Talaromyces stipitatus* KUFC 3357
Camera lucida drawings of A. asci; B. ascospores

10. ***Talaromyces trachyspermus*** (Shear) Stolk & Samson
(Figures 44-47)
Strains examined: KUFC 3355 forest soil, Chiang Mai; KUFC 3421 forest soil, Mae Hong Son
References: Stolk and Samson, 1972; Pitt, 1979a
Stat. Anam. ***Penicillium spiculisporum*** Lehman

Colonies on CZA growing rapidly, attaining a diameter 35-37 mm within 7 days at 25°C, plane, more or less funiculose, consisting of a compact basal

felt, producing numerous ascomata over the entire suface, white; conidiogenesis usually limited or developing in the mergins part of the colony; exudate absent; reverse Straw (R 46) to Sulphur Yellow (R 15) (Figures 44 A, a).

Colonies on CYA growing rather rapidly, attaining a diameter of 45-47 mm within 7 days, at 25°C, funiculose to floccose, plane, consisting of a thin basal felt with abundant developing ascomata, white; conidiogenesis spare and inconspicuous; exudates absent; margins entire; reverse Straw (R 46) to Luteous (R 12) (Figures 44 B, b).

Colonies on MEA growing moderately, attaining a diameter of 24-25 mm within 7 days at 25°C, plane, fasciculate, showing the same cultural characteristic as on CYA; margins entire and lower; reverse uncolored (Figures 44 C, c).

Colonies on CMA growing rapidly, attaining a diameter of 50-55 mm within 7 days at 25°C, plane, very thin, vegetative mycelium submerged or forming a sparse growth of white aerial hyphae; with scattered ascomata over the entire surface; conidiogenesis absent or sparse; margins submerged and broad; reverse uncolored (Figures 44 D, d).

Colonies on OMA growing rapidly, attaining a diameter of 35-40 mm within 7 days at 25°C, plane, thin, with vegetative mycelium submerged, producing abundant ascomata in the central area to subcentral area and very limited conidia, white; margins broadly submerged; reverse uncolored (Figures 44 E, e).

Colonies on G25N agar growing slowly, attaining a diameter of 10-15 mm within 7 days at 25°C, floccose, umbonate, producing only aerial mycelium, white; margins entire; reverse uncolored (Figures 44 F, f).

Ascomata often confluent, non-ostiole, globose, soft, white, in some strain creamish to yellowish are present, 350-400 (-500) µm in diameter, ripening within 14 days. Ascomatal wall consisting of a network of closely interwoven hyphae,

simulating a pseudoparenchymatous wall, surrounded by a loose weft of radiating hyphae. Ascomatal initials inconspicuous, growing as swollen side branches of aerial hyphae, which become strongly gnarled and branch profusely, then forming a compact structure of several brance (Figure 46 C). Asci subglobose to globose, 6.5-8.7 x 5.3-6.5 µm (Figures 45 B, 46 D). Ascospores ellipsoidal, 2.67-3.33 x 2-2.5 µm, spinulose (Figures 45 B-E, 46 D).

Conidiophores arising from aerial hyphae, usually short, 6.67-13.33 (-18) x 2.5-3 µm. Penicilli irregularly arranged, monoverticillate to biverticillate. Metulae, in small verticils of 2 to 3, 10-12 (-15) x 2 - 2.67 µm. Phialides about 3 to 5 in the verticil, lanceolate, 10-16 x 2.33-2.5 µm (Figures 45 A, 46 A-B). Conidia ellipsoidal to ovoidal, 3.3-3.5 x 1.67 -2 µm, smooth-walled, pale green in mass (Figures 45 A, 46 A-B).

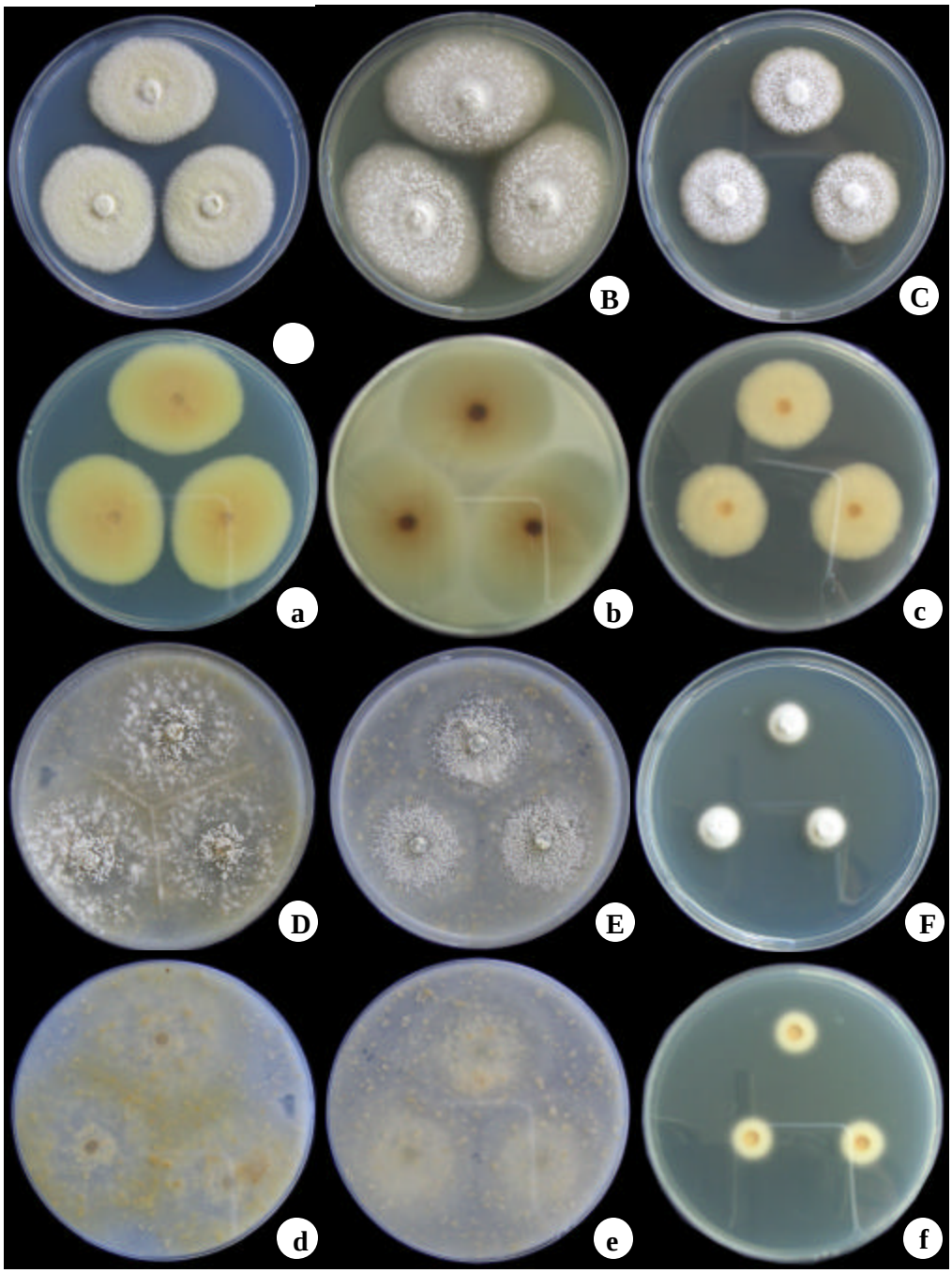


Figure 44 *Talaromyces trachyspermus* KUFC 3355. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

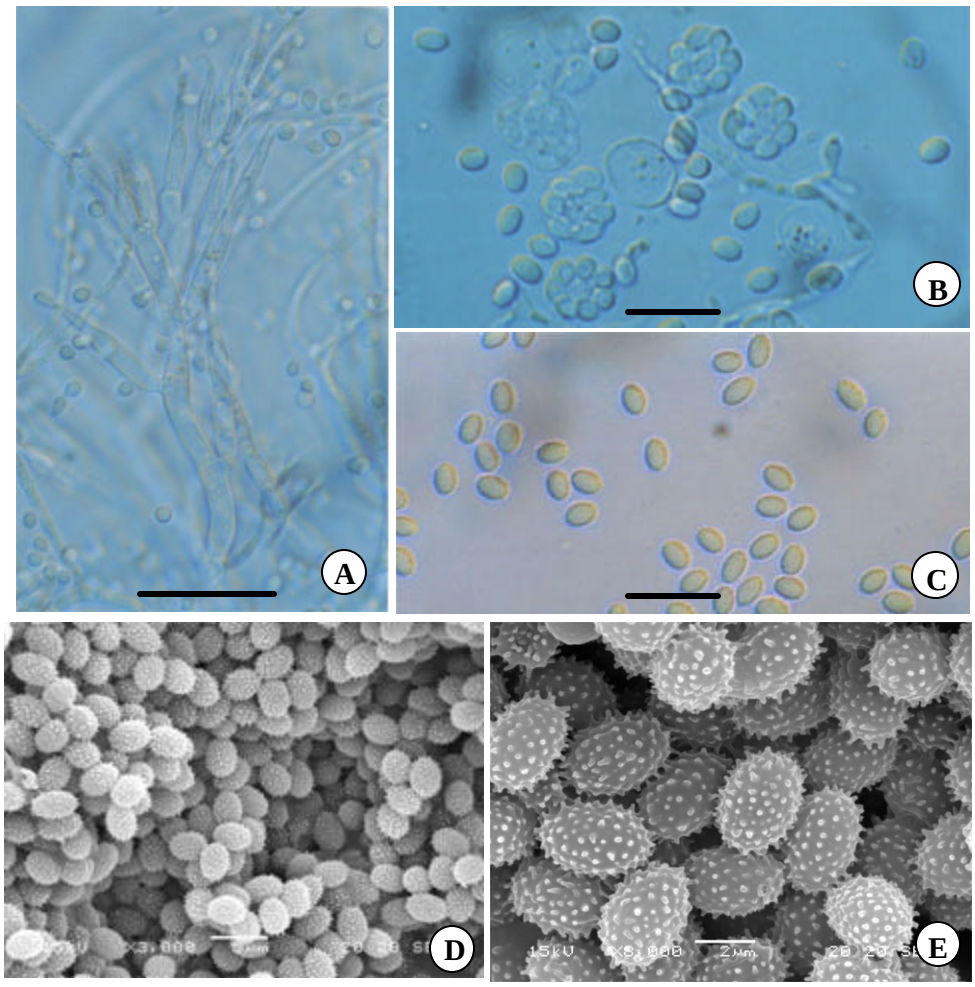


Figure 45 *Talaromyces trachysperus* KUFC 3355
A. penicilli; B-C. asci and ascospores; D-E. ascospores (SEM)
(Bars: A-C = 10 µm; D = 5 µm; E = 2 µm)

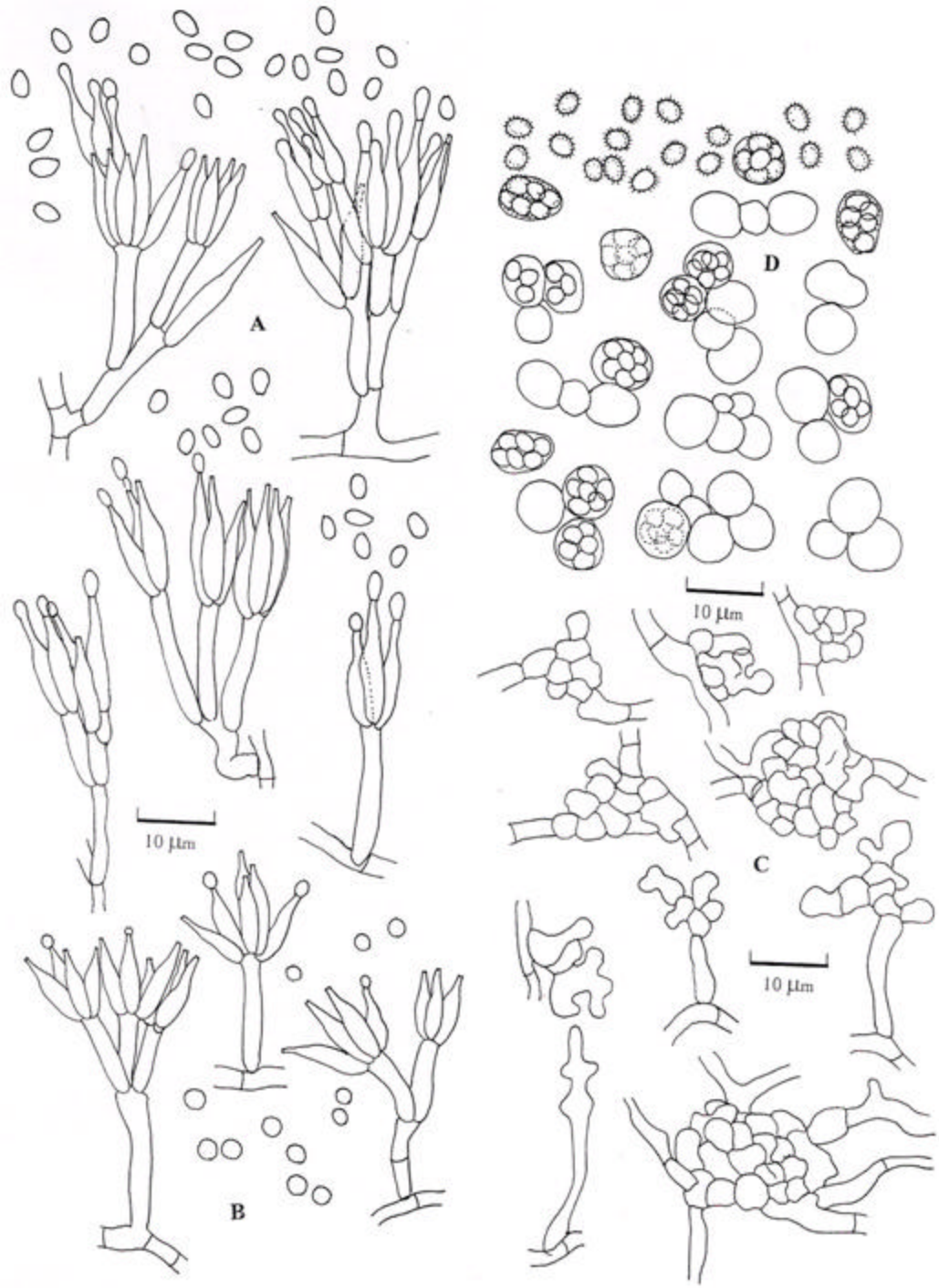


Figure 46 *Talaromyces trachyspermus*

Camera lucida drawings of A. penicilli and conidia KUFC 3355; B. penicilli and conidia KUFC 3421; C. ascomatal initials KUFC 3355; D. asci and ascospores KUFC 3355

11. ***Talaromyces wortmannii*** C.R. Benjamin (Figures 47-53)

Strains examined: KUFC 3333 forest soil, Mae Hong Son; KUFC 3354 nonagriculturalsoil, Krabi

References: Stolk and Samson, 1972; Pitt, 1979a

Stat. Anam. ***Penicillium wortmannii*** Klöcker

Colonies on CZA growing moderately, attaining a diameter of 20-22 mm within 7 days at 25°C, velvety, plane, consisting of a thin basal felt, Sulphur Yellow (R 15) or Pure Yellow (R 14); ascomata absent; conidiogenesis sparse and conspicuous; margins broad and lower, white; exudates and soluble pigment absent; reverse uncolored (Figures 47 A, a). Colonies on CZA at 28°C, reaching 32-35 mm in diameter within 7 days, umbonate, fasciculate, with abundant production of yellow ascomata; conidiogenesis inconspicuous or lacking; margins irregular; reverse Straw (R 46) (Figures 48-49 A, a).

Colonies on CYA growing moderately, attaining a diameter of 20-21 mm within 7 days at 25°C, velvety, consisting of a thin basal felt, ascomata absent; conidiogenesis abuntdant, Greyish Yellow-green (R 68) to Pale Greenish Grey (R 123); margins entire; exudates absent; reverse uncolored (Figures 47 B, b). Colonies on CYA at 28°C, reaching 30 mm in diameter within 7 days, velvety, sulcate, consisting of a compact basal felt, Pure Yellow (R 14) in color from the scattered ascomata which are embedded in the felt; intermixed with conidiogenesis abundant, showing Pale Greenish Grey (R 123) color; margins entire; exudates absent; reverse Straw (R 46) (Figures 48-49 B, b).

Colonies on MEA growing moderately, attaining a diameter of 21-22 mm within 7 days at 25°C, plane, velvety, more or less wrinkled or radially furrowed, consisting of a compact mycelial felt in which abundant yellow ascomata soon develop with ascompanying Greening Glaucous (R 91) color from the profuse conidia; margins entire; exudates absent; reverse Pale Luteous (R 11) to Luteous (R 12) (Figures 47 C, c). Colonies on MEA at 28°C, reaching 35 mm in diameter within 7 days, plane, velvety, central colony area lightly wrinkled, consisting of a compact

basal felt which numerous ascomata develop, showing Luteous (R 12) to Pale Orange (R 7) shade; conidiogenesis abundant, Pale Greening Glaucous (R 123) color from the profuse conidia; in some strain such as KUFC 3354, producing only umbonate, white mycelium; margins entire; exudates present as orange drops; reverse Pale Luteous (R 11) to Luteous (R 12) (Figures 48-49 C, c).

Colonies on CMA, attaining a diameter of 22-23 mm within 7 days at 25°C, plane, slightly zonate, consisting of a thin mycelial felt, granular in appearance due to the production of abundant ascomata, Sulphur Yellow (R 15) or Pure Yellow (R 14); conidiogenesis sparse and inconspicuous; exudates absent; reverse Straw (R 46) (Figures 47 D, d). Colonies on CMA at 28°C, reaching 40 mm in diameter within 7 days, plane, thin, consisting of a spreading, submerged vegetative mycelium, with limited development of funiculose aerial hyphae, producing yellow ascomata in limited numbers in central area; Pure Yellow (R 14); conidiogenesis sparse and conspicuous; exudates absent; reverse Pale Luteous (R 11) .

Colonies on OMA growing rather rapidly, attaining a diameter of 24-25 mm within 7 days at 25°C, velvety, plane, Sulphur Yellow (R 15) or Pure Yellow (R 14); ascomata usually absent; conidiogenesis profuse; reverse Straw (R 46) (Figures 47 E, e). Colonies on OMA at 28°C, reaching 45 mm in diameter within 7 days, plane, thin, Pure Yellow (R 14); ascomata slowly developing on the felt; conidiogenesis profuse; reverse Straw (R 46).

Colonies on G25N agar growing slowly; attaining a diameter of 10-12 mm within 7 days at 25°C, producing abundant penicIli, Glaucus Blue-Green (R 94); ascomata absent; mycelium at margins white; exudates absent; reverse Straw (R 46) (Figures 47 F, f).

Ascomata yellow to pale orange, soft, subglobose to globose, variable in size ranging 300-450 (-550) µm in diameter, discrete or confluent, ripening within 14 days. Covering composed of the thin network of loosely interwoven hyphae, surrounded by radiation, twisted hyphae (Figure 50 A). Ascomatal initials starts with

the production of intercalary or terminal cells which swell considerably, subsequent septation of hyphae. From these cells develop wide, somewhat irregularly coiling, gnarled branches, which continue to branch profusely, producing ascogenous hyphae (Figures 50 D, 53 A). Asci borne in chains, 8-spored, globose to ovoidal, 10-11.5 x 8-9.5 µm in diameter, evanescent (Figures 50 E, 53 B-C). Ascospores yellow, ellipsoidal, 4.67- 5.33 x 3.3-3.5 µm, spinulose. Some strains are smaller, asci 9.33-10 x 6.5-8.67 µm. Ascospores 4-4.7 x 3-3.5 µm, spinulose (Figures 50 E-H, 53 B-C).

Conidiophores arising from substratum hyphae, up to 250 µm long, 3-3.5 µm wide, smooth-walled, occasionally encrusted with yellow granules. Penicilli typically biverticillate (Figures 50 B-C), in some strains terverticillate or quaterverticillate (Figures 51, 52). Metulae in verticils of 4 to 6, measuring 10-13.33 x 2.67-3.33 µm. Phialides lanceolate, about 3 to 6 in the verticil, (8-)11.33-14 (-16.6) x 2.67-3.33 µm, gradually tapered to a fine tip. Conidia ellipsoidal or fusiform, pointed, 3.3-3.67 x 2-2.5 µm, smooth to finely spinulose, hyaline to greenish (Figures 50 B-C, 51-52).

Domsch *et al.*, (1993a, b) stated that *Talaromyces wortmannii* was the second most common soil-borne species of *Talaromyces* and has a worldwide distribution. It is easily recovered after brief soil steaming. In addition, to temperate latitudes where it is recorded from the British Isles, Sweden, Denmark, Germany, France and the USA. The mycelium contains mucilaginous polysaccharides resembling luteic acid. Metabolic products reported are the viridian-related wortmannin with antifungal but not antibacterial properties, wortmin, flavomannin, the anthraquinones skyrin and rugulosin. Dong *et al.*, (2006) reported wortmanilactones A, B, C and D from *Talaromyces wortmannii* isolated from soil in China. All compounds were screened for cytotoxic activity against a panel of five human cell lines (HCT-5, HCT-115, A549, MDA-MB-231, and K562). The IC_{50} values of the compounds range from 28.7 to 130.5 µM.

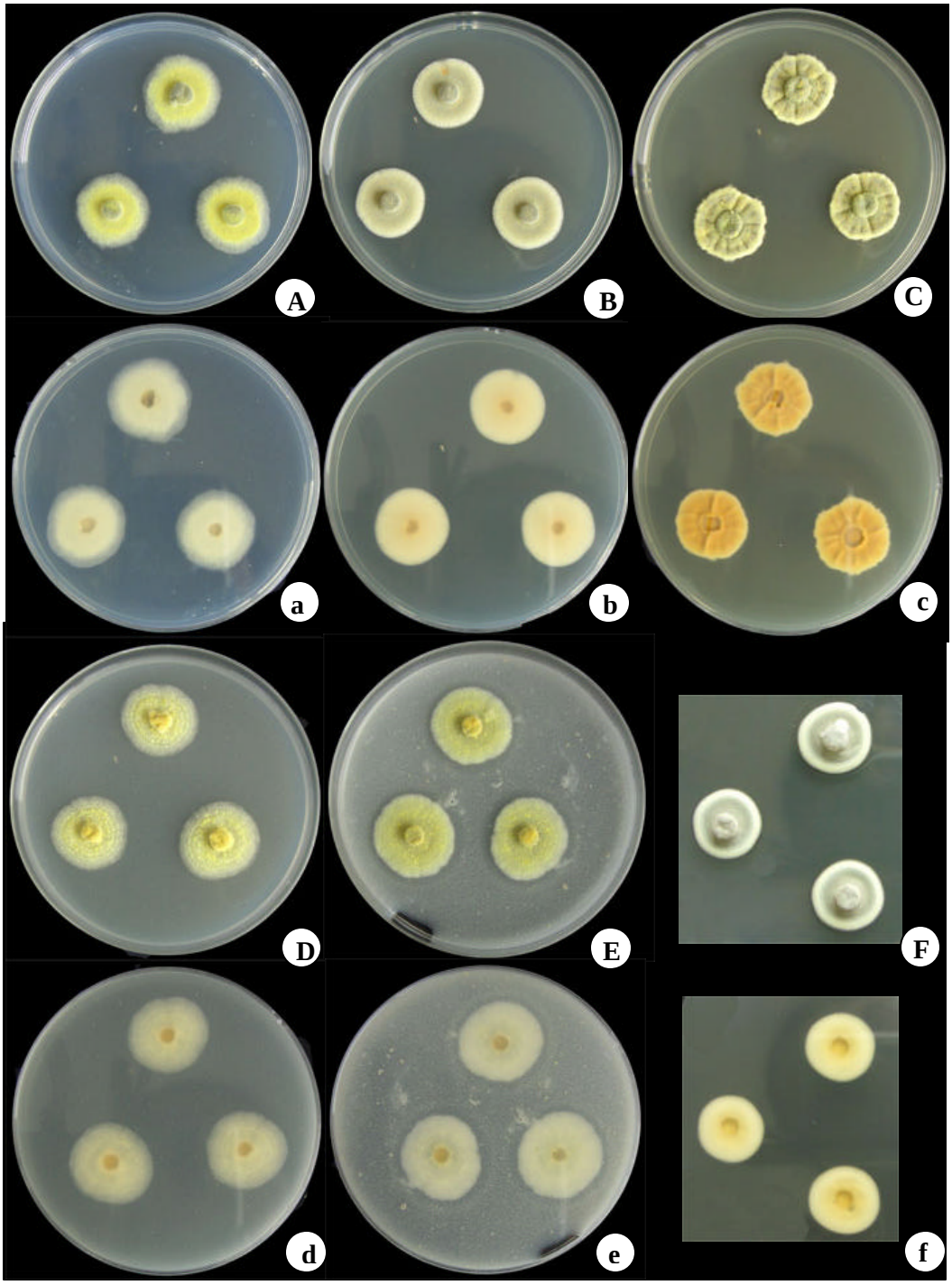


Figure 47 *Talaromyces wortmannii* KUFC 3333. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

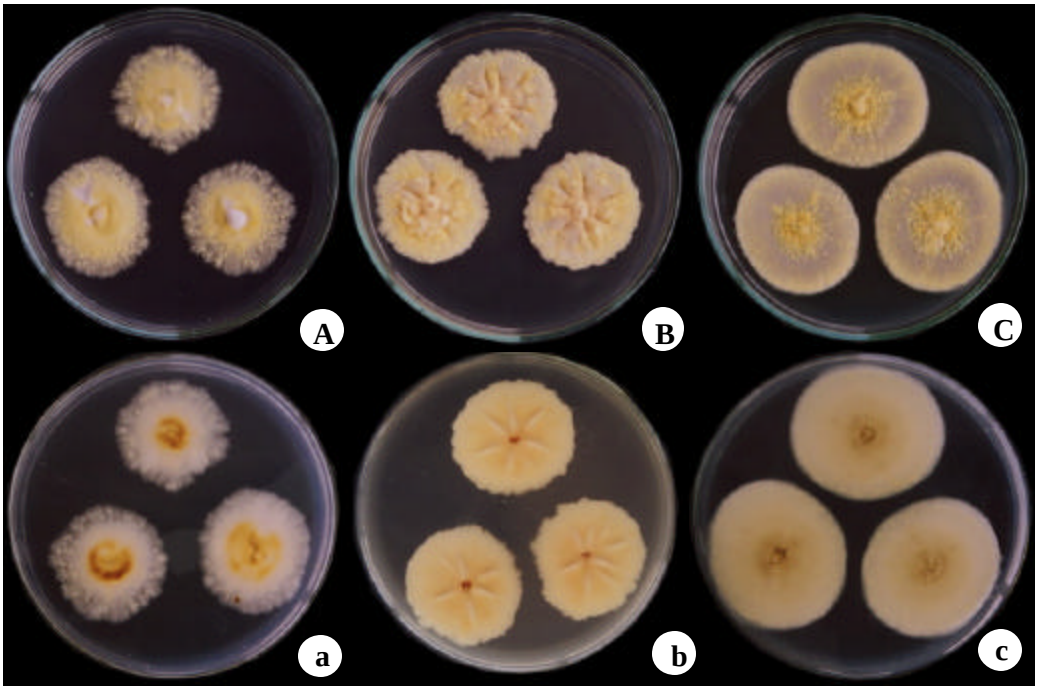


Figure 48 *Talaromyces wortmannii* KUFC 3333. Obverse and reverse views of colonies on different media, incubated for 7 days at 28°C; CZA (A, a), CYA (B, b), MEA (C, c)

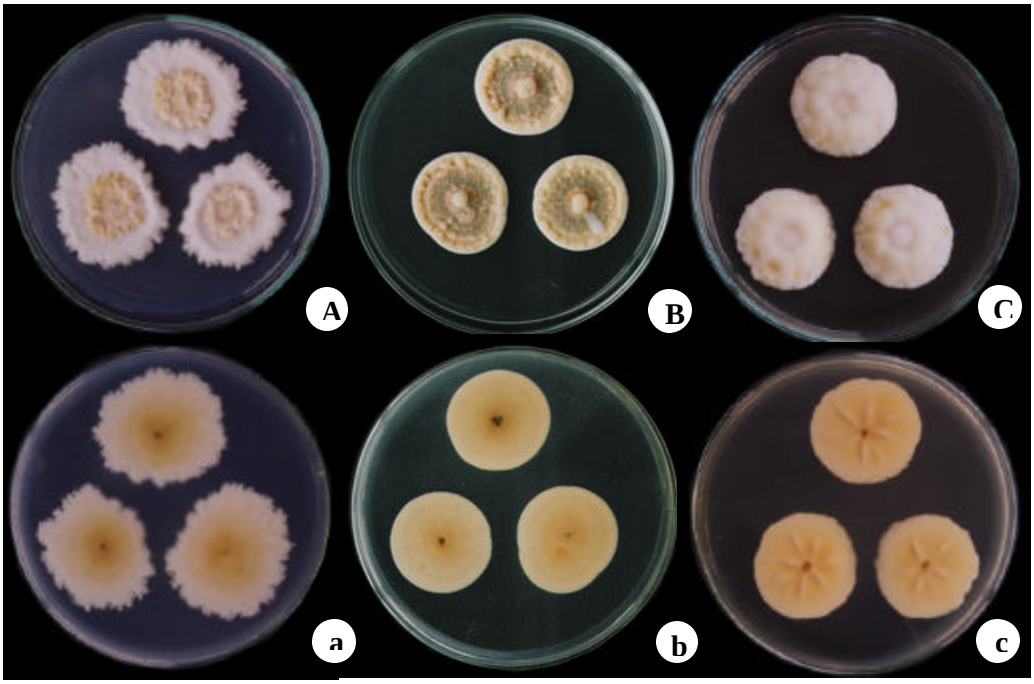


Figure 49 *Talaromyces wortmannii* KUFC 3354. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c)

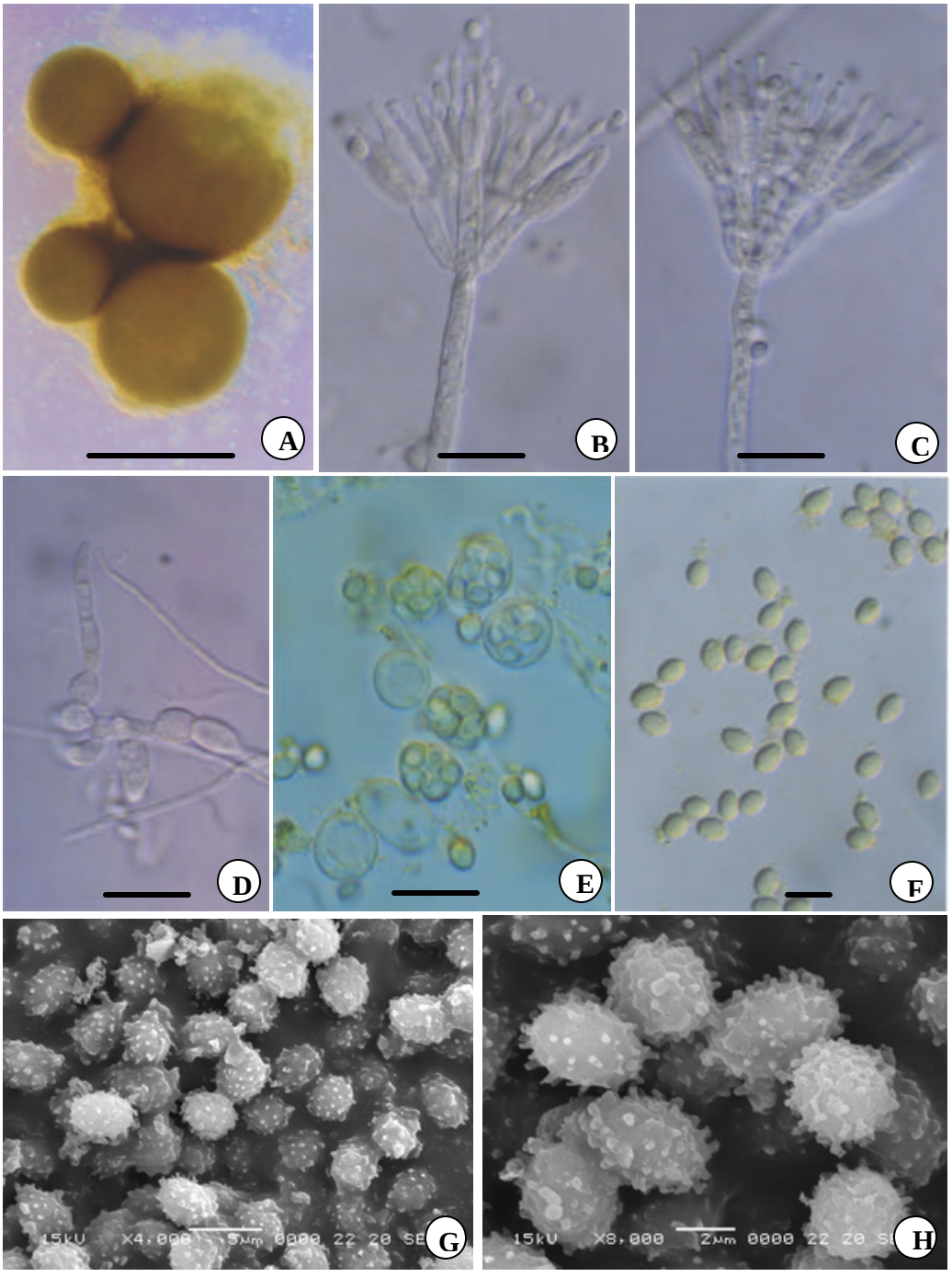


Figure 50 *Talaromyces wortmannii* KUFC 3333

A. ascomata; B-C. penicilli; D. ascomatal initials; E. asci and ascospores; F-H. ascospores (SEM)

(Bars: A = 200 µm; B-E = 10 µm; F-G = 5 µm; H = 2 µm)

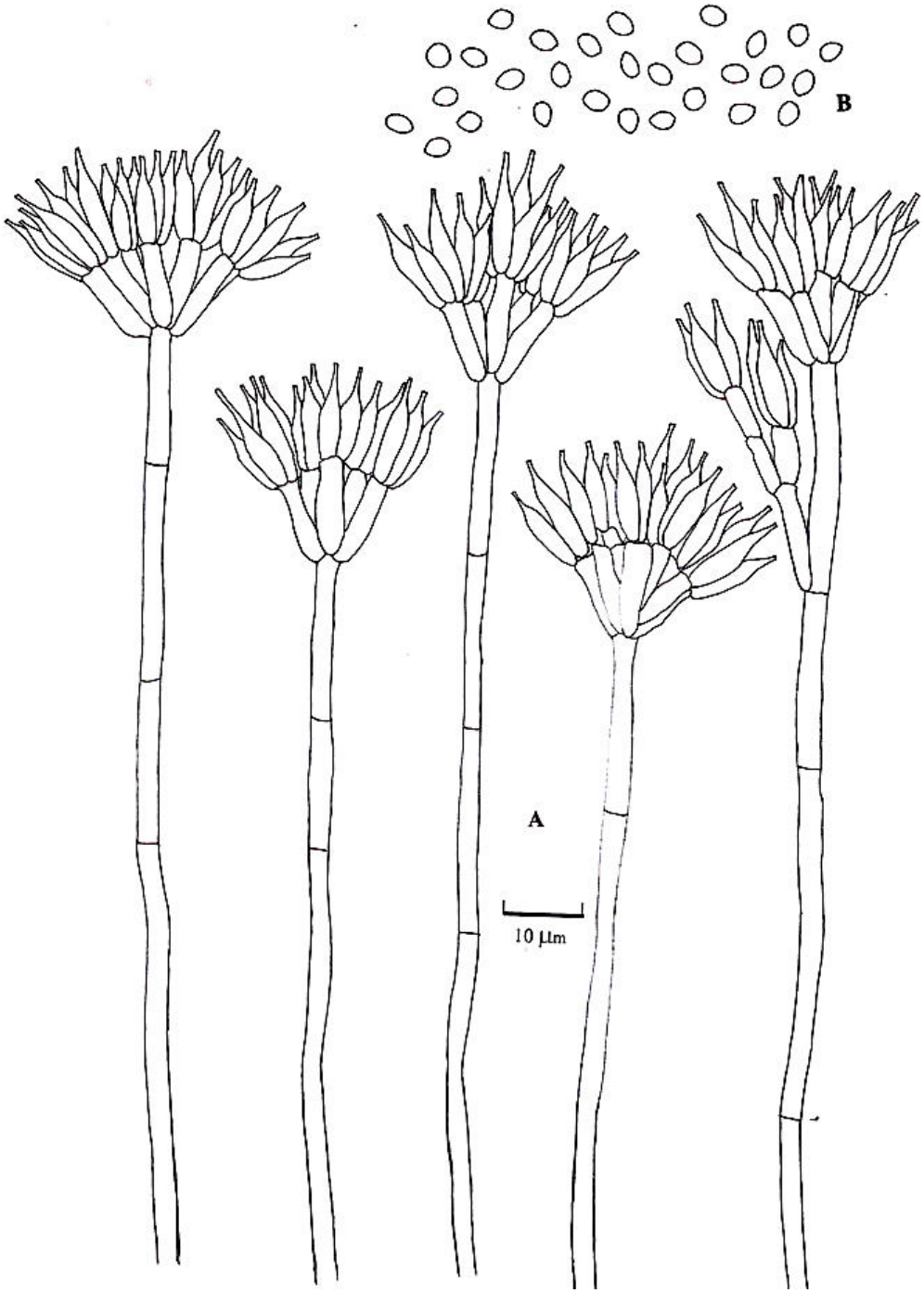


Figure 51 ***Talaromyces wortmannii*** KUFC 3333

Camera lucida drawings of A. penicilli and B. conidia

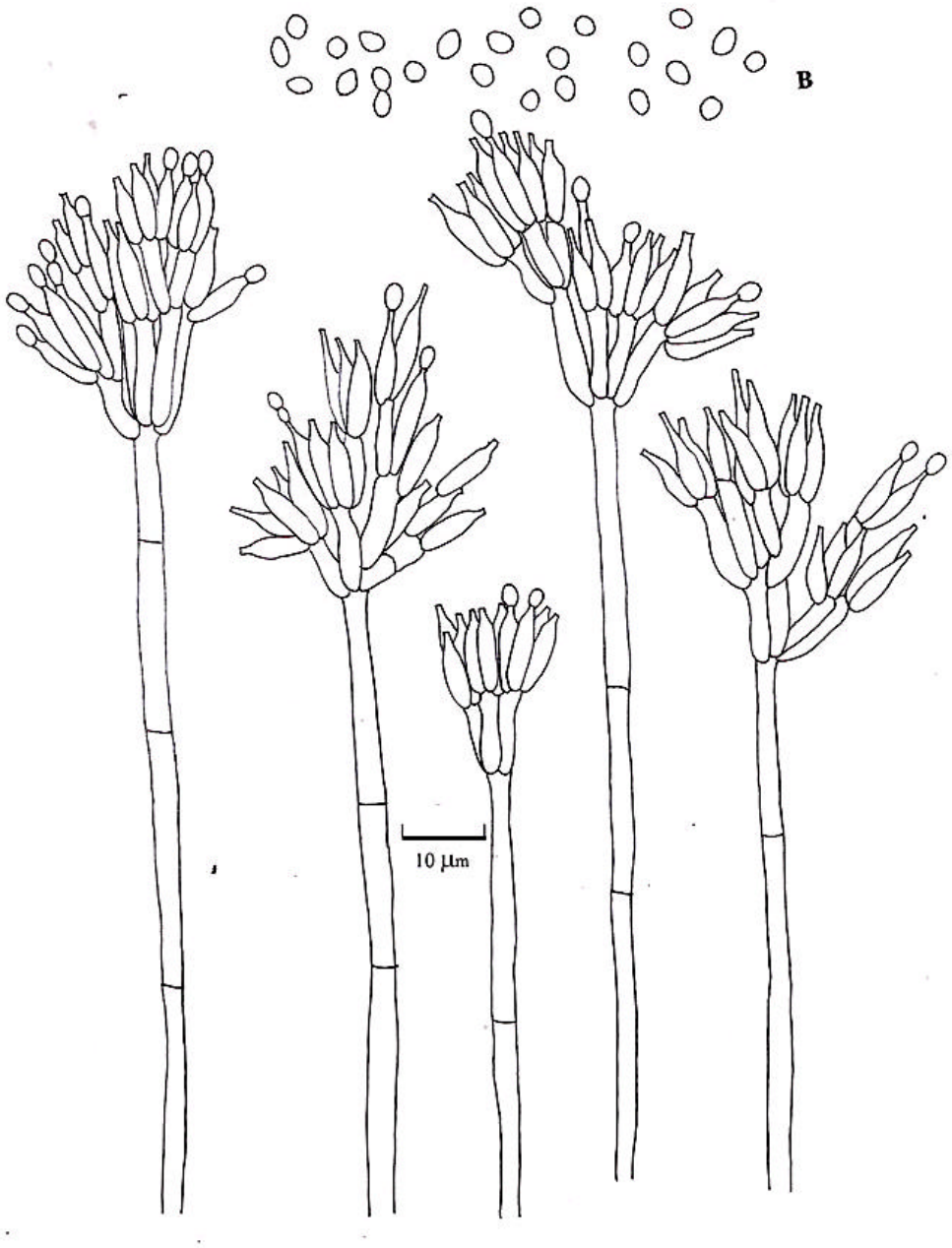


Figure 52 ***Talaromyces wortmannii*** KUFC 3354
Camera lucida drawings of A. penicilli and B. conidia

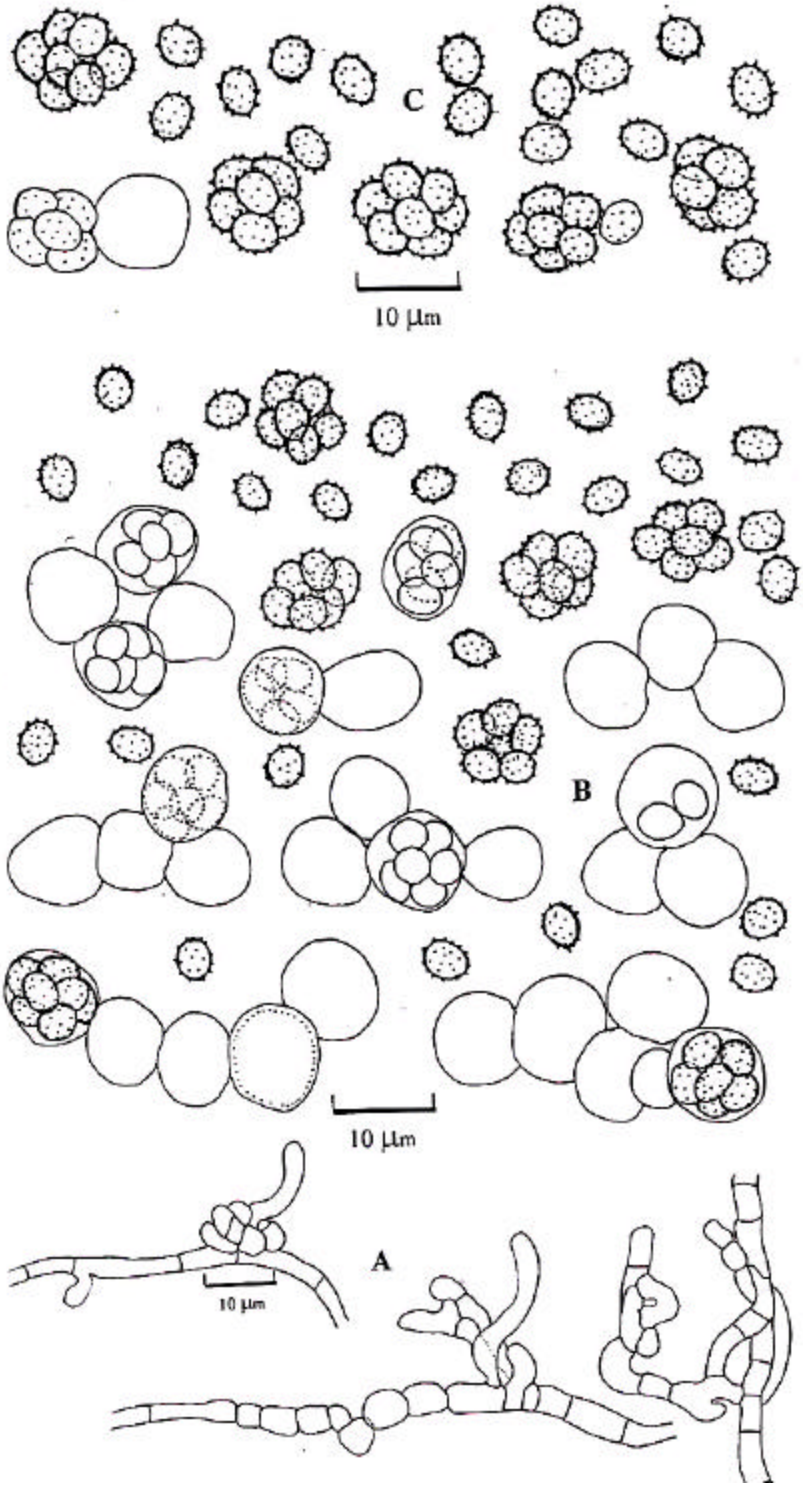


Figure 53 ***Talaromyces wortmannii***

Camera lucida drawings of A. ascomatal initials; B. ascus and ascospores KUFC 3333; C. ascus and ascospores KUFC 3354

12. *Talaromyces* sp. 1 (Figures 54-57)

Strain examined: KUFC 3399 forest soil, Trat

Reference: Stolk and Samson, 1972

Stat. Anam. *Penicillium* sp. 1

Colonies on CZA attaining a diameter of 20-22 mm within 7 days at 25°C, velvety, zonate, consisting of a thick basal felt, producing immature ascomata at central area, central area Apricot (R 42) or Umber (R 9), middle area Greenish Gray (R 110), margins Sulphur Yellow (R 15); conidiogenesis abundant; exudates absent; soluble pigment pale brown; margins entire; reverse Pure Yellow (R.14), with dark Amber (R 47) or Umber (R 9) in central area (Figures 54 A, a). Colonies on CZA at 28°C, attaining 25 mm and 35 mm in diameter within 7 and 14 days respectively, velvety, zonate, consisting of a thick basal felt, producing limited ascomata in central area, colored as colony on 25°C; conidiogenesis abundant; exudates absent; soluble pigment pale brown; margin entire; reverse Luteous (R12) to Umber (R 9) (Figures 55 A, a).

Colonies on CYA growing rapidly, attaining a diameter of 30-35 mm within 7 days at 25°C, velvety, plane, lightly radically sulcate, consisting of a compact basal felt which sparse aerial hyphae, Pale Luteous (R 11); ascomata absent; producing conidiogenesis abundant showing Pale Greenish Gray (R 123) color; exudates clear; reverse Umber (R 9) (Figures 54 B, b). Colonies on CYA at 28°C, reaching 35 mm and 45 mm in diameter within 7 and 14 days respectively, velvety, plane, sulcate, with central area raised up to 2-3 mm deep, consisting of a compact basal felt, producing only Luteous (R 12) aerial hyphae; ascomata very limited in number; conidiogenesis sparse and inconspicuous, Pale Greenish Gray (R 123); exudates clear; reverse Pale Luteous (R 11) to Apricot (R 42) (Figures 55 B, b).

Colonies on MEA growing moderately, attaining a diameter of 25-30 mm within 7 days 25°C, velvety, consisting of a thin basal felt, producing abundant conidiogenesis over the entire surface, Pale Olivaceous Grey (R 120); ascomata

limited; margins entire, broad and submerged; exudates absent; odor musty; reverse Straw (R 46) (Figures 54 C, c). Colonies on MEA at 28°C, reaching 30 mm and 45 mm in diameter within 7 and 14 days respectively, velvety, consisting of a thin basal felt, producing moderately ascomata at central area showing Pure Yellow (R 14) shade; conidiogenesis abundant, Pale Olivaceous Grey (R 120); margins entire; exudates absent; reverse Straw (R 46) to Luteous (R 12) (Figures 55 C, c).

Colonies on CMA growing rapidly, attaining a diameter of 30-35 mm within 7 days, 25°C, fasciculate, plane or somewhat zonate, consisting of a very thin basal felt, producing abundant ascomata in the central to subcentral areas, Pure Yellow (R 14) to Sulphur Yellow (R 15); conidiogenesis sparse and inconspicuous; exudates absent; reverse uncolored (Figures 55 D, d). Colonies on CMA at 28°C, growing rather rapidly, reaching 35 mm and 50 mm in diameter within 7 and 14 days respectively, plane, consisting of a very thin mycelial felt in which granular due to densely packed ascomata, Pure Yellow (R 14) to Sulphur Yellow (R 15); conidiogenesis sparse and conspicuous; exudates absent; reverse uncolored (Figures 55 D, d).

Colonies on OMA growing rapidly, attaining a diameter of 35-40 mm within 7 days, 25°C, plane, consisting of a thin basal felt, with surface appearing granular due to the production of abundant ascomata, Pure Yellow (R 14) to Sulphur Yellow (R 15); conidiogenesis moderately; exudates absent; reverse Luteous (R 12) (Figures 54 E, e). Colonies on CMA at 28°C, reaching 40 mm and 50 mm in diameter within 7 and 14 days respectively, plane, thin, with vegetative mycelium submerged, producing abundant ascomata intermixed with aerial hyphae and conidia, Pure Yellow (R 14) to Sulphur Yellow (R 15); exudates absent; reverse uncolored (Figures 55 E, e).

Colonies on G25N agar growing extremely slowly, attaining a diameter 10 mm and 13 mm within 7 days at 25°C and 28°C respectively, producing only sparse aerial growth (Figures 54-55 F, f).

Ascomata discrete or confluent, soft, non-ostiole, ripening within 14 to 21 days, subglobose to ellipsoidal, yellow to sulphur yellow, 200-540 µm in diameter. Ascomatal wall consisting of thin, branched, interwoven hyphae (Figure 56A). Ascomatal initials consisting of club-shaped ascogonia, around with thin antheridia coil tightly several times. At ascogonia apices, they produce dendroid or a few gnarled branches which continue to brance profused thus developing the ascogenous hyphae (Figures 56 D, 57 B). Asci in chains, 8-sporred, subglobose to globose, 7.5-9.5 µm, evanescent (Figures 56 E, 57 C). Ascospores hyaline, broadly ellipsoidal, 3.5-4 x 2-2.5 µm, spinulose (Figures 56 E-H, 57 C).

Conidiophores arising from the basal mycelium or aerial hyphae, erect; stipe hyalines 200-300 µm long, 3-3.5 µm wide. Penicilli often irregularly arranged, commonly biverticillate, rarely monoverticillate and terverticillate. Metulae appressed, in verticills of 4-6 (-8), 12-16.65 x 3.35-4.65 µm. Phialides lanceolate, 4-8 in the verticil, 10.5-8.6 x 2.6-4 µm, tapering to a pointed conidium-bearing tip (Figures 56 B-C, 57 A). Conidia hyaline, globose 2-3.3 µm, smooth-walled, born in chains (Figures 56 B-C, 57 A).

Talaromyces sp.1 (KUFC 3399) is closely related to *T. flavus* in the pattern of its ascomatal initials, size and morphology of the ascospores (Stolk and Samsom, 1972). But the two species differ significantly in the Pale Olivaceous Grey surface of producing abundance penicilli on both MEA and CZA, and typically biverticllate penicilli produced on relatively long conidiophores with globose conidia. *Talaromyces euchlorocarpius* somewhat resembles this species in the ascospores morphology and biverticillate penicilli, but differs in having greenish ascomata and the pattern of its ascomatal initials. This species is placed in the series *Flavi* of the section *Talaromyces* (Pitt 1979a) on the basis of growing rapidly on common media and its yellowish ascomata.

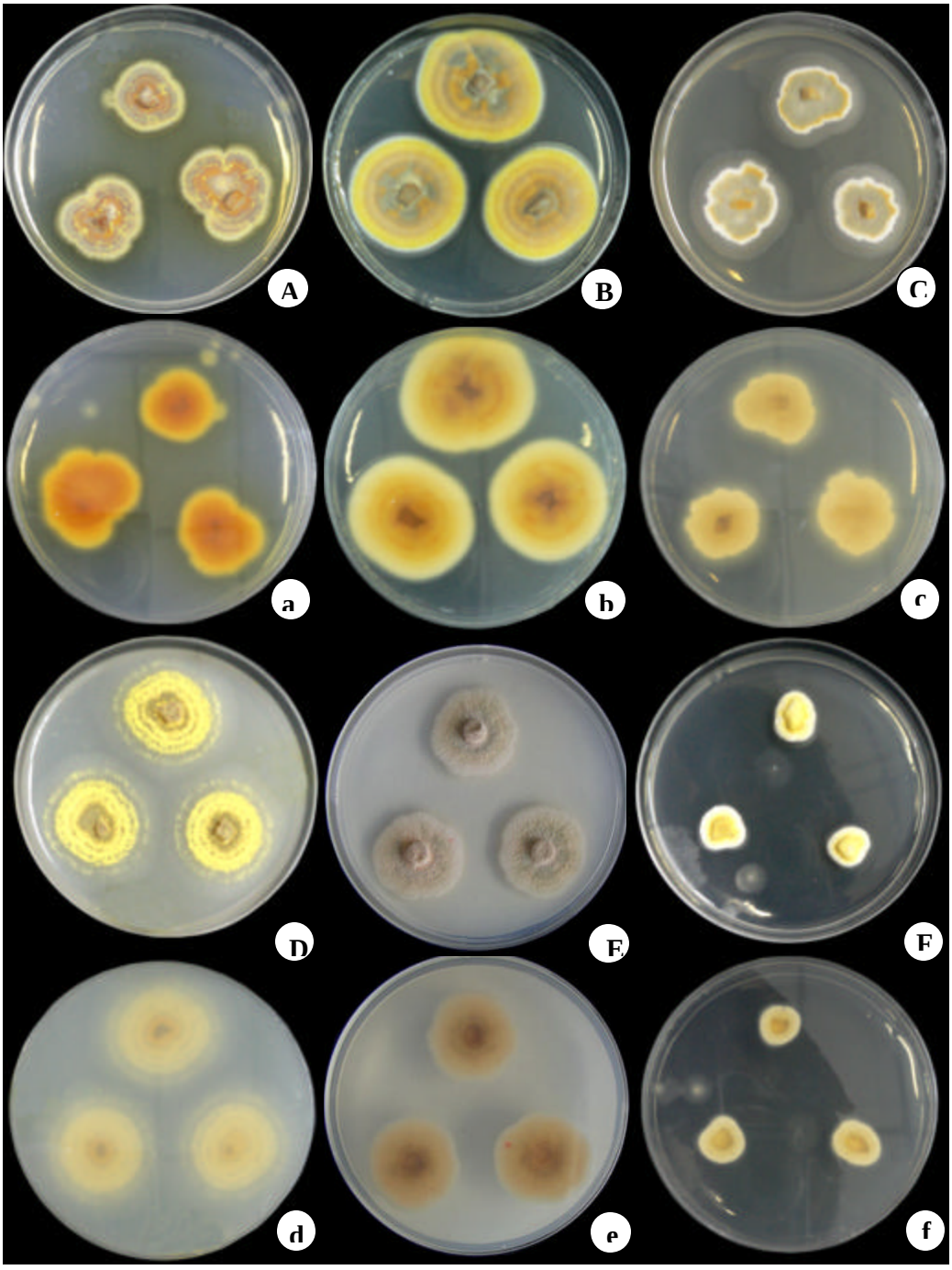


Figure 54 *Talaromyces* sp. 1 KUFC 3399. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

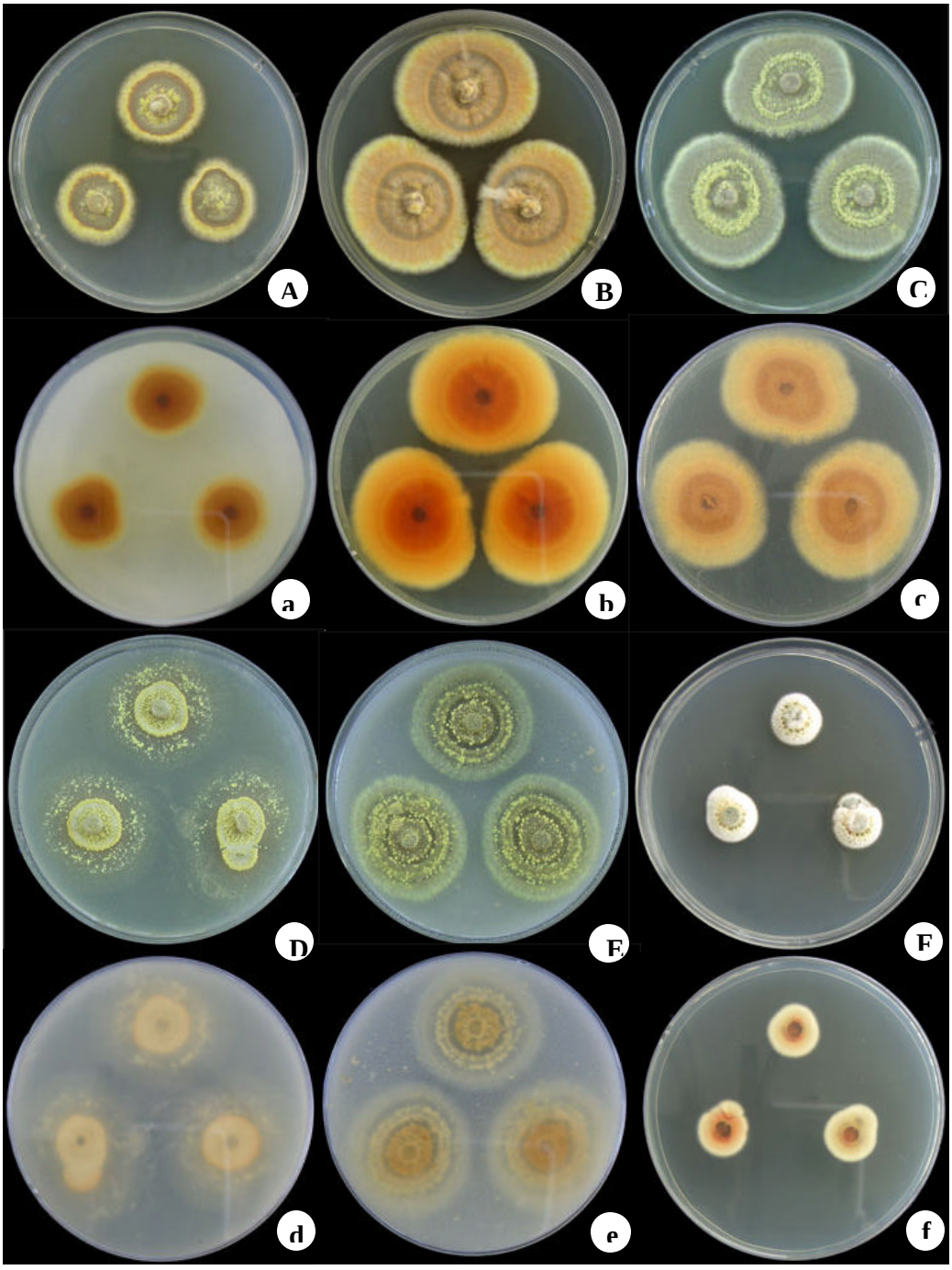


Figure 55 *Talaromyces* sp. 1 KUFC 3399. Obverse and reverse views of colonies on different media, incubated for 7 days at 28°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

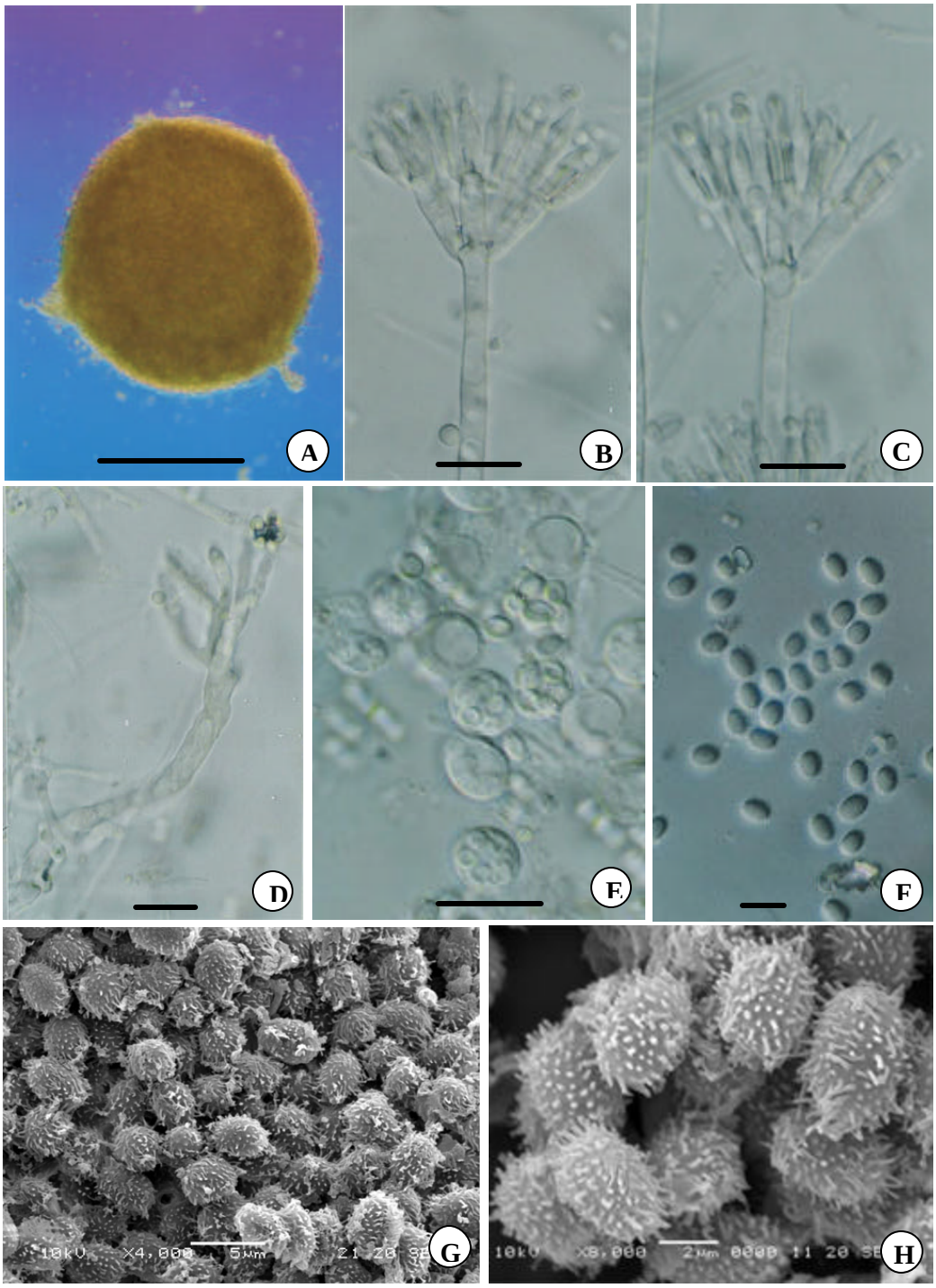


Figure 56 *Talaromyces* sp. 1 KUFC 3399

A. ascomata; B-C. Penicilli; D. ascomatal initial; E-F. asci and ascospores; G-H. ascospores (SEM)

(Bars: A = 200 µm; B-E = 10 µm; F-G = 5 µm; H = 2 µm)

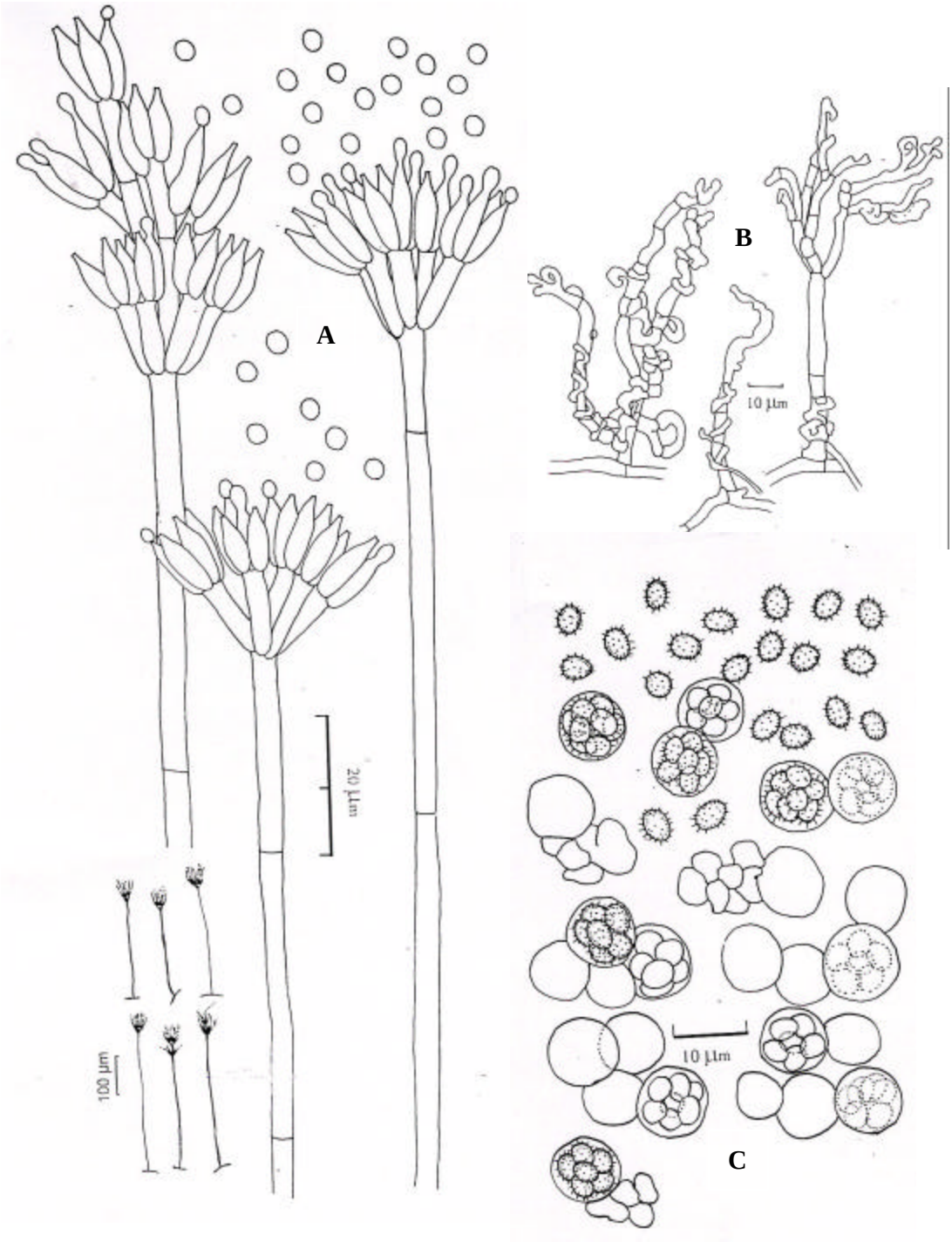


Figure 57 *Talaromyces* sp. 1 KUFC 3399

Camera lucida drawings of A. penicilli; B. ascomatal initials
C. asci and ascospores

13. ***Talaromyces*** **sp. 2** (Figures 58-60)

Strain examined: KUFC 3383 forest soil, Trat

Stat. Anam. ***Penicillium*** **sp. 2**

Colonies on CZA growing restrictively, attaining a diameter of 15-17 mm within 7 days at 25°C, velvety, plane, consisting of a thin basal felt in which abundant conidiogenesis, Pale Olivaceous Grey (R 120); ascomata limited; exudates absent; reverse Pale Luteous (R 11) (Figures 58 A, a).

Colonies on CYA grow ing restrictively, attaining 15 mm in diameter within 7 days at 25°C, umbonate, more or less wrinkled or radically furrow, raised in central area, consisting of a compact basal felt which abundant penicilli, Glaucus Grey (R 109) to Greenish Grey (R 110); ascomata absent; exudates clear; reverse Pale Luteous (R 11) (Figures 58 B, b).

Colonies on MEA growing moderately, attaining a diameter of 22-25 mm within 7 days 25°C, velvety to more or less funiculose, plane, slightly sulcate, consisting of a compact basal felt, producing moderately developing yellow ascomata in central area, Pure Luteous (R 11); conidiogenesis abundant produced at the periphery, Pale Greenish Grey (R 123); reverse Pale Luteous (R 11) to Luteous (R 12) (Figures 58 C, c).

Colonies on CMA growing moderately, attaining a diameter of 22-25 mm within 7 days at 25°C, consisting of a compact basal felt which surface appearing granular due to producing of abundant ascomata, Sulphur Yellow (R 15); conidiogenesis absent; exudates absent; reverse Pale Luteous (R 11), Luteous (R 12) to Umber (R 9) (Figures 58 D, d).

Colonies on OMA and G25N agar growing slowly, attaining a diameter of 15-18 mm within 7 days at 25°C, umbonate, zonate, consisting of a compact basal felt, producing only Pale Luteous (R 11) mycelium; ascomata moderately; conidiogenesis absent; exudates abundant; reverse uncolored (Figures 58 D-F, d-f).

Ascomata confluent or occasionally discrete, soft, non-ostiole, ripening within 14 days, subglobose to ellipsoidal, yellow, (170-) 200-300 µm in diameter, ascomatal wall composed of a loose network of branched, yellow, interwoven, septate, thick-walled and the smooth-walled hyphae (Figure 59 A). Ascomatal initials distinct, started as side branches of the swollen aerial hyphae, composed of large, terminally looped, swollen, septate hyphae, developing into loose coils in a helical pattern (Figures 59 D, 60 C). Asci in chains, 8-spored, subglobose to globose, 10 x 8-10 µm, evanescent (Figure 60 D). Ascospores pale yellow, broadly ellipsoidal, 4-4.5 x 3.5-3.8 µm, microtuberculate to tuberculate (Figures 59 E-G, 60 D).

Conidiophores arising from the basal mycelium or aerial hyphae, erect, stipe hyalines (100-)120-200 µm, Penicilli typically biverticillate, rarely monoverticillate or terverticillate. Metulae in verticils of 4-6, 11.33-13 x 3-3.5 µm. Phialides lanceolate, 3-6 in the verticil, 10-13.5 x 2.5-3 µm, with long collula (Figures 59 B-C, 60 A). Conidia hyaline, ellipsoidal, ovoidal to pyriform, 3-4.5 x 2-3 µm, smooth-walled, born in chains (Figures 59 B-C, 60 B).

From characteristics of growth rates, pure yellow to sulphur yellow colony and short swollen initials of the ascomata indicate *Talaromyces* sp. 2 (KUFC 3383) belongs in the series *Lutei* of the section *Talaromyces* (Pitt, 1979a). Growth rates, ascomatal initials pattern, ascomata size of *Talaromyces* sp. 2 (KUFC 3383) are similar *T. wortmannii* var. *wortmannii* and *T. wortmannii* var. *sublevisporus*, but differ in colony morphology. Colonies of both varieties of *T. wortmannii* are more or less floccose on CYA and MEA, while colonies of *Talaromyces* sp. 2 (KUFC 3383) are plane and velvety, which having abundant developing ascomata and penicilli. This fungus is more similar *T. wortmannii* var. *sublevisporus* in its shape and ornamentation of ascospores, but differs in having much larger ascospores and variable size of tuberculate on ascospores-wall.

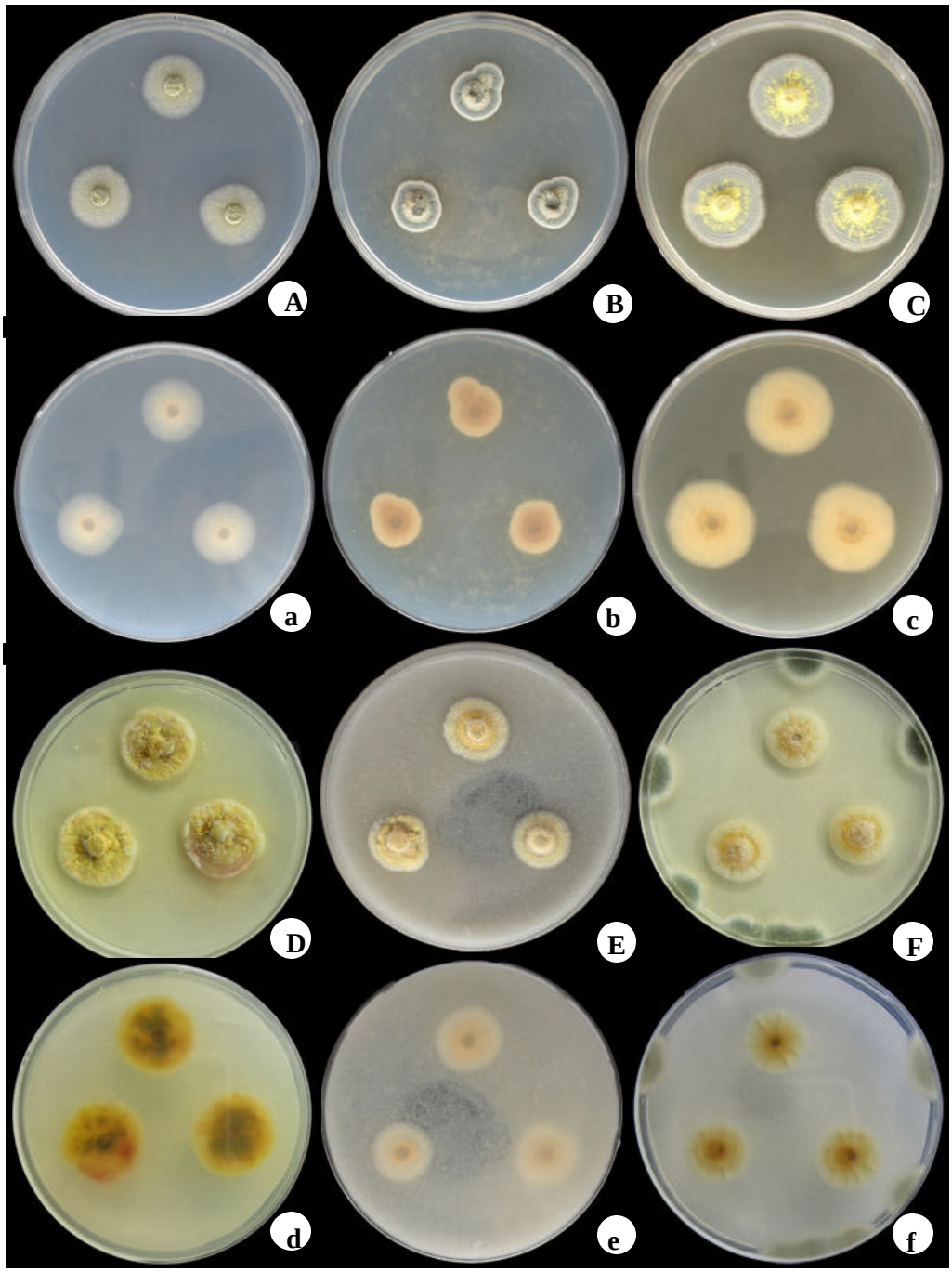


Figure 58 *Talaromyces* sp. 2 KUFC 3383. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

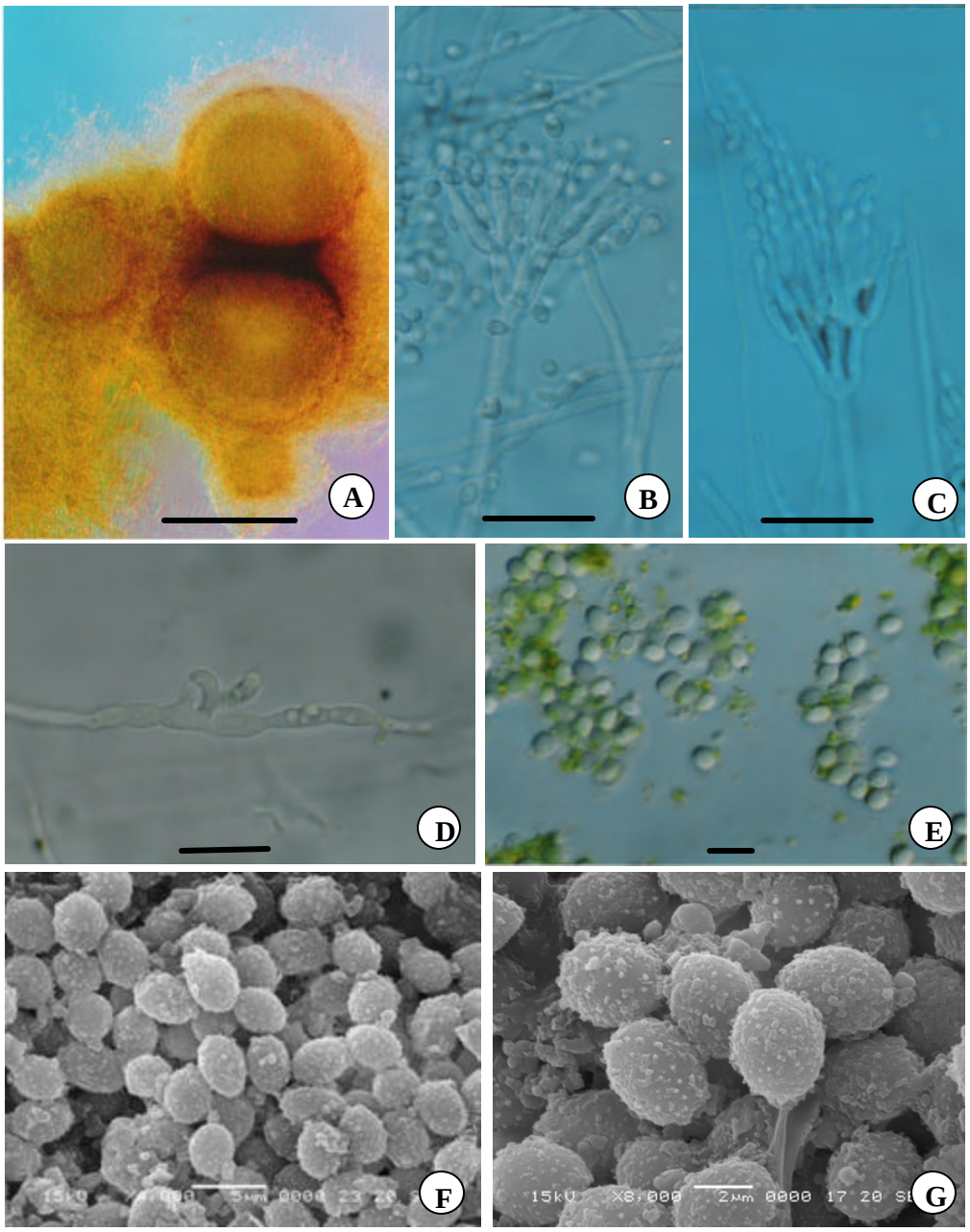


Figure 59 ***Talaromyces* sp. 2** KUFC 3383

A. ascomata; B-C. Penicilli; D. ascomatal initial; E. ascospores

F-G. ascospores (SEM)

(Bars: A = 200 µm; B-D = 10 µm; E-F = 5 µm; G = 2 µm)

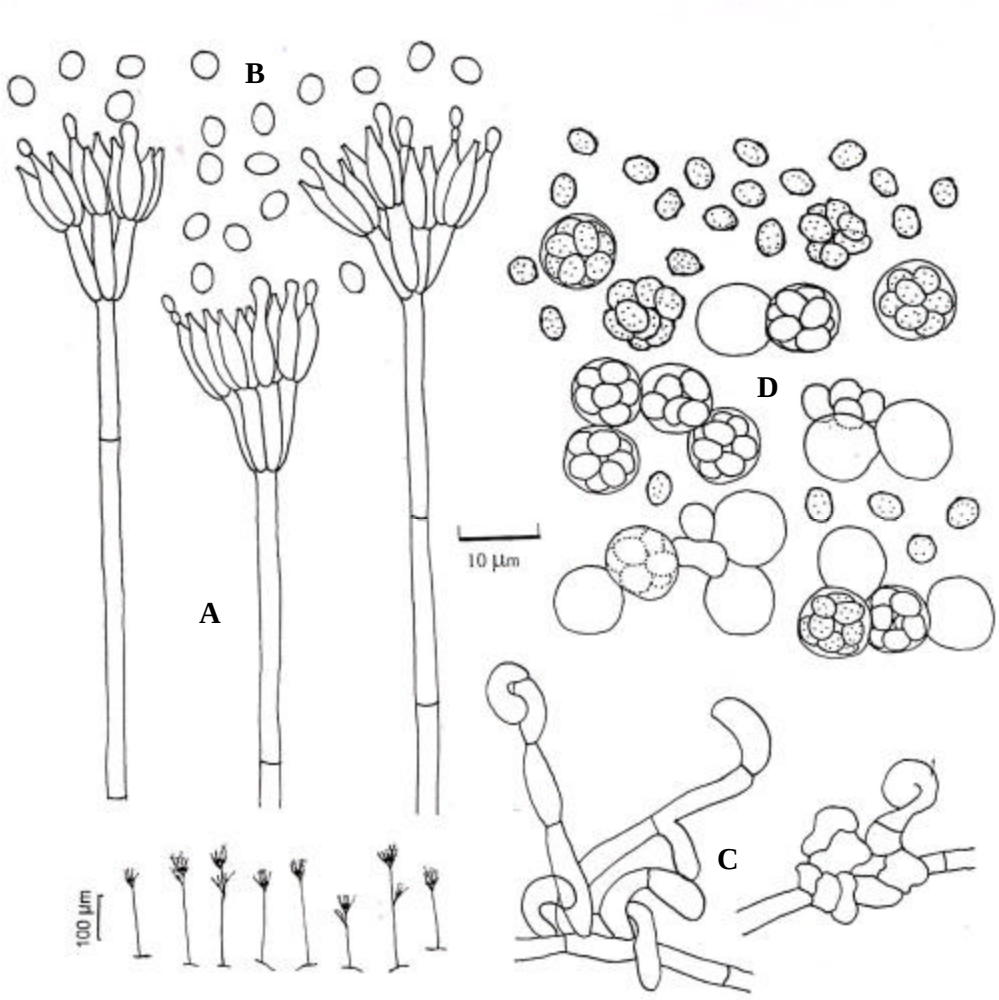


Figure 60 *Talaromyces* sp. 1 KUFC 3383

Camera lucida drawings of A. penicilli; B. conidia; C. ascomatal initials D. asci and ascospores

Thirteen species of *Talaromyces* have been reported consisting *Talaromyces austrocalifornicus, T. bacillisporus, T. flavus, T. macrospermus, T. helicus* var. *major, T. indigoticus, T. luteus, T. rotundus, T. stipitatus, T. trachyspermus, T. wortmannii* and two unidentified species (KUFC 3399, KUFC 3383). Two species are new recorded for Thailand (*Talaromyces austrocalifornicus* and *T. indigoticus*), whereas the other two unidentified species (KUFC 3383 and 3399) do not resemble to any described species. Pitt, (1979a) was used morphological study on standard media as the growth rate, color, texture, and temperature at 5°C, 25°C, 37°C for 7 days for identified species. In this study, the temperature range at 25°C, 28 °C and 37°C were employed. It was found that most species grew well at 28°C for 7 or 14 days and produced abundant ascomata within 10-14 days at 28°C. No growth occurred at 37°C, except *Talaromyces bacillisporus, T. indigoticus* and *T. stipitatus*.

Morphological characteristics in term of sizes and shapes of ascomata, asci, ascospores, penicilli and conidia are resemble or close to those described by previous reported (Stolk and Samson, 1972; Pitt, 1979a; Takada and Udagawa, 1988; Yaguchi *et al.*, 1992; Yaguchi *et al.*, 1993a, b; Udagawa, 1993; Yaguchi *et al.*, 1994a, b; Yuguchi *et al.*, 1996; Udagawa *et al.*, 1993; Udagawa and Suzuki, 1994). In some case, only slightly different in size and shape of ascospores, for example *Talaromyces bacillisporus, T. rotundus* and *T. wortmannii*. In addition, *Talaromyces flavus* and *T. macrosporus* were the dominant species and were found from all soil samples. They are also extremely variable species, in color and texture of colonies, in the amount of red pigment produced, in the number of penicilli and ascomata, and in size and color of ascospores.

3. Phylogenetic study of *Talaromyces*

Phylogenetic analyses were conducted using polymorphic microsatellites of 21 fungi comprising 18 species of *Talaromyces* and 3 other Trichocomaceae isolated in Thailand (Figures 61-62). *Talaromyces* species included in the analysis comprise of species representing the 2 sections, section *Talaromyces* and section *Emersonii*, 3 series, series *Flavi*, series *Lutei* and series *Trachyspermus* as defined by Stolk and Somson, 1972, and Pitt, 1979a (Table 4).

The analysis of the phylogenetic relationships among *Talaromyces* and other Trichocomaceae indicated that the majority of *Talaromyces* species clustered in one major clade with minor branch supported by 100% of the bootstrapped data set.

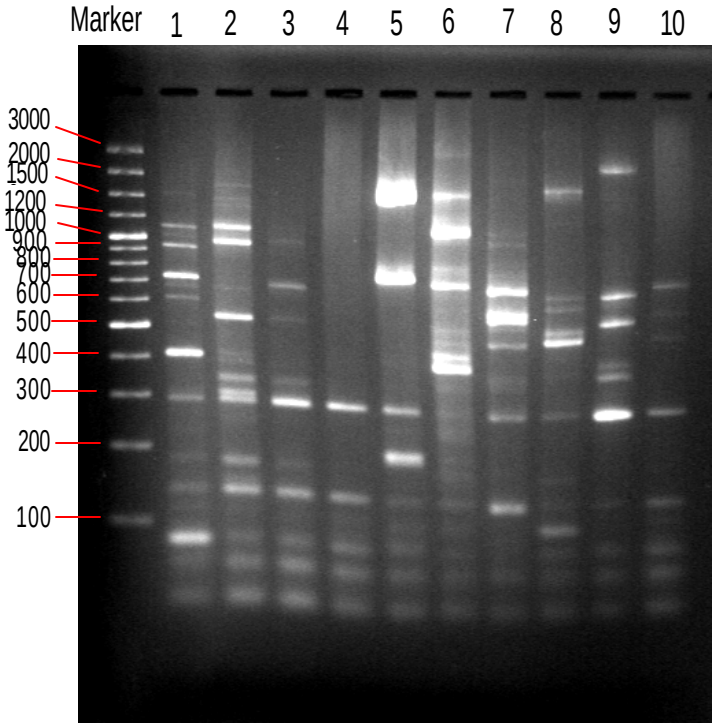


Figure 61 Electrophoresis of PCR products using PM7
1 = *T. bacillisporus* 2 = *T. austrocalifornicus* 3 = *T. macrosporus*
4 = *T. stipitatus* 5 = Trichocamaceae (*Penicillium* anamorph)
6 = *Talaromyces* sp. 1 7 = *T. roduntus* KUFC 3446 8 = *T. wortmannii*
9 = *T. flavus* 10 = *T. rotundus* KUFC 3359
M = marker 1kb 100bp ladder plus (Fermentas)

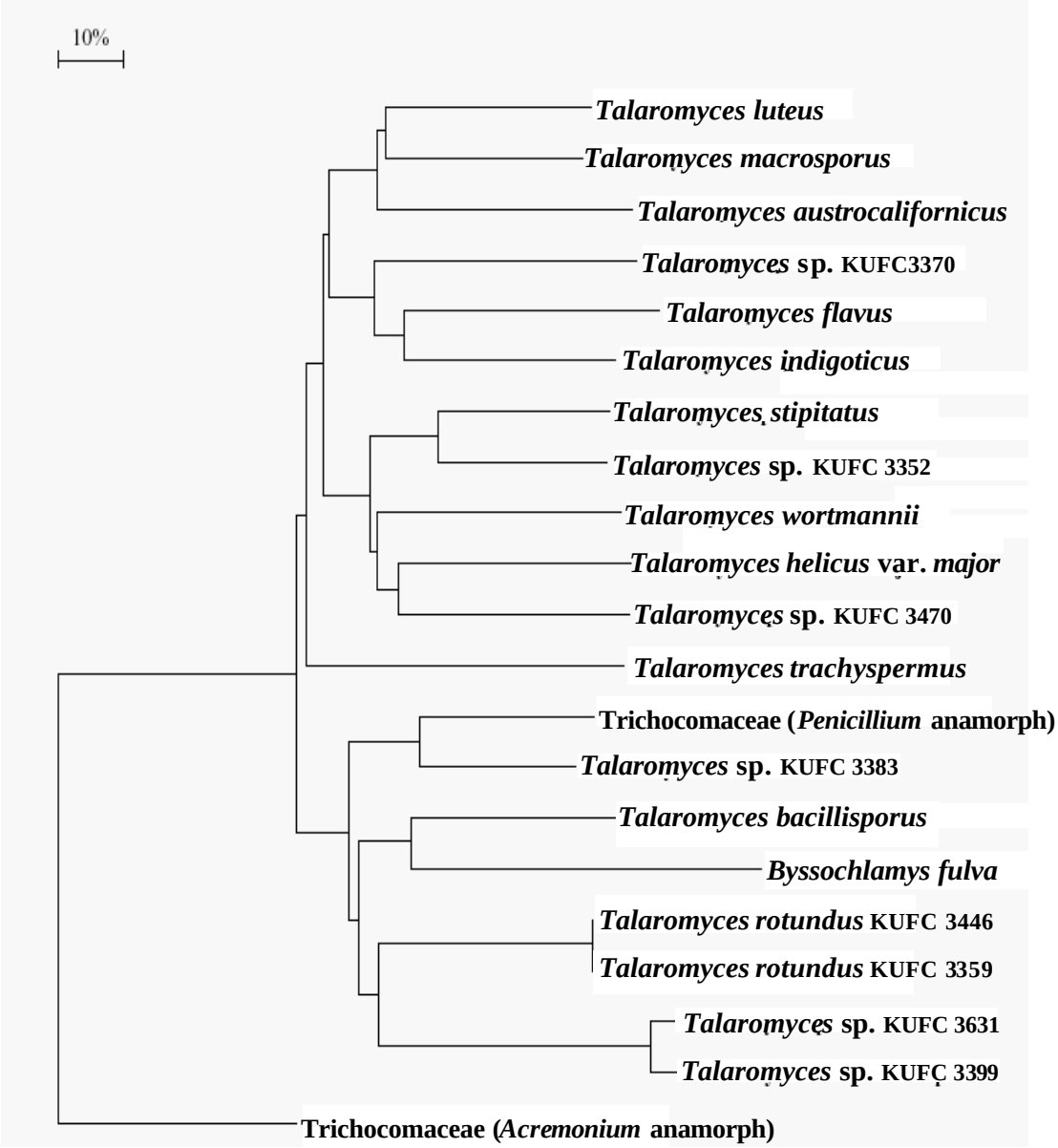


Figure 62 Phylogenetic relationships of *Talaromyces* species and related Trichocomaceae based on SSR gene

In the monographic treatment by Stolk and Samson (1972), *Talaromyces* comprised 4 sections with 18 species, most of which were described as having a *Penicillium* anamorph, but which also included two species with a *Paecilomyces* state. In his treatise on the genus *Penicillium*, Pitt (1979a) divided the 16 *Talaromyces* species with *Penicillium* anamorphic states into three sections and five series including section *Talaromyces* (series *Flavi* Pitt; *Lutei* Pitt and *Trachyspermus* Pitt), section *Purpureus* Stolk et Samson (series *Purpurei* Pitt) and section *Thermophilus* Stolk et Samson (series *Thermophili* Pitt).

The classification of species in 3 series of section *Talaromyces* (Pitt, 1979a) does not fully correlate with phylogenetic analysis of the data in this study, because species of same series do not cluster together (Figure 62). Among the 5 *Talaromyces* species in the series *Flavi* and 3 species in the series *Lutei* shared the clade together. The other two species within the series *Lutei*, *T. roduntus* and the unidentified species, *Talaromyces* sp. KUFC 3399 were found on same clade which occupies a basal position to the main *Talaromyces* clade. The SSR phylogeny of my study included species of section *Talaromyces* and section *Emersonii*, with *Penicillium* and *Geosmithia* anamorphs. The results also showed no correlated of the divisions with the phylogenetic analysis, similar to findings of LoBuglio *et al.* (1993) and Luangsa-ard (2004).

Interestingly, *Talaromyces flavus* and *T. macrosporus* form different sub-clade. based on the phylogenetic tree. These two holomorphic species are difficult to be distinguished according to morphological characters with in the series *Flavi*. The key differences between them are the size of ascospores. First, *Talaromyces macrosporus* had been treated as a variety of *T. flavus*, then gained the species status by its different heat-resistance and secondary metabolites (Frisvad *et al.*, 1990). But even though with these differences, they are very closely related in many respects and should be in the same clade rather than in different ones. The morphology of *Talaromyces* sp. KUFC 3399 is very similar *T. flavus* and *T. macrosporus* but also lies in the different clade. This is prelimentary test base open SSR which did not involve in sequences analtsis.

Talaromyces trachyspermus is a distinctive species according to morphological and molecular characters. It has white to creamish mycelia and ascomata consisting of closely interwoven hyphae, while other, are usually bright colored-yellow, or neatly so; its initials are swollen hyphae producing gnarled branches. This species is also well separated from others in the studied of LoBuglio and Taylor (1993).

Trichocomaceae (*Penicillium* anamorph) has several distinctive morphological characteristics relative to other *Talaromyces* species (including production of a pale brown conidiophore with big and tuberculate ascospores. It was also clustered with *Tala romyces* sp. KUFC 3383 that has strictly Biverticilllium *Penic illium*-anamorph with 5% distance. *Byssochlamys fulva* (*Paecilomyces* anamorph) is found on the same clade with *T. bacillisporus* that has a *Geosmithia*- anamorph.

4. Antagonism against plant pathogenic fungi by *Talaromyces*

4.1 *In vitro* inhibition growth of plant pathogenic fungi

Twenty isolates of *T. flavus* effectively inhibited mycelial growth of the three Oomycetes plant pathogenic fungi including *Phytophthora palmivora*, *P. parasitica* and *Peronophythora litchii* on PDA, at 28°C, but failed to inhibit *Pythium aphanidermatum in vitro* (Table 10, Figures 63-64).

Twenty isolates of *Talaromyces flavus* inhibited mycelial growth of the three Coelomycetes plant pathogenic fungi including *Colletotrichum capsici*, *C. gloeosporioides* and *Pestalotiopsis guepinii*, but could not control *Lasiodiplodia theobromae in vitro* (Table 11, Figures 63-64).

Percent inhibition of mycelial growth for the five Hyphomycetes plant pathogenic fungi by *T. flavus* strains varied with the plant pathogen by isolate combination tested. Twenty isolates inhibited more than 70% of the radial growth of *Helminthosporium maydis* and *H. oryzae*. Two isolates of *T. flavus*, KUFC 3530 and KUFC 3528, provided 69 % inhibition of the radial growth of *Curvularia lunata* (Table 12), whereas eighteen 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 12, Figures 63-64). However, twenty isolates of *T. flavus* did not inhibit plant pathogenic fungi in the Class Agonomycetes, *Sclerotium rolfsii* and *Rhizoctonia solani in vitro*.

Table 10 Percent inhibition on mycelial growth of four oomycetous plant pathogenic fungi by twenty isolates of *Talaromyces flavus* cultivated on PDA as dual culture at 28 ºC for 14 days

Talaromyces flavus KUFC	**Inhibition (%)**			
	Phytophthora palmivora	*Phytophthora parasitica*	*Pythium aphanidermatum*	*Peronophythora litchii*
3334	76.92	75.00	10.27	77.50
3363	75.00	74.62	19.20	76.27
3381	78.05	70.59	0*	76.27
3388	78.05	75.00	9.45	75.00
3395	78.75	77.32	0	77.50
3397	69.56	72.50	0	75.32
3400	75.20	76.65	0	74.78
3485	72.39	71.26	7.32	75.00
3446	70.79	68.45	4.67	78.32
3450	77.27	75.00	0	76.47
3473	81.93	71.26	0	79.51
3483	70.59	70.59	0	75.55
3501	76.47	70.59	11.89	70.58
3506	70.45	67.77	4.78	68.89
3508	73.78	73.65	0	74.34
3523	83.24	79.56	0	80.45
3525	78.22	75.00	0	76.16
3528	80.69	80.48	12.80	79.54
3530	79.01	77.50	19.24	79.50
3550	75.02	73.76	7.42	75.00

* plant pathogenic fungi overgrew the colony of *T. flavus*

Table 11 Percent inhibition on mycelial growth of five coelomycetous plant pathogenic fungi by twenty isolates of *Talaromyces flavus*, each isolate of *T. bacillisporus* and *Talaromyces* sp. 1 (KUFC 3399) cultivated on PDA as dual culture test at 28 °C for 14 days

Talaromyces flavus KUFC	**Inhibition (%)**				
	Colletotrichum capsici	*Colletotrichum gloeosporioides*	*Pestalotiopsis quepinii*	*Lasiodiplodia theobromea*	*Phyllosticta* sp.
3334	70.56	65.45	55.46	10.34	79.01
3363	69.56	60.34	52.32	0*	62.75
3381	70.45	65.00	50.54	0	63.64
3388	67.03	68.75	51.12	0	64.80
3395	67.76	65.77	52.38	0	72.73
3397	69.50	65.89	42.78	0	68.18
3400	71.35	67.34	59.31	7.32	63.78
3485	72.78	65.00	52.44	0	60.77
3446	73.45	66.07	54.80	0	65.19
3450	74.24	66.67	55.00	0	60.67
3473	76.49	69.57	50.38	6.75	57.89
3483	77.06	66.67	55.90	7.32	67.52
3501	75.67	67.06	60.67	7.34	72.41
3506	67.34	55.00	58.40	0	77.49
3508	74.00	58.71	57.47	10.45	72.84
3523	76.88	68.31	59.61	0	85.00
3525	74.19	65.00	61.33	0	73.65
3528	78.36	67.41	62.32	11.40	80.00
3530	79.45	69.90	60.82	0	79.11
3550	70.22	67.34	60.34	0	70.00
T. bacillisporus	70.87	60.45	55.56	0	68.32
Talaromyces sp. KUFC 3399	70.13	55.89	54.78	0	65.19

* plant pathogenic fungi overgrew the colony of *T. flavus*

Table 12 Percent inhibition on mycelial growth of four hyphomycetous plant pathogenic fungi by twenty isolates of *Talaromyces flavus*, each isolate of *T. bacillisporus* and *Talaromyces* sp. 1 (KUFC 3399) cultivated on PDA, as dual culture at 28 ^{o}C for 14 days

Talaromyces flavus KUFC	**Inhibition (%)**			
	Curvularia lunata	*Fusarium oxysporum* f.sp. *lycopersici*	*Helminthosporium maydis*	*Helminthosporium oryzae*
3334	60.00	-	72.45	70.67
3363	54.67	67.82	70.31	-
3381	65.54	71.21	75.34	72.32
3388	60.42	72.32	70.69	70.55
3395	62.00	73.33	72.51	71.89
3397	65.37	-	73.39	70.34
3400	60.36	75.14	74.27	75.98
3485	61.19	75.06	76.43	76.14
3446	55.34	70.69	70.42	75.89
3450	57.38	-	78.36	74.56
3473	61.45	77.14	75.56	76.78
3483	62.41	75.85	76.64	-
3501	60.37	-	75.00	74.34
3506	56.11	68.71	74.80	72.14
3508	66.91	75.11	75.67	70.45
3523	-	76.02	78.65	77.50
3525	65.90	-	79.21	76.76
3528	69.20	79.31	79.88	79.08
3530	69.39	78.35	80.31	78.32
3550	65.03	75.26	79.41	70.77
T. bacillisporus	60.59	73.39	70.46	-
Talaromyces sp. KUFC 3399	58.45	76.91	72.54	61.90

- Contamination

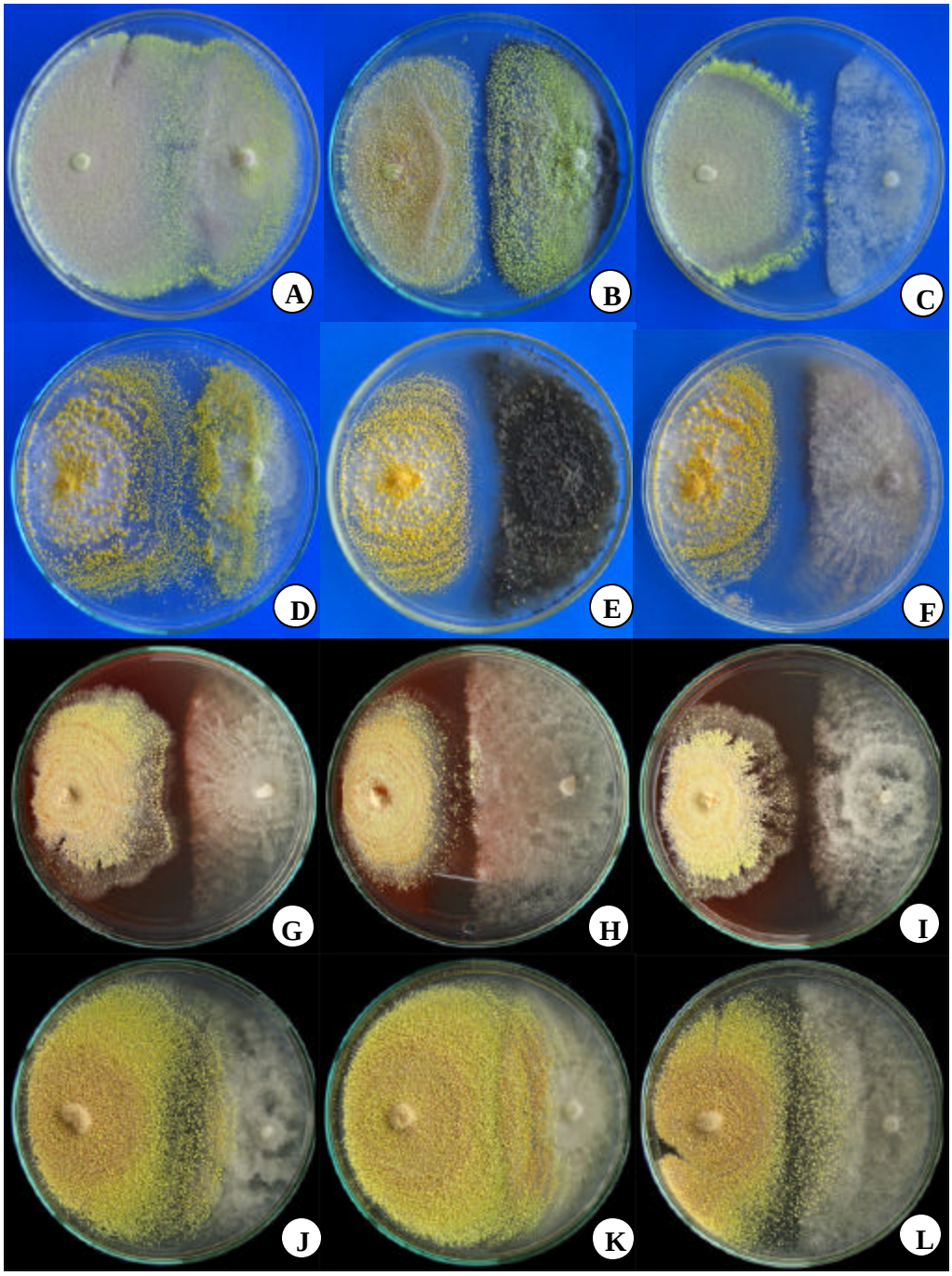


Figure 63 Antagonistic tests as dual cultures of different *Talaromyces flavus* isolates (left) and plant pathogenic fungi (right) on PDA incubated for 14 days at 28°C

T. flavus (KUFC 3523) vs *Phytophthora palmivora* (A), *Curvularia lunata* (B), *P. parasitica* (C)

T. flavus (KUFC 3528) vs *Phytophthora palmivora* (D), *Colletotrichum capsici* (E), *Fusarium oxysporum* (F)

T. flavus (KUFC 3531) vs *Phytophthora palmivora* (G), *P. parasitica* (H), *Peronophythora litchii* (I)

T. flavus (KUFC 3334) vs *Phytophthora palmivora* (J), *P. parasitica* (K), *Peronophythora litchii* (L)

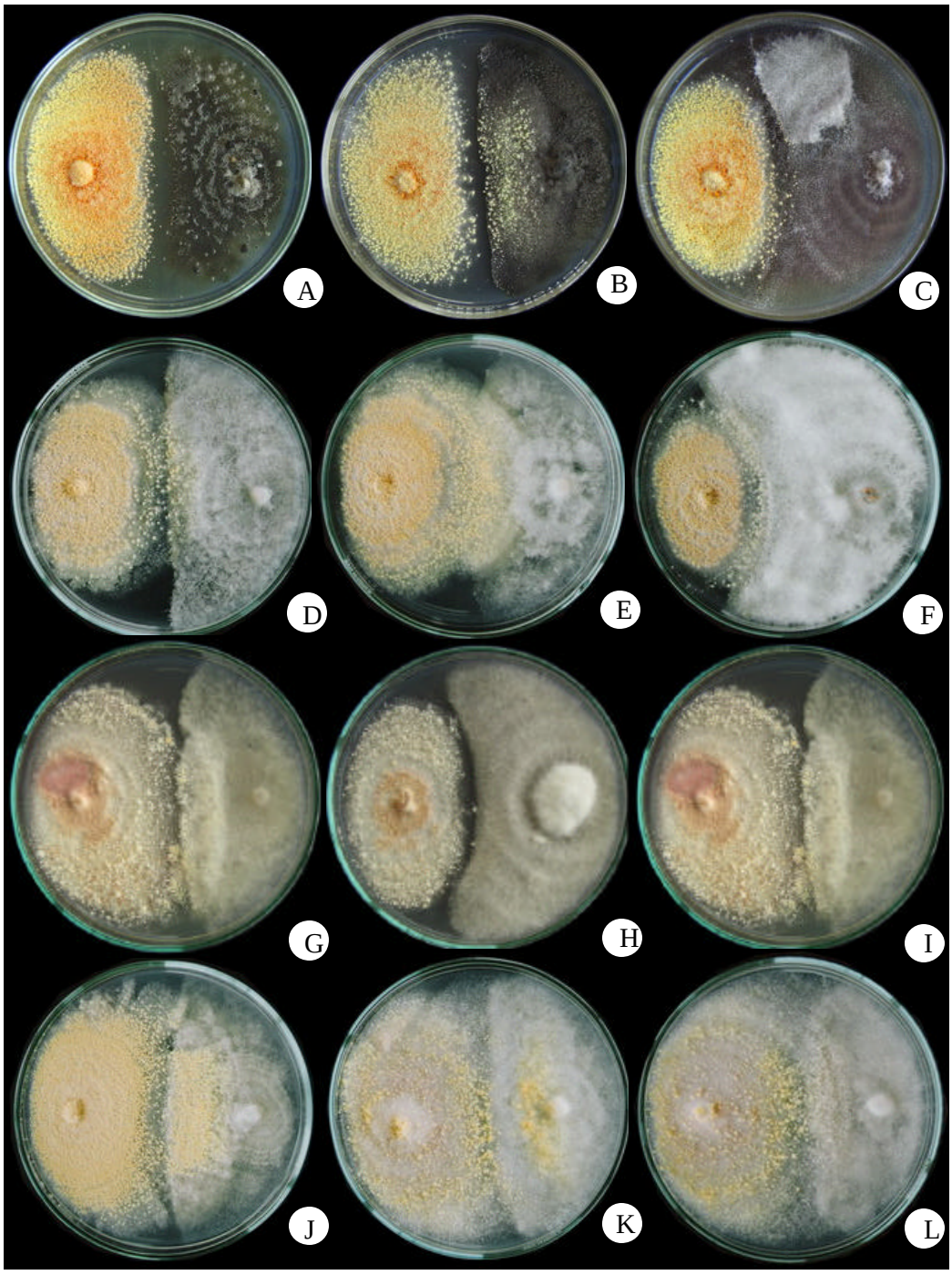


Figure 64 Antagonistic tests as dual cultures of different *Talaromyces flavus* isolates (left) and plant pathogenic fungi (right) on PDA incubated for 14 days at 28°C

T. flavus (KUFC 3363) vs *Colletotrichum capsici* (A), *Phyllosticta* sp. (B), *Fusarium oxysporum* (C)

T. flavus (KUFC 3395) vs *Phytophthora palmivora* (D), *P. parasitica* (E), *Colletotrichum gloeosporioides* (F)

T. flavus (KUFC 3450) vs *Phytophthora palmivora* (G), *Colletotrichum gloeosporio ides* (H), *P. parasitica* (I)

T. flavus (KUFC 3550) vs *Phytophthora palmivora* (J), *P. parasitica* (K), *Peronophythora litchii* (L)

4.2 Antagonistic activity tests of twenty isolates of *Talaromyces flavus* against *Sclerotium rolfsii* in the greenhouse

The efficacy of *T. flavus* in the greenhouse as the biological control agent against *Sclerotium rolfsii*, the causal agent of bean stem rot, indicated that the highest seedling survival was 93, 88 and 87% in the treatment with ascospore suspension of *T. flavus* isolates KUFC 3523, 3528 and 3530, respectively, at 7 days after planting (Figures 65-66). In contrast seedlings survival was 38% 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, 82 and 80%, respectively, at 14 days after planting (Table 13).

The highest percentage of seedling survival at 30 days after planting were 45 and 41% 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 13).

None of the *T. flavus* isolates gave high inhibition of *S. rolfsii in vitro*, because *S. rolfsii* thoroughly colonized *T. flavus* in the petridishes. However, in the greenhouse experiment, isolates KUFC 3530 and 3334 controlled *S. rolfsii*, as well or better than *Trichoderma harzianum*, which showed 34% of seedlings survival 30 days after inoculation (Table 13). These results indicates that mechanisms other than antibiotic production may be responsible for the disease control of *S. rolfsii* in the greenhouse experiment.

Table 13 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 ®)

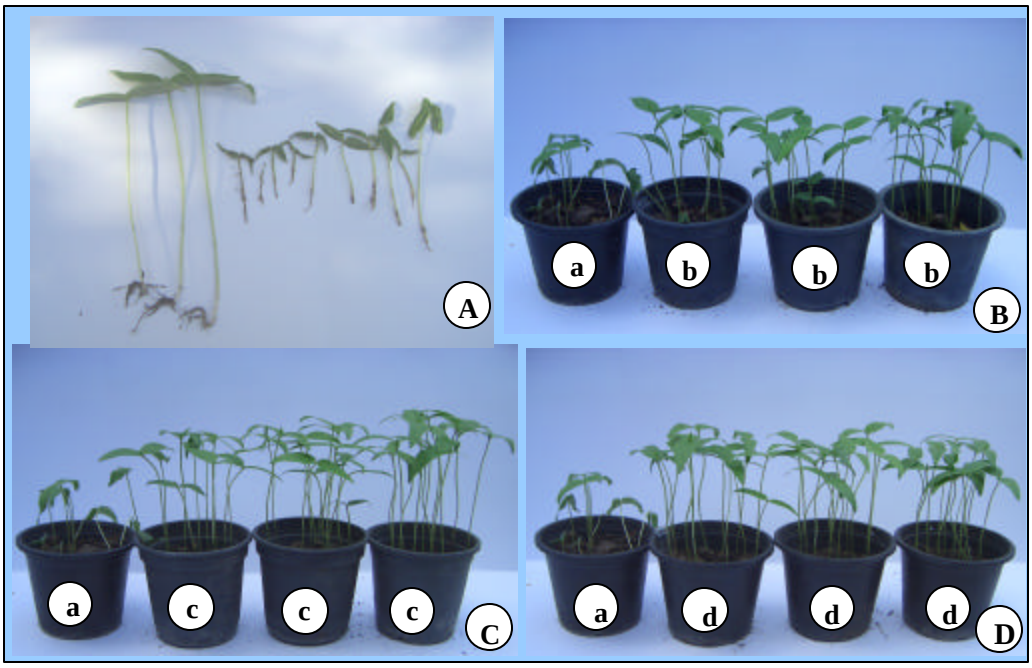


Figure 65 **A**. Mungbean seeds + distilled water (left) and mungbean seeds + *Sclerotium rolfsii* (right), 7 days after planting (Control), **B.** mungbean seeds + *S. rolfsii* (**a**), mungbean seeds + *Talaromyces flavus* KUFC 3523 + *S. rolfsii* (**b**), **C.** mungbean seeds + *T. flavus* KUFC 3550 + *S. roflsii* (**c**), **D.** mungbean seeds + *T. flavus* KUFC 3528 + *S. rolfsii* (**d**), 7 days after planting

Figure 66 Mungbean seeds inoculated with *Sclerotium rolfsii* for 7 days; the collapsed mungbean seedlings and sclerotia (circled) are shown

Among 122 isolates of *Talaromyces flavus* found in this study, 20 isolates were used for antagonistic activity test against 15 species of plant pathogenic fungi *in vitro* (Tables 10-12). The results showed that all strains of *T. flavus* could effectively control oomycetes plant pathologenic fungi *in vitro*, including *Phytophthora palmivora, P. parasitica* and *Peronophythora litchii in vitro*. All strains of *T. flavus* could moderately control *Fusarium oxysporum* f.sp. *lycopercisi, F. semitectum, Colletotrichum capsici*, and *C. gloeosporioides*, but could not control *Lasiodiplodia theobromae, Rhizoctonia oryzae* and *Sclerotium rolfsii in vitro*. This study was supported by report of Jun *et al*., (1999) as they found that *T. flavus* isolate 0-5-1 could effectively control plant pathogenic fungi of cotton *in vitro, Verticillium dahiae, Fusarium oxysporum* f.sp. *vasinfectum, Rhizoctonia solani* and *Colletotrichum gossypii.*

The efficacy of twenty isolates of *T. flavus* to control bean stem rot caused by *Sclerotium rolfsii* was examined in the greenhouse. The greatest disease reduction was 92.59% when treated seeds with ascospores suspension of *T. flavus* KUFC 3523, followed by *T. flavus* KUFC 3528 and 3334 at 88.37 and 87.54%, respectively. The remaining isolates could moderate control the disease, ranging from 70.45 to 80.22%. Madi *et al*., (1997) reported that 64% was greatest reduction in this disease by *T. flavus* strains in the greenhouse.

Talaromyces 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, 1995, 1997) and parasitizes *Sclerotinia sclerotiorum* (McLaren *et al*., 1986, 1989, 1996; 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, 1990a, b; Stosz *et al.*, 1996), and competition (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*.

Talaromyces flavus produces four antibiotics: vermiculine (Fuska *et al*., 1972), vermistatin (Fuska *et al*., 1979a, 1986), 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*., (1990a, b) 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).

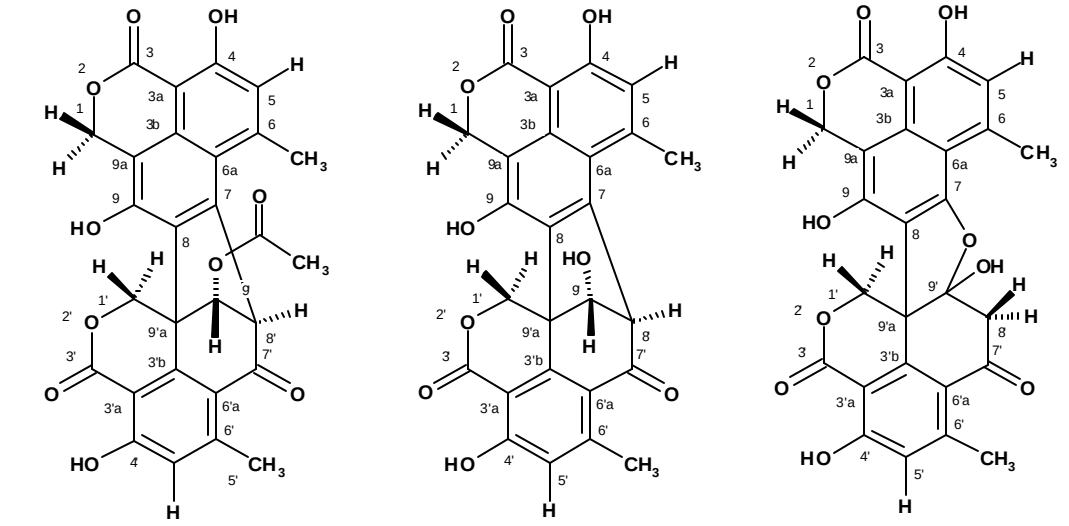
4. Secondary metabolites isolated from *Talaromyces bacillisporus* and *Talaromyces* sp. 1 (KUFC 3399)

4.1 Secondary metabolites isolated from *Talaromyces bacillisporus*

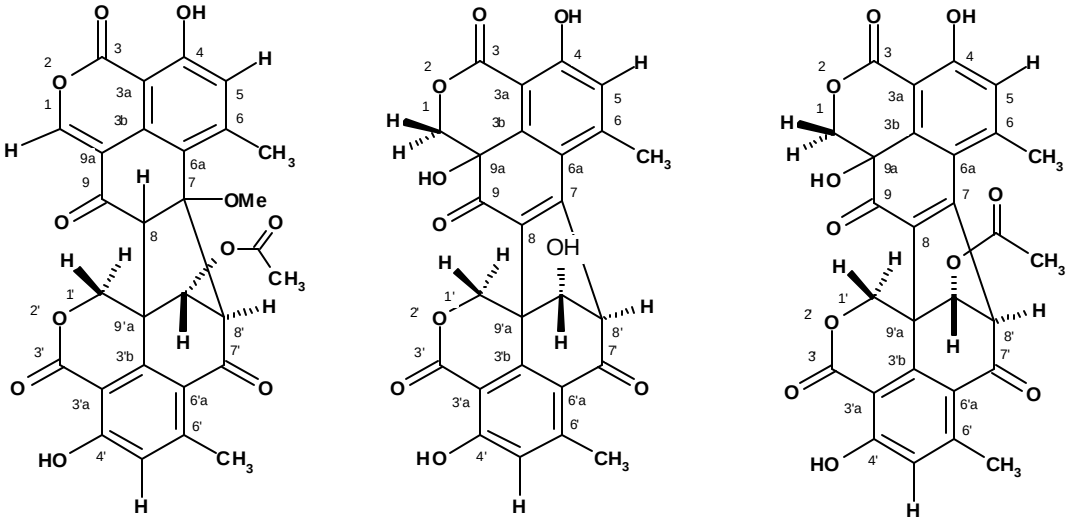
The ethyl acetate extract of the culture of *Talaromyces bacillisporus* furnished, besides bacillisporins A (**4**), B (**5**), C (**6**) and duclauxin (**50**), previously isolated from the Japanese collection of *Talaromyces bacillisporus* (Yamazaki and Okuyama, 1980), two new oxyphenalenone derivatives which have named bacillisporins D (**69**) and E (**70**). The structures of these new compounds have been established by spectrosacopic methods (^{1}H, ^{13}C NMR, COSY, HSQC and HMBC) and HRMS as well as comparison of their proton and carbon chemical shift values with those of bacillisporins A, B, C and duclauxin (Tables 14 and 15).

An earlier article on the chemistry of the fungus *Talaromyces bacillisporus* (Stolk and Samson, 1972) described three new oligophenalenone dimmers (Yamazaki and Okuyama, 1980), which because the authors misspelled the name of the fungus were misnamed bacillosporins A, B and C (Cooke and Edwards, 1981; Stolk and Samson, 1972; Yamazaki and Okuyama, 1980; Ishii *et al*., 1995) instead of more properly bacillosporins A-C, a designation which will use henceforth. A new xanthone pinselin was also reported in the same article. In addition, Ishii *et al*., (1995) have also isolated a cytotoxic pyrrolizidinedione derivative from the same fungus. The effect of bacillisporin A on mitochondrial respiration has been studied (Shiojiri *et al*., 1984).

Duclauxin has been previously reported from three other *Talaromyces* species including *T. flavus*, *T. macrosporus* and *T. stipitatus* (Ogihara *et al*., 1965; Shibata *et al*., 1965; Frisvad *et al*., 1990) and several articles have described various biological activities of this substance (Fuskova *et al*., 1977; Kuhr and Fuska, 1973; Kovac *et al*., 1978; Kawai *et al*., 1982; Shiojiri *et al*., 1983).



4 **5** **6**



50 **69** **70**

Figure 67 Structure of compounds isolated from the culture of *T. bacillisporus*, collected from Kasetsat University, Bangkok

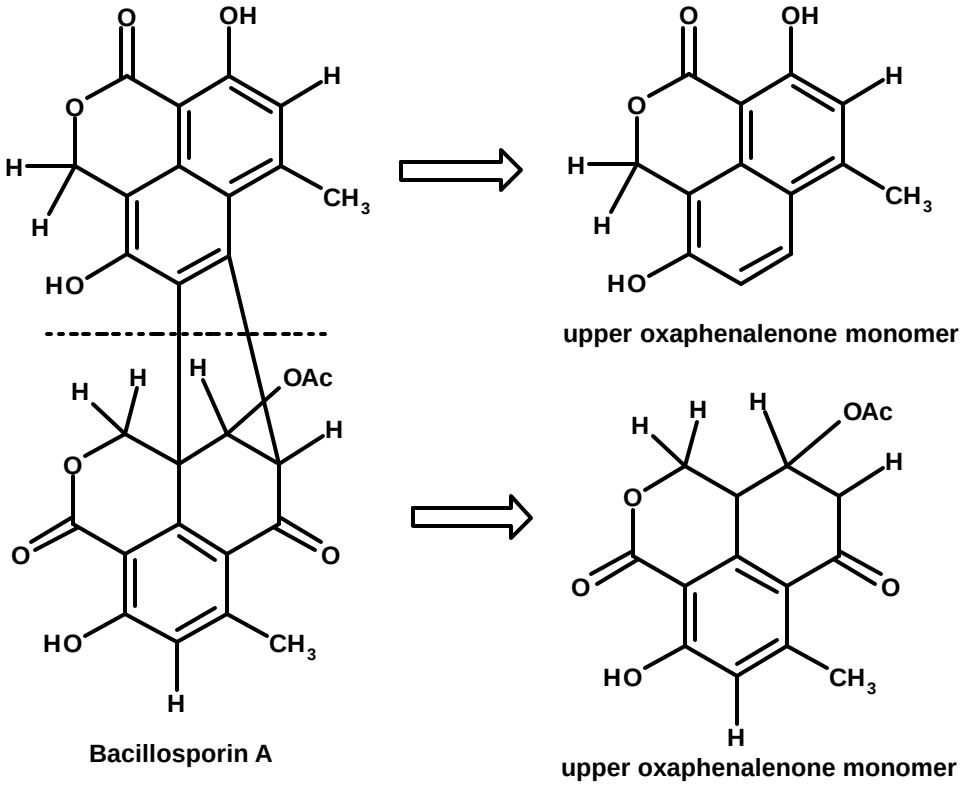
Table 14 ^{1}H NMR of bacillisporins A (**4**), B (**5**), C (**6**), D (**69**), duclauxin (**50**) (DMSO, 500 MHz), and bacillisporin E (**70**) ($CDCl_3$, 300 MHz)

^{1}H	**4**	**5**	**6**	**69**	**50**	**70** ($CDCl_3$)
1a	5.72d (15.2)	5.73d (15.0)	5.64d (14.3)	7.72s	4.54d (12.4)	4.90d (13.0)
1b	5.64d (15.2)	5.65d (15.0)	5.47d (14.3)	----	4.61d (12.4)	4.82d (13.0)
5	6.93s	6.96s	7.00d (0.6)	6.91s	6.97s	6.92s
1'a	5.13d (12.3)	5.14d (12.4)	4.93d (11.2)	5.10d (12.3)	5.00d (12.4)	4.88d (12.5)
1'b	5.05d (12.3)	4.99d (12.4)	4.63d (11.2)	4.79d (12.3)	4.66d (12.4)	4.76d (12.5)
5'	6.88s	6.83d (0.7)	6.81d (0.9)	6.65s	6.88s	6.82d (0.7)
8'	5.00brs	4.83 brs	3.27d (15.5)	4.15brs	4.53brs	4.73d (1.0)
	----	-----	3.10d (15.5)	-----	----	-----
9'	5.82 brs	4.77d (4.5)	------	5.21brs	4.64d (5.5)	5.69d (0.9)
OH-4	11.65s	11.86s	11.66s	10.67s	11.3s	11.26s
OH-9	10.14 brs	9.99s	9.89s	-----	-----	-----
OH-4'	11.96 brs	12.00s	11.86s	11.71s	11.87s	11.88a
OH-9'	----	6.28d (4.9)	8.68s	-----	6.37d (5.5	-----
CH_3-6	2.93s	2.97s	2.10s	2.75s	2.84s	2.88s
CH_3-6'	2.50s	2.48s	2.75s	2.12s	2.51s	2.62s
OMe-7	-----	-----	-----	2.98	------	-----
CH_3-Ac	2.01s	-----	-----	2.23s	------	2.15s

Table 15 ^{13}C NMR of bacillisporins A (**4**), B (**5**), C (**6**), D (**69**), duclauxin (**50**) and bacillisporin E (**70**) (125.77MHz)

^{13}C	**4** (DMSO)	**5** (DMSO)	**6** (DMSO)	**69** (DMSO)	**50** (DMSO)	**70** ($CDCl_3$)
1	68.73	66.84	66.67	148.69	71.05	71.54
3	169.21	169.44	169.55	163.80	167.29	167.53
3a	97.47	97.54	109.58	101.35	107.94	108.02
3b	131.46	131.31	131.92	132.83	144.82	143.30
4	161.61	161.52	162.84	161.73	160.96	162.98
5	119.38	119.15	119.98	120.79	120.67	122.44
6	145.76	145.97	148.69	151.97	147.44	148.05
6a	118.06	119.15	121.01	118.18	117.44	116.78
7	134.23	137.29	155.08	88.71	154.22	155.45
8	135.97	134.76	113.72	63.96	146.70	145.59
9	148.27	148.92	150.01	193.68	192.71	191.38
9a	110.18	109.68	102.12	113.19	65.12	65.81
1'	66.36	70.10	73.30	71.32	69.13	68.06
3'	167.36	167.94	168.97	167.26	167.60	167.32
3'a	103.72	103.82	96.83	104.74	104.41	104.05
3'b	146.52	147.77	144.15	142.82	136.68	135.81
4'	163.25	163.16	162.54	164.74	163.23	164.85
5'	120.17	119.68	117.05	121.33	119.57	121.23
6'	152.66	152.38	145.90	151.95	152.31	153.95
6'a	116.40	116.78	108.67	120.88	117.37	116.90
7'	191.21	192.81	193.22	190.81	190.62	188.13
8'	61.22	64.59	48.45	67.30	66.40	63.64
9'	85.33	85.31	111.49	78.80	84.26	83.85
9'a	47.98	49.57	48.49	51.04	48.57	47.93
Me-6	24.27	24.45	23.01	22.16	23.71	24.77
Me-6'	23.19	23.28	23.08	22.62	23.29	23.96
Ac	20.73	-----	-----	20.94	------	20.87
	170.10	-----	-----	169.50	------	170.04

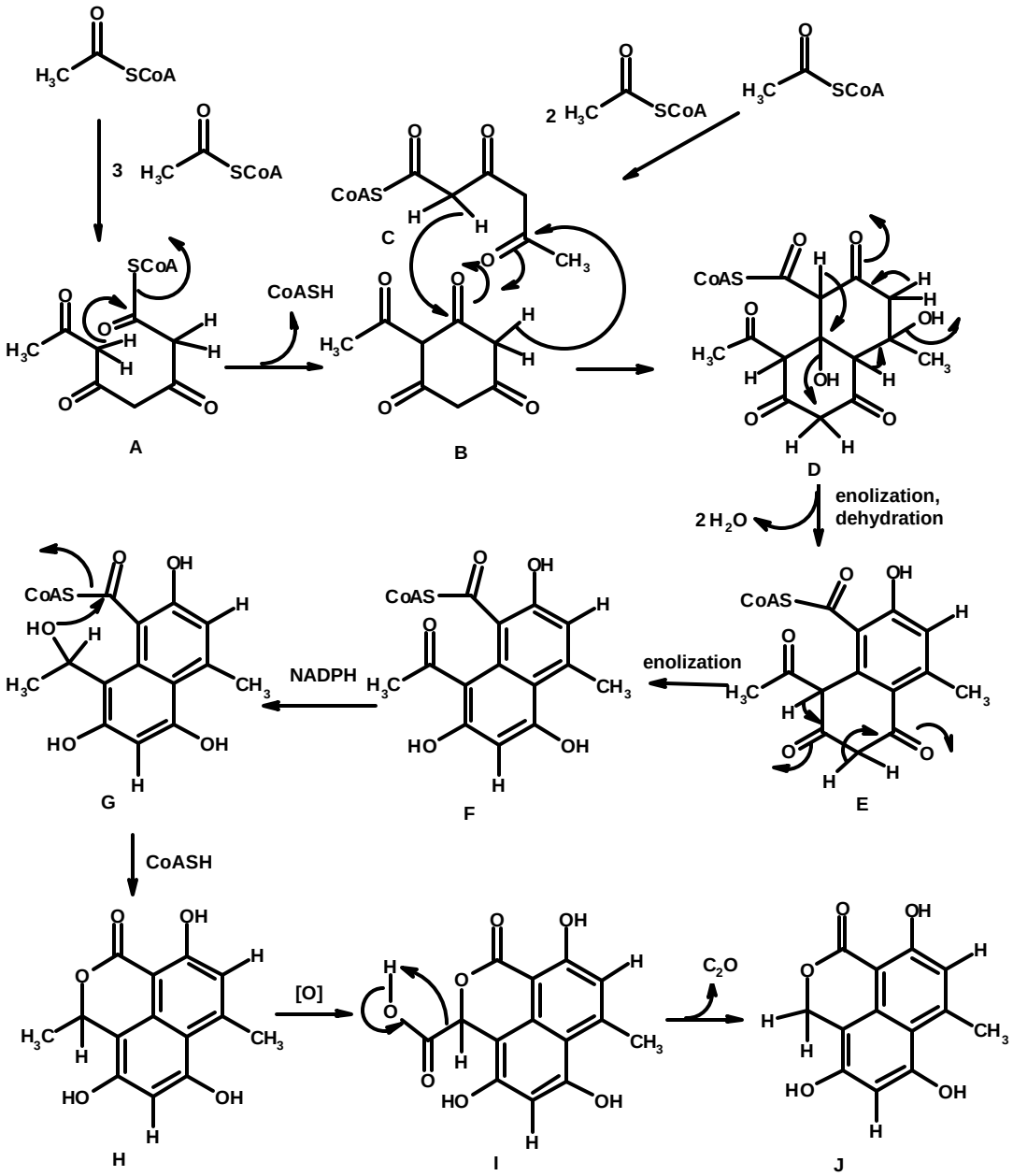
The molecule of bacillosporins and duclauxin can be considered as a dimer of oxapnenalenone derivatives. Consequently, they are derived from the coupling of two oxaphenalenone monomers:



Thus, it is convenient to delineate first the biogenetic pathway of the two monomers before considering their coupling. It is clear that both of the monomers are derived from the acetate pathway. The formation of the upper monomer (**J**) from the tetraketide (**A**) and triketide (**C**) chains is shown in **Scheme 1** .

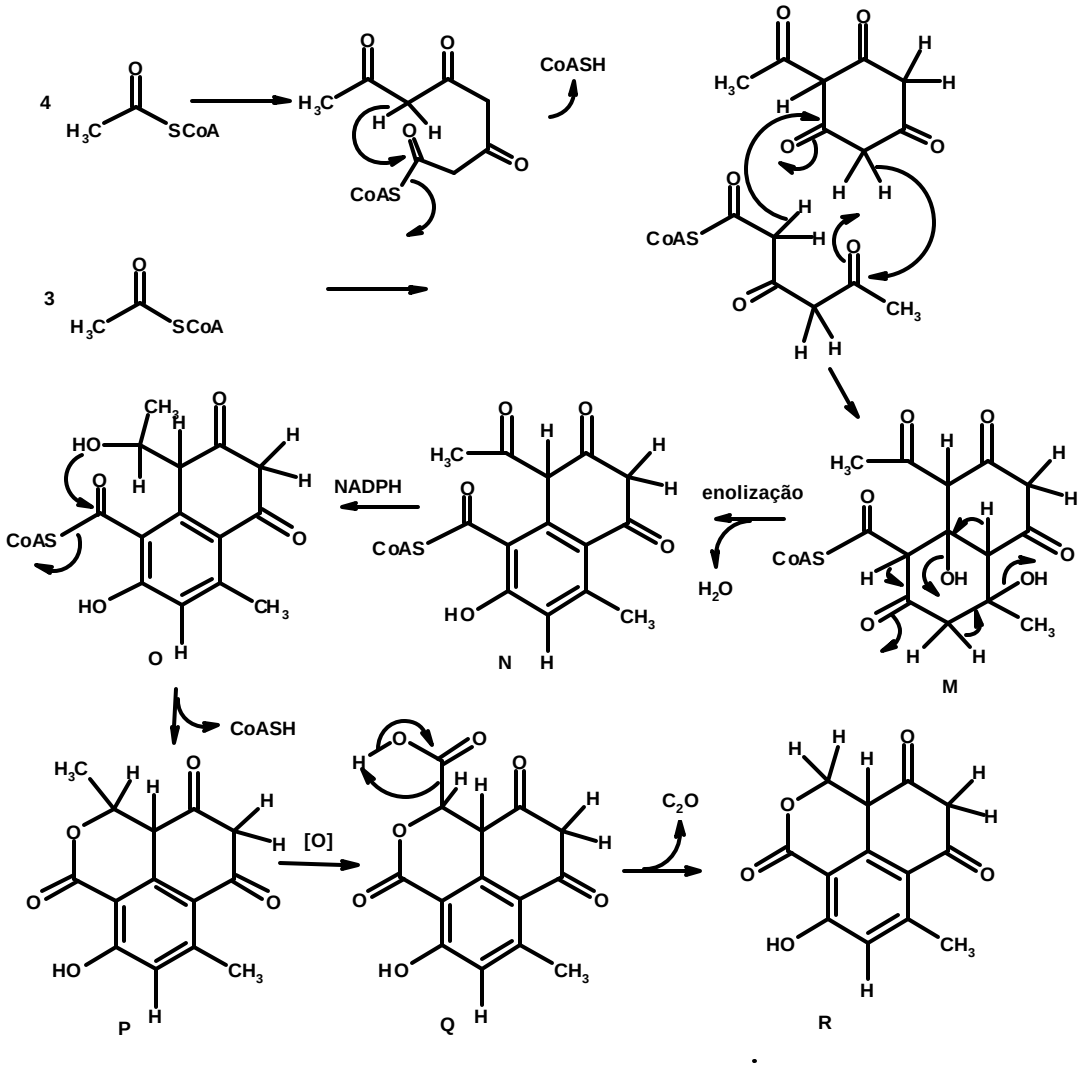
Before condensing with the triketide chain (**C**), the tetraketide chain (**A**) is thought to suffer a Claisen condensation to give the cyclic form (**B**). Aldol condensation between the tetraketide **B** and triketide **C** results in the intermediate **D**, which, after enolization and dehydration, gives the intermediate **F**, through the intermediate **E**. Reduction of the ketone function of the intermediate **F** by NADPH gives the alcohol function in **G**. Claisen condensation of the hydroxyl group with the thioester of acetyl Co A results in the formation of the lactone ring in **H**. Oxidation of

the methyl group of **H** gives the carboxyl function in **I**, followed by decarboxylation to give the oxyphenalenone **J**, a monomer of bacillosporins.



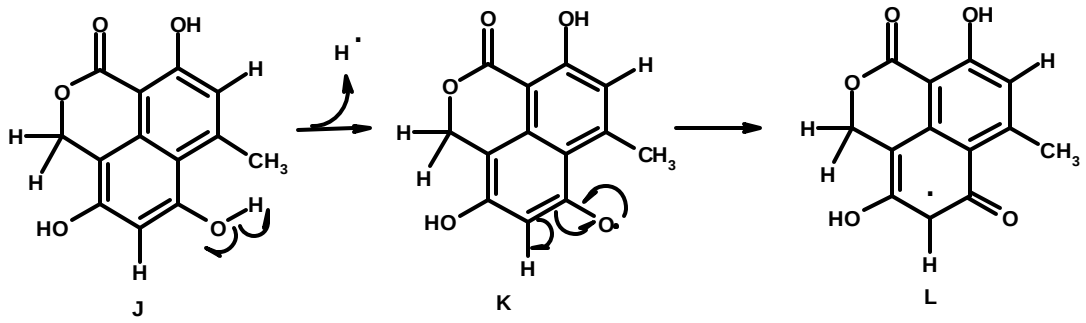
Scheme 1 Formation of the upper oxaphenalenone monomer

Formation of the lower oxaphenalenone unit (**R**) also proceeds via the condensation of the tri- and tetraketide units through the intermediates **M**, **N**, **O**, **P** and **Q** (**Scheme: 2**). However, the manner in which the two polyketide chains are disposed is different from the formation of the upper unit.



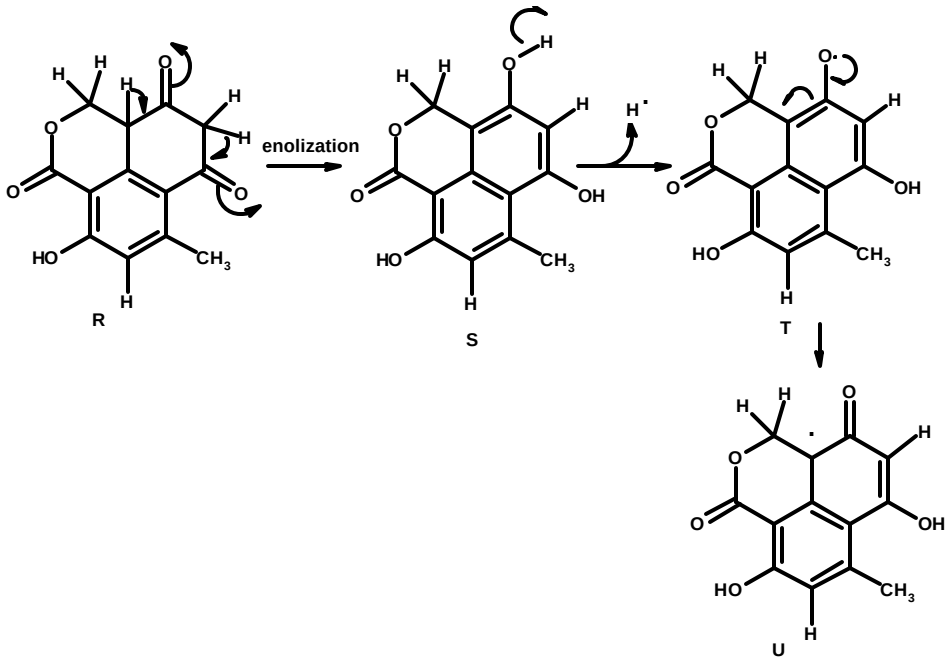
Scheme 2 Formation of the lower oxaphenalenone monomer

Through the action of the peroxidase enzymes, the monomer **J** can form the free radical **L**, through the oxyradical **K** (**Scheme 3**).



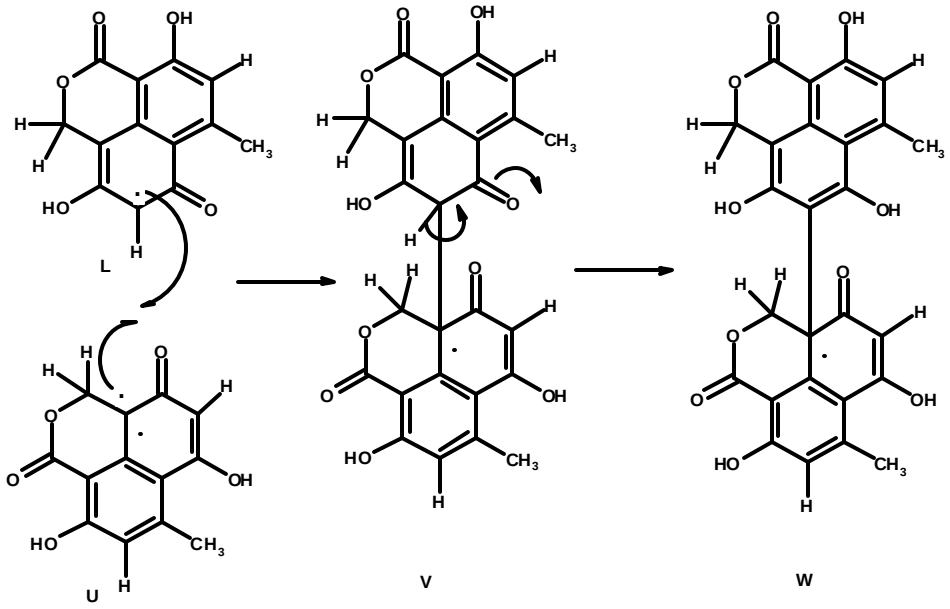
Scheme 3 Formation of the free radical of the upper oxaphenalenone monomer

In the same manner, the lower oxaphenalenone unit (**R**) can also form the free radical **U**, through the action of peroxidase enzymes (**Scheme 4**).



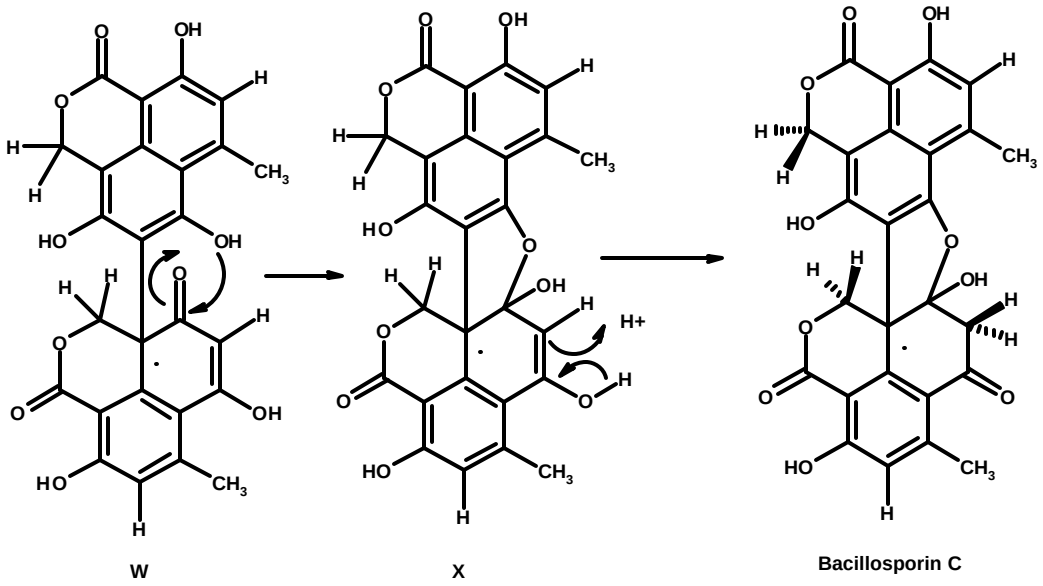
Scheme 4 Formation of the free radical (**U**) of the second oxaphenalenone monomer (**R**)

Oxidative coupling between the free radicals of the upper (**L**) and lower (**U**) oxyphenalenone units results in the intermediate **V**, which, after enolization, gives the dimer **W** (**Scheme 5**).

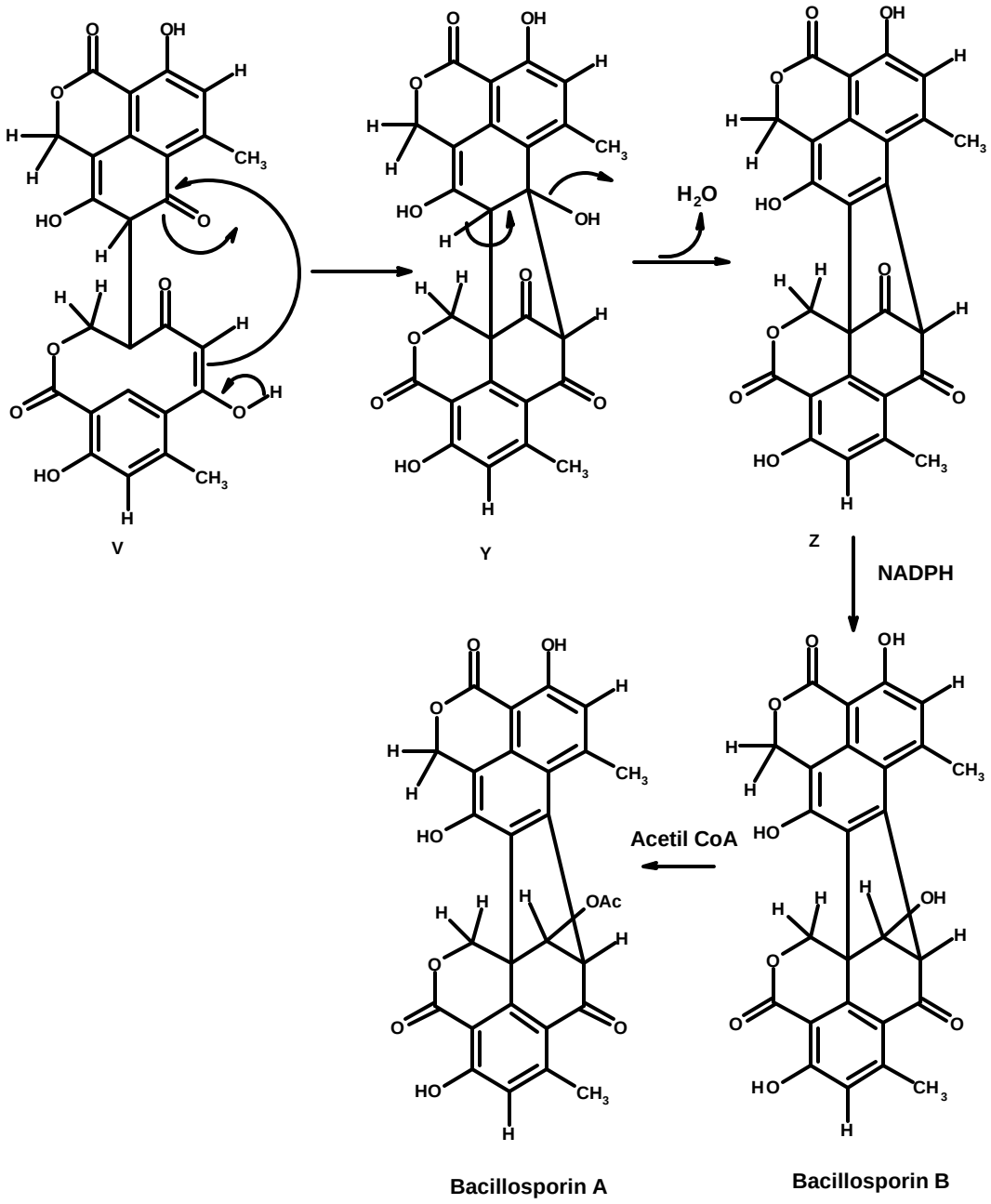


Scheme 5 Oxidative coupling of two oxaphenalenone units

Nucleophilic addition of the ketone function of the lower oxyphenalenone unit by the phenolic hydroxyl group of the upper oxyphenalenone unit of the intermediate **W**, yeilds a hemiketal derivative **X**. Keto-enol tautomerism of the lower unit in **X** results in the more stable keto form in **bacillosporin C** (**Scheme 6**).



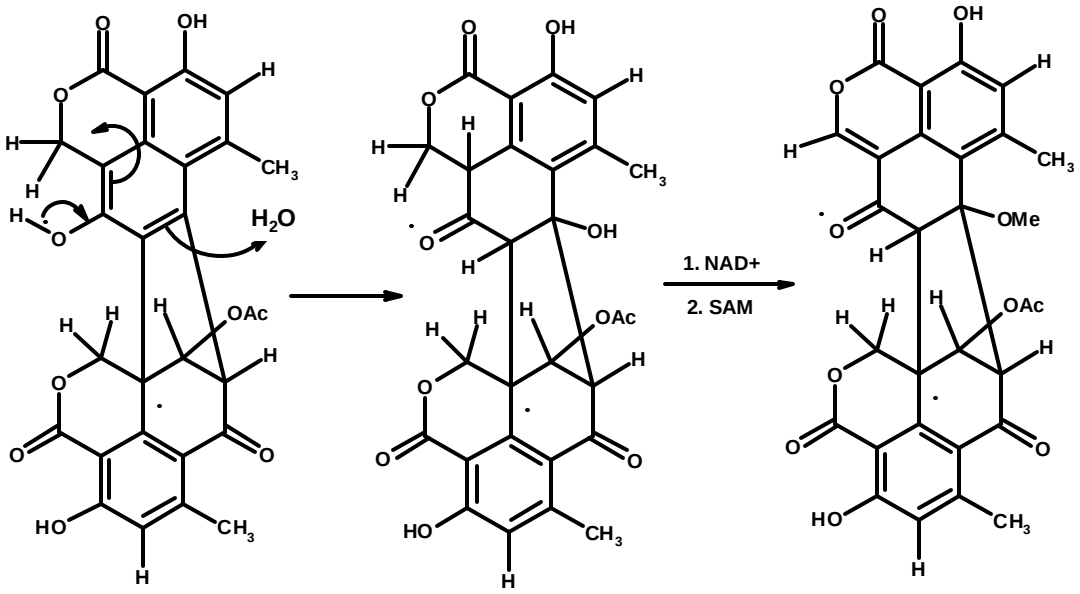
Scheme 6 Formation of **bacillosporin C**



Scheme 7 Formation of **bacillosporin A** and **bacillosporin B**

The biogenesis of **bacillosporins A** and **B** can be considered to derive from the intermediate **V**. Instead of undergoing enolization to form the intermediate **W** for the route to **bacillosporin C**, the ketone funtion of the upper unit in the intermediate **V**

undergoes a nucleophilic attack by the enol group of the lower unit. This results in formation of the second carbon-carbon bond between the two units in the intermediate **Y**. Elimination of the water molecule in **Y** to give the double bond in the upper unit in **Z**. Reduction of the keto function of the lower unit, probably by NADPH, gives **bacillosporin B** and, acetylation of the resulting hydroxyl group, yields **bacillosporin A (Scheme 7)**.



Scheme 8 Formation of duclauxin from bacillosporin A

The formation of **duclauxin** can be considered as the continuation of the reaction sequence of **bacillosporin A** Keto-enol tautomerism and hydration of the double bond of the upper unit of **bacillosporin A** to give the hydroxyketo intermediate. Dehydrogenation of the pyrone ring and methylation of the resulting hydroxyl by **SAM** yields **duclauxin** (**Scheme 8**).

4.2 Secondary metabolites isolated from *Talaromyces* sp. 1 (KUFC 3399)

Chemical investigation of *Talaromyces* sp. 1 (KUFC 3399) furnished the two new merodrimanes thailandolides A (**71**) and B (**72**), an O-methylated derivative (**73**) of the aromatic fragment incorporated in thailandolide B, and three known closely related 1(*3H*)-isobenzofuran derivatives, penisimplicissin (**74**), vermistatin (**75**) and hydroxydihydrovermistatin (**76**). The unusual peptide analogue *N*-benzoylphenylalanyl-*N*-benzoylphenyl alaninate (**77**) and 2-glyceryl palmitate (**78**) were also found.

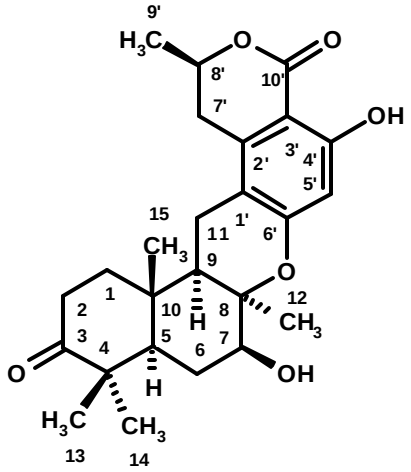
Thailandolide A (**71**) was a 3-oxo-7ß–hydroxydrimane linked through a tertiary oxygen to an aromatic moiety incorporating a lactone function in the manner characteristic of merodrimanes known from fungi of the genus *Stachybotrys* (Sawadjoon *et al.*, 2004), but with the lactone function closed to C-8 of the drimane portion as in the kampanols from *Stachybotrys kampalensis* (Singh *et al.*, 1998), was deduced from the ^{1}H and ^{13}C NMR spectra, listed in Table 16. The probable stereochemistries of C-8 and C-8' and the location of the phenolic hydroxyl group on C-4' of the aromatic ring deduced from chemical shifts and coupling constants in Table 16 as well as COSY and NOESY data were confirmed by an X-ray analysis. The ORTEP diagram of **71** (Figure 68) led to the relative configuration shown in formula **71**.

^{1}H and ^{13}C NMR spectroscopic data of compound **72** (thailandolide B) demonstrated that it differed from **71** in being the extract was **73**, an *O*-methylated derivative of the aromatic fragment incorporated in **72**. The ring conformation of **73** is such that the methyl group is quasiequatorial, as can be gleaned from the H-3/H-4a,b coupling constants and conforms to the stereochemistry of the C-8'-methyl group in **71**. The location of the hydroxyl group on C-6 and the methoxy group on C-8 is shown by the presence of hydrogen bonding to the carbonyl, as evidenced by a broad –OH singal at d 10.29, the COSY spectrum, which exhibited cross-peaks between H-5 and H-1'a,b and between H-3 and the OMe group, and the HMBC spectrum with cross-

peaks between H-5 and CH_2-1', C-3, C-1 and C-0, between H-3 and C-1, C-5, and CO, and between –OMe and CO.

Interestingly, the dipeptide *N*-benzoylphenylalanyl-*N*-benzoylphenyl alaninate (**77**) has been previously reported as a constituent of a higher plant *Croton hieronymi* (Euphorbiaceae) was also found from this fungus (Catalan *et al*, 2003).

Three further constituents of the extract were three 1(*3H*)-isobenzofuranone derivatives, **74**-**76**. Compound **75** was identical with vermistatin, which has been isolated previously from cultures of *Penicillium verticulatum* (Fuska *et al*., 1986; Massias *et al*., 1989), *P. verruculosum* (Murtaza *et al*., 1997) and *P. simplicissimum* (Komai *et al*., 2005) as well as from fungal cultures related to *Talaromyces flavus* (Arai *et al*., 2002; Komai *et al*., 2004, 2005), while compounds **74** and **76** were identical with penisimplicissin and a hydrated analogue of vermistatin, both recently reported from *Pencillium simplicissimum* (Komai *et al*., 2005). The structures of **75** was comfirmed by an X-ray analysis (Figure 69). The ^{1}H and ^{13}CNMR data of vermistatin (**63**), hydroxydihydrovermistatin (**64**) and penisimplissin (**65**) are in Tables 17 and 18.



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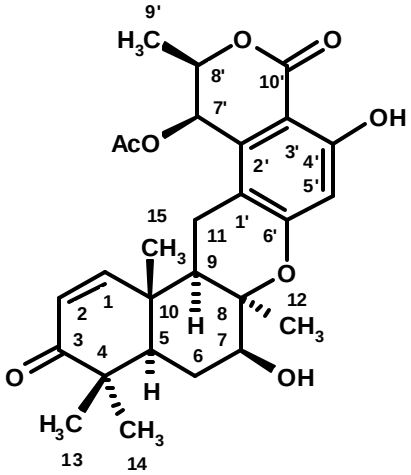

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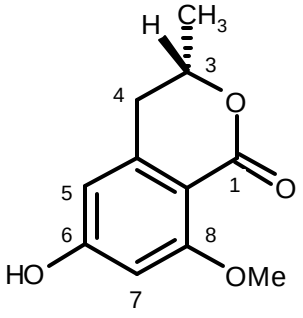

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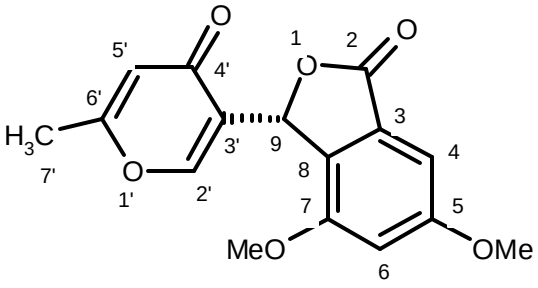

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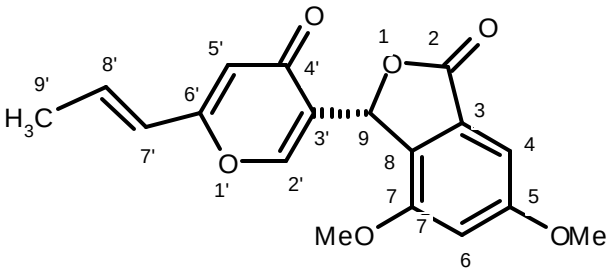

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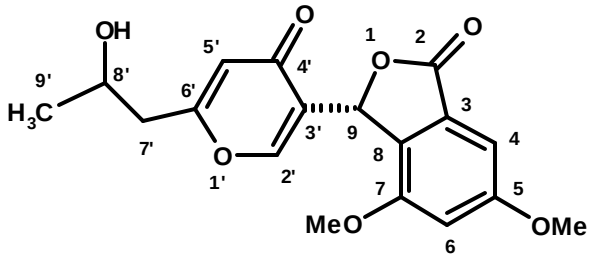

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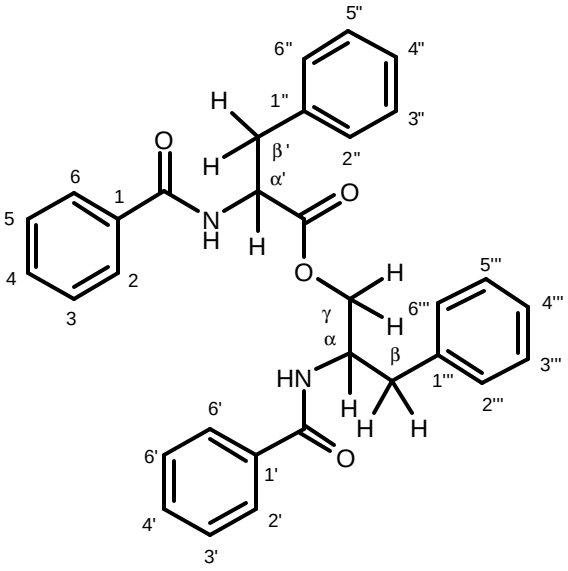

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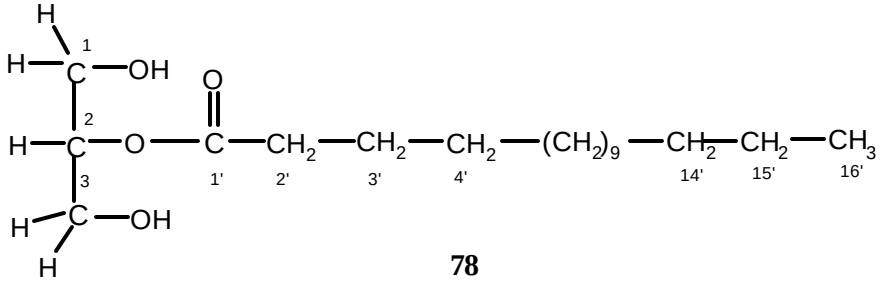

78

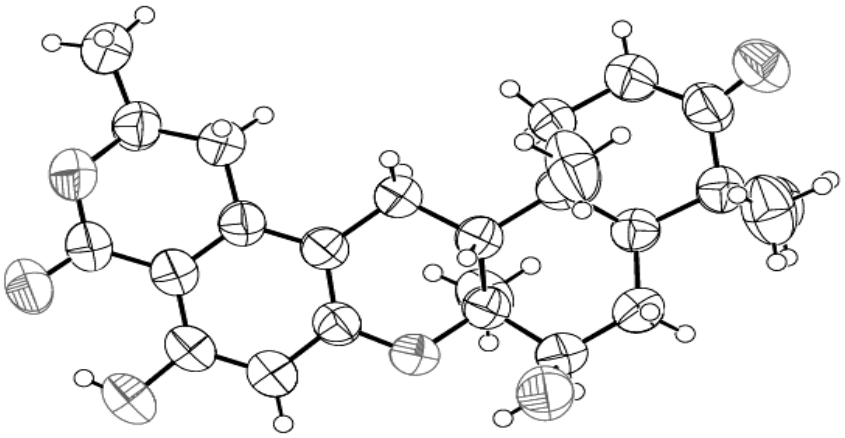

Figure 68 ORTEP view of thailandolide A (**71**)

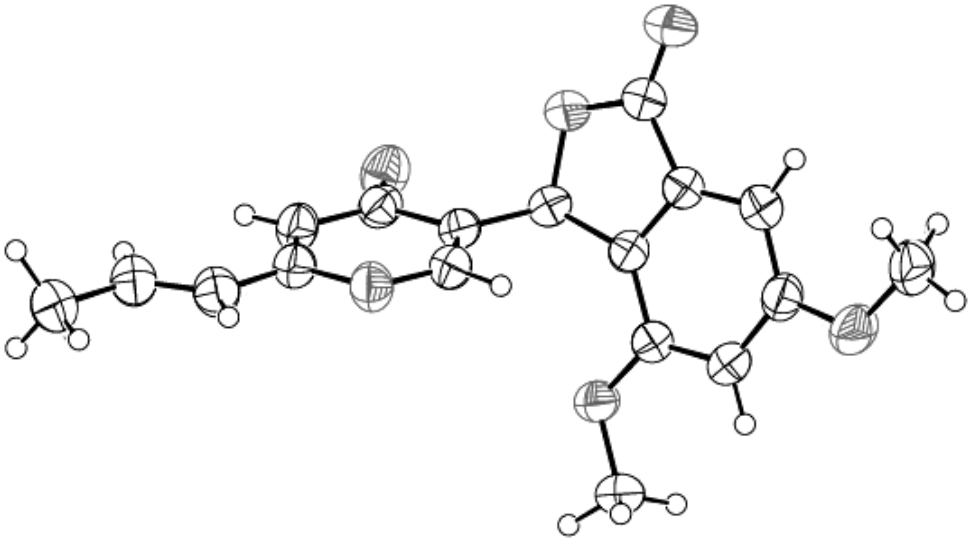

Figure 69 ORTEP view of vermistatin (**75**)

Table 16 NMR Data of compound **71** and **72** in $CDCl_3$ (^1H 300 MHz, ^{13}C 75 MHz)

	71		**72**	
position	d_H (*J* in Hz)	d_C (DEPT)	d_H	d_C
1α	2.01c	32.1t	7.16d(10.3)	156.2d
1ß	1.7c			
2α	2.49c	33.6t	6.00d (10.3)	127.5d
2ß	2.65td (11, 4.8)			
3		207.3s		203.7s
4		46.9s		44.6s
5	1.95dd (14,3)	43.6d	2.18dd (14, 4.6)	42.4d
6α	1.59	28.2t	2.2-2.3c	26.5t
6ß	2.18ddd (14, 7.5, 3)		1.85ddd (14, 14, 2.4)	
7	4.03t (7.5)	73.0d	4.18dd (8.6, 2)	71.8d
8		78.3s		79.7s
9	2.05dd (12, 6.2)	40.8d	2.27dd (14, 5)	41.8d
10		35.7s		38.4s
11α	2.5c	19.5t	2.62dd (15, 14)	21.5t
11ß	2.5c		2.96dd (15, 5)	
12[a]	1.37s	23.1q	1.27s	21.4q
13[a]	1.11s	20.0q	1.11s	21.3q
14[a]	1.12s	28.6q	1.12s	27.6q
15[a]	1.05s	22.88q	1.36s	27.4q
1’		102.2s		102.2s
2’		139.1s		135.8s
3’		109.9s		112.3s
4’		162.3s		162.2s
5’	6.35s	103.1d	6.50s	106.3d
6		158.6s		159.6s
7’α	2.8dd (16.5, 3.5)	31.5t	6.17d (1.7)	64.1d
7ß	2.72dd (16.5, 12)			
8’	4.65ddq (12, 3.5, 6.3)	74.7d	4.73qd (6.6, 1.7)	76.0d
9’	1.56d (6.3)	21.0q	1.49d (6.6)	16.4q
10		170.0s		168.8s
7-OH	3.11brs			
4’-OH	11.09s		11.06s	
Ac			2.17S[a]	20.7q
				170.6s

[a] Intensity three proton.

Table 17 ^{1}H NMR of vermistatin (**75**), hydroxydihydrovermistatin (**76**) and penisimplissin (**74**) (300 MHz, $CDCl_3$)

^{1}H	**75**	**76**	**74**
H-4	6.98d (2.0)	6.92d (1.8)	6.98d (2.0)
H-6	6.69d (2.0)	6.84d (1.8)	6.68d (2.0)
H-9	6.46s	6.26s	6.45s
H-2'	6.16s	6.14s	6.20s
H-5'	7.43s	8.14s	7.43s
H-7'	6.07dd (15.6, 1.6)	2.58m	2.27s
H-8'	6.61dd (15.6, 6.9)	3.99m	-----
H-9'	1.93dd (16.9, 1,6)	1.14d (6.2)	-----
OCH_3-5	3.88s	3.87s	3.88s
OCH_3-7	3.79s	3.78s	3.79s
OH-8'	----	8.24s	-----

Table 18 ^{13}C NMR of vermistatin (**75**), hydroxydihydrovermistatin (**76**) and penisimplissin (**74**) (75.47 MHz, $CDCl_3$)

^{13}C	**75**	**76**	**74**
2	170.3	169.6	170.0
3	129.3	128.9	129.3
4	98.9	98.8	98.9
5	163.0	162.3	163.0
6	105.1	104.7	105.1
7	154.8	154.7	154.8
8	127.6	127.4	127.6
9	73.6	74.4	73.5
2'	112.8	115.3	115.0
3'	123.3	122.0	123.3
4'	177.3	176.1	176.9
5'	153.9	156.1	154.4
6'	162.1	167.4	166.0
7'	123.0	43.4	19.7
8'	136.0	63.9	----
9'	18.5	23.3	----
OCH_3-5	56.0	55.8	56.0
OCH_3-7	55.8	55.9	55.8

CONCLUSIONS

A total of 342 isolates of *Talaromyces* were obtained from soil, comprising 13 species and 1 varieties including *Talaromyces austrocalifornicus, T. bacillisporus, T. flavus, T. macrospermus, T. helicus* var. *major, T. indigoticus, T. luteus, T. rotundus, T. stipitatus, T. trachyspermus, T. wortmannii, Talaromyces* sp. 1 (KUFC 3399) and *Talaromyces* sp. 2 (KUFC 3383). *Talaromyces austrocalifornicus* and *T. indigoticus* are new records for Thailand.

Talaromyces flavus and *T. macrosporus* were the dominant species followed by *T. stipitatus, T. trachyspermus, T. wortmannii, T. bacillisporus, T. rotundus, T. indigoticus, T. helicus* var. *major, T. austrocalifornicus, T. luteus, Talaromyces* sp. 1 (KUFC 3399) and *Talaromyces* sp. 2 (KUFC 3383).

Phylogenetic analyses were conducted using polymorphic microsatellites of 21 fungi comprising 18 species of *Talaromyces* and 3 other Trichocomaceae (KUFC 3576, 5642, 5655) from Kasetsart University Culture Collection isolated in Thailand. The results showed that the *Talaromyces* used in this study did not show any congruence to the division either done by Stolk and Samson, 1972 or Pitt, 1979. The unidentified species, *Talaromyces* sp. 1 (KUFC 3399) was found on same clade with *T. roduntus* which occupies a basal position to the main *Talaromyces* clade and both of them belong to the Series *Lutei*.

The antagonistic activity tests revealed that 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, little inhibition was observed for *Pythium aphanidermatum, Lasiodiplodia theobromae, Rhizoctonia solani* and *Sclerotium rolfsii in vitro*. The greenhouse experimental 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* could inhibit *S. rolfsii* at 30 days after inoculation.

For secondary metabolites investigation, the oligophenalenone dimer duclauxin and two new analogues, bacillisporins D and E, were isolated from *Talaromyces bacillisporus* in addition to the previously reported bacillisporins A, B and C.

Chemical study of an unidentified *Talaromyces* sp. 1 (KUFC 3399), furnished the two new merodrimanes thailandolides A and B, an *O*-methylated derivative of the aromatic fragment incorporated in thailandolide B, and three known closely related 1(*3H*)-isobenzofuran derivatives, penisimplicissin, vermistatin, and hydroxydihydrovermistatin. Structures were established by spectroscopic measurements and confirmed by X-ray analyses of compounds thailandolides A and vermistatin. The unusual peptide analogue *N*-benzoylphenylalanyl-*N*-benzoylphenyl alaninte isolated earlier from a higher plant was also found.

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APPENDIX

Culture media for isolating fungi

1. Gauchnaur's glucose ammonium nitrate agar (GAN)

NH_4NO_3	1.0	g
K_2HPO_4	1.0	g
$MgSO_4.7H_2O$	0.5	g
Rose bengal	0.03	g
Yeast extract	1.0	g
Glucose	5.0	g
Agar	15.0	g
Streptomycin solution *	30.0	ppm
Distilled water	1,000	ml

* Add after media autoclaved and decrease temperature for 45-50^{o}C

2. Water agar (0.2% WA)

Agar	20.0	g
Distilled water	1,000	ml

Culture media for cultivating fungi

1. Potato Dextrose Agar (PDA)

Potato	200.0	g
Dextrose	20.0	g
Agar	15.0	g
Distilled water	1,000	ml

2. Potato Dextrose Broth (PDB)

Potato	200.0	g
Dextrose	20.0	g
Distilled water	1,000	ml

3. Cornmeal Agar (CMA)

Add 60 g freshly ground cornmeal to1 litre water, heat to boiling and simmer gently for 1 hour. Strain through cloth and sterilize for 15 min at 121 ^{o}C overpressure. Fill up to 1 litre and add 15 g agar and sterilize at 121^{o}C for 15 min.

4. Czapek's Agar (CZA)

Sucrose	30.0	g
$NaNO_3$	3.0	g
K_2HPO_4	1.0	g
KCl	0.5	g
$MgSO_4.7H_2O$	0.5	g
$FeSO_4.7H_2O$	0.01	g
Agar	15.0	g
Distilled water	1,000	ml

5. Czapek Yeast Autolysate Agar (CYA)

KH_2PO_4	1.0	g
Czapek concentrate *	10.0	ml
Yeast autolysate or extract	5.0	g
Sucrose	30.0	g
Agar	15.0	g
Distilled water	1,000	ml

6. 25% Glycerol Nitrate Agar (G25N)

KH_2PO_4	0.75	g
Czapek concentrate *	7.5	ml
Yeast autolysate or extract	3.7	g
Glycerol, analytical grade	250.0	g
Agar	15.0	g
Distilled water	1,000	ml

7. Malt Extract Agar (MEA)

Malt Extract Agar	20.0	g
Peptone	1.0	g
Glucose	20.0	g
Agar	15.0	g
Distilled water	1,000	ml

8. Oatmeal Agar (OMA)

Heat 30 g oat flakes in 1 litre water to boiling and simmer gently for 2 hours. Filter through cloth and fill up to 1 litre. Add 15 g agar to one litre and sterilize by autoclaving at 121^{o}C for 15 min.

***CZA Concentrate**

$NaNO_3$	30.0	g
KCl	5.0	g
$MgSO_4.7H_2O$	5.0	g
$FeSO_4.7H_2O$	0.1	g
Distilled water	100	ml

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