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).

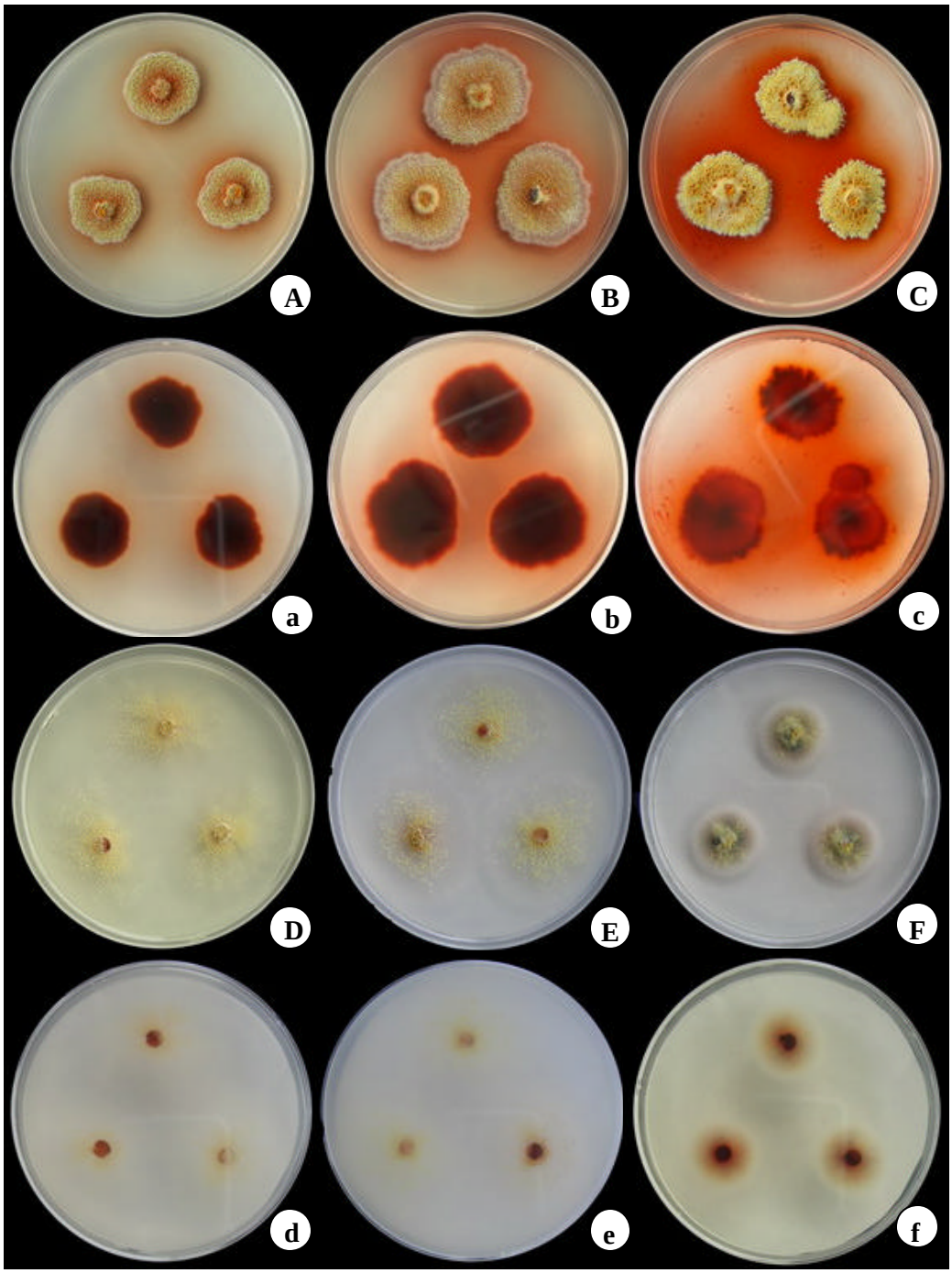


Figure 26 *Talaromyces macrosporus* KUFC 3381. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

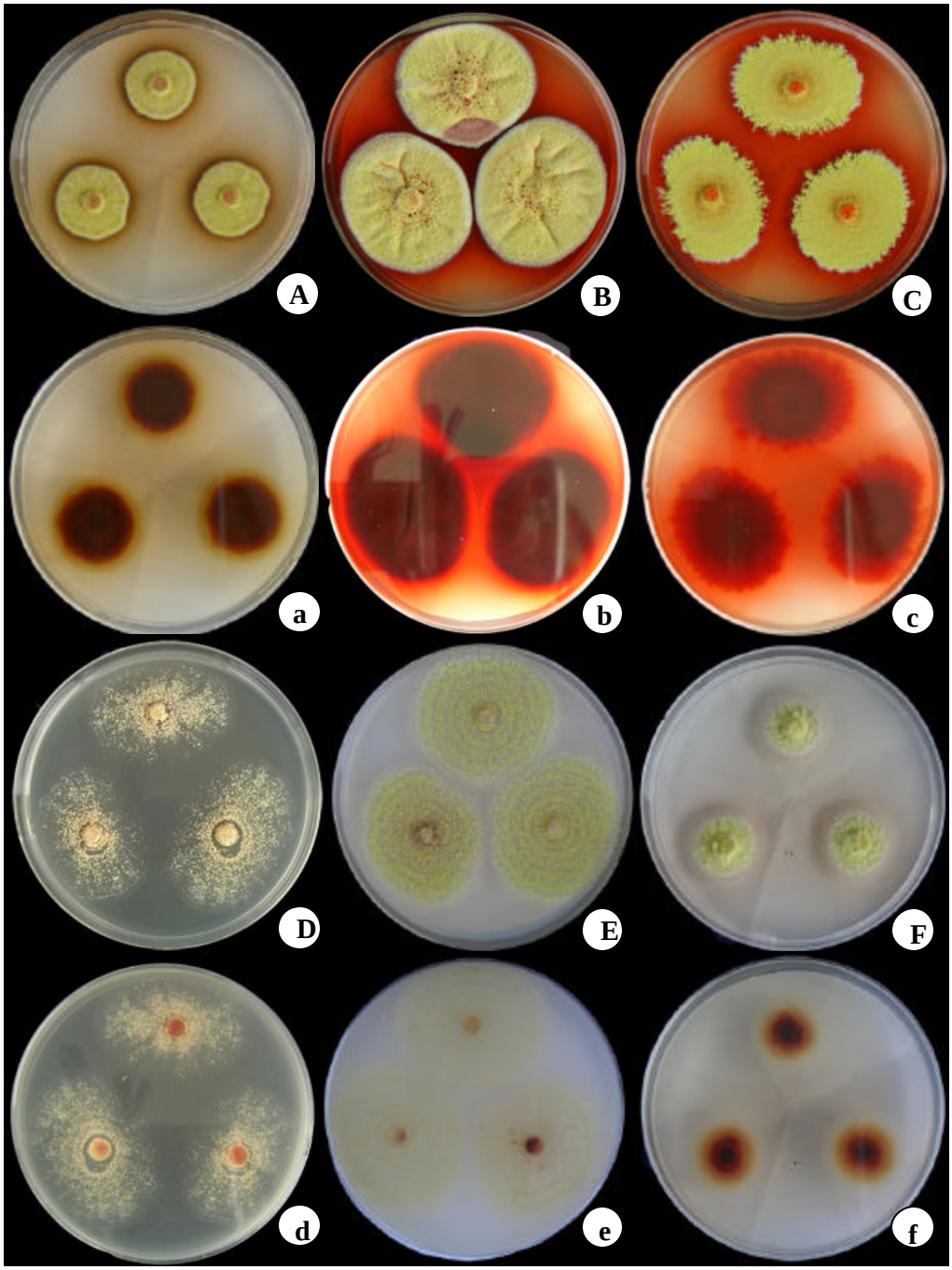


Figure 27 *Talaromyces macrosporus* KUFC 3363. Obverse and reverse views of colonies on different media, incubated for 7 days at 28°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

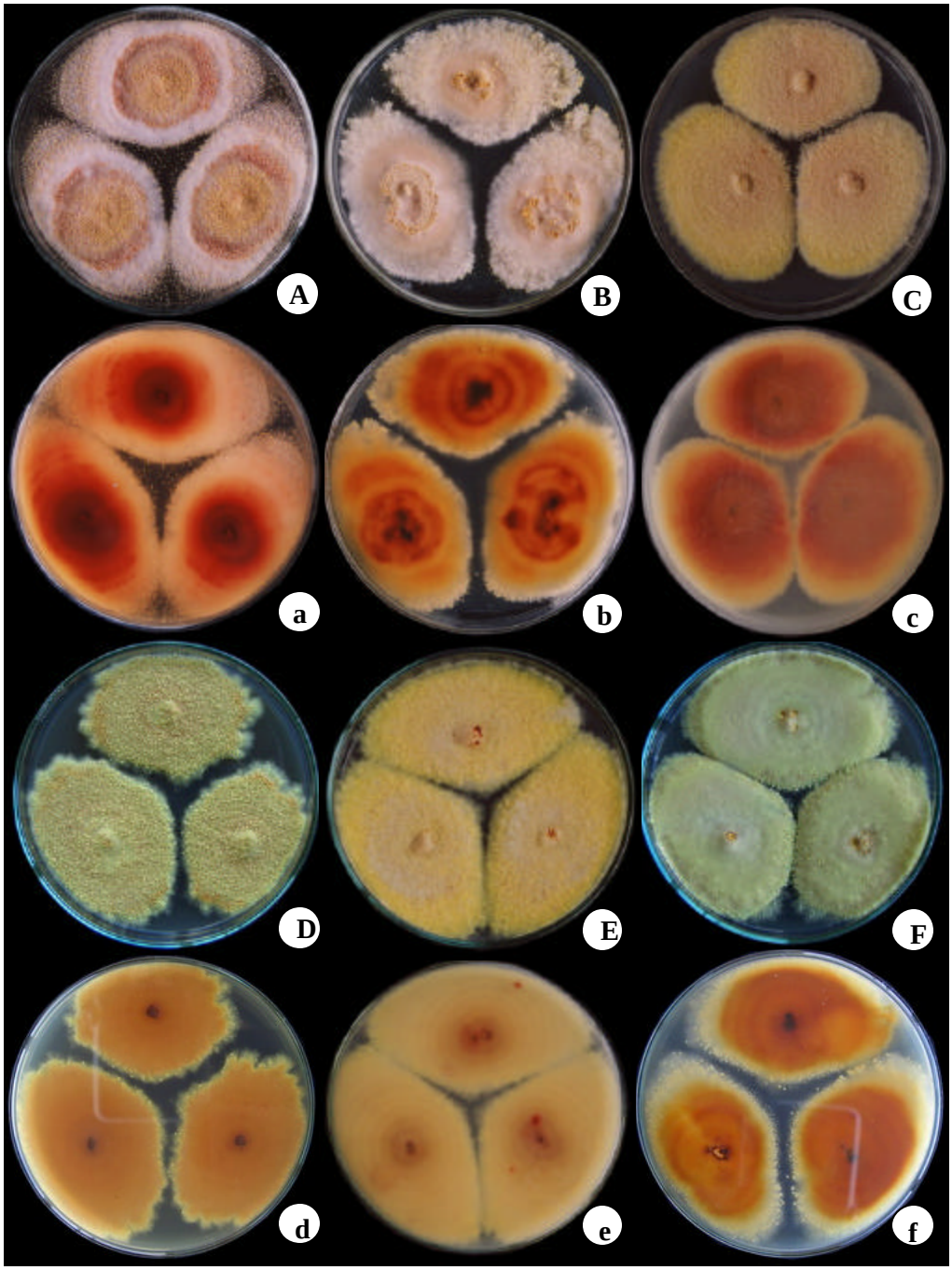


Figure 28 Variations of colony pattern showing obverse and reverse views of various isolates of *T. macosporus* on MEA at 28°C, 14 days

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

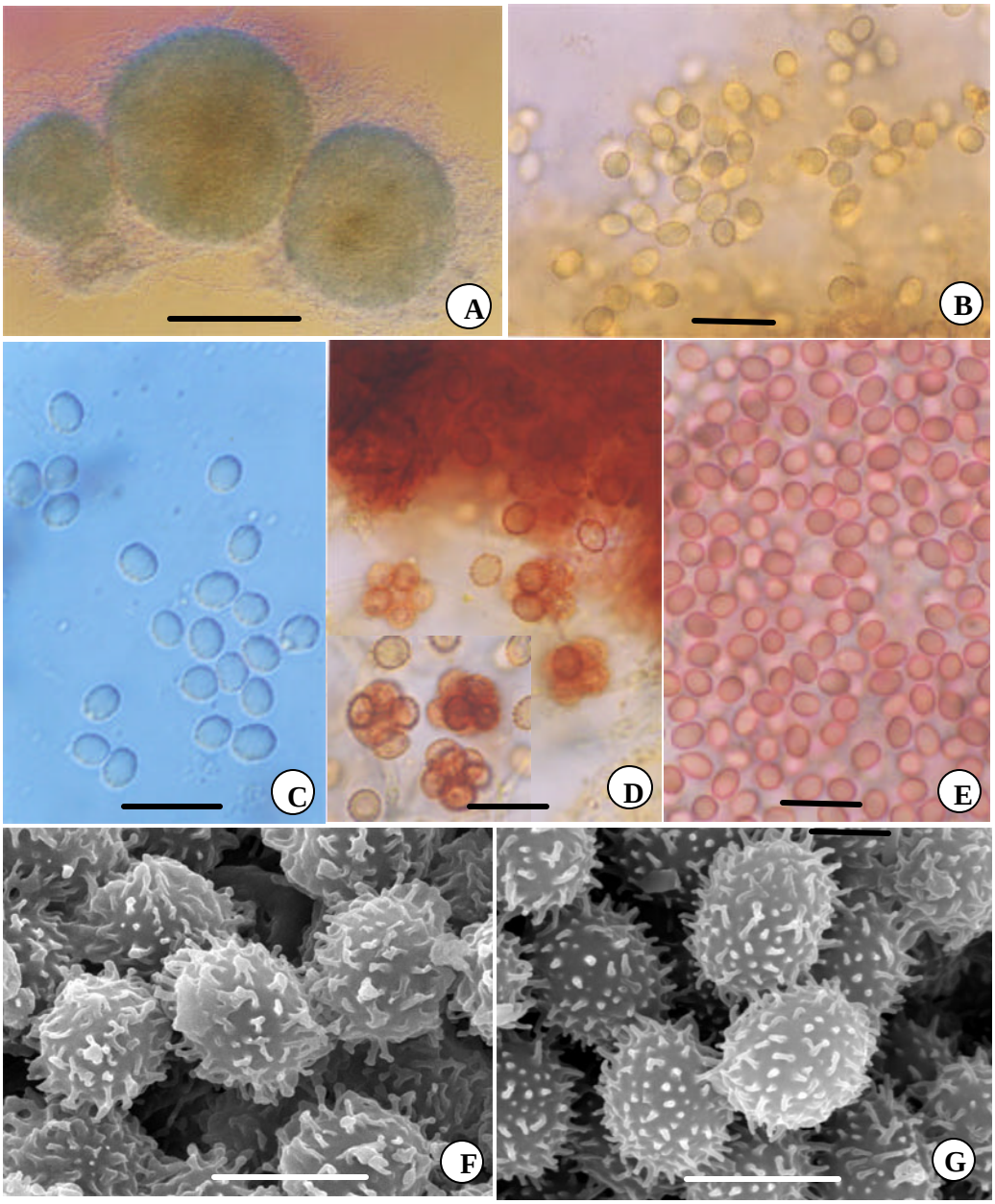


Figure 29 *Talaromyces macrosporus* KUFC 3363

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

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

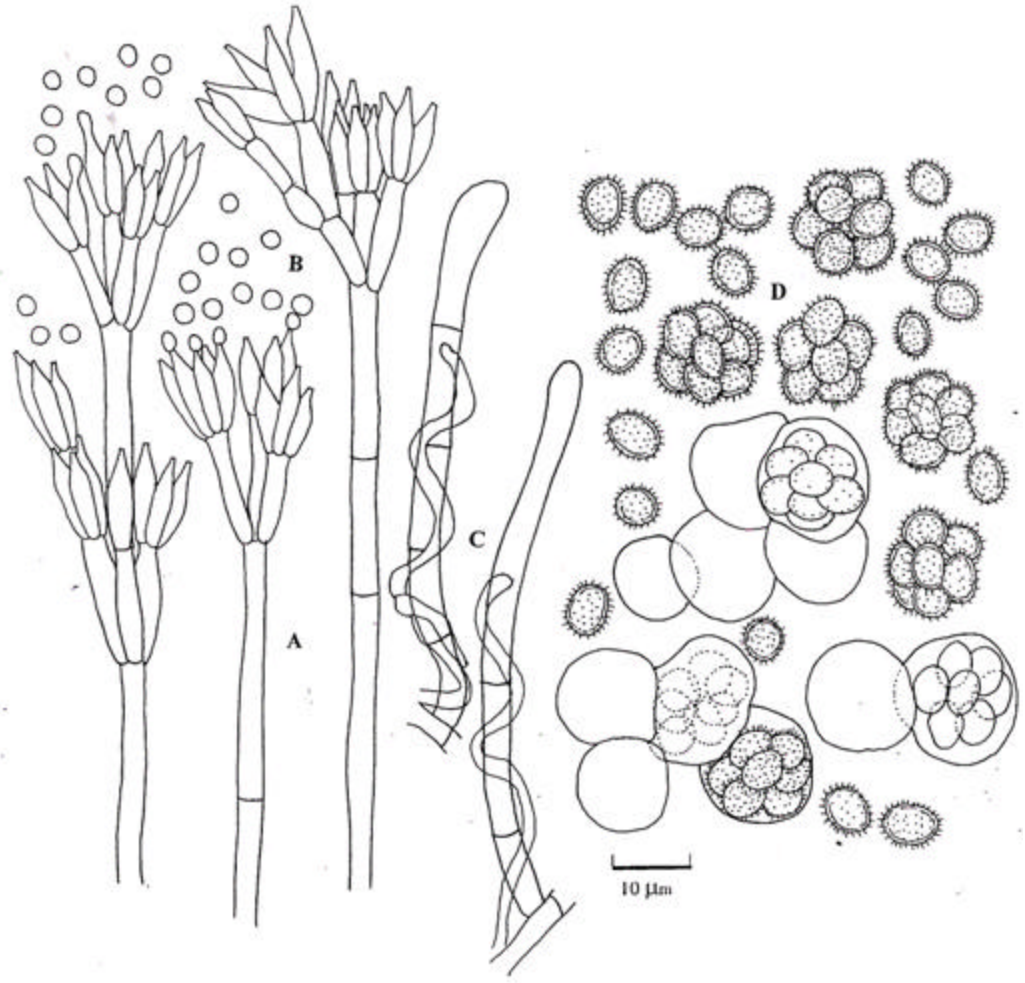


Figure 30 *Talaromyces macrosporus* KUFC 3363
Camera lucida drawings of A. penicilli; B. conidia
C. ascomatal initials; D. asci and ascospores

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).

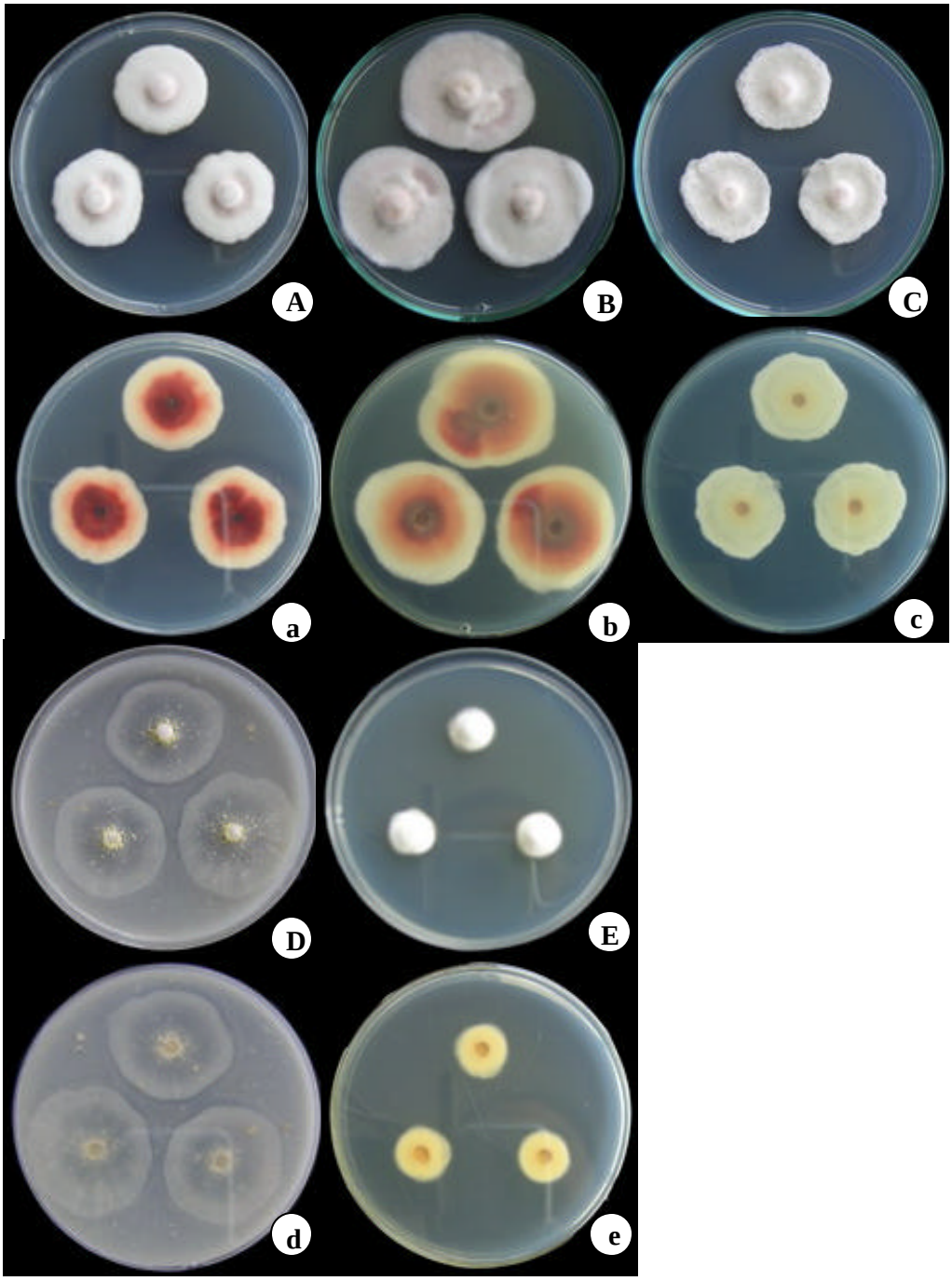


Figure 31 *Talaromyces indigoticus* KUFC 3366. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c), OMA (D, d), G25N (E, e)

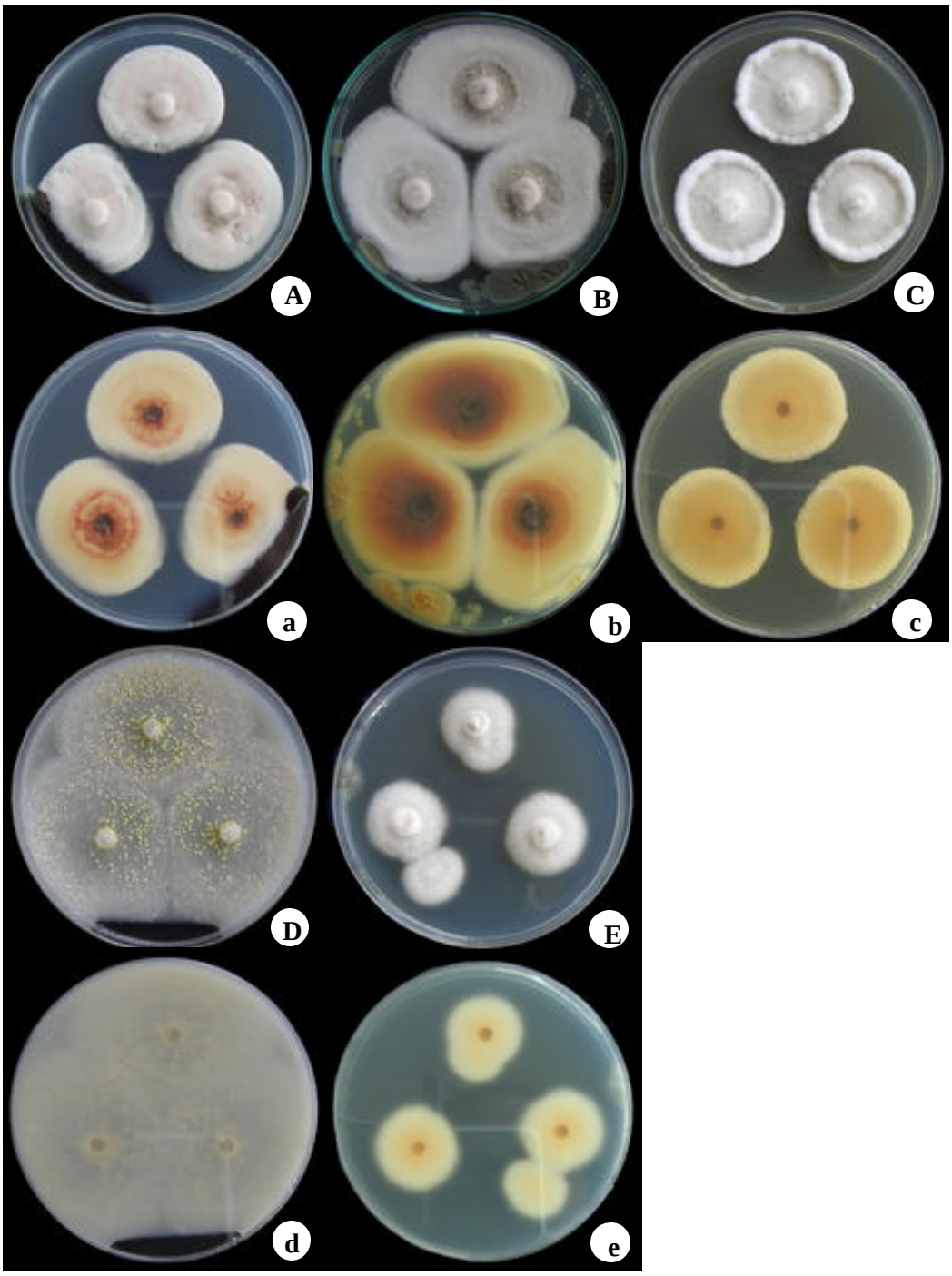


Figure 32 *Talaromyces indigoticus* KUFC 3366. Obverse and reverse views of colonies on different media, incubated for 7 days at 28°C; CZA (A, a), CYA (B, b), MEA (C, c), OMA (D, d), G25N (E, e)

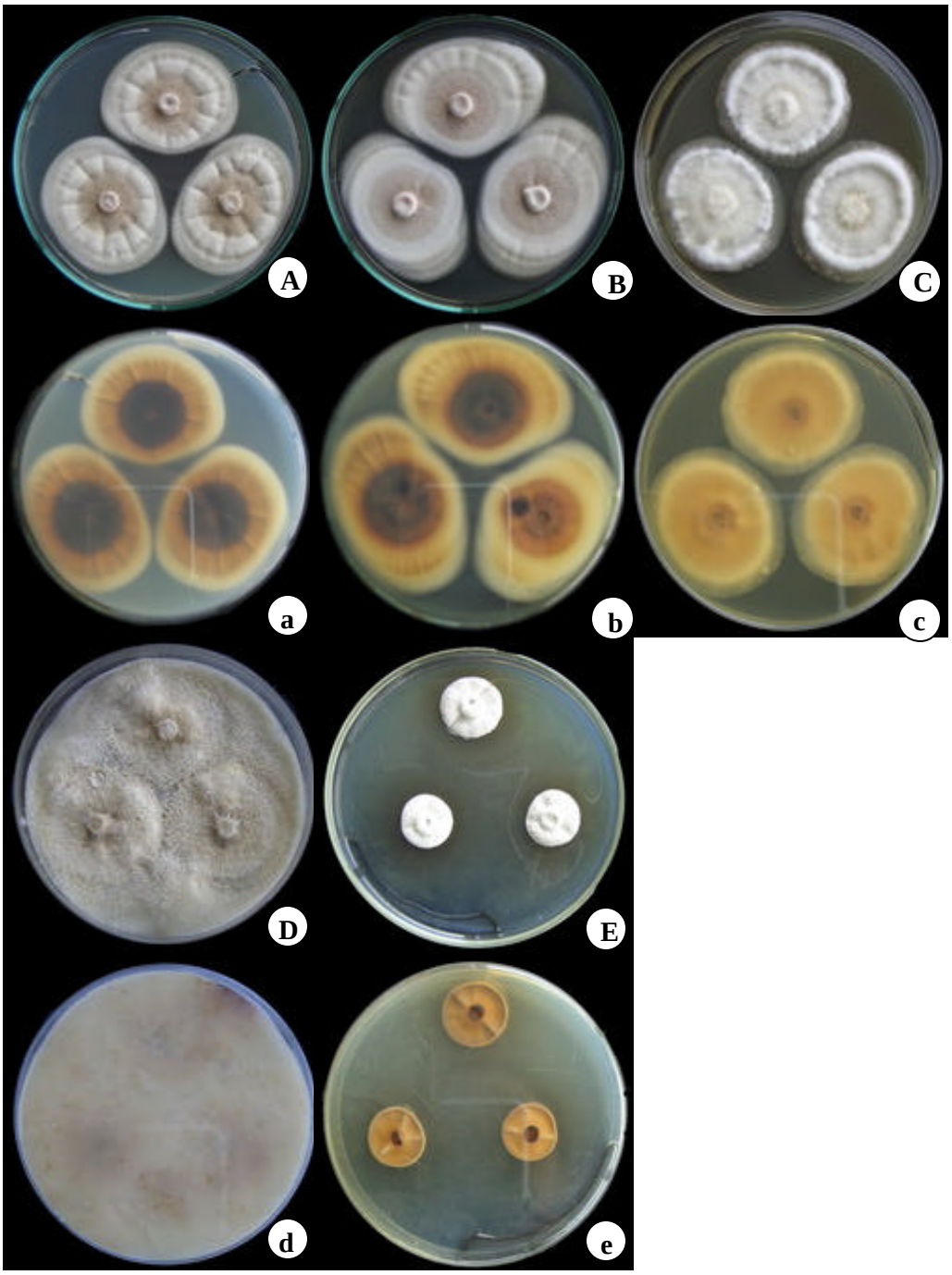


Figure 33 *Talaromyces indigoticus* KUFC 3366. Obverse and reverse views of colonies on different media, incubated for 14 days at 37°C; CZA (A, a), CYA (B, b), MEA (C, c), OMA (D, d), G25N (E, e)

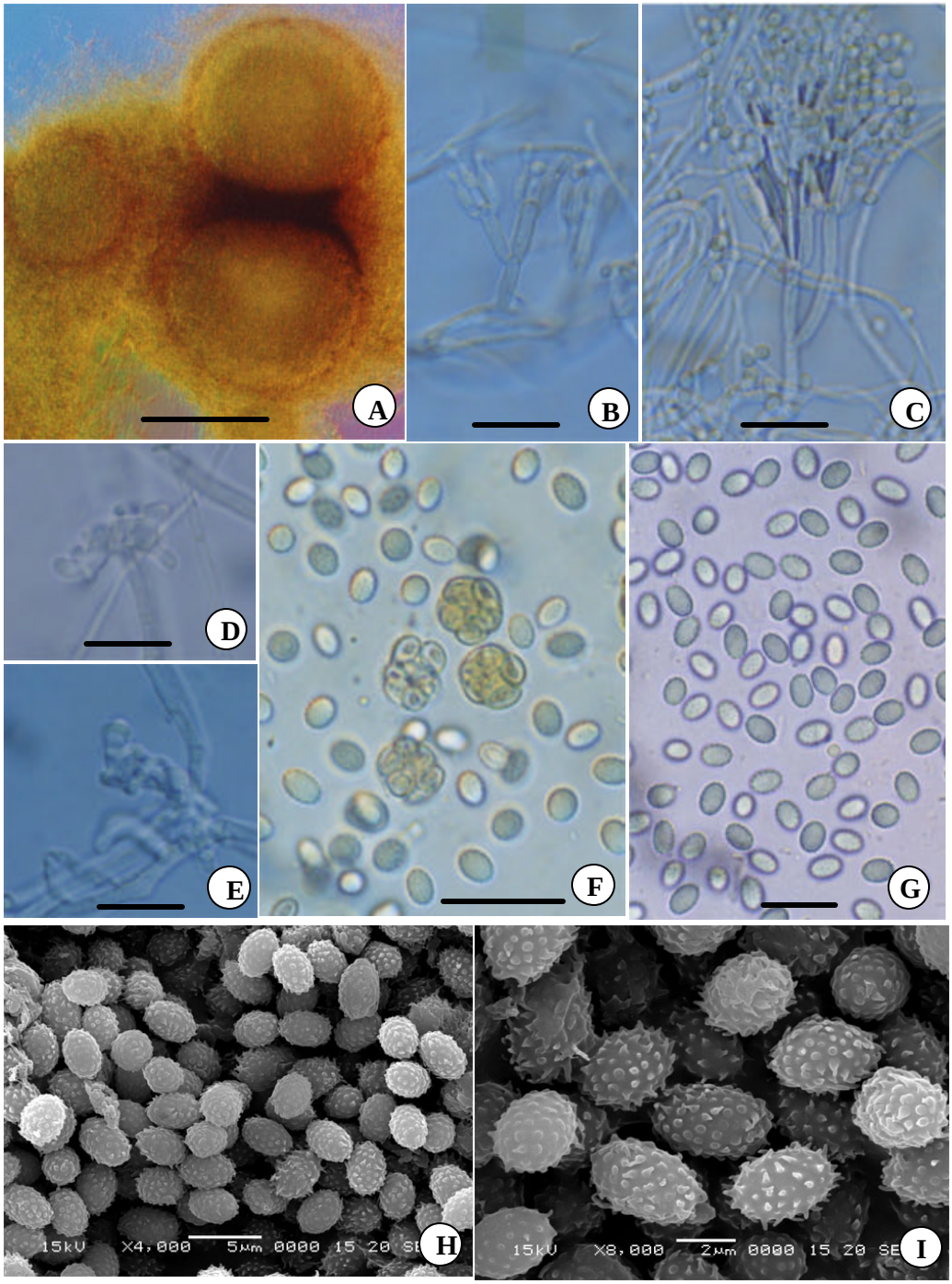


Figure 34 *Talaromyces indigoticus* KUFC 3366

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)

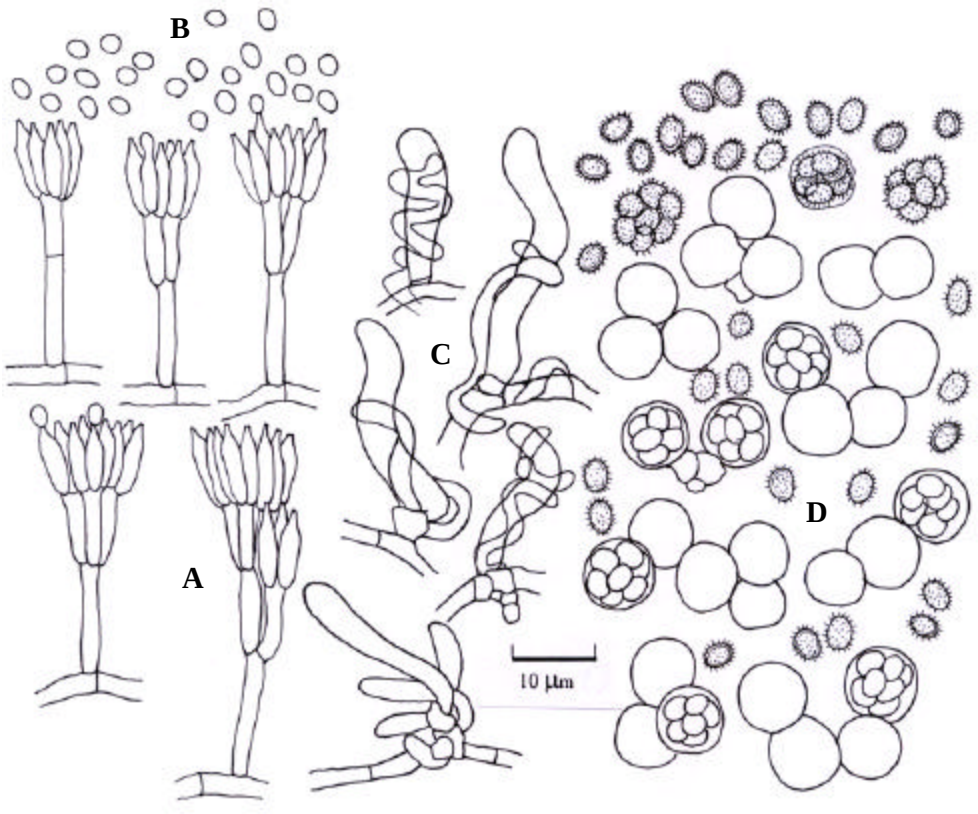


Figure 35 *Talaromyces indigoticus* KUFC 3366

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).

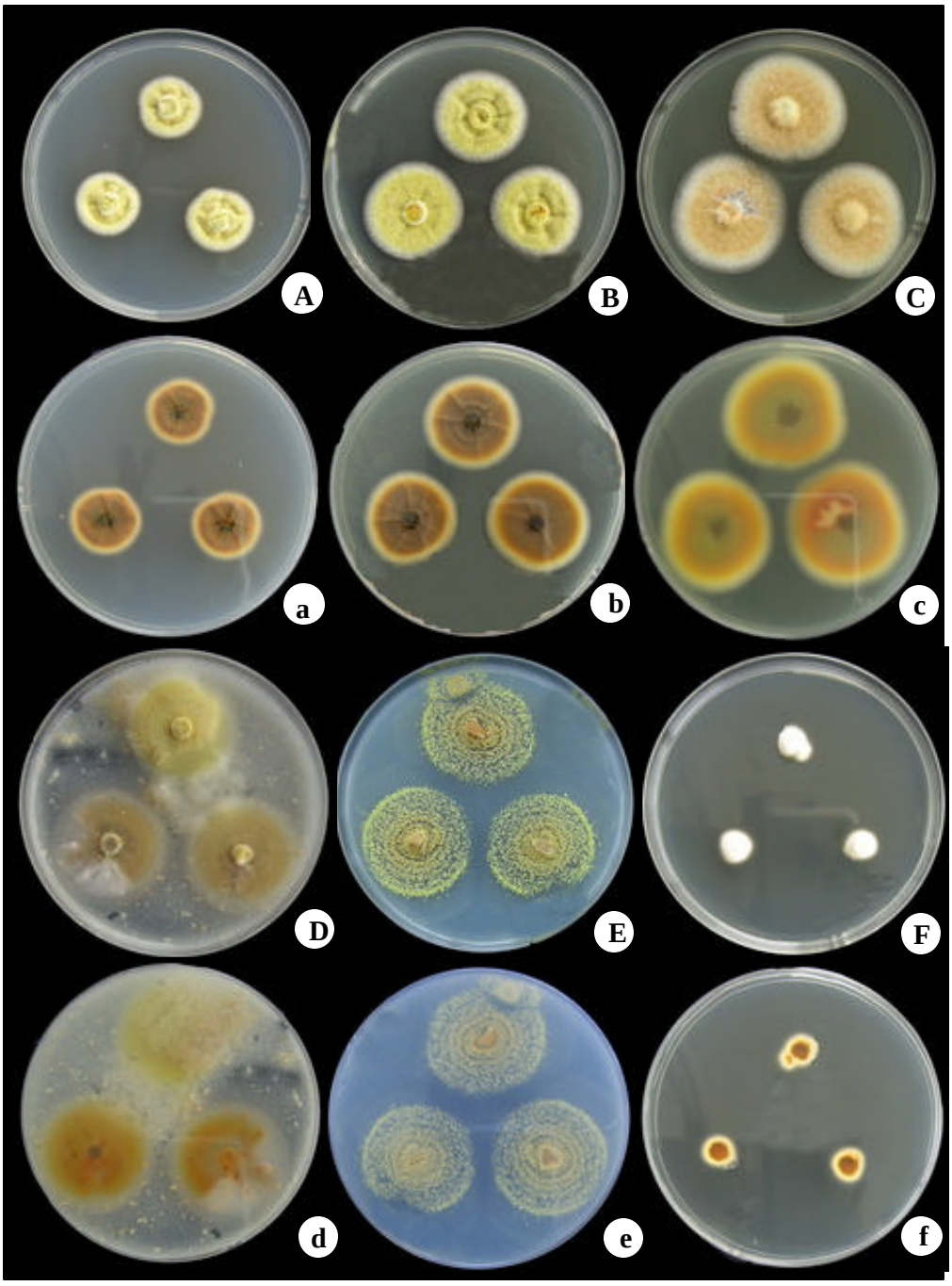


Figure 36 *Talaromyces rotundus* KUFC 3359. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

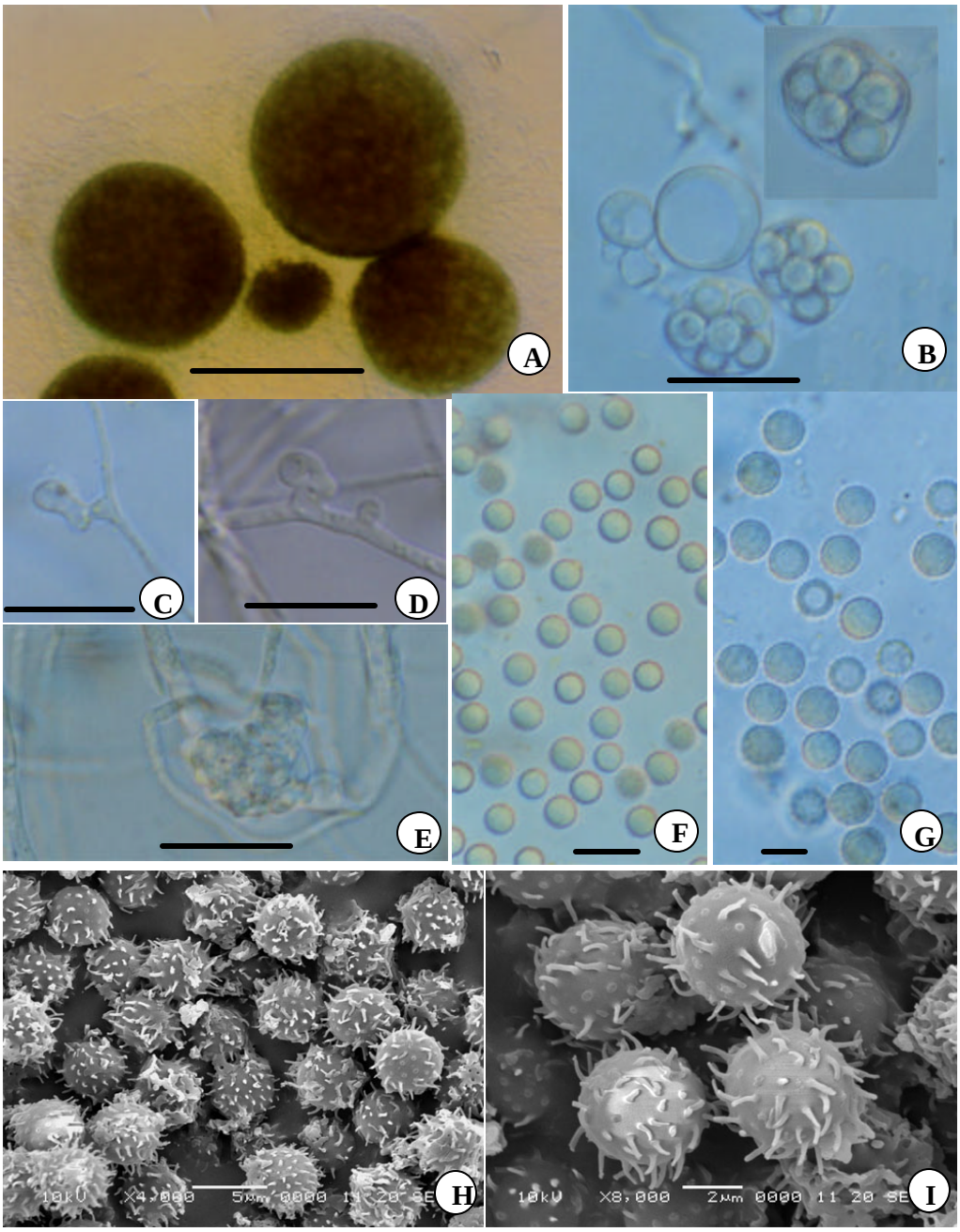


Figure 37 *Talaromyces rotundus* KUFC 3359

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)

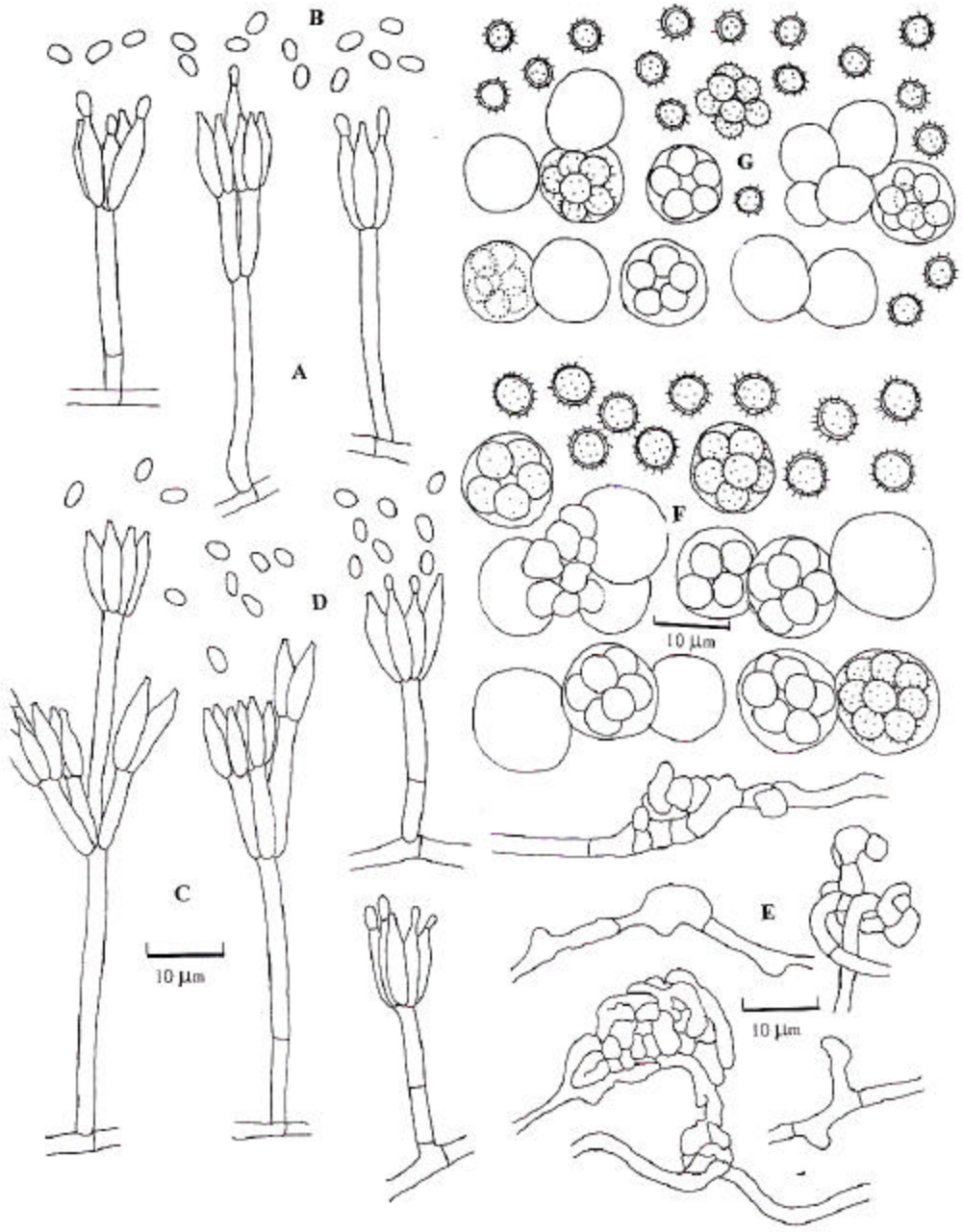


Figure 38 *Talaromyces rotundus*

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).

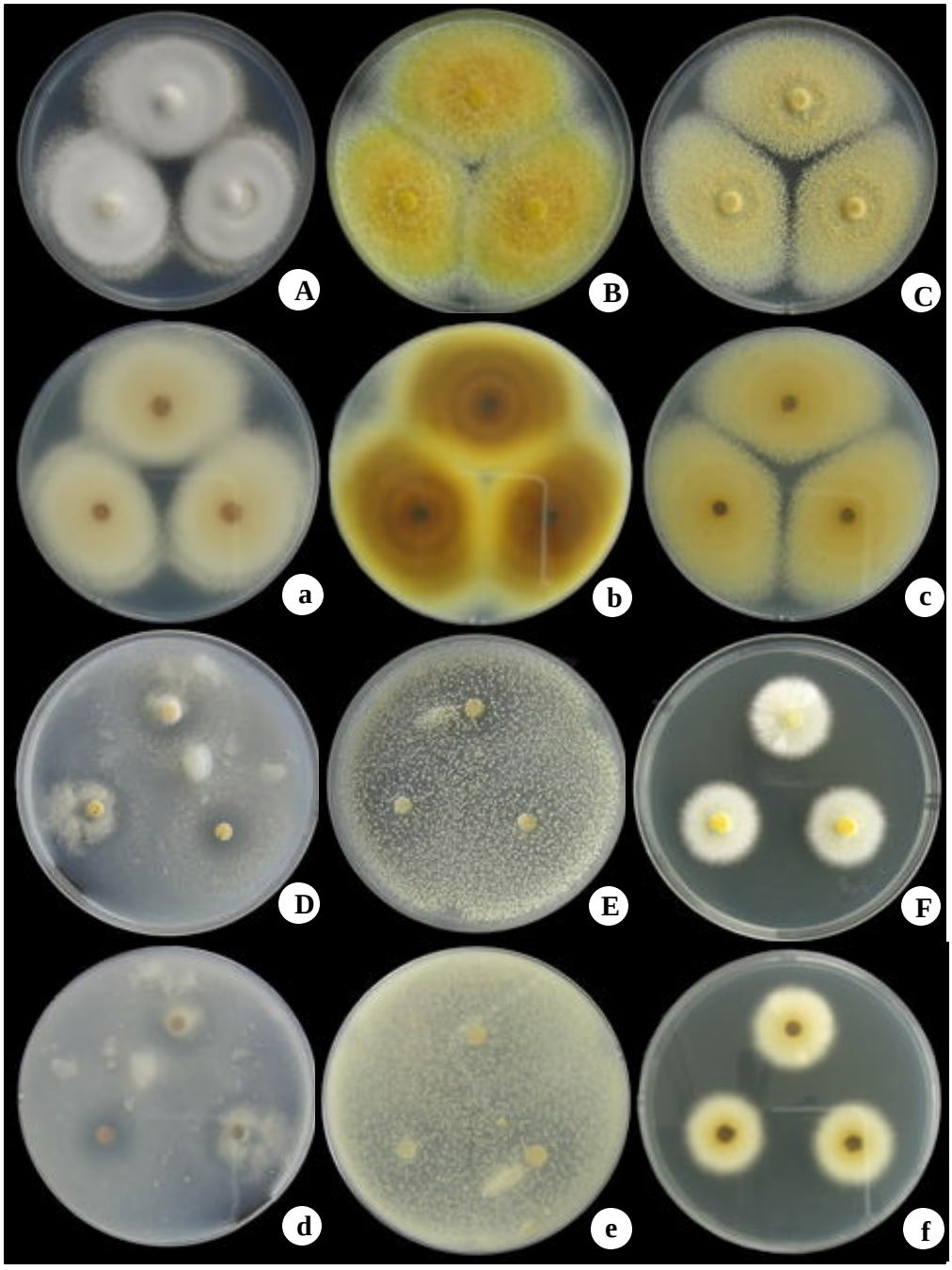


Figure 39 *Talaromyces stipitatus* KUFC 3357. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

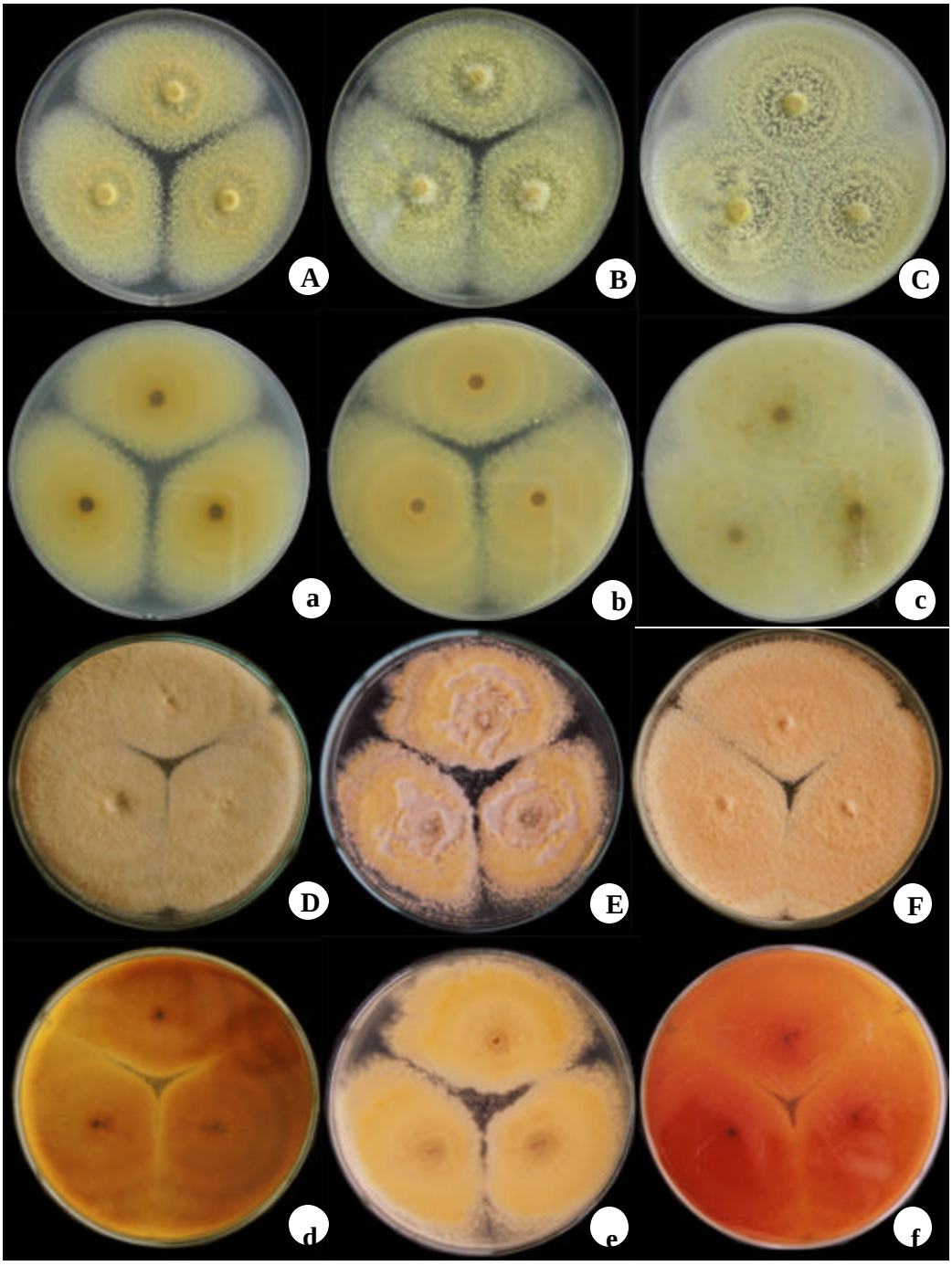


Figure 40 *Talaromyces stipitatus* KUFC 3357. Obverse and reverse sides of colonies on different media, incubated for 7 days at 28°C: CZA (A, a), CYA (B, b), MEA (C, c), incubated for 14 days at 28°C: CZA (D, d), CYA (E, e), MEA (F, f)

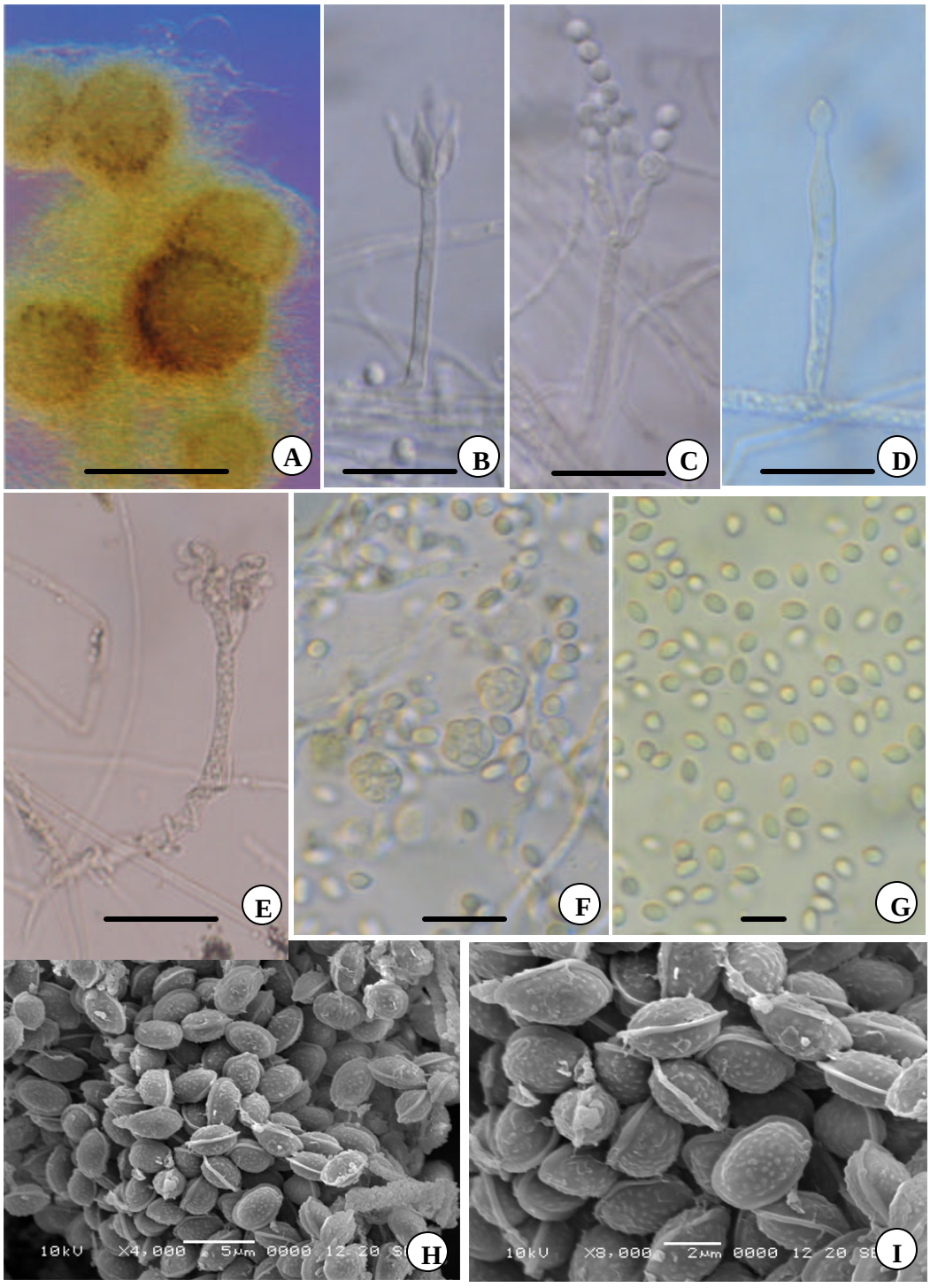


Figure 41 *Talaromyces stipitatus* KUFC 3357

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)

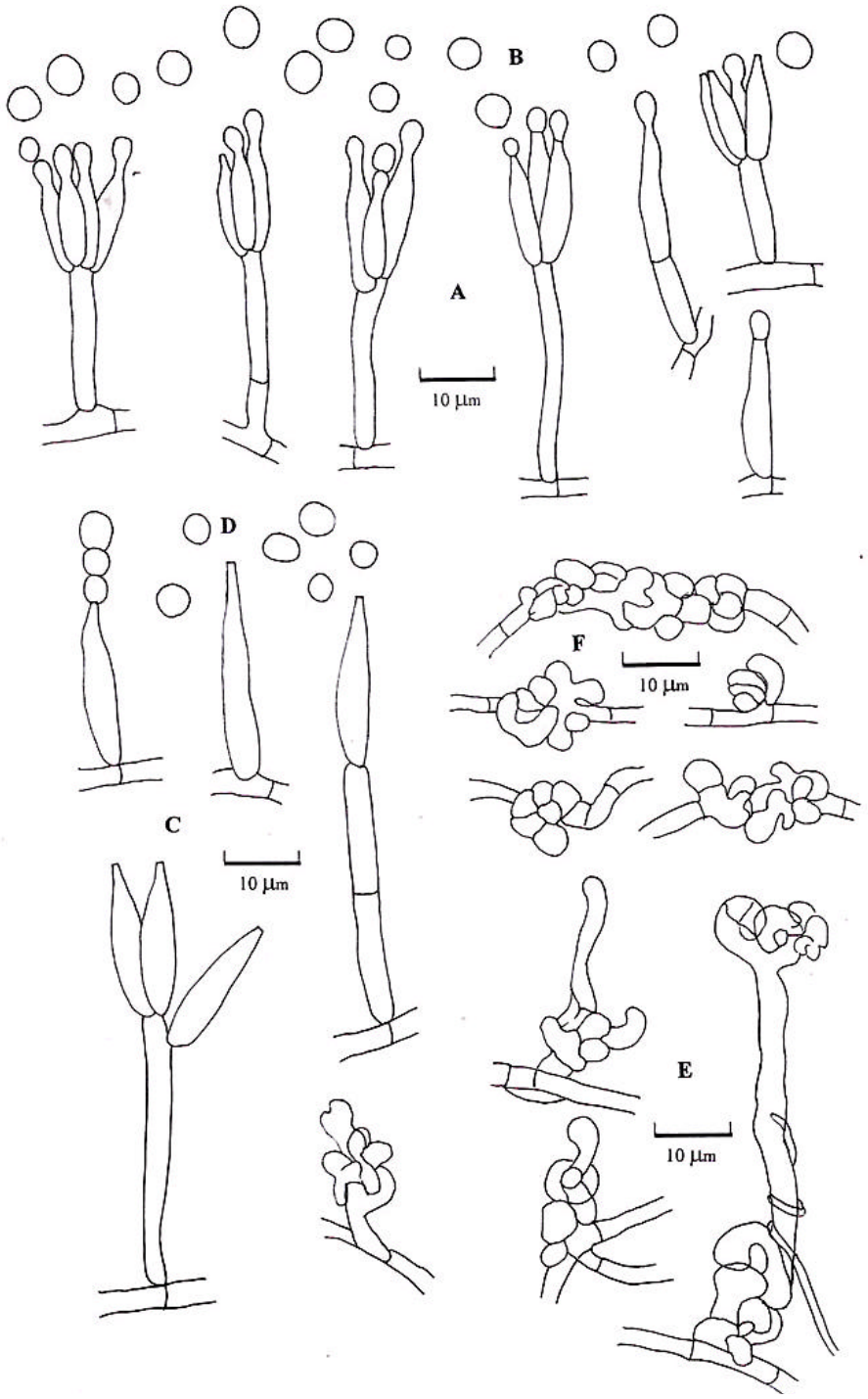


Figure 42 *Talaromyces stipitatus*

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

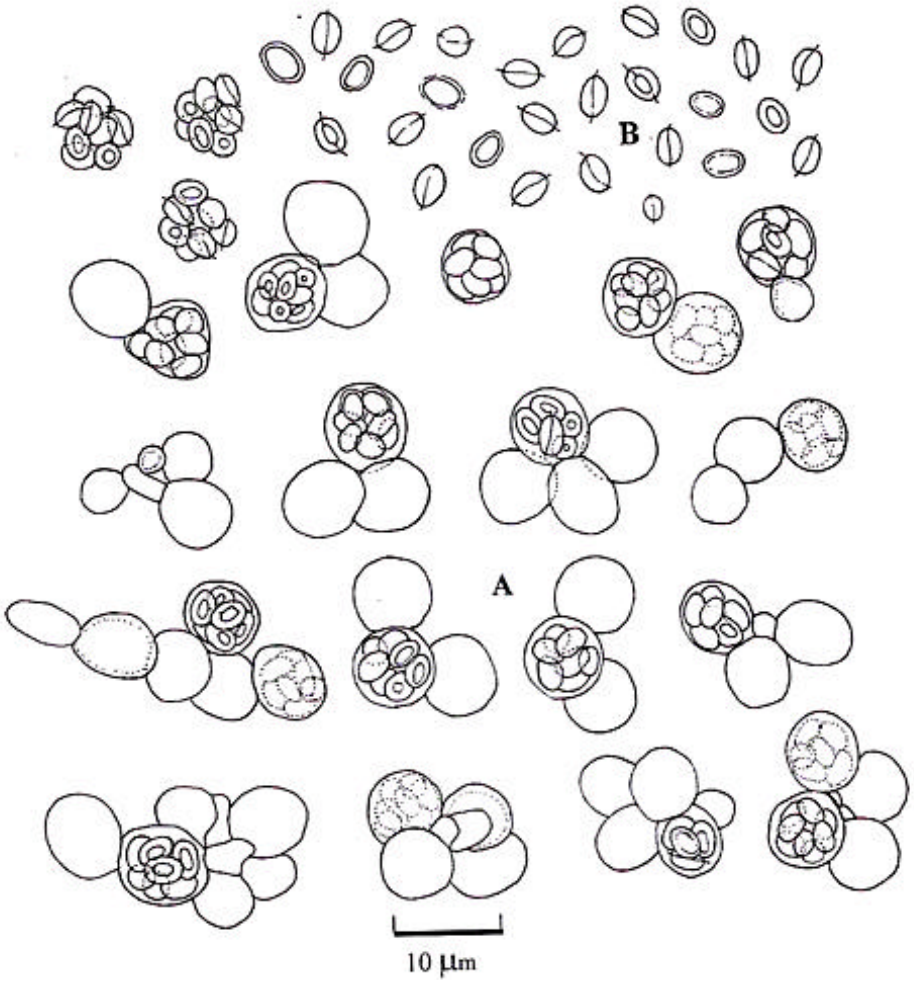


Figure 43 *Talaromyces stipitatus* KUFC 3357
Camera lucida drawings of A. asci; B. ascospores

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).

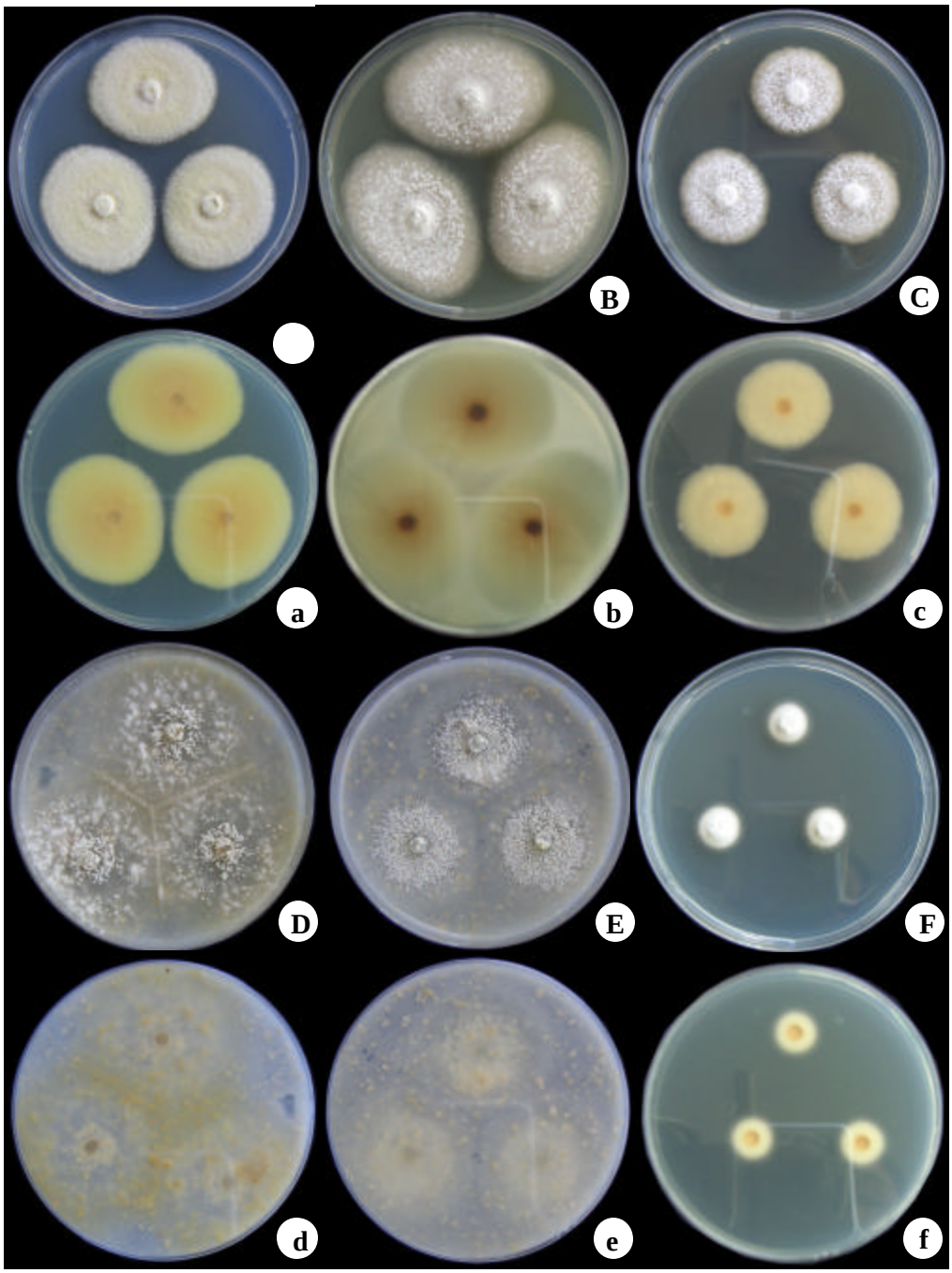


Figure 44 *Talaromyces trachyspermus* KUFC 3355. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

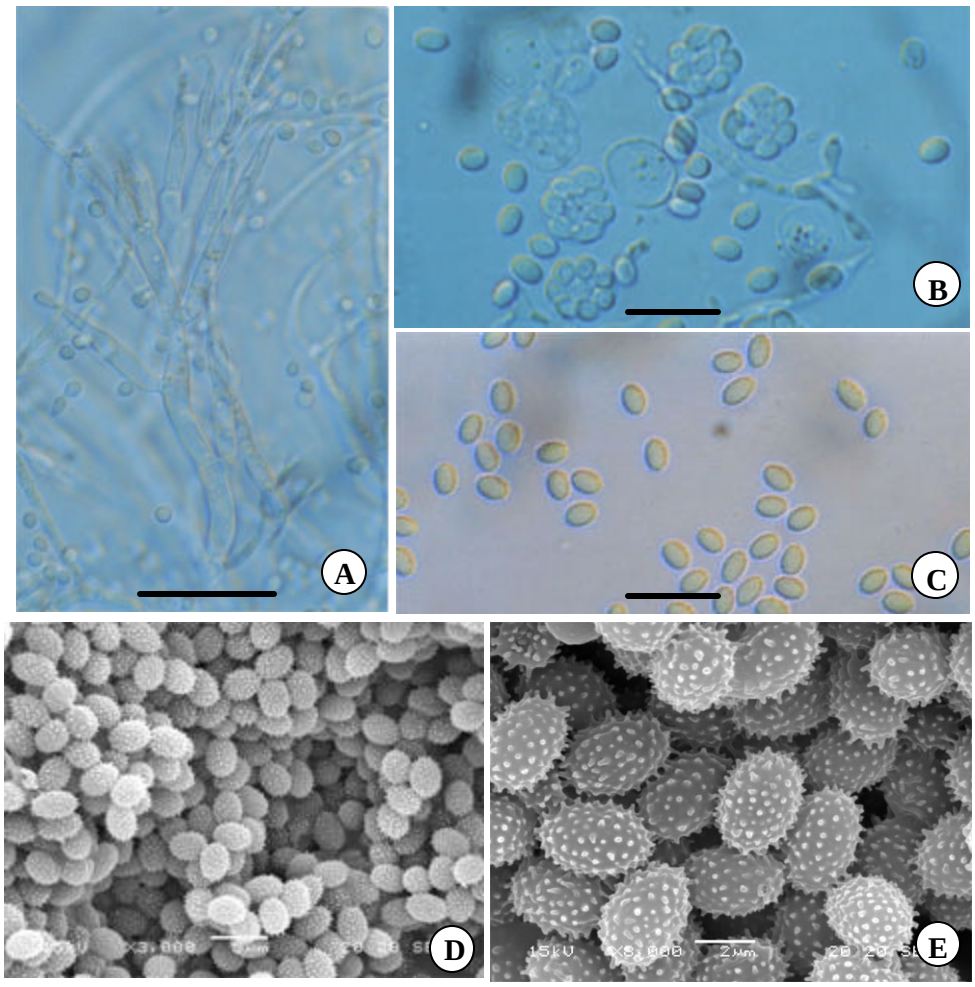


Figure 45 *Talaromyces trachysperus* KUFC 3355
A. penicilli; B-C. asci and ascospores; D-E. ascospores (SEM)
(Bars: A-C = 10 µm; D = 5 µm; E = 2 µm)

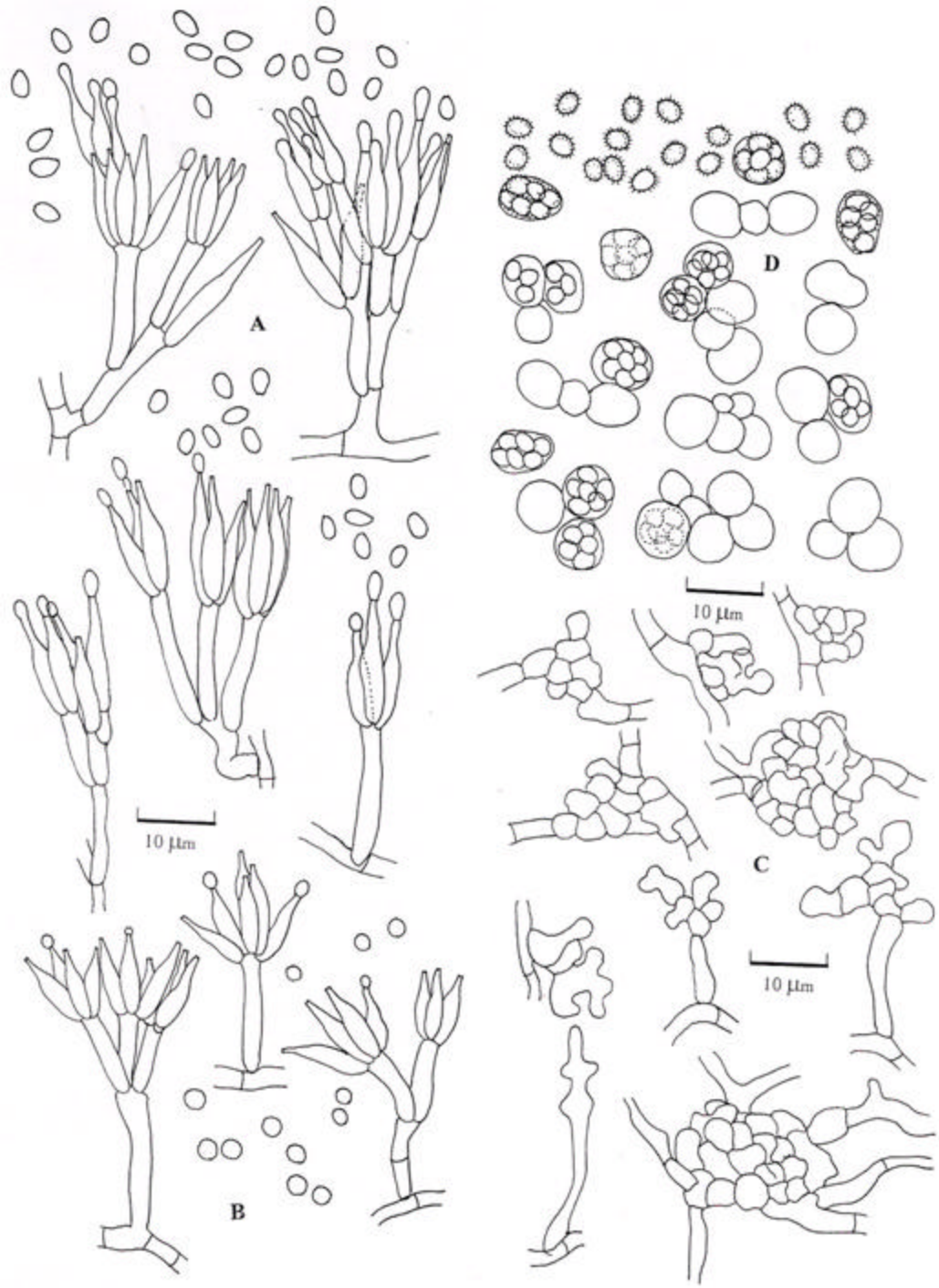


Figure 46 *Talaromyces trachyspermus*

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.

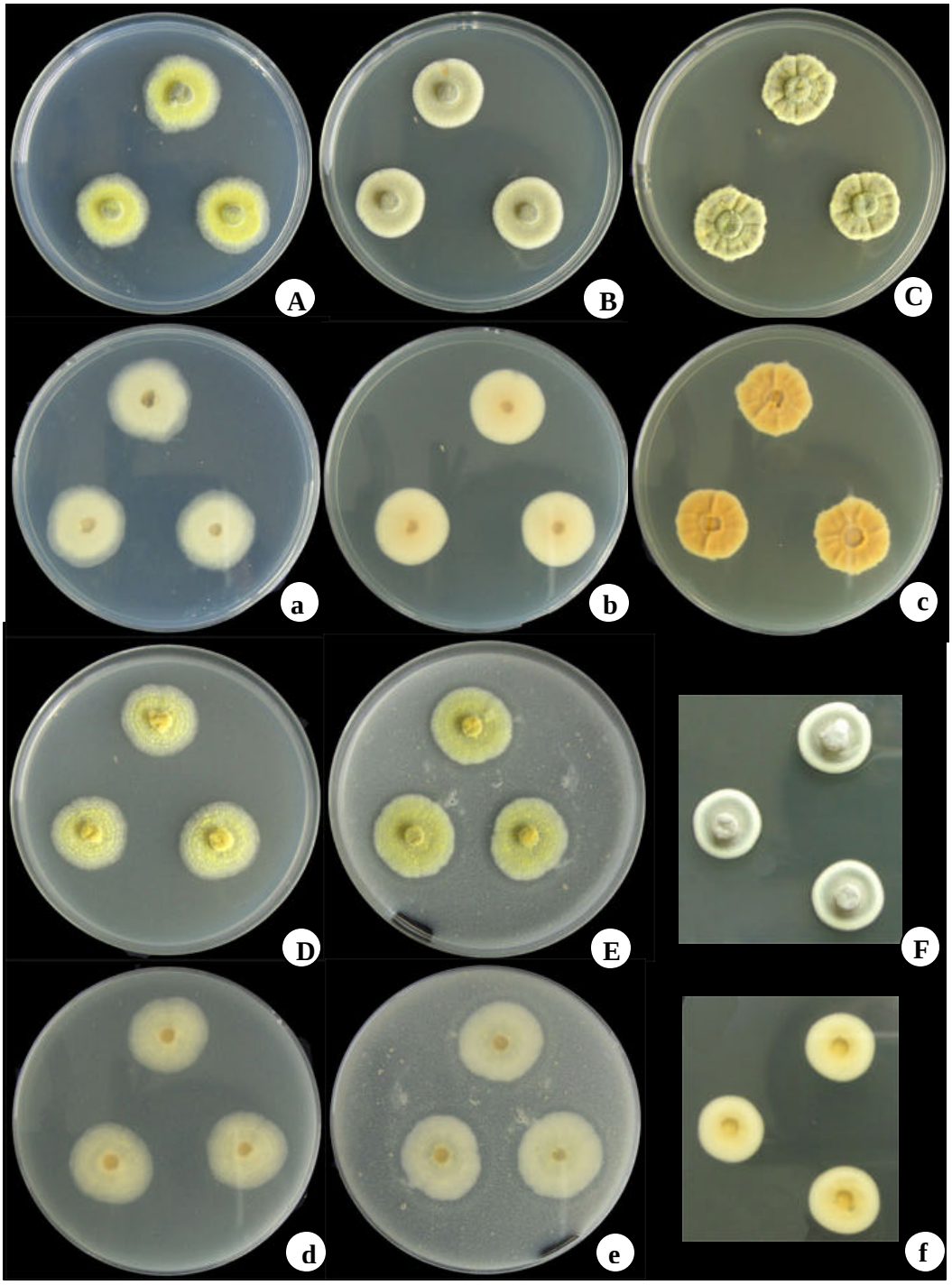


Figure 47 *Talaromyces wortmannii* KUFC 3333. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

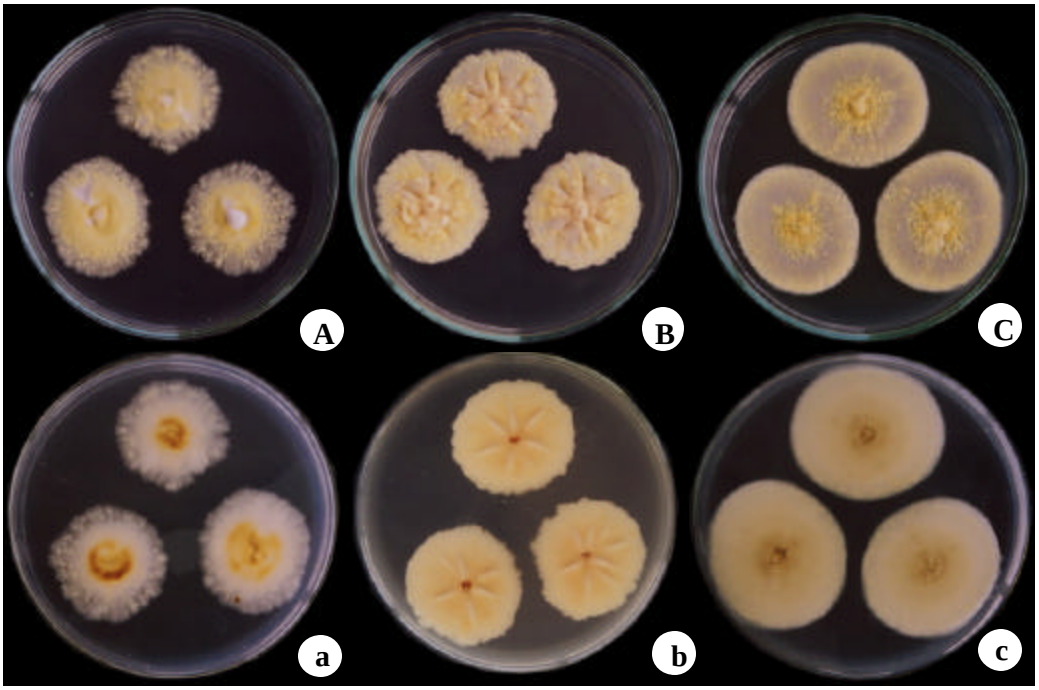


Figure 48 *Talaromyces wortmannii* KUFC 3333. Obverse and reverse views of colonies on different media, incubated for 7 days at 28°C; CZA (A, a), CYA (B, b), MEA (C, c)

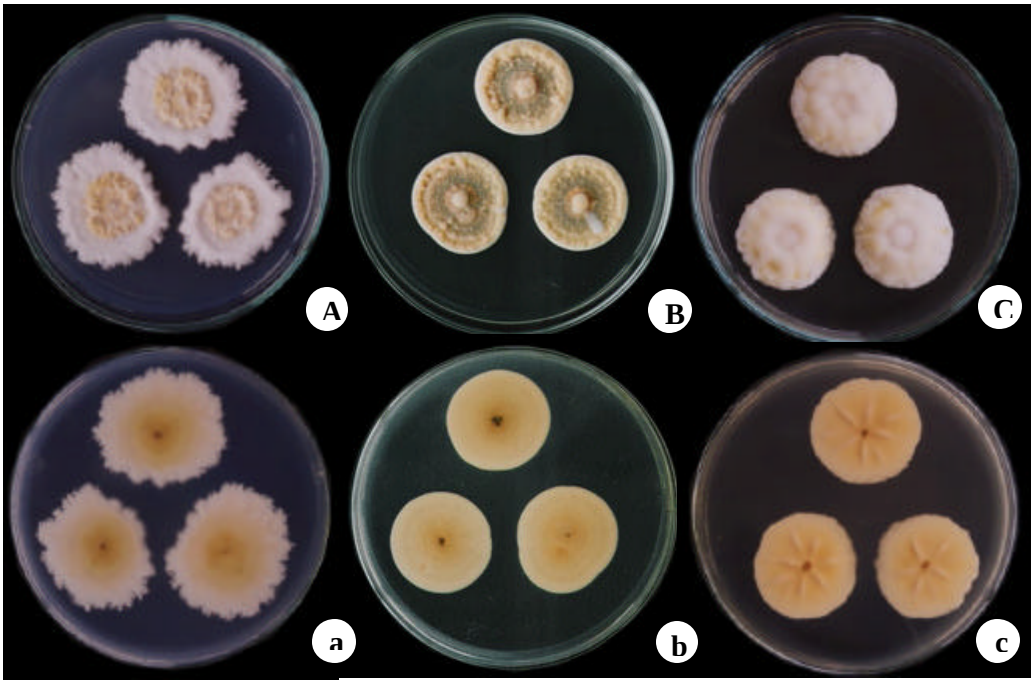


Figure 49 *Talaromyces wortmannii* KUFC 3354. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c)

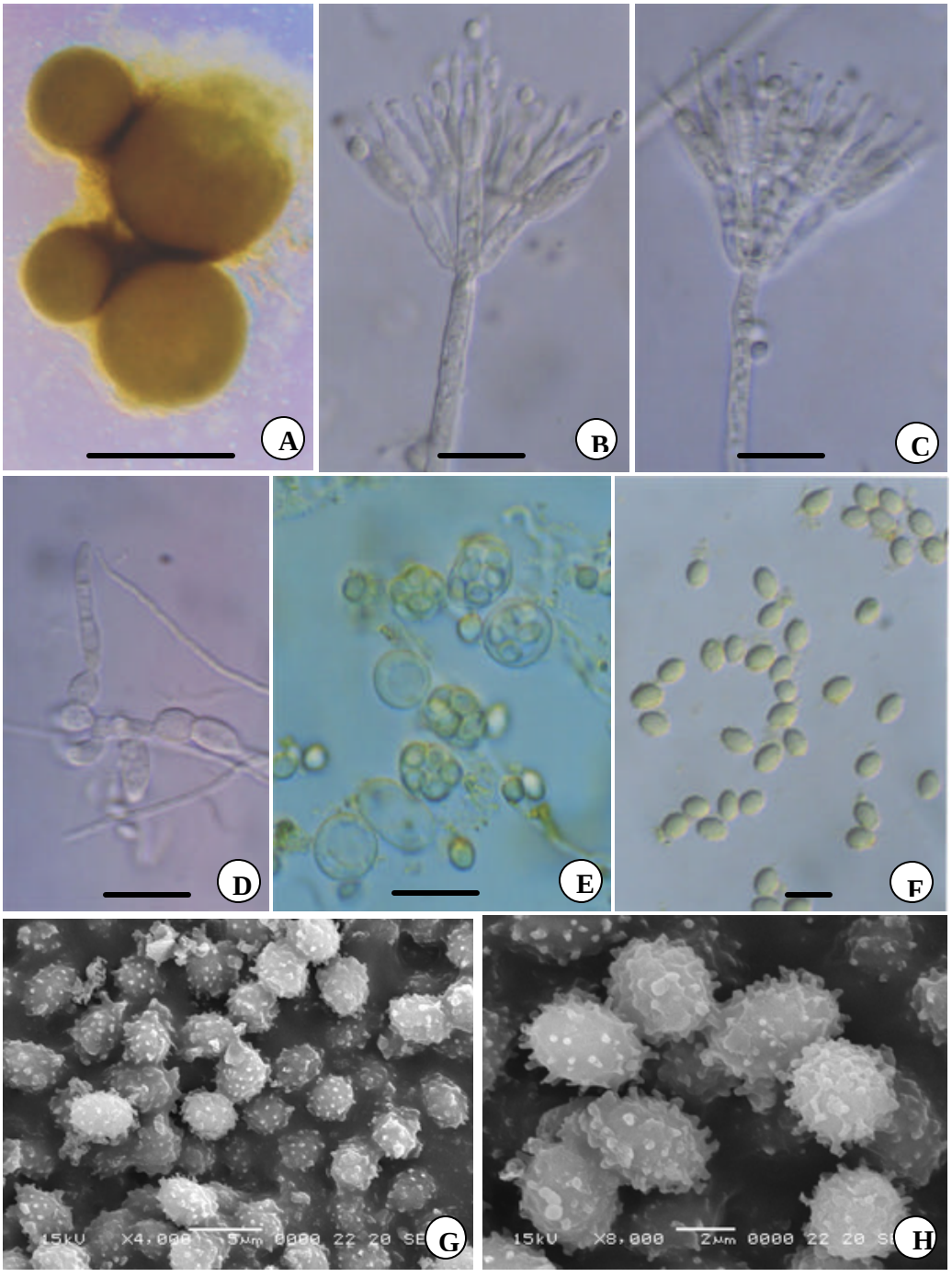


Figure 50 *Talaromyces wortmannii* KUFC 3333

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)

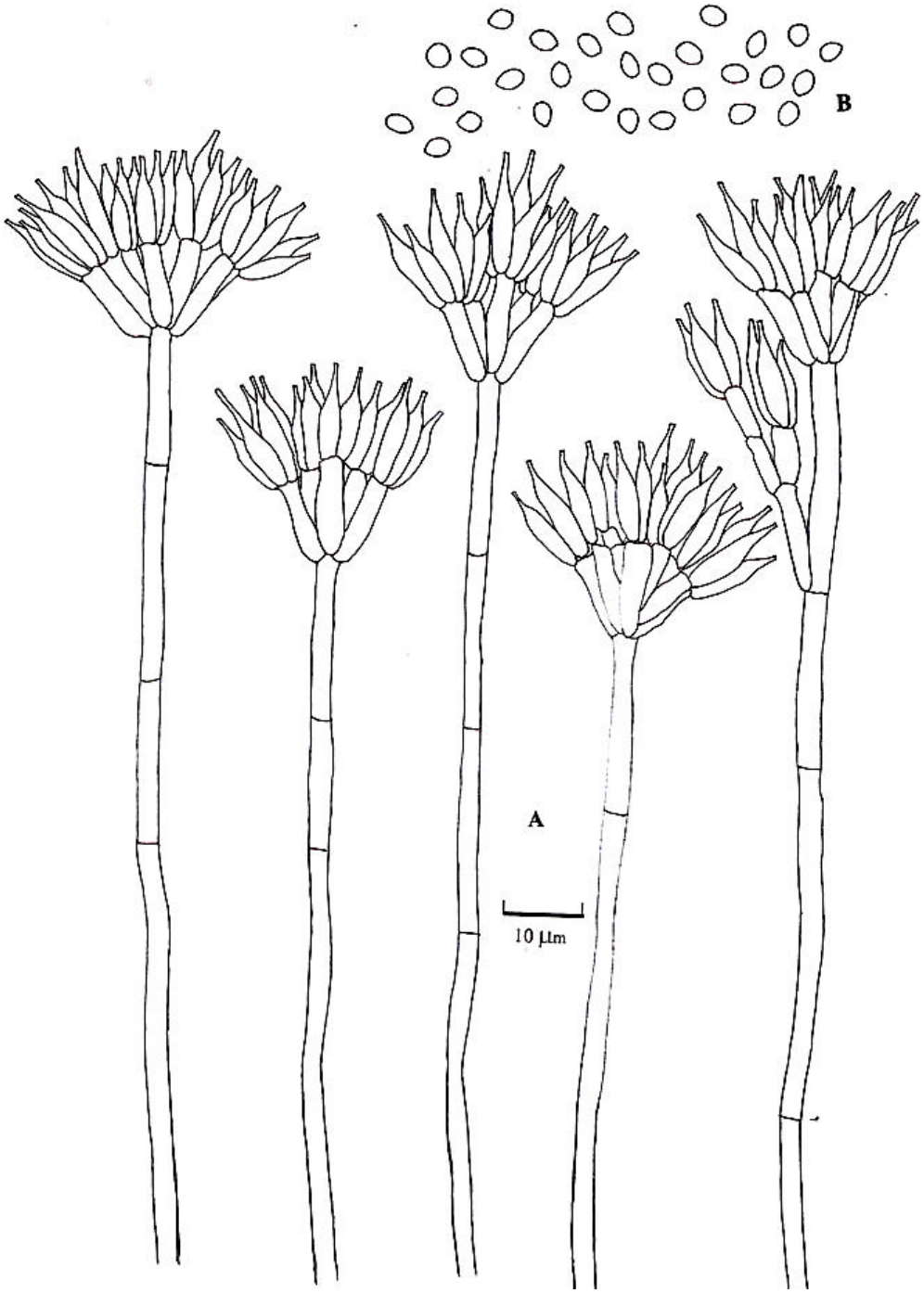


Figure 51 ***Talaromyces wortmannii*** KUFC 3333

Camera lucida drawings of A. penicilli and B. conidia

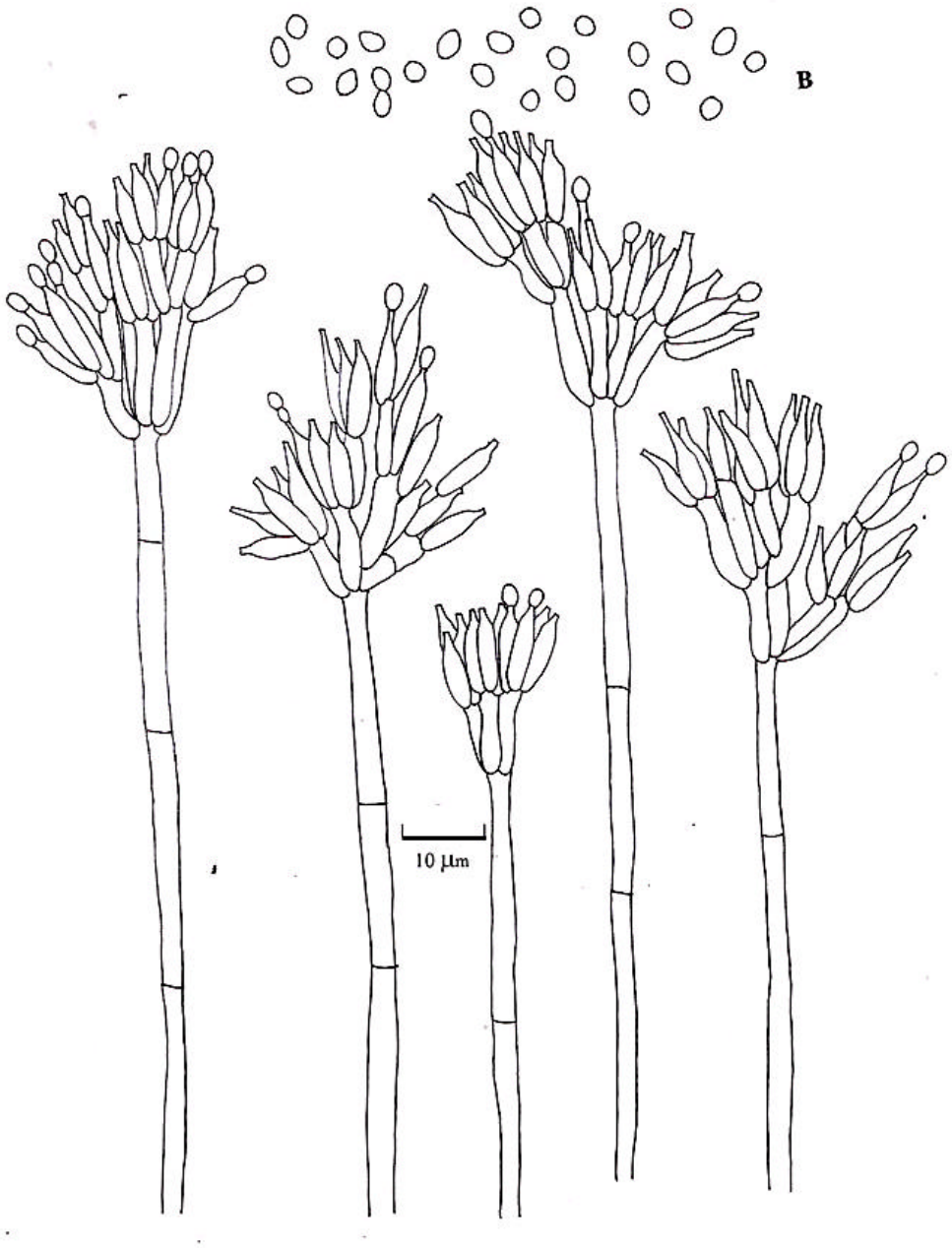


Figure 52 ***Talaromyces wortmannii*** KUFC 3354
Camera lucida drawings of A. penicilli and B. conidia

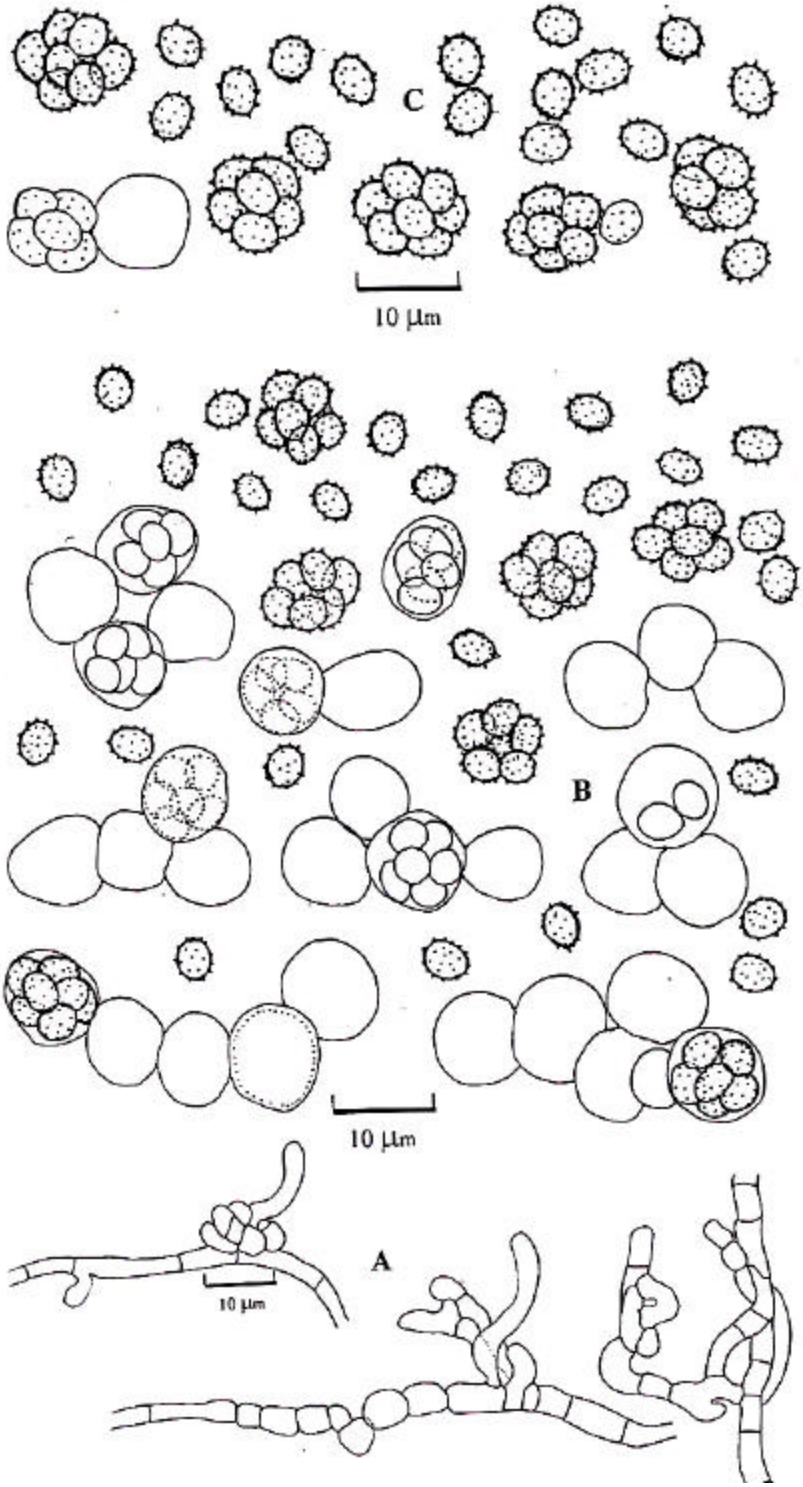


Figure 53 ***Talaromyces wortmannii***

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.

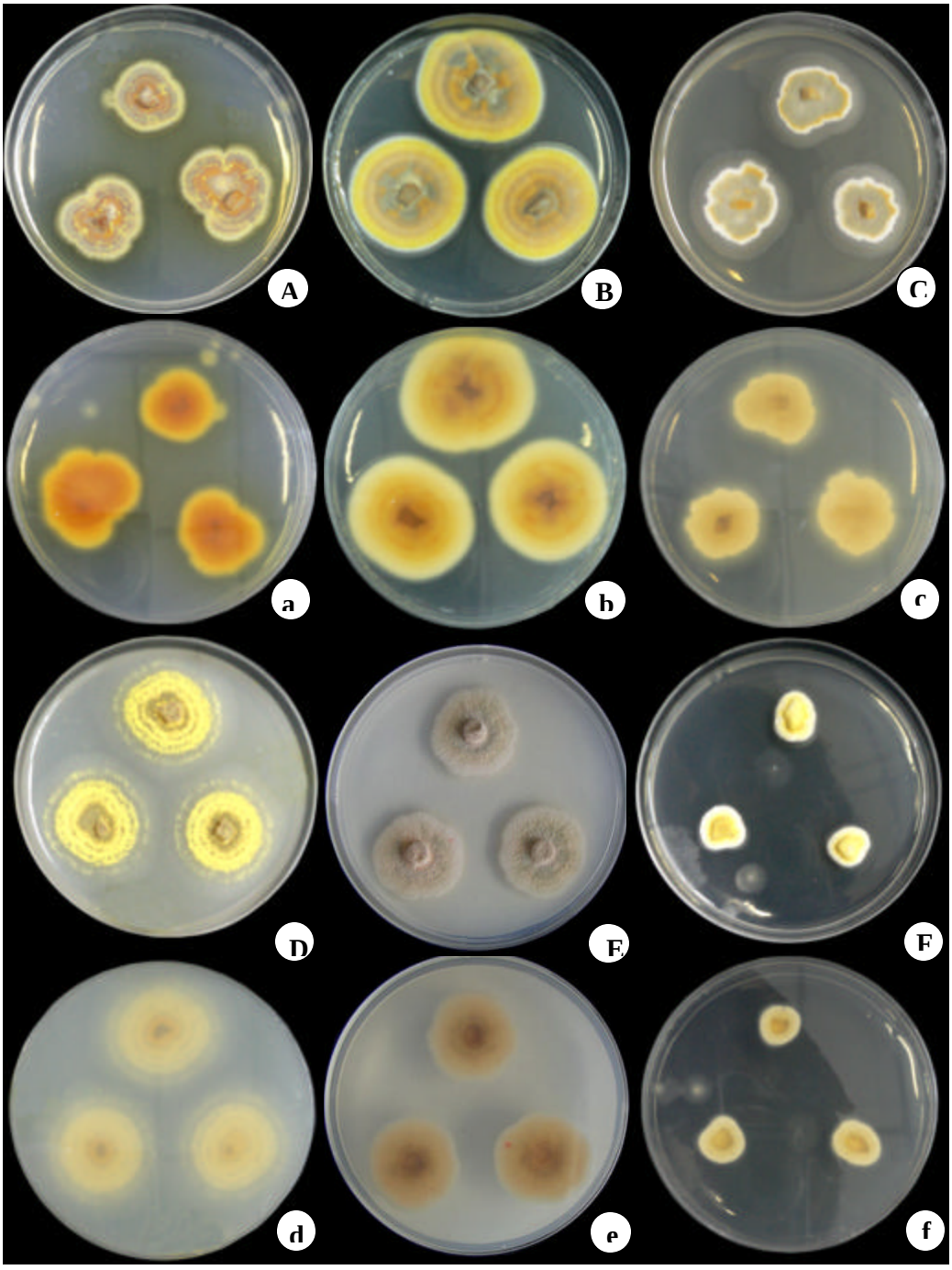


Figure 54 *Talaromyces* sp. 1 KUFC 3399. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

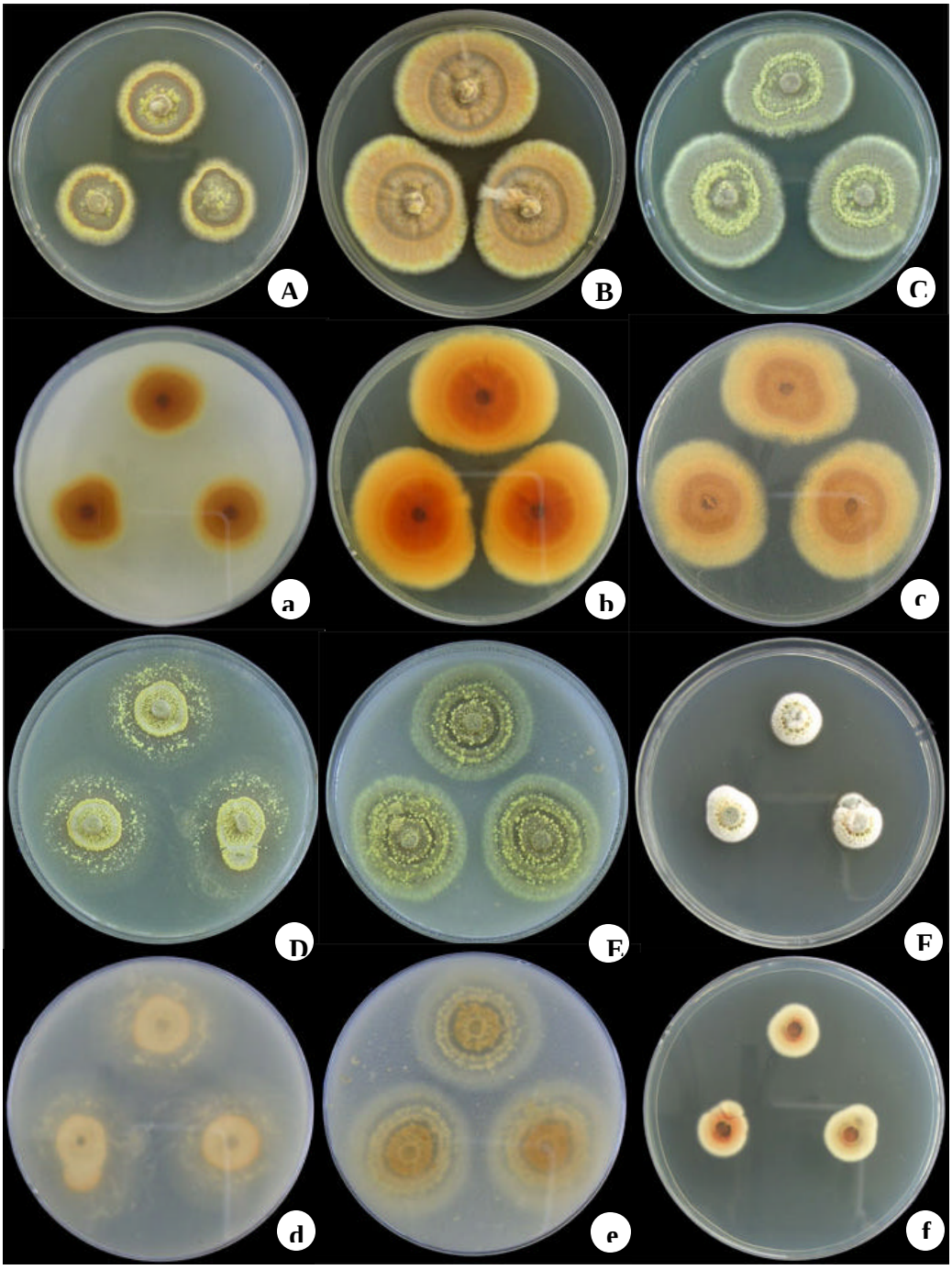


Figure 55 *Talaromyces* sp. 1 KUFC 3399. Obverse and reverse views of colonies on different media, incubated for 7 days at 28°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

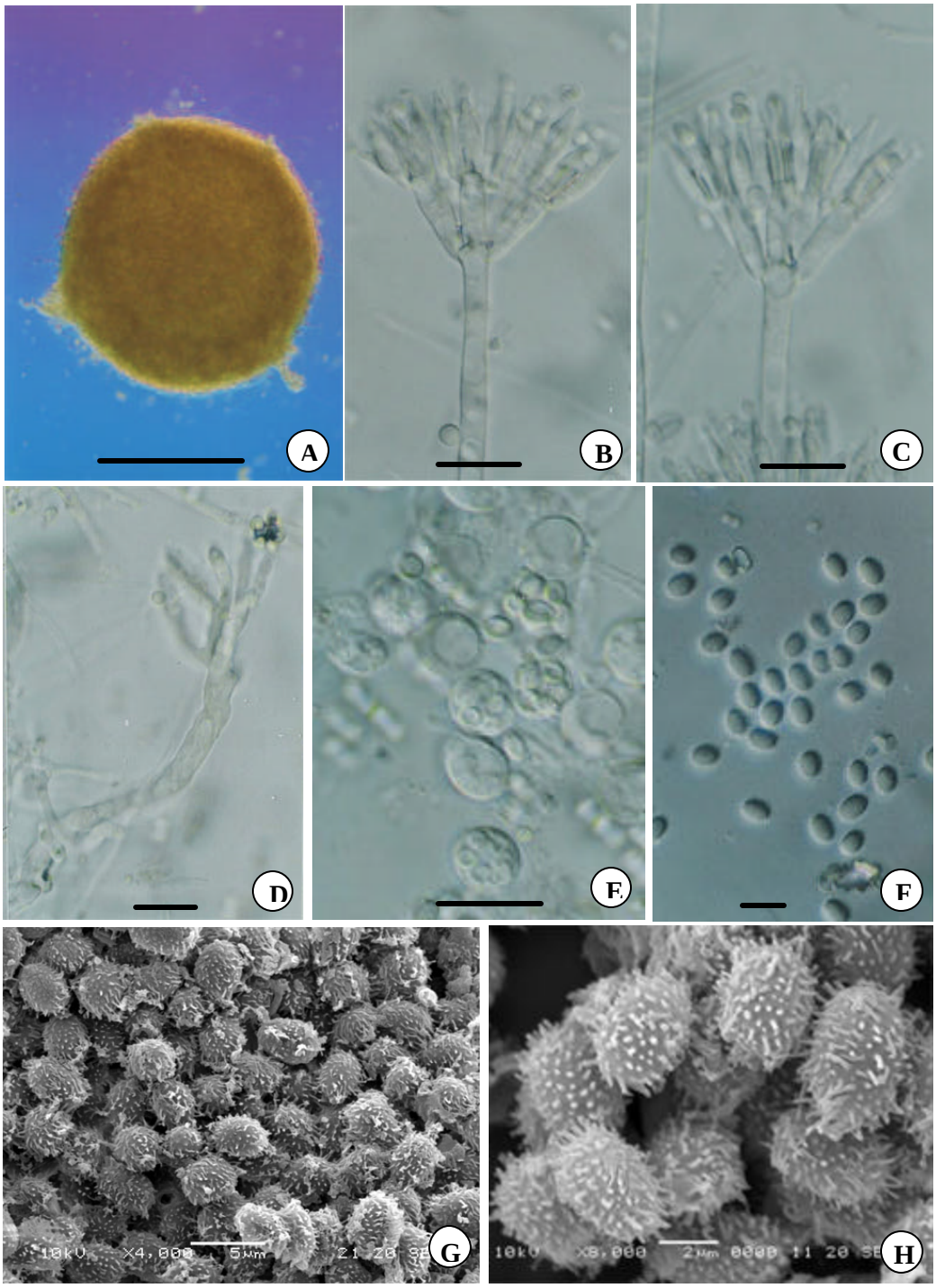


Figure 56 *Talaromyces* sp. 1 KUFC 3399

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)

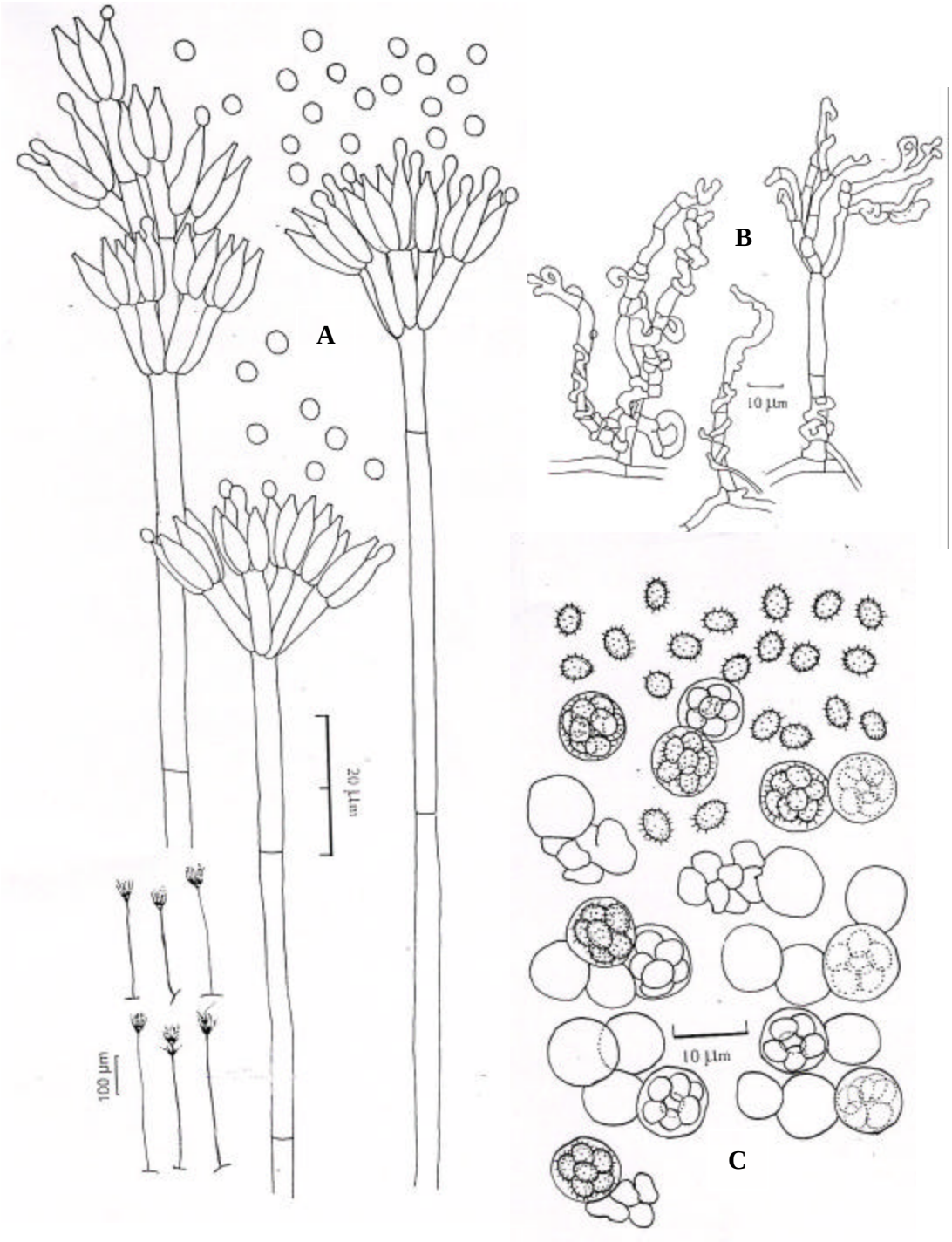


Figure 57 *Talaromyces* sp. 1 KUFC 3399

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.

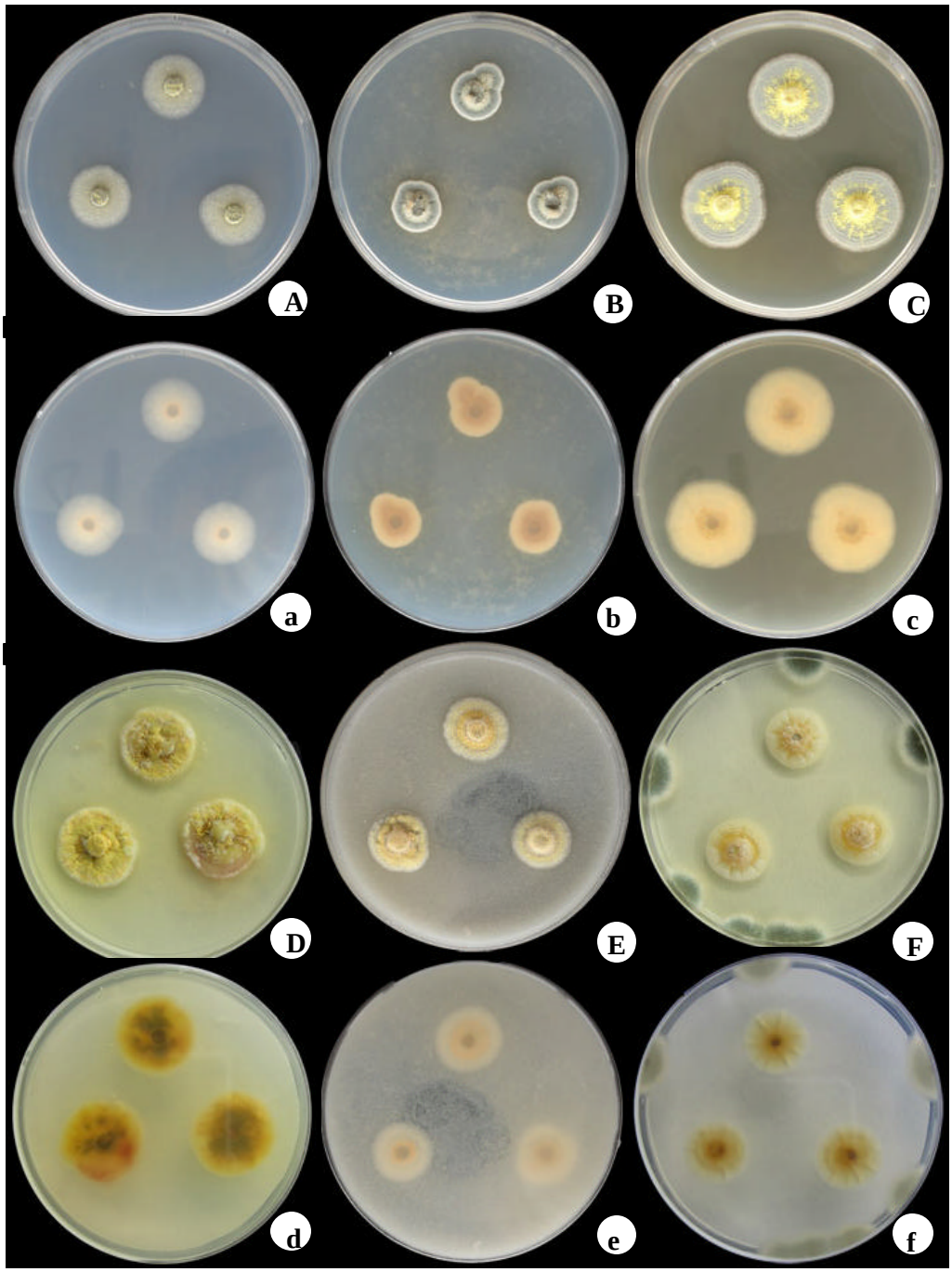


Figure 58 *Talaromyces* sp. 2 KUFC 3383. Obverse and reverse views of colonies on different media, incubated for 7 days at 25°C; CZA (A, a), CYA (B, b), MEA (C, c), CMA (D, d), OMA (E, e), G25N (F, f)

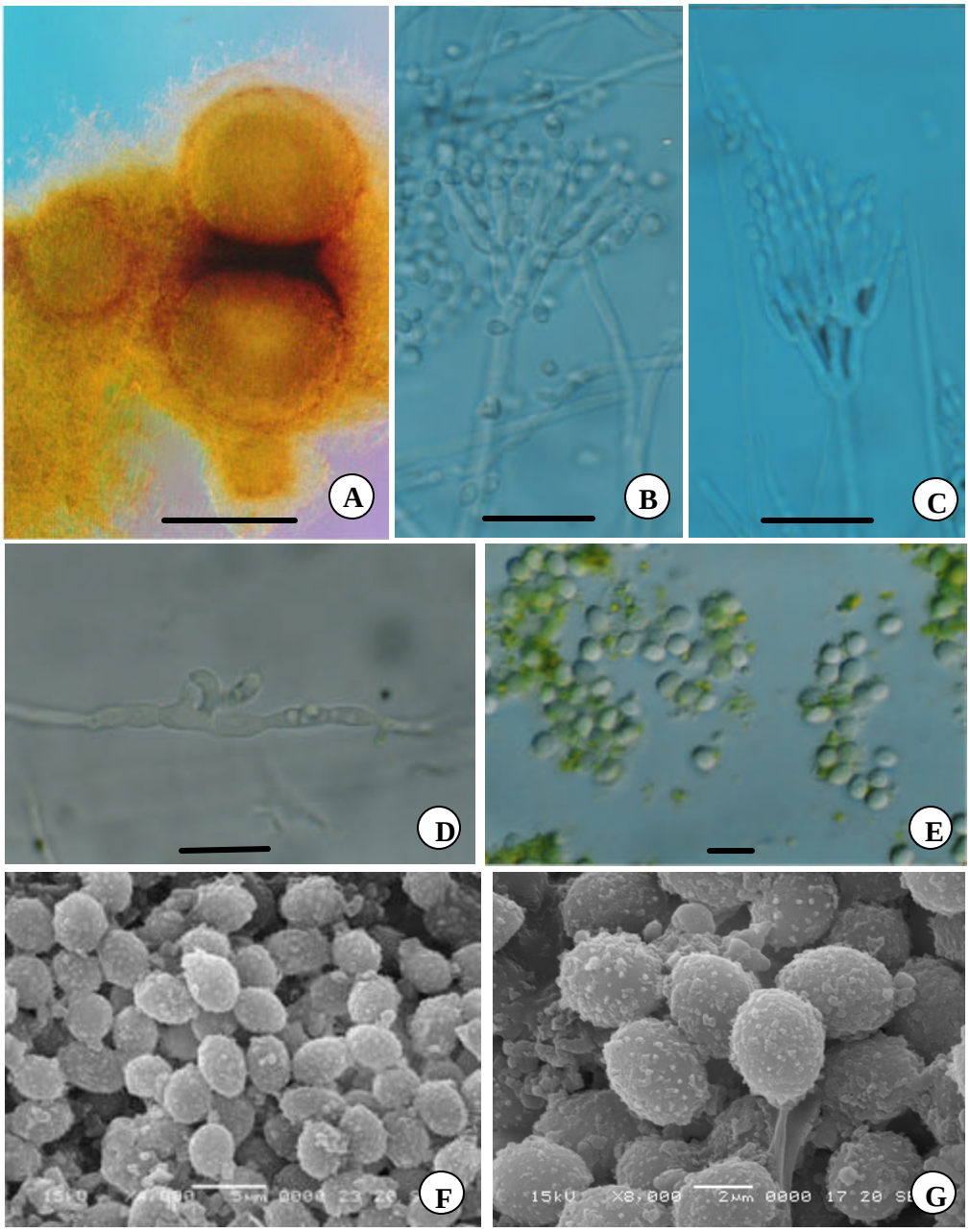


Figure 59 ***Talaromyces* sp. 2** KUFC 3383

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)

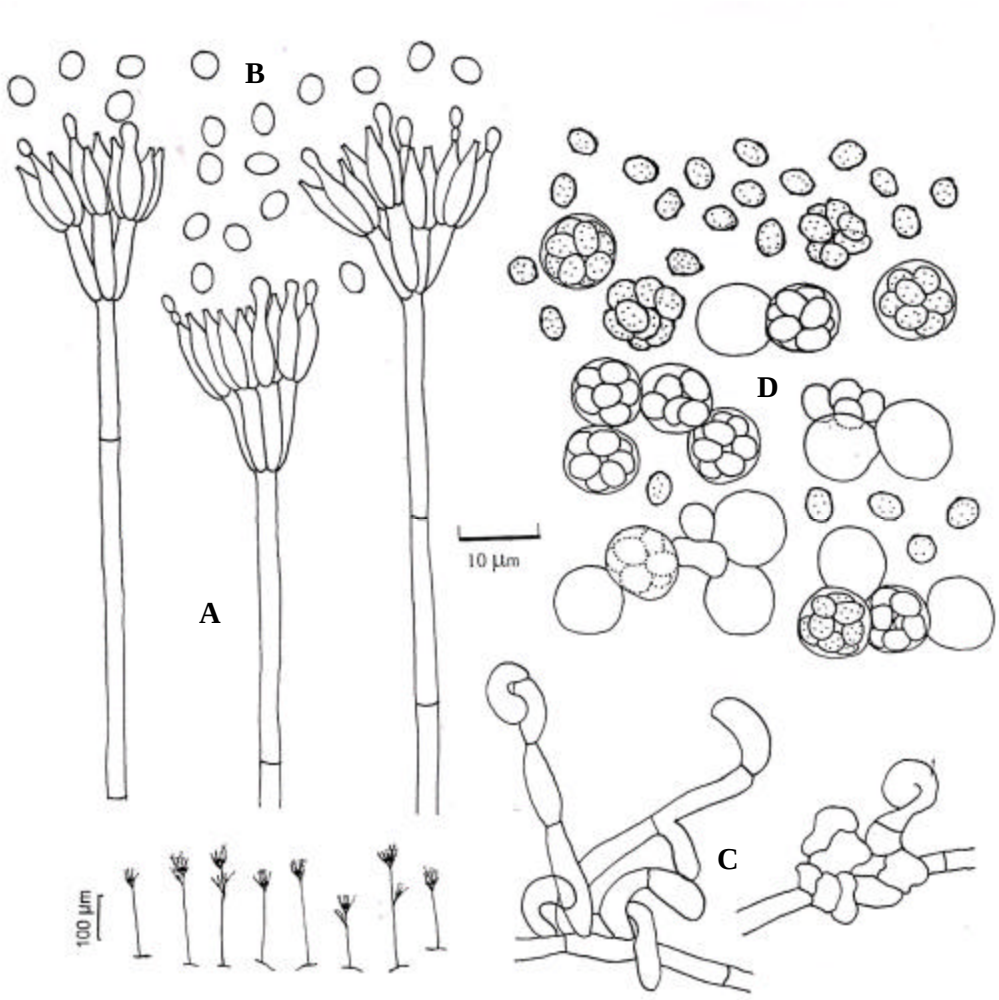


Figure 60 *Talaromyces* sp. 1 KUFC 3383

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.

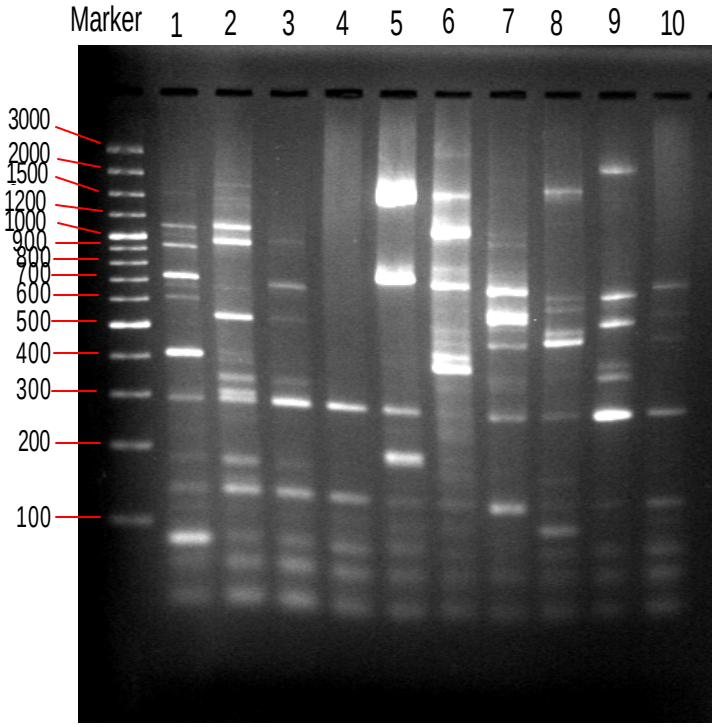


Figure 61 Electrophoresis of PCR products using PM7
1 = *T. bacillisporus* 2 = *T. austrocalifornicus* 3 = *T. macrosporus*
4 = *T. stipitatus* 5 = Trichocamaceae (*Penicillium* anamorph)
6 = *Talaromyces* sp. 1 7 = *T. roduntus* KUFC 3446 8 = *T. wortmannii*
9 = *T. flavus* 10 = *T. rotundus* KUFC 3359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

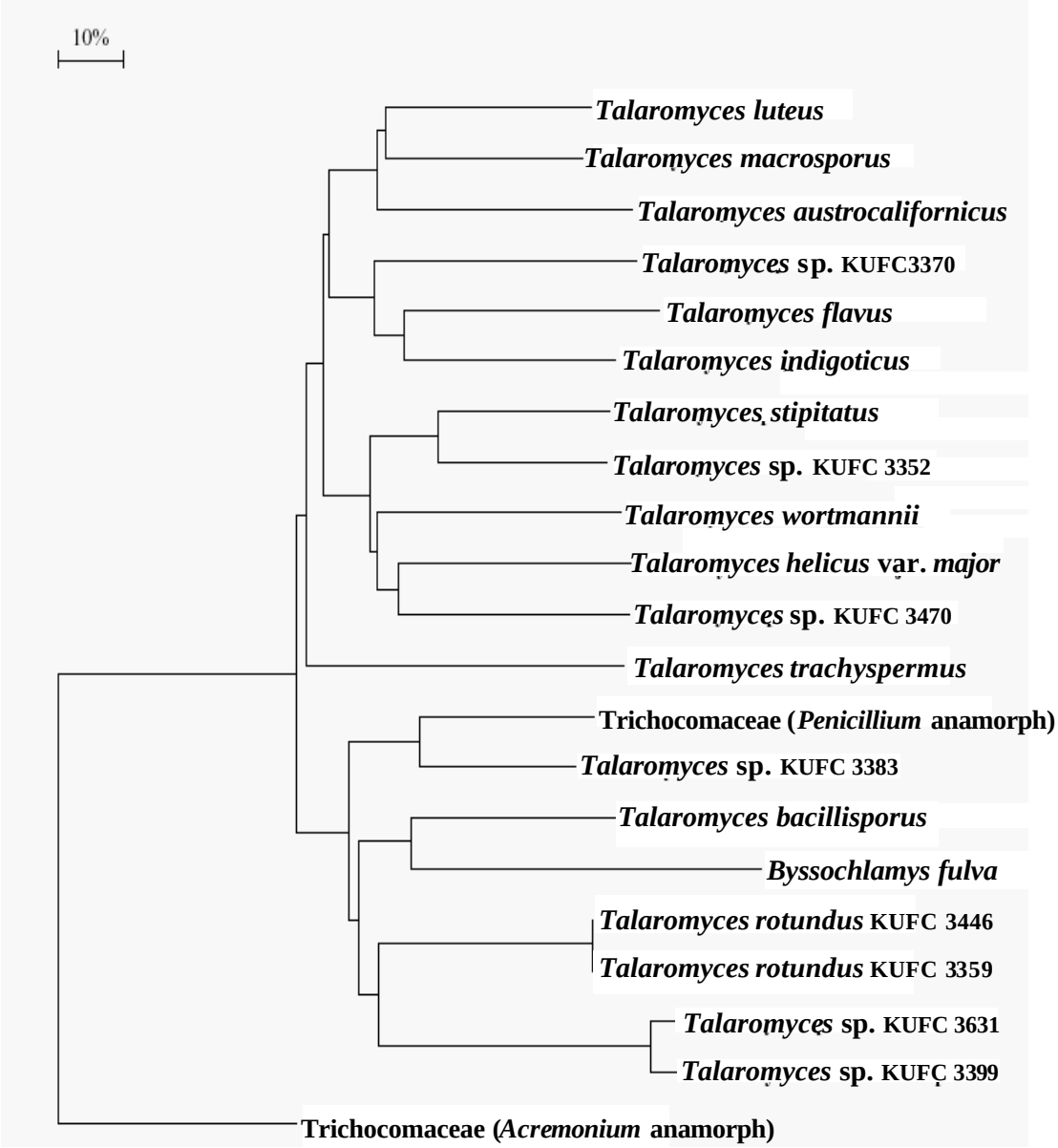


Figure 62 Phylogenetic relationships of *Talaromyces* species and related Trichocomaceae based on SSR gene

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

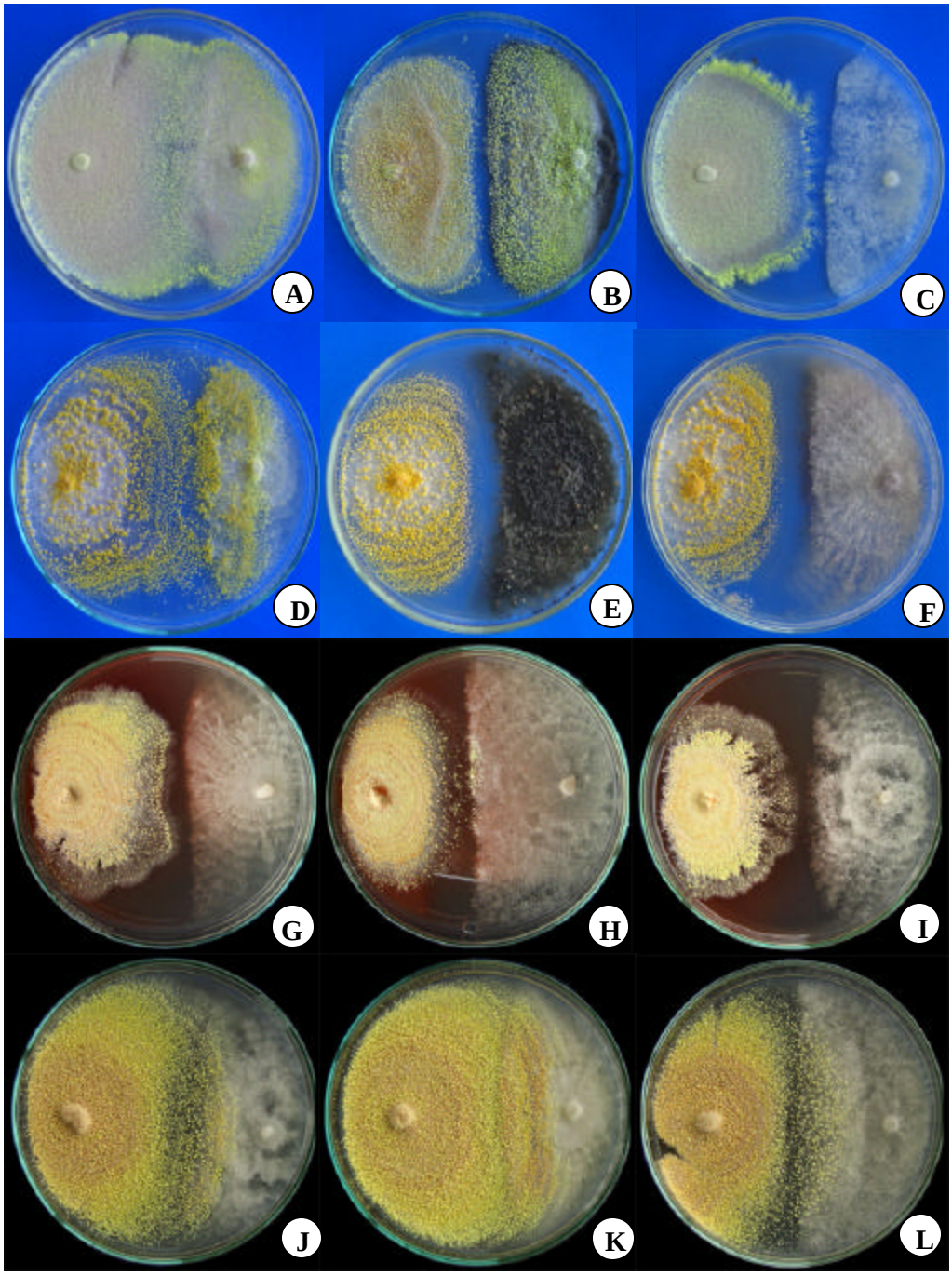


Figure 63 Antagonistic tests as dual cultures of different *Talaromyces flavus* isolates (left) and plant pathogenic fungi (right) on PDA incubated for 14 days at 28°C

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)

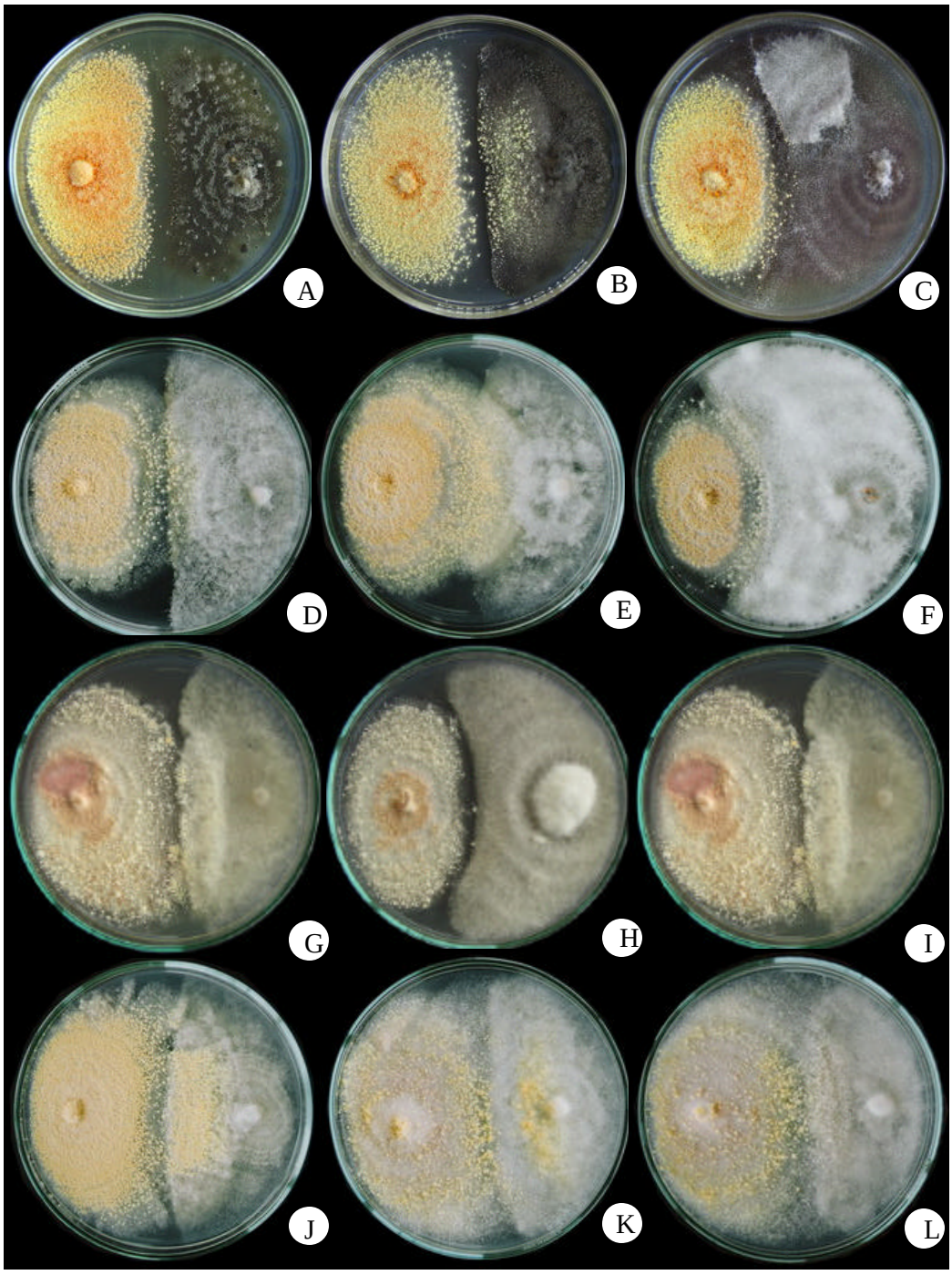


Figure 64 Antagonistic tests as dual cultures of different *Talaromyces flavus* isolates (left) and plant pathogenic fungi (right) on PDA incubated for 14 days at 28°C

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 ®)

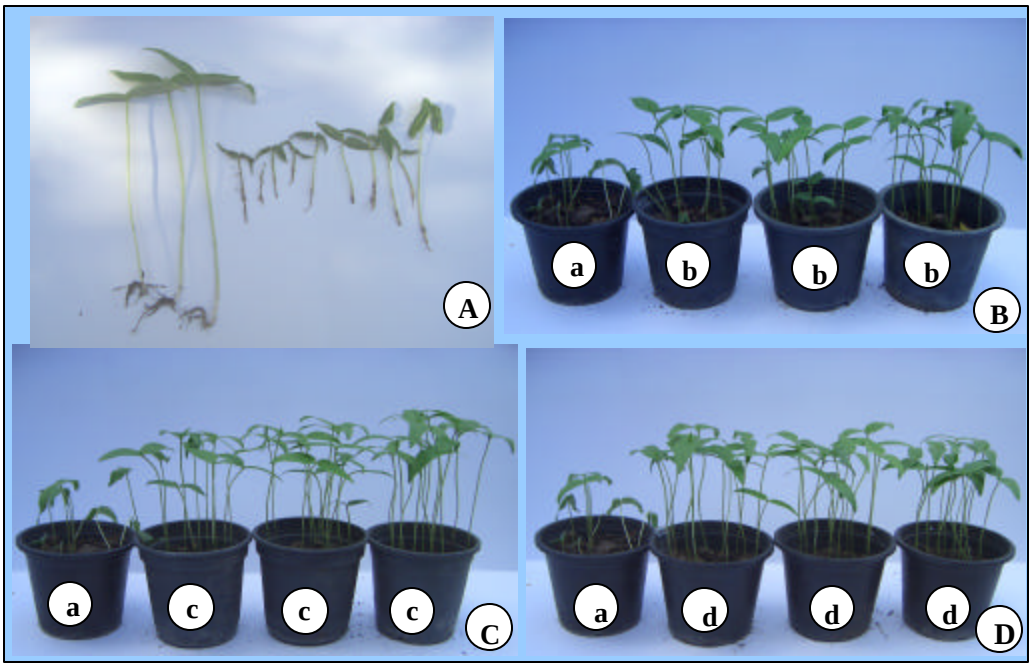


Figure 65 **A**. Mungbean seeds + distilled water (left) and mungbean seeds + *Sclerotium rolfsii* (right), 7 days after planting (Control), **B.** mungbean seeds + *S. rolfsii* (**a**), mungbean seeds + *Talaromyces flavus* KUFC 3523 + *S. rolfsii* (**b**), **C.** mungbean seeds + *T. flavus* KUFC 3550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

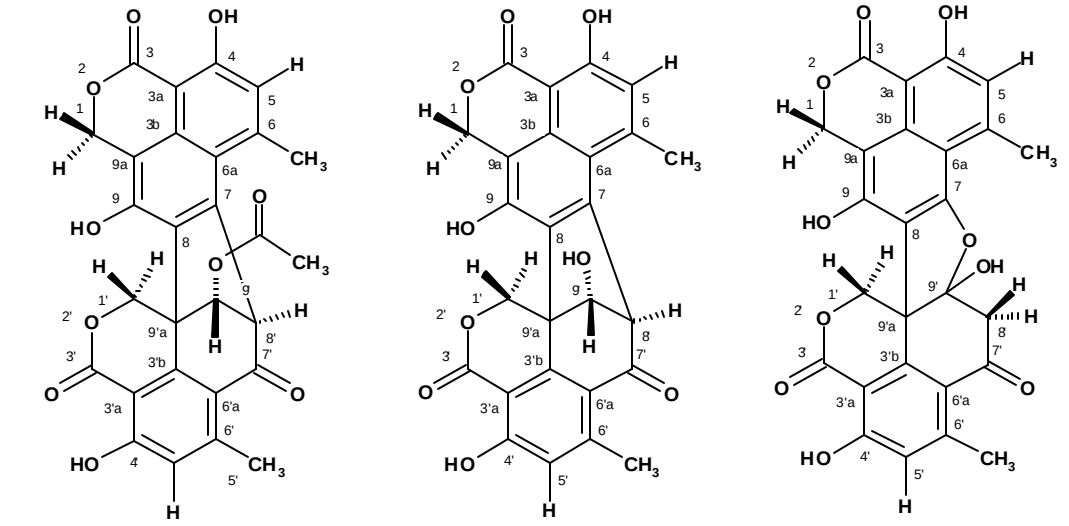
4. Secondary metabolites isolated from *Talaromyces bacillisporus* and *Talaromyces* sp. 1 (KUFC 3399)

4.1 Secondary metabolites isolated from *Talaromyces bacillisporus*

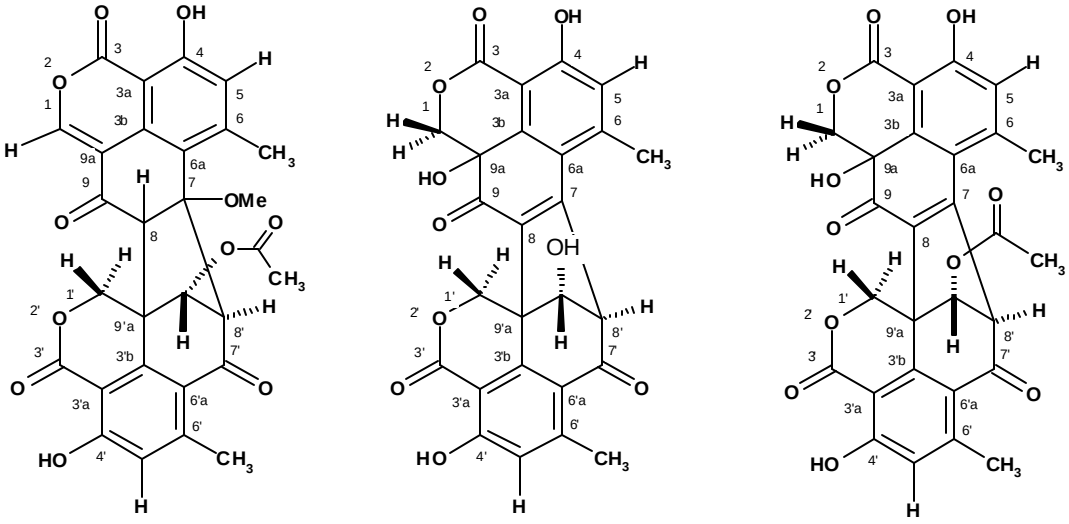
The ethyl acetate extract of the culture of *Talaromyces bacillisporus* furnished, besides bacillisporins A (**4**), B (**5**), C (**6**) and duclauxin (**50**), previously isolated from the Japanese collection of *Talaromyces bacillisporus* (Yamazaki and Okuyama, 1980), two new oxyphenalenone derivatives 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

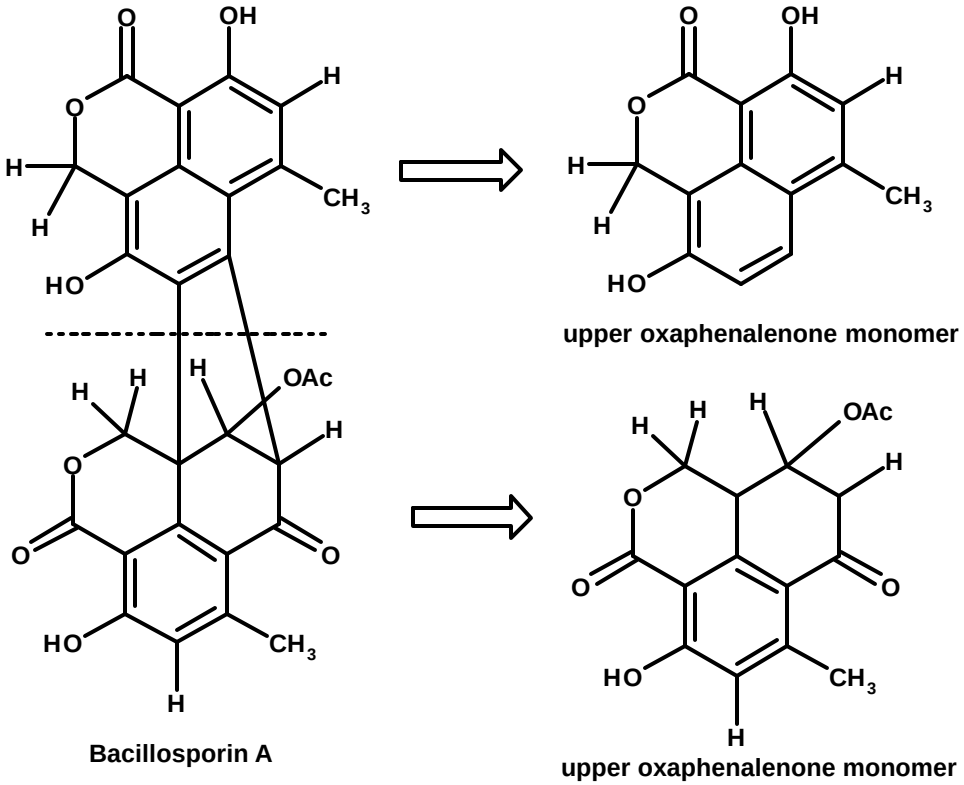
Table 14 ^{1}H NMR of bacillisporins A (**4**), B (**5**), C (**6**), D (**69**), duclauxin (**50**) (DMSO, 500 MHz), and bacillisporin E (**70**) ($CDCl_3$, 300 MHz)

^{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

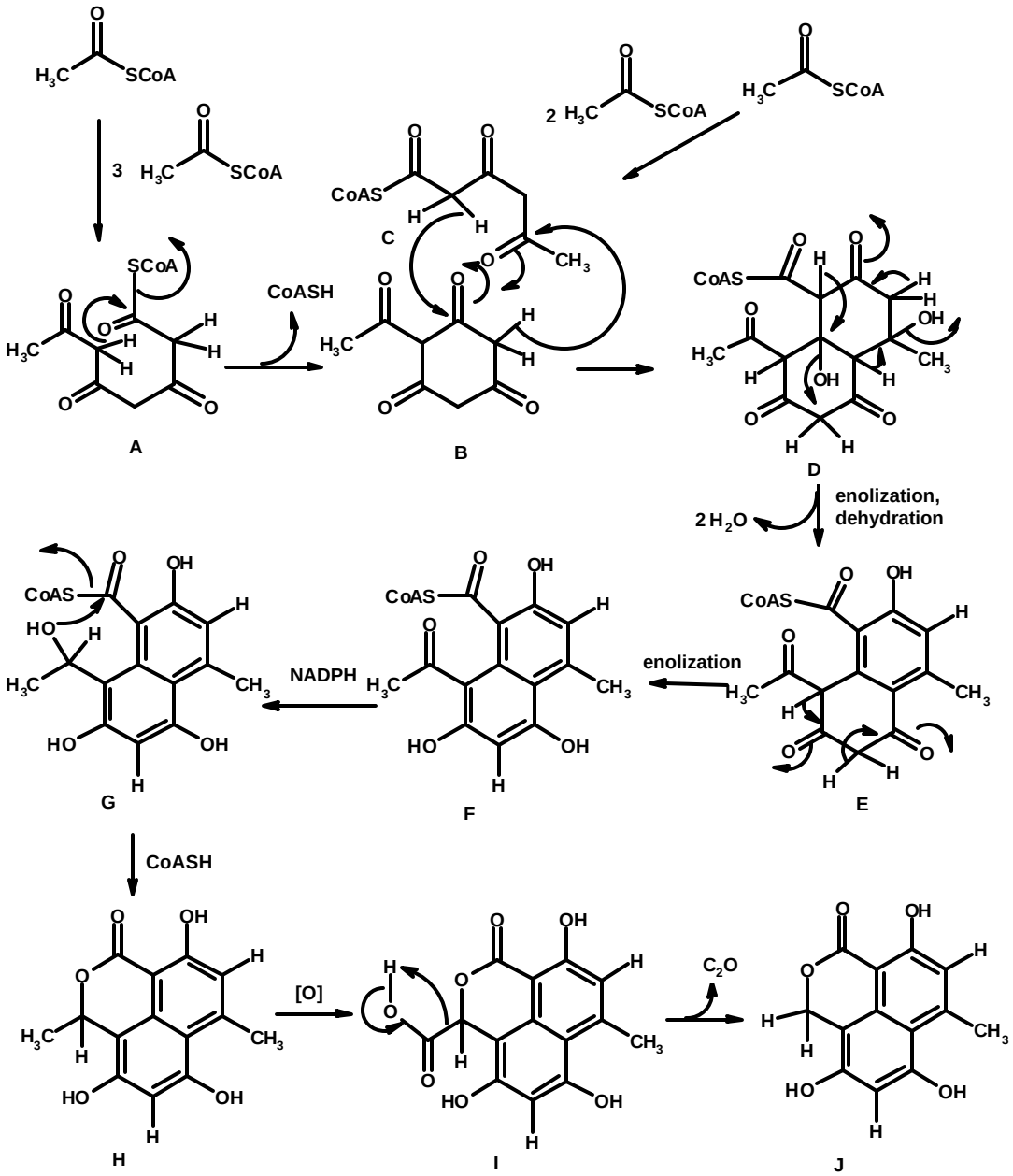
The molecule of bacillosporins and duclauxin can be considered as a dimer of oxapnenalenone derivatives. Consequently, they are derived from the coupling of two oxaphenalenone monomers:



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** .

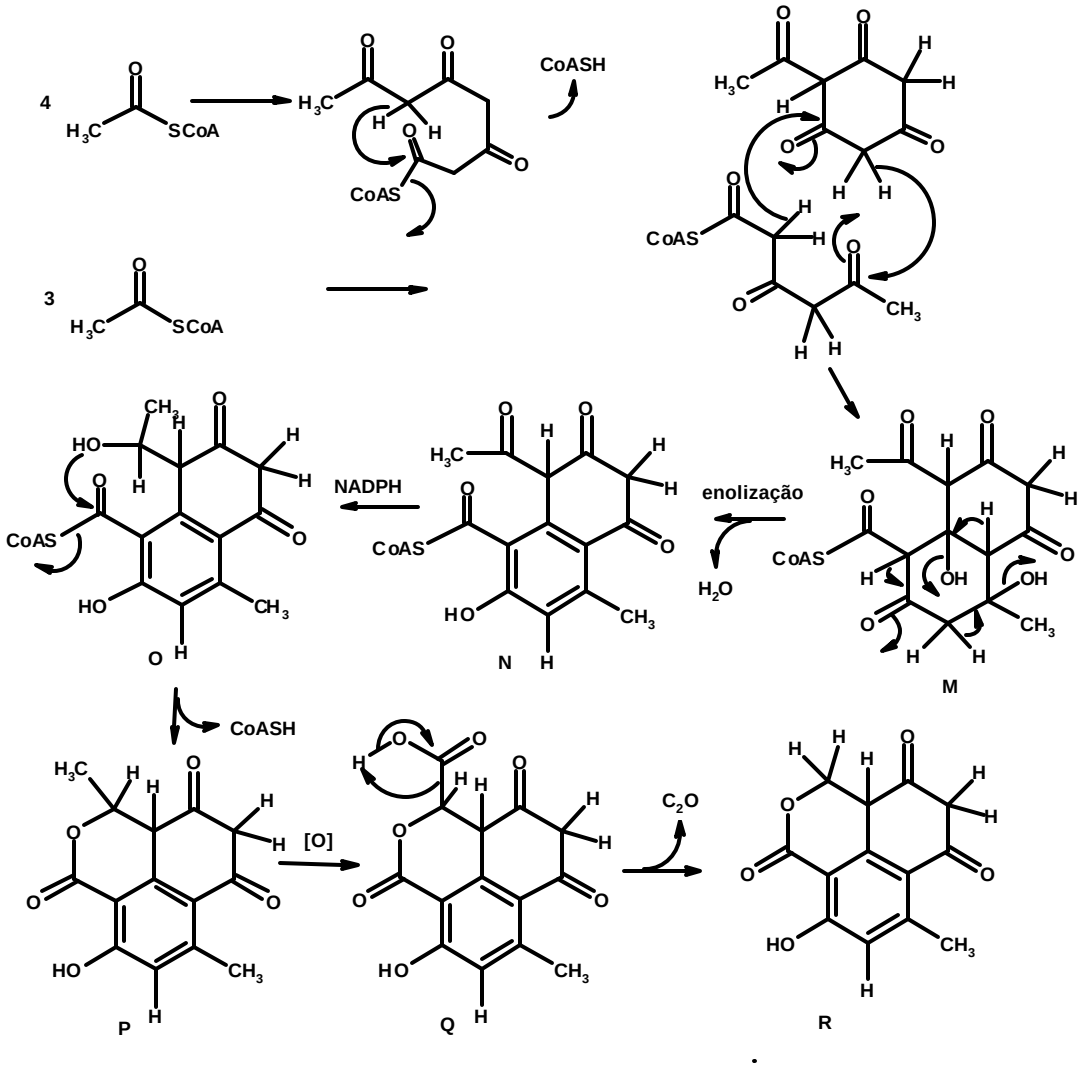
Before condensing with the triketide chain (**C**), the tetraketide chain (**A**) is thought to suffer a Claisen condensation to give the cyclic form (**B**). Aldol condensation between the tetraketide **B** and triketide **C** results in the intermediate **D**, which, after enolization and dehydration, 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.



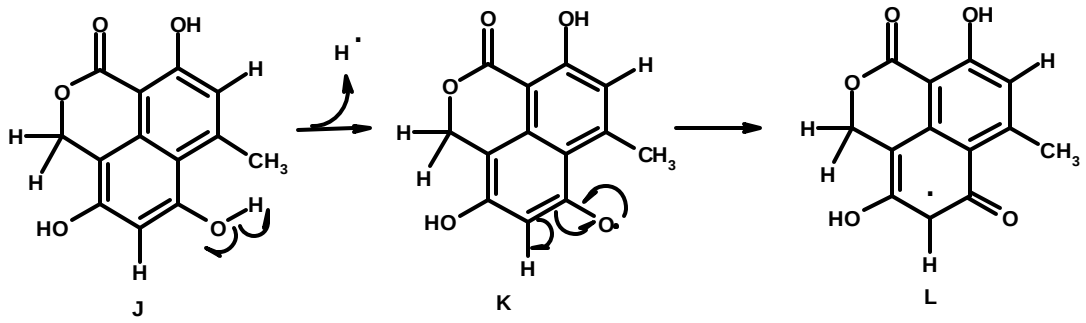
Scheme 1 Formation of the upper oxaphenalenone monomer

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.



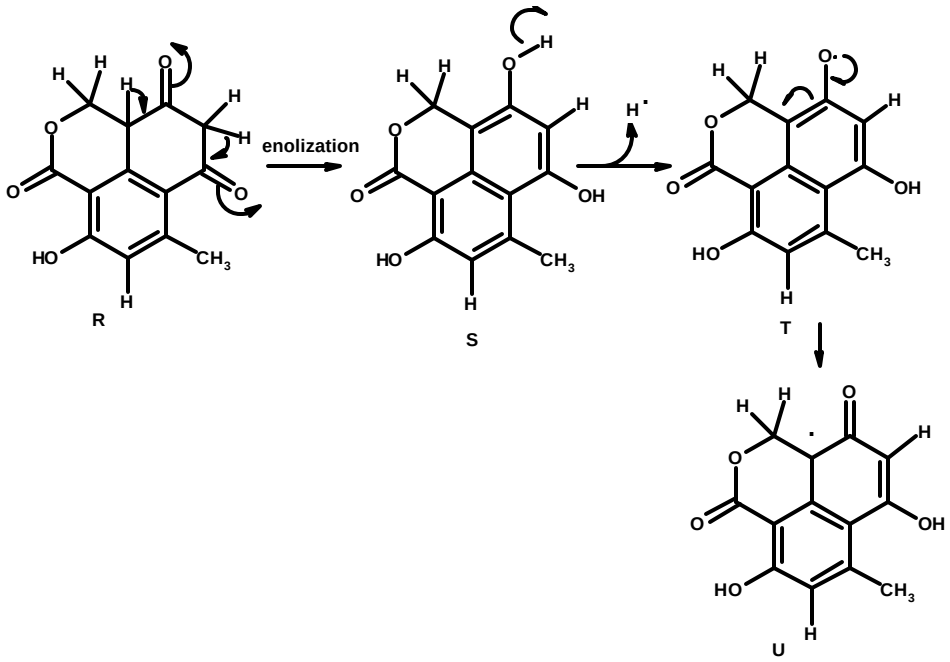
Scheme 2 Formation of the lower oxaphenalenone monomer

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



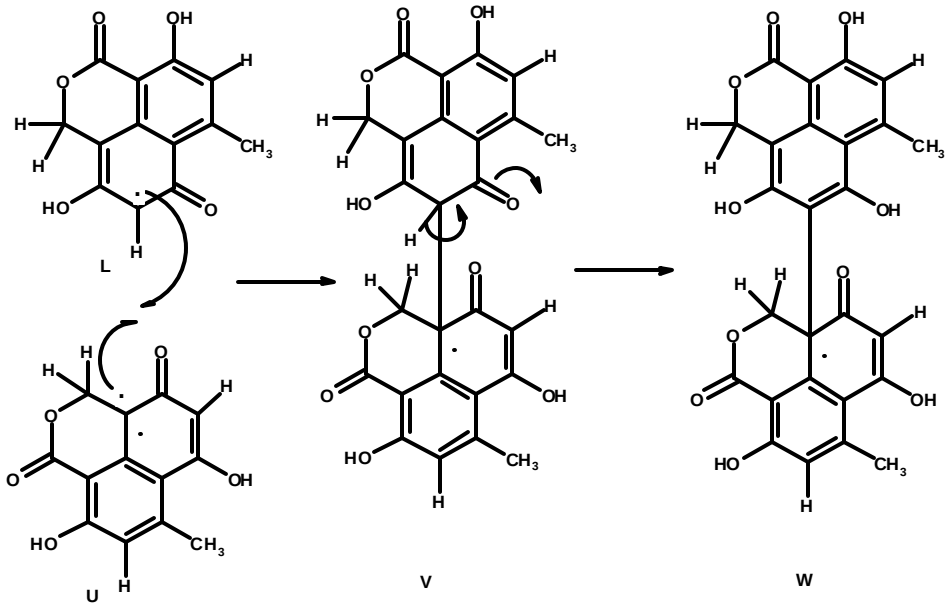
Scheme 3 Formation of the free radical of the upper oxaphenalenone monomer

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



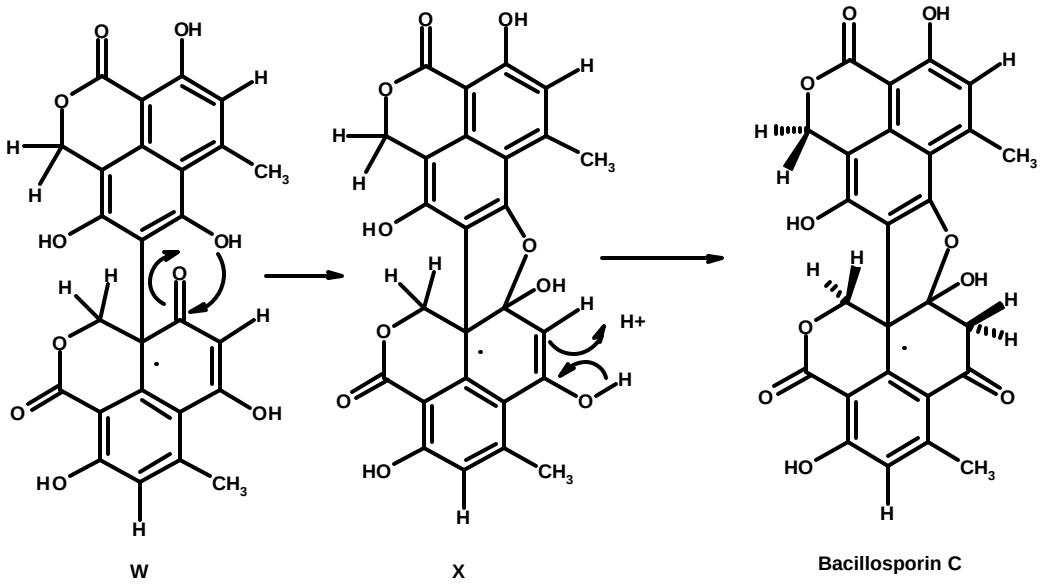
Scheme 4 Formation of the free radical (**U**) of the second oxaphenalenone monomer (**R**)

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**).

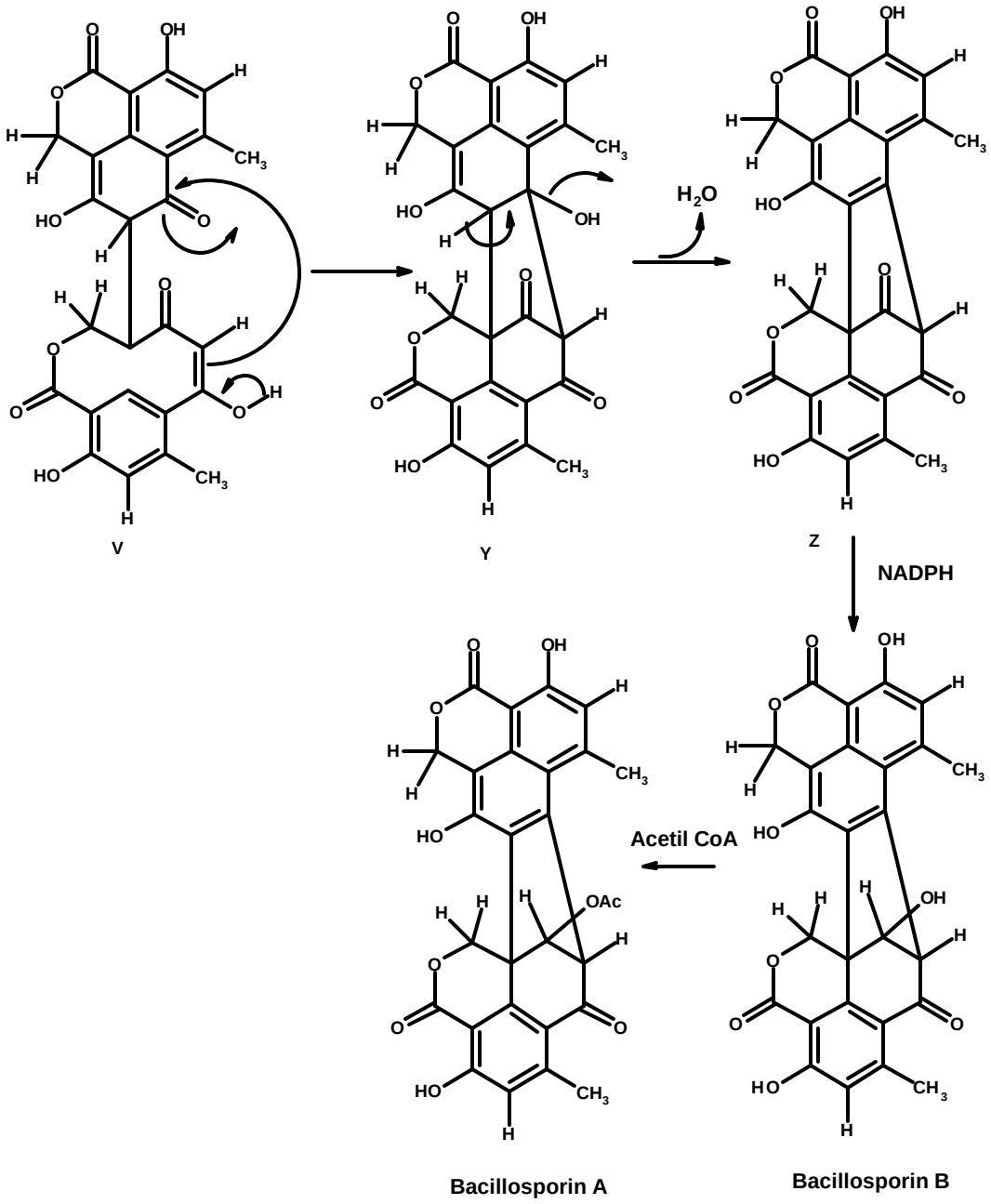


Scheme 5 Oxidative coupling of two oxaphenalenone units

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**).



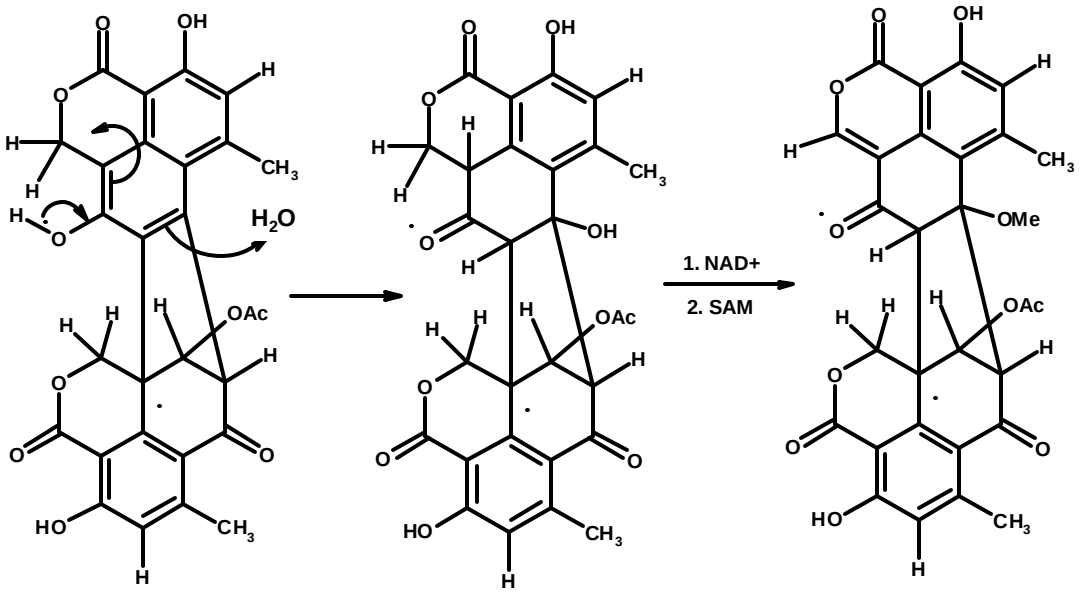
Scheme 6 Formation of **bacillosporin C**



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.

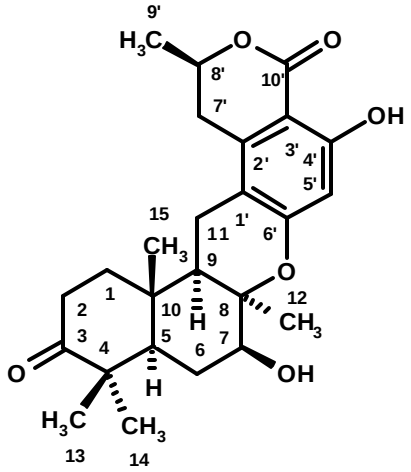
Thailandolide A (**71**) was a 3-oxo-7ß–hydroxydrimane linked through a tertiary oxygen to an aromatic moiety incorporating a lactone function in the manner characteristic of merodrimanes known from fungi of the genus *Stachybotrys* (Sawadjoon *et al.*, 2004), but with the lactone function closed to 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-

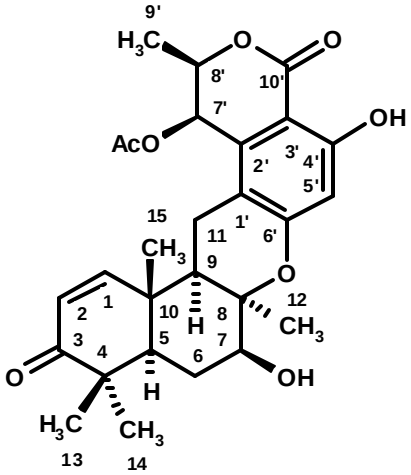
peaks between H-5 and CH_2-1', C-3, C-1 and C-0, between H-3 and C-1, C-5, and CO, and between –OMe and CO.

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).

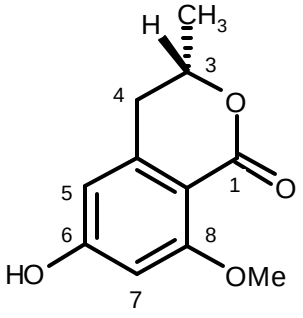
Three further constituents of the extract were three 1(*3H*)-isobenzofuranone derivatives, **74**-**76**. Compound **75** was identical with vermistatin, which has been isolated previously from cultures of *Penicillium verticulatum* (Fuska *et al*., 1986; Massias *et al*., 1989), *P. verruculosum* (Murtaza *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.



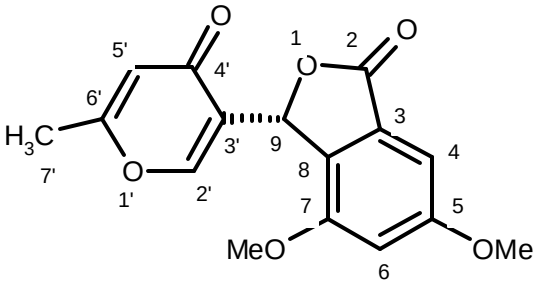
71



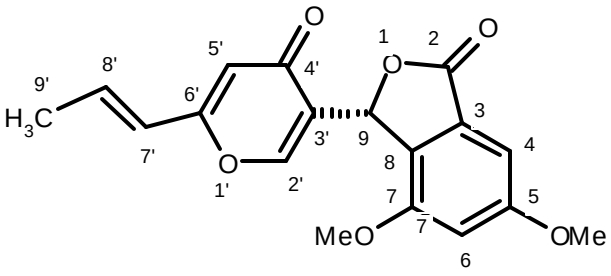
72



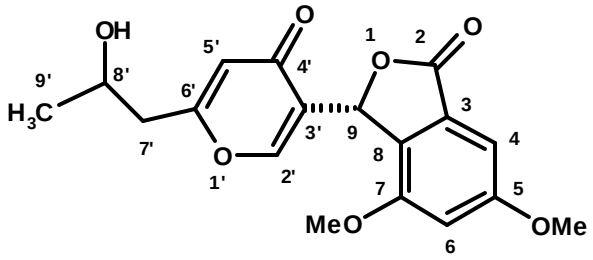
73



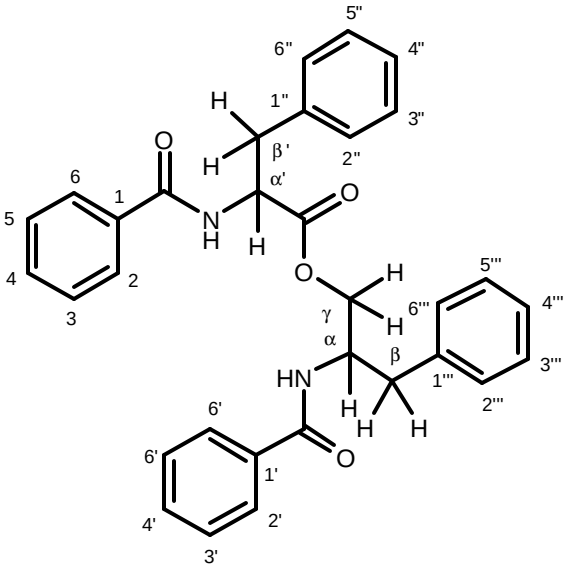
74



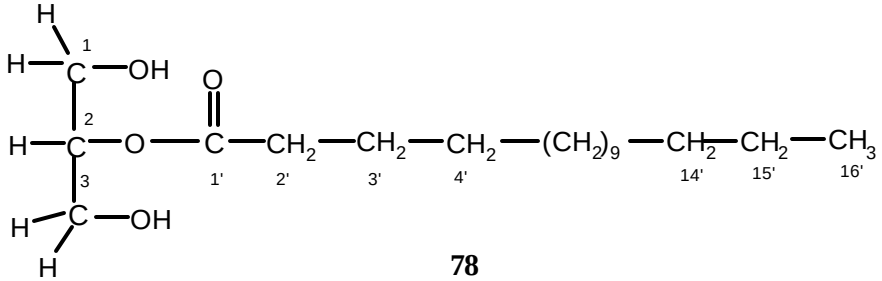
75



76



77



78

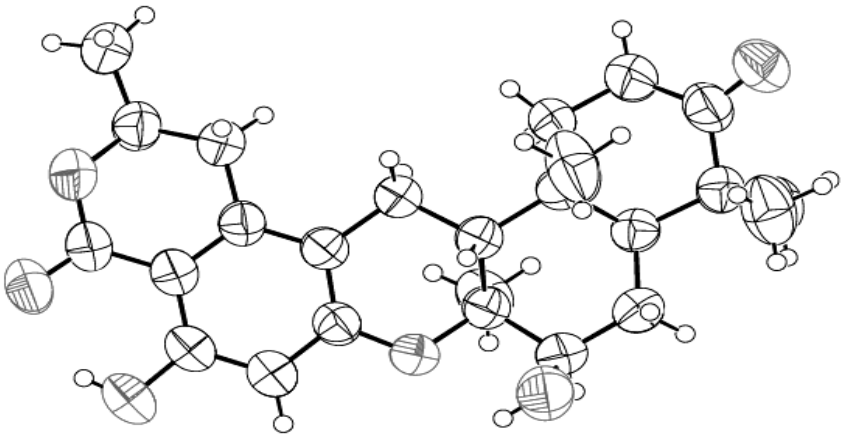


Figure 68 ORTEP view of thailandolide A (**71**)

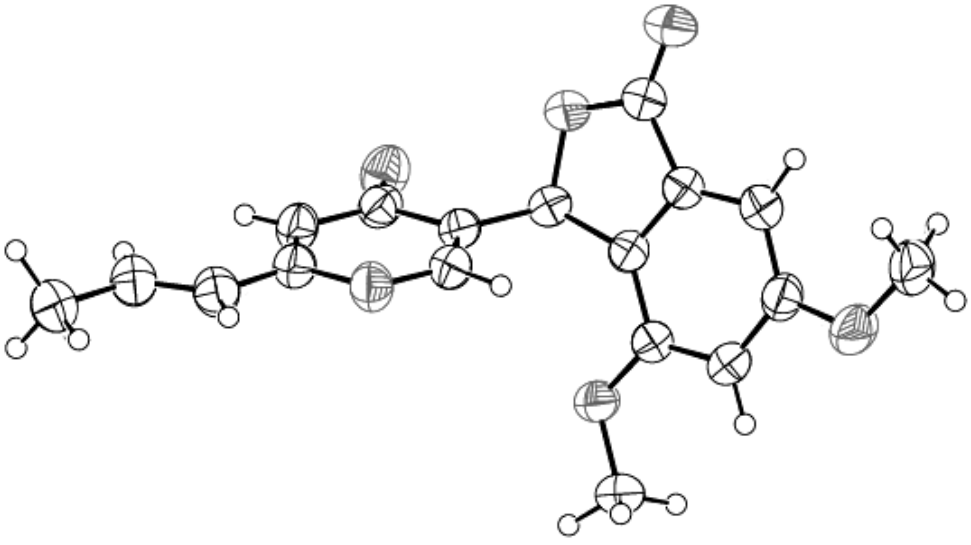


Figure 69 ORTEP view of vermistatin (**75**)

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