

## Descriptive Reports on Some Soil-Inhabiting Fungi in Korea

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During the study of microbial structures in root-regions of tomato and red pepper from fields, various soil-inhabiting fungi were isolated with the dilution plate technique. Among them an ascomycete, *Emericellopsis mirabilis* and three hyphomycetes, *Gliocladium solani*, *Humicola veronae* and *Verticillium chlamydosporium* are presented for the first time in Korea along with *Talaromyces trachyspermus*, *Chaetomium globosum* and *Doratomyces microsporus*.

**KEYWORDS:** *Chaetomium globosum*, *Doratomyces microsporus*, *Emericellopsis mirabilis*, *Gliocladium solani*, *Humicola veronae*, *Talaromyces trachyspermus*, *Verticillium chlamydosporium*, Soil-inhabiting fungi, Taxonomy

As a part of analytic studies on microbial structures in soil and crop root system (Kim *et al.*, 1999; Lee *et al.*, 2000), fungal inhabitants in soils of both *Phytophthora*-infested and non-infested tomato and red pepper fields were studied with mineral soils of Mt. Yeogi. Twenty-five fungal species isolated were consisted of mycelia sterilia, ascomycetes and hyphomycetes from each different field. All the isolates presented in this study are reported for the first time in Korea except for *Talaromyces trachyspermus* with its anamorph, *Chaetomium globosum* and *Doratomyces microsporus* which were previously reported by Kim (1979), Min *et al.* (1982) and Min *et al.* (1980), respectively. All the isolates are deposited at the culture collection center, KACC of National Institute of Agricultural Science and Technology.

### Materials and Methods

Root-free soils, rhizospheres and rhizoplanes of tomato and red pepper were collected from greenhouses in Jinju, Kyungsangnam-do, Korea on April, 1999. Mineral soils as a control were collected from Mt. Yeogi in Kyunggi-do, Korea at the same time. Serial dilutions were made of 1:100, 1:1000 and 1:10000. One ml of the water suspension was spread onto each of rose bengal agar plates. Individual colonies growing on RBA plates were transferred onto 2% malt extract agar or corn meal agar plates. Triplicate isolates were cultured on corn meal agar at 25°C for a growth rate study. General observations and measurements of characteristic structures were made on fresh materials mounted in water. Sizes represent 25 measurements. Photographs were taken with a Nikon FX-35DX on an Olympus BH2 stereo microscope.

### Results and Discussion

#### Ascomycetes.

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*Chaetomium globosum* Kunze ex Steud., 1824.

Colonies on corn meal agar reaching 53 mm in diameter in 10 days at 25°C, with aerial mycelia, margins mostly lobed, growth patterns in culture various among isolates. Ascospores globose, perithecioid, superficial on aerial mycelia in culture, gregarious, dark brown to black, clothed with hairs. Lateral hairs dark brown, straight, simple. Terminal hairs dark olivaceous brown, septate, undulated upper part, 3.3~6.6 µm in width. Ascospore wall translucent, textura intricata. Asci fasciculate, stalked, multiserial, clavate to broadly clavate, with evanescent walls, 8-spored, 55.5~80.0×11.1~15.5 µm. Ascospores 1-celled, biapiculate, with small oil droplets, initially hyaline to light brown, dark brown at maturity, flattened lemon-shaped, with an apical germ pore, pushed out of ostiole in a cirrus, 10.0~11.5×7.8~8.9 µm. Anamorph not observed.

Microhabitat: Root-free soils of both *Phytophthora*-infested and non-infested greenhouse of red pepper (*Cap-sicum annuum* L.) and tomato (*Lycopersicon esculentum* Mill.) in Jinju, Kyungsangnam-do, April, 1999, J.-S. Kim. Isolate deposited in KACC (#40863).

The genus *Chaetomium* is a representative of cellulolytic fungi and characterized by ascospores, deliquescent asci and limoniform ascospores. As a type species of the genus, *C. globosum* has been described so many times and resulted in many synonyms (Arx *et al.*, 1986). So far, six species in the genus have been described in Korea from arable soils and bamboo-growing soil (Kim, 1979; Lee *et al.*, 1998; Min *et al.*, 1982).

*Emericellopsis mirabilis* (Malan) Stolk, *Trans. Brit. Mycol. Soc.* 38: 419, 1955.

≡ *Peyronellula mirabilis* Malan, *Mycopath. Mycol. Appl.* 6: 165, 1952.

Figs. 1, 4, 6.

Colonies on corn meal agar reaching 22 mm in diameter

in 9 days at 25°C, white buff to faintly pinky creamy with center grey to dark with ascomata. Ascomata cleistothecoid, developing within 2 weeks and abundantly produced, partially or entirely immersed either in the media or in aerial mycelium, smooth, globose, translucent, 44.1~176.4 µm in diameter. Cleistothecial wall subhyaline, 3~4 µm thick, consisting of 2 to several layers of flattened cells. Asci globose to subglobose, scattered, 8-spored, 17.8~26.6 µm in diameter. Ascus wall thin, hyaline, soon dissolving, liberating the ascospores already inside the ascoma. Ascospores hyaline at first and becoming olivaceous dark brown, ellipsoid to oval, 1-celled, mostly containing one large oil drop, 11.1~13.3×5.6~7.1 µm, initially surrounded by a wide gelatinous sheath which collapses at maturity forming a number of longitudinal wings, surrounded by (4~)5~6 longitudinal wings. Wings hyaline, continued or discontinued, straight or curved, smooth or undulate rim, not lacinate, 0.7~1.8 µm in width.

Anamorphic state *Acremonium*-like hyphomycete. Conidiophores scarce, mostly simple phialides. Conidia ellipsoid to cylindrical, slightly attenuated at the base, hyaline, adhering in slimy heads, 6.2~8.9×2.7~4.4 µm.

Microhabitat: Root-free soil of *Phytophthora*-infested red pepper (*Capsicum annuum* L.) growing greenhouses in Jinju, Kyunsangnam-do, April, 1999, J.-S. Kim. Isolate deposited in KACC (#40864).

Species identification was made following Gams (1971) and Beljakova (1974). The type species, *E. terricola* is the only species reported in Korea from paddy field by Min *et al.* (1982), which has relatively smaller ascospores compared to *E. mirabilis*. The spore range of this isolate is narrower than that described by Stolk (1955).

*Talaromyces trachyspermus* (Shear) A. C. Stolk & R. A. Samson, *Stud. Mycol., Baarn* 2: 32, 1972.

≡ *Arachniotus trachyspermus* Shear, *Science, N. Y.* 16: 138, 1902.

= *Talaromyces spiculispurus* (Lehman) C.R. Benj., *Mycologia* 47: 683, 1955.

Figs. 2, 3, 5.

Colonies on corn meal agar reaching 47 mm in diameter in 12 days at 25°C, white to slightly creamy, peripheral areas usually sparse, with wide subsurface margins, margin 5 mm with concentric growth, centrally deep, floccose and quite dense. Ascomata cleistothecoid, slowly developing, white or creamy, superficial or partially embedded in mycelium, when mature colored yellowish white, globose, translucent, 188.8~334.6(~351.8) µm in diameter. Cleistothecial wall subhyaline, consisting of 2 to several layers of flattened cells. Asci globose to subglobose, scattered, 8-spored, 7.0~9.7×6.2~8.8 µm, borne in short chains. Ascus wall thin, hyaline, soon dissolving,

liberating the ascospores already inside the ascoma. Ascospores slightly pigmented, ellipsoid to oval, 1-celled, mostly containing one large oil drop, 3.6~4.5×2.7~3.2 µm, smooth when young and becoming spinose.

Anamorphic state *Penicillium*, rarely formed. Conidiophores borne from aerial hyphae, scarce. Stipes very short, commonly (9~)15~38~2~3 µm, bearing terminal penicilli, monoverticillate. Metulae appressed, in verticils of 2~3, 9.0~10.0×2.5~3.0 µm. Phialides 3~6 per metula, acerose, 9.0~15.0×1.5~3 µm, with long collula. Conidia ellipsoid to cylindrical, smooth walled, borne in short disordered chains, 2.5~4.9×2.0~2.5 µm.

Microhabitat: Root-free soil of *Phytophthora*-infested red pepper (*Capsicum annuum* L.) growing greenhouses in Jinju, Kyunsangnam-do, April, 1999, J.-S. Kim. Isolate deposited in KACC (#40865).

Species identification was made following Pitt (1979) and Domsch *et al.* (1980). Eight *Talaromyces* species were reported from paddy fields and forest soils by Kim (1979), Min *et al.* (1982, 1987) and Song and Min (1991).

### Hyphomycetes

*Doratomyces microsporus* (Sacc.) Morton & G. Sm., *Mycol. Pap.* 86: 77, 1963.

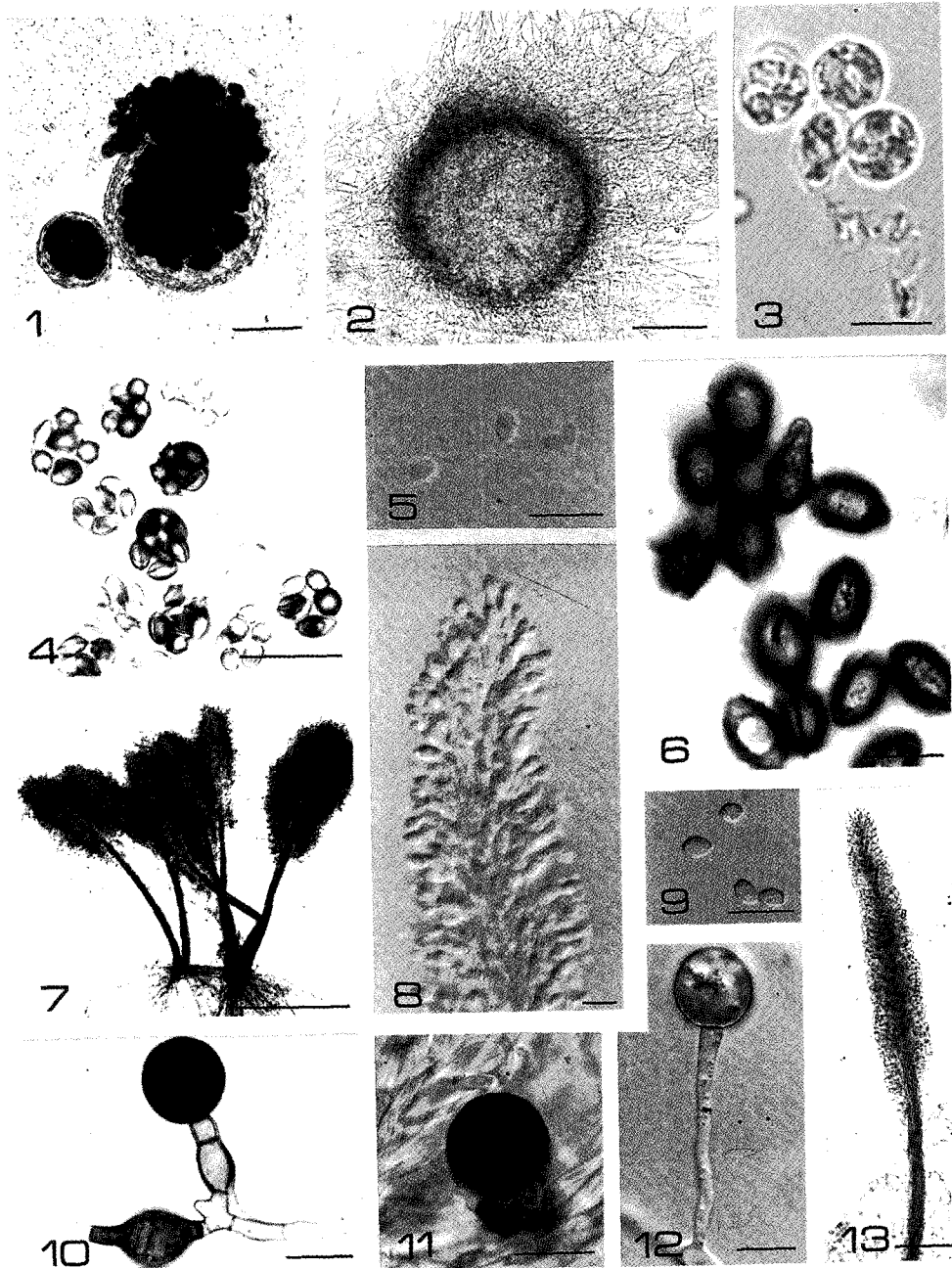
≡ *Stysanus microsporus* Sacc., *Michelia* 1: 274, 1878.

= *Graphium graminum* Cooke, *Grevillea* 16: 11, 1887.

Figs. 7-9, 13.

Colonies on corn meal agar reaching 23.5 mm in diameter in 10 days at 25°C, white and transparent becoming ash grey in sporulating area, edges lobed, with synnemata mostly scattered. Mycelia mostly immersed. Conidiophores synnematos. Conidiogenous cells annellides. Synnemata indeterminate, singly or in groups, 369.6~440 µm tall, (11.1~)15.5~17.8 µm at the base, fertile portion the upper two-fifths to two-thirds, composed of parallel hyphae. Synnemata composing hyphae hyaline at first and becoming dark brown, smooth, thicker than vegetative hyphae, straight to slightly twisted, septate, 29.7~44.1×1.8~2.7 µm. Spore heads dry, broadly cylindrical at maturity, 176.0~290.4 µm long, white at first but soon turning grey and dark greyish brown with age. Annellides 4.4~7.1×1.8~2.6 µm with swollen base, tapering to the annellated zone. Annellide supporting structures semi-cylindrical, hyaline, smooth, 5.3~6.2×2.6~3.5 µm. Conidia slightly pigmented, ovate to mitriform, truncate at the base and acuted at the apex, thick-walled, smooth, 1-celled, produced in basipetal succession, left the terminal end marked by transverse bands or annellations, 3.6~5.4×2.7~3.2 µm.

Microhabitat: Rhizoplanes and root-free soils of *Phytophthora*-infested and non-infested tomato (*Lycopersicon esculentum* Mill.) growing in greenhouses in Jinju, Kyun-

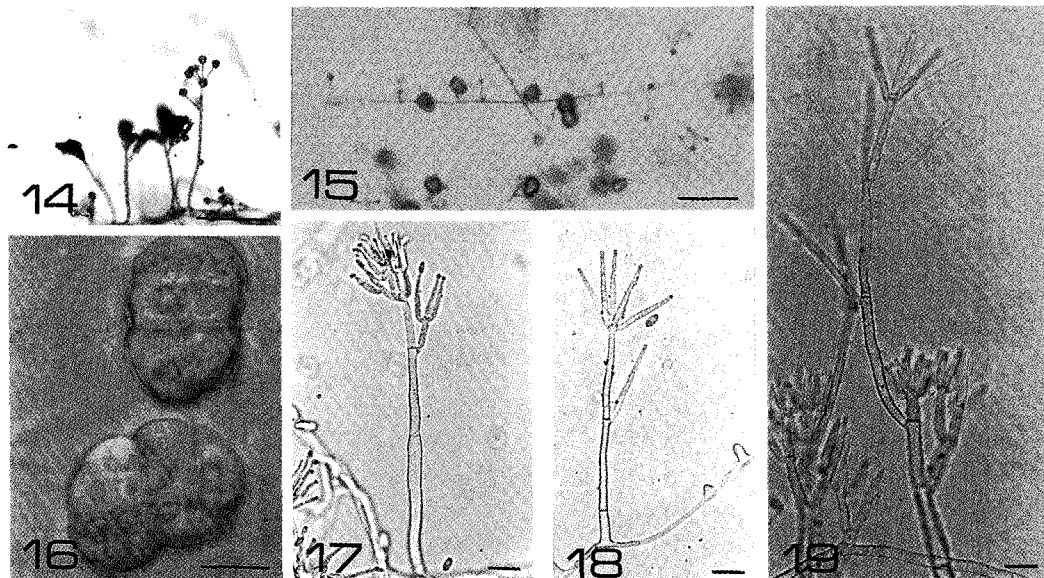


**Fig. 1.** Light micrographs of *Emericellopsis mirabilis* (KACC 40864), *Talaromyces trachyspermus* (KACC 40865), *Doratomyces microsporus* (KACC 40862), *Humicola veronae* (KACC 40860). 1. Ascoma with ascospores extruded showing layered translucent ascomatal wall (*E. mirabilis*). 2. Close view of ascoma with indistinct ascomatal wall (*T. trachyspermus*). 3. Asci in short chain (*T. trachyspermus*). 4. Scattered globose asci containing 8 spores (*E. mirabilis*). 5. Spinulose ascospores and conidia (*T. trachyspermus*). 6. Ascospores surrounded by longitudinal wings (*E. mirabilis*). 7. Cluster of synnemata (*D. microsporus*). 8. Close view of synnematal head showing annellides (*D. microsporus*). 9. Conidia with truncate base and apiculate end (*D. microsporus*). 10. Intercalary conidium and dark, globose conidium on septate conidiogenous cell (*H. veronae*). 11. Mature conidium having pigmented melanin sheath (*H. veronae*). 12. Immature conidium with long and 1-celled conidiogenous cell (*H. veronae*). 13. Conical or narrow cylindrical spore head at young stage (*D. microsporus*). Scale bars: 3, 5-6, 8-12 = 10  $\mu\text{m}$ ; 1, 4, 13 = 50  $\mu\text{m}$ ; 2, 7 = 100  $\mu\text{m}$ .

sangnam-do, April, 1999, J.-S. Kim. Isolate deposited in KACC (#40862).

Species identification was made following Domsch *et al.* (1980) and Morton and Smith (1963). This genus is

regarded as a synnematal form of *Scopulariopsis*. *Doratomyces* species are known to be found relatively commonly in soils, particularly with a high organic content. A high xylanase activity and cellulase activity of *D.*



**Fig. 2.** Light micrographs of *Gliocladium solani* (KACC 40858) and *Verticillium chlamydosporium*. (KACC 40859). 14. Top view of verticillate-type conidiophores with slimy drops of conidia and penicillate-type conidiophores with dry conidial chains (*G. solani*). 15. Top view of chlamydospores and conidiophores with slimy droplets of conidia (*V. chlamydosporium*). 16. Chlamydospores (*V. chlamydosporium*). 17. Biverticillate penicillate-type conidiophore (*G. solani*). 18. Unbranched verticillate conidiophore (*G. solani*). 19. Conidiophore having both of branching types (*G. solani*). Scale bars: 16, 18 = 10  $\mu\text{m}$ ; 17, 19 = 20  $\mu\text{m}$ ; 14-15 = 100  $\mu\text{m}$ .

*microsporus* were reported by Domsch and Gams (1969).

*Gliocladium solani* (Hartig) Petch, *Trans. Brit. Mycol. Soc.* 27: 149, 1944.

$\equiv$  *Spicaria solani* Harting, *Nieuwe Verh. Kon. Inst. Wetensch. Amsterdam* 12: 203, 1846.

= *Verticillium candidum* Sacc. var. *solani* Sacc., *Michelia* 2: 637, 1882.

Figs. 14, 17-19.

Colonies on corn meal agar reaching 54 mm in diameter in 10 days at 25°C, floccose, sectored, white to creamy, showing concentric growth with each type of conidiophores in turn. Conidiophores dimorphic with verticillate and penicillate branching types. Verticillate conidiophores smooth, hyaline, erect, (62.1-~)128.8-217.6(~244.2) $\times$ 2.2~4.4  $\mu\text{m}$ , rarely branched, if branched, phialide supporting structures cylindrical, 24.4-31.1 $\times$ 2.2  $\mu\text{m}$ . Phialides hyaline, cylindrical to subulate, collarettes inconspicuous, in 2 to 4 whorls, 22.2-35.5 $\times$ 2.2  $\mu\text{m}$ . Conidia hyaline, white to creamy in mass, navicular formed, slightly curved, dry, catenate, 1-celled, 4.0-6.7 $\times$ 1.8-3.3  $\mu\text{m}$ . Penicillate conidiophores smooth, biverticillately branched, 84.4-155.4 $\times$ 2.5-4.4  $\mu\text{m}$ . Branches cylindrical, smooth, hyaline, in 2 to 4 whorls, bearing (2-~)3-4 phialide supporting structures, (17.8-~)20.0-26.6 $\times$ 2.2-3.3  $\mu\text{m}$ . Phialide supporting structures, cylindrical, 13.3-20.0 $\times$ 2.2  $\mu\text{m}$ . Phialides subulate, in terminal whorls of 2 to 5, 11.1-17.8 $\times$ 2.2  $\mu\text{m}$ . Conidia hyaline, white to creamy in mass, in slimy heads, oblong

to ellipsoidal, 6.6-11.1 $\times$ 2.2-4.4  $\mu\text{m}$ .

Microhabitat: Mineral soil of Mt. Yeogi in Kyunggi-do, April, 1999, J.-S. Kim. Isolate deposited in KACC (#40858).

Species identification was made following Domsch *et al.* (1980). They described verticillate conidiophores in appressed shape, which feature was not clearly observed in this isolate. Although the range of spore size and overall morphology reminds *G. roseum*, its creamy colony which was persisted with age and never turned into the pink did not match with its colony characteristic of *G. roseum*. In early descriptions of this fungus by Hartig in 1848 and de Bary and Reinke in 1879, two types of conidiophores were not mentioned. Teleomorphic state of this species is known as *Nectria solani* Reinke & Berth. reported on potato forming erumpent fleshy stroma. Booth (1959) tried to get teleomorphic structures using a variety of media including sterilized potatoes and elm twigs. He had perithecial-like structures similar to those of *N. solani*, but no asci or ascospores were formed. Historic review of taxonomic status was discussed by Petch (1944). A common soil fungus *G. catenulatum* was close to this isolate, but its general appearance of colony and size of conidia are definitely distinguished it from *G. solani*.

*Humicola veronae* De Bert., *Canad. J. Bot.* 54: 2755, 1976.

Figs. 10-12.

Colonies on corn meal agar reaching 54 mm in diameter

in 10 day at 25°C, white to pale grey at first and becoming dark grey with age, lanose, zonate, reverse similarly colored. Conidiophores inconspicuous, consisting of 1 to 2 cells, irregularly branched or reduced to a single conidiogenous cell, light to dark brown but brighter than spores. Conidiogenous cells monoblastic, determinate, cylindrical to dolliform, 4.4~11.1×2.2~4.4(~6.6) μm. Conidia terminal or scarcely intercalary, single or rarely in 2 to 3 chain, globose, slightly pigmented to golden brown, smooth, 1-celled, 11.1~15.5 μm in diameter, sometimes surrounded by pigmented sheath. Phialidic conidia absent.

Microhabitat: Root-free soil of tomato (*Lycopersicon esculentum* Mill.) growing in greenhouses in Jinju, Kyunsangnam-do, April, 1999, J.-S. Kim. Isolate deposited in KACC (#40860).

Species identification was made following Ellis (1971), Nicoli and Russo (1974) and Bertoldi (1976). The absence of phialidic spores, scarcity of intercalary chlamydospores and mostly globose-shaped spores are characteristic figures distinguishing this isolate from both *H. grisea* and *H. nigrescens*. The size of spore range described by Bertoldi (1976), 13.5~14.3 μm, is narrower than this isolate but included in the scope of Korean isolates.

*Verticillium chlamydosporium* Goddard, *Botan. Gaz.* 56: 249, 1913.

≡ *Diheterospora chlamydosporia* (Goddard) G. L. Barron & Onions, *Canad. J. Bot.* 44: 861, 1966.

= *Stemphyliopsis oorum* Petch, *Trans. Brit. Mycol. Soc.* 23: 147, 1939.

= *Diheterospora heterospora* Kamyschko, *Bot. Mater.* 15: 138, 1962.

Figs. 15-16.

Colonies on corn meal agar reaching 35 mm in diameter in 11 day at 25°C, white, lanose, reverse remaining white. Conidiospores conspicuous, erect, slender, mostly not branched, bearing 2~3(~6) whorls of divergent phialides over most of their length, each whorls 24.4~77.7(~88.8) μm apart. Phialides single or in 2 to 6 whorls, slender to aculeate, 20.0~33.3 μm long, 2.2 μm at the base and tapering to 1 μm. Conidia forming slimy heads, ellipsoidal to short cylindrical, hyaline to slightly pigmented, 1-celled, with apiculate base, 3.6~5.6×1.8 μm. Chlamydospores dictyosporous, slightly pigmented, abundantly produced in the aerial mycelium with age, produced on stalks of 8.9~26.6 μm long, consisting of a cluster of 6~9 thick-walled cells, (19.8~)24.3~29.7×16.2~20.7(~26.1) μm.

Microhabitat: Rhizoplane of *Phytophthora*-infested red pepper (*Capsicum annuum* L.) growing in greenhouses in Jinju, Kyunsangnam-do, April, 1999, J.-S. Kim. Isolate deposited in KACC (#40859).

Species identification was made following Domsch *et al.* (1980). This genus was worldwide reported from vari-

ous soils. The cellulose and chitin degradation ability, an antibiotic activity against several bacteria and the parasitic ability in the oospores of *Phytophthora cactorum* were reported (Sneh *et al.*, 1977; Gochenaour, 1975). Hughes (1953) suggested that the name of dimorphic fungus should be applied to the one that is most frequent, constant in occurrence, most conspicuous or most readily identifiable. Barron and Onions (1966) considered the above suggestion but rather introduced a new genus, *Diheterospora*, for this species because of difficulty to fit this genus into existent fungal groups. The term used for thick-walled spores in this isolate, which had been obscurely called as either aleuriospores or chlamydospores was defined as dictyosporous chlamydospores by Campbell and Griffiths (1975) after their observations on the ultrastructural deposition of secondary walls which defines the entity of chlamydospore. *Verticillium catenulatum* shows close similarity except for globose spore shape and phialospores cohering in chains.

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