FIVE SPECIES OF *PYTHIUM*, TWO SPECIES OF *PYTHIOGETON* NEW FOR TAIWAN AND *PYTHIUM AFERTILE*^(1,2)

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Abstract

Five species of Pythium, i.e., P. dissotocum, P. gracile, P. marsipium, P. irregulare and P. elongatum, and two species of Pythiogeton, i.e., P. autossytum and P. uniforme, new for Taiwan were described. Some Characteristic features of Pythium afertile were also described and discussed.

Introduction

Pythium spp. have been well known as plant pathogens causing damping off and root rot of seedlings of flowering plants. Sawada (1927, 1943) had made some studies on this genus of fungi during his nearly forty years extensive survey of Taiwan fungal flora before the Second World War, since then few studies on genus Pythium have been carried out (Hsu 1963, 1965; Hsu & Chu 1962; Hsu & Liu 1966). Recently Watanabe (1974) reported his study on the fungi from underground parts of sugarcane and described five species and 3 species complexes of Pythium and 1 species of Pythiogeton.

We started our study on genus *Pythium* of this island, in June of 1974, since then 12 species have been identified. Among identified species, 5 species are new for Taiwan and 1 species *P. afertile* which was reported by Watanabe (1974) are described and discussed in this report. Two species of *Pythiogeton* new for Taiwan are also described.

Materials and Methods

The fungi were isolated from soil samples, which were collected from various places in Taiwan, by baiting technique. We used corn seeds which were autoclaved for 15 minutes at 121°C as baiting materials. Every soil sample

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was placed in glass containers to a depth of 2 cm and added distilled water to a depth of 1.5 cm over the surface of soil. Two to three autoclaved corn seeds were placed in each container. After incubation at 20°C for 6-12 hr, the seeds were washed with distilled water and the excessive water on seeds was absorbed by sterilized tissue paper, then were place on water agar plates. The plates were incubated at 20°C for 1-3 days. Hyphal tips of emerging fungi were cut off and transfered to potato sucrose agar (PSA) slants.

For identification, the fungi were grown on corn-meal agar (20 g corn meal, 20 g agar and 1000 ml distilled water) or V-8 juice agar (20 ml V-8 juice, 20 g agar and 1000 ml distilled water). For observation of formation of vesicles and zoospore discharge, the fungi were grown on boiled pieces of grass blades in sterilized water. Both were incubated at 20°C.

Descriptions

In this study, besides the species described here, we have also isolated many other pythiaceous fungi, among them included *Pythium spinosum*, *P. aphanidermatum*, *P. catenulatum*, *P. deliense* and *P. myriotylum*. All these species have been described by other workers in Taiwan. Furthermore, many isolates produced only filamentous, lobulate or spherical sporangia and no sexual organs were observed. They are usually unidentifiable.

Seven isolates of the genus *Pythiogeton* were isolated. Among them 3 isolates are *P. uniforme*, 2 isolates are *P. autossytum* and 2 isolates are *P. ramosum*. The species of *P. ramosum* was described by Watanabe in 1974 in Taiwan.

Pythium dissotocum Drechsler (Plate 1, Figs. 1-6)

Jour. Wash. Acad. 20: 398-418, 1930.

Phytopathology 30: 189-213, 1940.

Pythium oryzae Ito and Tokunaga, Jour. Fac. Agr. Res. Hokkaido Imper. Univ. 32: 201-233, 1933.

Hyphae measuring 2 to 7μ in diameter. Sporangia typically undifferentiated hyphae elements, but at times including slightly swollen dactyloid lateral elements. Zoospores several to many in a vesicle, 8 to 9μ in diameter when encysted. Oogonia terminal or intercalary, spherical to subspherical, measuring 12 to 29μ , average about 20μ in diameter, smooth. Antheridia usually monoclinous though also diclinous, 1 to 4, mostly 1 to 2 per oogonium, often sessile or originating very near the oogonium when monoclinous. Oospores aplerotic though almost filling oogonial cavity, smooth, measuring 10 to 25μ in diameter, with a moderately thick wall which measuring about 1.5 to 2μ in thickness.

This fungus was isolated many times from rice field soil, garden soil and other soil samples in many places of Taiwan. (1974-1975).

The sporangia of this fungus are filamentous and usually are indistinguishable from the vegetative hyphae. The oogonia are frequently intercalary though also are terminal. The antheridium of this fungus is a distinctive feature from other related filamentous species; it is crook-necked, inflated, breviform, and borne on a relatively short antheridial stalk or sessile. The antheridia are usually monoclinous though at times are also diclinous. When the antheridia are monoclinous origin, they are often sessile or originate closely to the oogonia; so sometimes the oogonia of *P. dissotocum* look like those of *P. ultimum* (both have thick-wall and aplerotic oospores).

Pythium gracile Schenk (Plate 1, Figs. 7-10)

Verhandl. Phy.-Med. Ges. Wurzburg 9: 12-31, 1859.

Pythium diclinum Tokunaga, Trans. Sapporo Nat. Hist. Soc. 14: 11-33, 1935. Hyphae measuring 1.5 to $6\,\mu$ in diameter. Sporangia filamentous, indistinguishable from the vegetative hyphae. Zoospores from 3 to many in a vesicle, reniform, about $6-9\,\mu\times10-13\,\mu$ in size, when encysted measuring 8 to $11\,\mu$ in diameter. Oogonia spherical to subspherical, smooth, mostly terminal or subterminal, occasionally intercalary, measuring 15 to $23\,\mu$ in diameter. Antheridia usually one, rarely two per oogonium, diclinous origin. Oospore single, aplerotic, wall thick, measuring $13\,\mu$ to $21\,\mu$ in diameter.

This fungus was isolated from soil of rice field in Mei-San.

This isolate was identified as *P. gracile* on the basis of the formation of strictly filamentous sporangia, smooth oogonia, 1 or 2 diclinous antheridia and aplerotic oospores with relatively thick wall.

Pythium marsipium Drechsler (Plate 2, Figs. 15-24)

Phytopathology 31: 505-506, 1941.

Hyphae measuring 2 to 7μ in diameter. Appressoria abundant on agar plate, measuring $4\text{--}9\times15\text{--}32\,\mu$ in size. Sporongia spherical, subspherical or asymmetrical utriforn, papillate or not, proliferous, generally by production of the secondary sporangium within the primary; when spherical or subspherical measuring 17 to $45\,\mu$ in diameter, when utriform measuring $14\text{--}34\times17.5\text{--}48.5\,\mu$ in size. Zoospores reniform, measuring about $9\text{--}12\,\mu$ in diameter when encysted. Oogonia spherical or subspherical, terminal, subterminal or intercalary (usually intercalary), measuring 20 to $37\,\mu$ in diameter. Antheridia typically diclinous, 1 to 5, rarely 6 or 7 per oogonium, broadly contact with oogonia. Oospores aplerotic, spherical, measuring 17 to $32\,\mu$ in diameter, with moderately thickened wall, 1 to $2.5\,\mu$ thick.

This fungus was isolated from soil of rice field in Taichung.

Our isolate possesses spherical, subspherical or asymmetrically utriform

sporangia which are proliferous, smooth oogonia, 1-5 antheridia of diclinous origin and aplerotic oospoeres with moderately thick wall. The sporangia of this fungus are usually broadly rounded at one end and skewly beaked at the other. They resemble those found in *Pythiogeton* in being utriform, the long axis generally attached transversely or obliguely to the sporangiophore rather than parallel to it. Drechsler (1941) originally described that the number of antheridia is 1 to 4, but our isolate generally has 1 to 5 and occasionally with 7 in number. The sex organs rarely formed on agar plate cultures, but formed relatively abundantly when grew on boiled grass leaf blades in water.

Pythium irregulare Buisman (Plate 2, Figs. 25-32)

Med. Phytopath. Lab. 11: 1-51, 1927.

Hyphae measuring 2 to $8\,\mu$ in diameter. Sporangia of various shapes, spherical, subspherical, obvate to pyriform, terminal or intercalary, measuring 11-31 μ , mostly 18-24 μ in diameter, forming zoospores in vesicles or germinating directly as conidia. Oogonia spherical or subspherical, varying in shapes, terminal or intercalary, oogonia wall smooth or with one to several irregular projections which may or may not be cut off by a cross wall, measuring 11 to 27 μ , mostly 17 to 21 μ in diameter without projection. Antheridia 1 to 3, usually only one, monoclinous or diclinous (typically monoclinous). Oospores aplerotic, spherical, smooth, measuring 9 to 24 μ in diameter, oospore wall about 1.5 μ thick.

This fungus was isolated from garden soil and orchard soil in Taipei, Yeong-Ho, Ho-Li and Tainan (1974-1975).

The sporangia of *Pythium irregulare* vary in shape from spherical to subspherical, elliptical to ovoid, pyriform to truncate. The oogonial walls are smooth or irregularly echinulate. When oogonial wall are echinulate, the spines are 1 to numerous, of irregular lengths, of irregular shapes and irregularly arranged on the oogonia. According to these characteristics, we can distinguish this species from other related species.

Pythium afertile Kanouse and Humphrey (Plate 1, Figs. 11-14)

Papers Mich. Acad. Sci. Arts and Letters 8: 129-140, 1927.

Hyphae measuring 2 to $5.5\,\mu$ in diameter, branching, nonseptate when young. Sporangia hypha-like. Vesicles at the tips of long emission tubes up to $300\,\mu$ or more in length, measuring 15 to $35\,\mu$ in diameter. Zoospores several to more than 30 in a vesicle, measuring 7 to $9\,\mu$ in diameter when encysted. Spherical hypha-bodies resembling chlamydospores, spherical or ellipsoidal, terminal or intercalary, measuring 10 to $30\,\mu$ in diameter, germinating by germtubes. Sexual reproduction unknown,

This fungus was isolated from soil of rice field and rotted rice roots in Taipei (1974).

This fungus was identified as *P. afertile* based on the formation of filamentous or lobulate sporangia, chlamydospores, vesicles formed on the tips of long emission tubes and the absence of sex organs. We isolated 4 isolates from rice root and 1 isolate from soil of rice field. The sporangia of two isolates are strictly filamentous that agreed with the description of Middleton (1943), other isolates had lobulate inflated sporangia agreed with the description of Watanabe (1974). However, 5 isolates are morphologically similar to one another among other characteristics, such as: vesicles formed on long emission tubest, chlamydospores and absence of sex organs.

Pythium elongatum Matthews (Plate 3, Figs. 33-38)

Studies on the genus Pythium. 1931.

Hyphae measuring 2 to 4μ in diameter, branched. Sporangia terminal or intercalary, spherical, subspherical, pyriform, cylindrical or curved, when spherical measuring 11 to 35μ in diameter, when cylindrical up to 55μ long; zoospores several to many in a vesicle borne on a long emission tube (up to 150μ long); zoospores about $6\times12\mu$ in size. Sporangia also germinate by means of germ tubes. Sexual reproduction unknown.

This fungus was isolated from root of rice seedling in Taipei (1974).

This isolate was identified as P. elongatum based on the formation of spherical, cylindrical or curved sporangia and vesicles borned on the tips of relatively long emission tubes (up to 150μ long). These characteristics were observed and agreed with the original description of Matthews (1931).

Pythiogeton autossytum Drechsler (Plate 3, Figs. 44-49)

J. Wash. Acad. Sci., 22: 447, Figs. 1-5, 1932.

Hyphae branching mostly at rather wide angles, measuring 2-7 μ in diameter. Sporangia terminal or intercalary, typically markedly ventricose, utriform, reniform or bursiform, but sometimes spherical, subspherical or ellipsoidal, measuring 20-220 μ in length and 20-50 μ in greatest width when bursiform, 30-50 μ in diameter when spherical. Emission tube often arising from opposite attachment of surporting hyphae and directed in approximate alignment with that hypha, measuring 20-250 μ in length and 3-9 μ in diameter, in some cases the tubes branched. Sporangial content discharged through the emission tube into water, then mature to form zoospores. Zoospores broadly reniform, measuring about $12\times18~\mu$ in motile state, $15~\mu$ in diameter when encyst. Sexual reproduction unknown.

This fungus was isolated from soil of rice field in Taipei and orchard soil

PLATE I

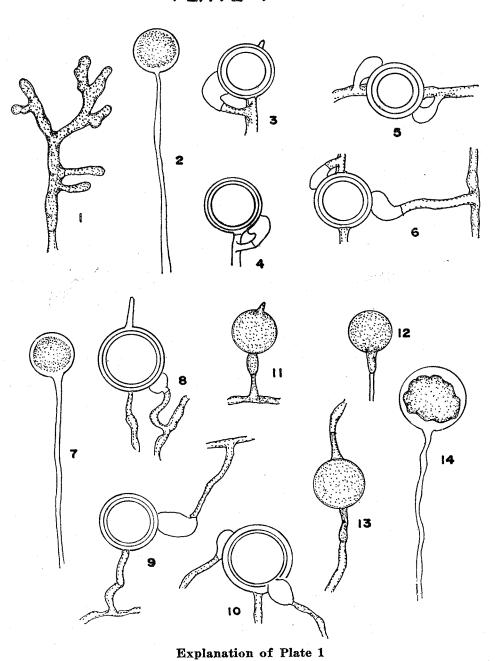


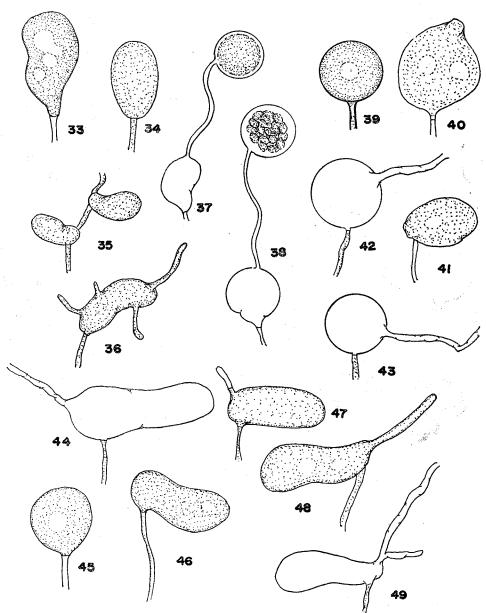
Fig. 1-6. Pythium dissotocum. Fig. 1. Sporangia showing slight inflation. Fig. 2. Vesicle formed on emission tube from filamentous sporangium. Fig. 3-6. Sex organs. Figs. 7-10. Pythium gracile. Fig. 7. Vesicle formed on tip of emission tube from filamentous sporangium. Figs. 8-10. Sexual organs, Figs. 11-14. Pythium afertile. Figs. 11-13. Chlamydospores. Fig. 14. Vesicle.

PLATE 2 30 31

Explanation of Plate 2

Figs. 15-24. Pythium marsipium. Figs. 15-18. Sporangia. Figs. 19-21. Empty sporangia, secondary sporangia formed within them. Figs. 22-24. Sexual organs. Figs. 25-32. Pythium irregulare. Figs. 25-29. Sporangia. Figs. 30-32. Sexual organs. Figs. 30-31. Oogonia with echinulated wall. Fig. 32. Oogonium with smooth wall.

PLATE 3



Explanation of Plate 3

Figs. 33-38. Pythium elongatum. Figs. 33-35. Sporangia. Fig. 36. Sporangium germinating by germ tubes. Figs. 37-38. Vesicles and emission tubes from sporangia. Figs. 39-43. Pythiogeton uniforme. Figs. 39-41. Sporangia. Figs. 42-43. Empty sporangia with emission tubes. Figs. 44-49. Pythiogeton autossytum. Fig. 44. Empty sporangia. Fig. 45. Spherical sporangium. Figs. 46-47. Bursiform sporangia. Fig. 48. Germinating sporangia. Fig. 49. Empty sporangia with branched emission tubes.

in Kuan-Si.

Our isolates were identified as *Pythiogeton autossytum* on the basis of the formation of both two types of sporangia: bursiform type and spherical type, in water. Sometimes this fungus may produce many spherical or subspherical sporangia in water, especially when cultured for a period of time.

This fungus grow poorly on PSA, corn-meal agar and V-8 juice agar, but grow relatively well on grass blades in water.

Pythiogeton uniforme Lund (Plate 3, Figs. 39-43)

Kgl. Danske Vidensk. Selsk. Skrift. Naturv. Math., Afd. IX, 6(1): 64, fig. 28 a-c, 1934.

Hyphae measuring 2.5-6 μ in diameter, branched. Sporangia terminal, rarely intercalary, spherical, subspherical, pyriform or slightly oval, measurin 35-60 \times 25-45 μ or 30-50 μ in diameter when spherical. Sporangial content discharged through emission tube into water, then mature to form zoospores. Emission tube measuring 30-120 μ in length and 5-7 μ in diameter. Sexual reproduction unknown.

This fungus was isolated from soil of rice field in Taipei (Sept. 19, 1974). This fungus, like *Pythiogeton autossytum*, grow poorly on PSA, corn-meal agar or V-8 juice agar, but grow relatevely well on grass blades in water.

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臺灣產 Pythium 屬腐黴菌及其相關屬之菌類

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本文描述六種 Pythium 屬廢黴菌,其中五種爲新記錄者。另有二種 Pythiogeton 屬的菌,亦爲臺灣之新記錄者。