土壌病菌Pythiumの生態および分類第X報ビート苗立枯病を起因する数種Pythium菌

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Ecologic and Taxonomic Studies on *Pythium* as Pathogenic Soil Fungi

X. Several *Pythium* causing damping-off of sugar beet seedlings

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高橋 実*・田中 寛**・一谷多喜郎*・R. V. ALICBUSAN***: 土壌病菌 *Pythium* の生態および分類 第X報 ビート苗立枯病を起因する数種 *Pythium* 菌

Abstract

Several species of *Pythium* were isolated from damped off sugar beets and considered as the pathogens. *Pythium betae* n. sp. was identified as a new species. *P. aphanidermatum* (Edson) Fitz., *P. spinosum* Sawada, and *Pythium* spp. were also isolated. Sporangia of *P. betae* n. sp. are spherical, often oval. Germination is either by zoospores or by germ tube. Oogonia are rarely smooth, usually 2–3 spines, spherical, 14.3–21.3 μ in diameter. Oospores filling the oogonium, rarely not filling. Antheridia one or two in an oogonium and attached at the lateral side.

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Introduction

Sugar beets have been cultivated in Hokkaido, the subfrigid zones, in Japan. Damping-off of sugar beets was one of the most serious diseases when their cultivation had been introduced in such warmer region as Kyushu. *Phoma*, *Rhizoctonia*, *Aphanomyces*, *Fusarium* and *Pythium* have already been reported as the pathogens causing damping-off of the beets. *Phoma* is the most important pathogen in Germany[12] and England[16]. In the United States, Reddy[23] reported that *Pythium debaryanum* is the cause of damping-off of sugar beets and relatively few is caused by *Phoma*. But the black rot disease of beets in Michigan and Ohio was caused by *Aphanomyces*[3,4]. Warren[31] and Afanasiev[1] working independently arrived at the same conclusion that seedling diseases of beets in the United States are generally caused by *Aphanomyces*. Drechsler[7,9] identified *Aphanomyces cochlioides* as the pathogen causing root rot of sugar beets. In Hokkaido, Tanaka and Narita[28] reported that *Phoma betae* is the main cause of damping-off of sugar beets, and *Pythium debaryanum* and *Rhizoctonia solani* are also isolated. Ishizuka[17] estimated that 75% of the damage on sugar beets are due to *Phoma* and he isolated *Alternaria*, *Fusarium*, *Rhizoctonia* and other fungi as a pathogen. Kuwayama *et al.*[18] found *Fusarium* as the pathogen causing damping-off of beets. *Aphanomyces cochlioides*, *Phoma betae*, *Rhizoctonia solani* and *Pythium* were considered to be the important pathog-

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ens causing damping-off of sugar beets in Hokkaido[30].

Several species of *Pythium* such as *P. debaryanum*, *P. ultimum*, *P. aphanidermatum* were reported as pathogens of sugar beet seedlings[2,5,12-15,21,23,28,29,31-33]. Yoshii *et al.*[35] emphasized that *Pellicularia filamentosa* was the cause of damping-off of sugar beets in warmer regions.

Though several *Pythium* have been isolated and considered as the causal fungi, the ecologic and taxonomic studies have never been conducted. The present paper deals with the identification of the *Pythium* causing damping-off sugar beets isolated from Aomori, Fukuoka, Kagawa, Kagoshima, Mie, Osaka, Shizuoka and Tokushima prefectures.

Materials and methods

Diseased seedlings of sugar beets caused by *Pythium* were mainly obtained from beet growing regions such as Kadoma city in Osaka, Aikui-cho and Naruto city in Tokushima.

It is difficult to isolate *Pythium* selectively from contaminating bacteria and other soil fungi. Specific isolation of *Pythium* was done by using corn-meal agar with 1,000 ppm penicillin and 50 ppm of streptomycin[27]. *Pythium* can also be isolated from the other organisms by the differences in growth rate. Four pieces or sections sliced from the disinfected diseased stem were placed on corn-meal agar with 50 ppm of streptomycin and they were incubated at 28°C for 24 hours. Bacterial growth suppressed while *Pythium* grew considerably. Other species of fungi scarcely grew. By successive transfers of the hyphal tips to another plates with the same medium a pure culture of *Pythium* could be obtained[26].

The formation of reproductive organs as well as mycelial growth of *Pythium* is good on such decoction agar media as corn-meal, oat-meal and potato dextrose agars but especially abundant on corn-meal agar[26]. The optimum temperature for mycelial growth was generally from 28°C to 30°C, with the exception of 36°C in *P. aphanidermatum*. The formation of conidia is abundant in relatively high temperature (about 28–30°C), while oospores are formed considerably in lower temperature (20°C)[26].

Results

1. *Pythium aphanidermatum* (Edson) Fitzpatrick (Plate 1, A) Mycelium well developed on many culture media and aerial mycelium moderately abundant on corn-meal agar. Sporangia are inflated filamentous on agar media, and germinate by extending long tube and forming vesicle with many zoospores. Zoospores formed 15–40 or more to a vesicle, 8.6–11.4 μ in diameter. Oogonia terminal, spherical, smooth, 16.0–34.0 μ (average 24.5 μ) in diameter, with a thick wall. Oospores spherical smooth, not filling the oogonium, 12.0–28.0 μ (average 19.6 μ) in diameter. In the mature stage, the oospores are thick walled with a central reserve globule surround by a granular layer of protoplasm in which a small refractive body is embedded. Antheridia stalked or intercalary, usually diclinous, rarely monoclinous, commonly 1–2 to an oogonium and attached either at the base or at the lateral side.

Several strains of this fungus were isolated from sugar beets in Osaka, Kagawa, and Tokushima prefectures. Their sporangia were composed of lobulate inflated element and formed vesicle with many zoospores. There were sometimes slight differences in size of conidia and oogonia, shape and number of antheridia and their mode of attachment, but they can not be considered as different species.

The shapes of sporangia and oogonia of this fungus have a close resemblance to *P. inflatum* Matthews, *P. arrhenomanes* Drech., *P. graminicolum* Subram. and *P. aphanidermatum* (Edson) Fitz. Matthews[20] reported that *P. inflatum* forms abundant toruloid hyphae and they may be sporangia

although not producing zoospores. This fungus, therefore, is different from *P. inflatum* because of easy formation of zoospores. *P. arrhenomanes* has many antheridia to an oogonium and its oospore is sometimes completely filling the oogonium. Thus this fungus is different species from *P. arrhenomanes*. *P. graminicolum* has many antheridia to an oogonium and oospore is filling oogonium, and this fungus can not be the same as *P. graminicolum*. The sporangia of *P. aphanidermatum* are filamentous, composed of a lobulate inflated mass of branches, cut off by a cross wall from the remainder of the mycelium, with a long or short tube of discharge. Oospores are not filling the oogonium. Number of antheridia is one, rarely two to an oogonium and their form is large. This fungus has a close resemblance to the description of *P. aphanidermatum*. Considering also the physiological characteristics and pathogenicity of this fungus, it can be identified as *P. aphanidermatum*.

Most of *Pythium* isolated from south-western Japan is *P. aphanidermatum* and relatively few of this species is isolated from other regions. This suggests the possibility that *P. aphanidermatum* will be important pathogen of sugar beets in warmer regions.

2. *Pythium spinosum* Sawada (Plate 1, B) Mycelium well developed on corn-meal agar medium and other media forming an aerial growth. Sporangia formed abundantly, usually spherical, rarely elliptical, 16.3–29.9 μ in diameter, average 25.1 μ. Sporangia germinate by germ tube and never by zoospores. Oogonia usually terminal, rarely intercalary, spherical, having 10–20 spines, 16.3–24.3 μ in diameter, average 21.2 μ. Oospore usually filling rarely not filling the oogonium in mature stage, 15.0–24.4 μ in diameter, average 17.3 μ. Antheridia monoclinous rarely diclinous, 1–2 to an oogonium and attached either at the base or at the lateral side.

The main characteristics of this fungus are the inability to form zoospores and oospore not filling the oogonium. This fungus resembles the following fungi: *P. mamillatum* Meurs., *P. echinulatum* Matthews, *P. polymastum* Drech., *P. spinosum* Sawada. The oogonium of *P. mamillatum* has many spines and oospore is filling the oogonium. Single antheridium arises from the oogonial hypha. Though *P. mamillatum* causes damping-off sugar beet seedlings its oogonium has considerably more spines and different characteristics of the antheridium, and, therefore, this fungus can not be regarded as *P. mamillatum*. The formation of zoospores makes this fungus different from *P. echinulatum*. The many antheridia and oospore not filling the oogonium makes it different from *P. polymastum*.

P. spinosum does not produce zoospore. Abundant sporangia are produced in water culture but few within the host. Oogonium has many spines and oospore is either filling or not filling the oogonium. These characteristics are also found in this fungus. However, this fungus usually filling and produces few sporangia in water culture. Emphasizing the facts that this fungus does not produce zoospores and oospores rarely not filling could make the identification as *P. spinosum*. In general, *P. spinosum* resembles *P. mamilatum*.

This fungus was isolated from damped off seedlings of sugar beets at Shizuoka Prefectural Agricultural Experiment Station in the spring of 1959. *P. spinosum* Sawada was originally isolated from rice plant but at present this fungus is isolated from many other hosts.

3. *Pythium* spp. (Plate 2, C) Mycelium, measuring 1.5 to 7.3 μ in diameter, well developed and aerial mycelium grew well on common agar media. Sporangia are intercalary, rarely terminal, spherical to oval, 11.2–22.8 μ in diameter, average 17.1 μ. Sporangia germinate with either formation of zoospores or germ tubes. Oogonia spherical, often oval or spindle-shaped, having usually 10 spines, 14.7–22.8 μ in diameter, average 18.7 μ. Oospores completely filling the oogonium, 11.4–19.9 μ in diameter, average 15.7 μ. Antheridia monoclinous, rarely diclinous. One antheridium usually attached to an oogonium, rarely two or three attached to basal septum.

This fungus is similar to *P. spinosum*, *P. mamillatum* and *P. echinulatum*. *P. echinulatum* looks more closely to this fungus than the other two because its general habit is similar except for the characteristics of sporangium and size of oogonium. The oospores of *P. echinulatum* is either filling or not filling the oogonium while oospores of this fungus is completely filling the oogonium. Thus this fungus is different from *P. echinulatum* and, therefore, this fungus is considered unidentified *Pythium* (*Pythium* spp.).

4. *Pythium betae* M. Takahashi n. sp.* (**Plate 2, D**) Mycelium, measuring 1.0 to 6.4 μ in diameter, well developed and aerial mycelium grew well on common agar medium. Hyphae are non-septated and branched. Sporangia are spherical, often oval, intercalary. Sporangia germinate with germ tubes and rarely produce 6–10 zoospores in a vesicle. Conidia are 11.4–19.9 μ in diameter, average 13.1 μ. Oogonia are produced as much as sporangia, but not so abundant on corn-meal agar. Oogonia, spherical, terminal or intercalary, usually having 2–3 spines, rarely 5–6 spines. Spines are relatively long. Oogonia rarely smooth, 14.3–21.3 μ in diameter, average 18.3 μ. Oospores with a thin wall, spherical, filling the oogonium, rarely not filling. Interior of oospores is granulated, 12.8–21.4 μ in diameter, average 16.8 μ. Antheridia diclinous, rarely monoclinous, 1–2 to an oogonium and attached at the lateral side.

Oogonia are characterized by relatively few spines. This fungus is similar to *P. mamillatum* due to filling the oogonium and its size. However, *P. mamillatum* differs from this fungus in having oogonium with numerous spines and oogonium with usually one antheridium. *P. spinosum* differs from this fungus in not forming zoospores with monoclinous antheridium, and having oogonium with numerous spines. Both *P. polymastum* and *P. megalacanthum* are morphologically similar to this fungus, but they differ in having large oogonium.

P. echinulatum is closely resembling this fungus because of its conidial germination and oospore filling the oogonium. However, *P. echinulatum* differs from this fungus in having so many spines (14–30 spines) and monoclinous antheridia. Since this fungus has different characteristics from *P. echinulatum* which is most closely resembled to this fungus, it is considered that this fungus is a new species.

This fungus was isolated from damped off sugar beets in Mie prefecture by Agricultural Experiment Station and highly pathogenic to sugar beets.

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* Mycelium hyalinum, ramosum, 1.1–6.4 μ latum. Zoosporangium spheroideis, saepe ovoideis, intercale, 11.4–19.9 μ diam. Zoosporangium germinatum cum hypha, raro 6–10 zoospora in una vesicula producentibus. Oogonium, 14.3–21.3 μ diam., spheroideus interjucctum vel terminale, 1–3 sinis relative longis, 5–6 spinis rarius. Oogonium blandus rarius. Oosporum impleta in oogonium, rarius non impleta, spheroideus membrana tenua, 12.8–21.4 μ diam. Antheridium 1–2 ad oogonium, haerentibus lateris. Generatim heterothallismum, raro homothallismum.

Hab. Causing seedling blight of sugar beet (*Beta vulgaris* L.). Collected from the field of Mie Agricultural Experiment Station in Ejima-cho, Suzuka city, Mie prefecture, in 1959.

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和文摘要

土壌病菌 *Pythium* の生態および分類

第X報 ビート苗立枯病を起因する数種 *Pythium* 菌

高橋 実・田中 寛・一谷多喜郎・R. V. ALICBUSAN

ビートの栽培はわが国では北海道において，古くから行なわれている。以前暖地への導入が試みられたことがあり，その時苗立枯病が主な病害であった。その病原菌として，*Pythium aphanidermatum*, *P. spinosum*, *Pythium* spp. など数種の *Pythium* が分離され，新種として *Pythium betae* M. Takahashi n. sp. を同定した。

P. betae の胞子のうは通常球形で発芽管を生ずるが，まれに球のう内に6–10個の遊走子を形成する。蔵卵器は球形，頂生，通常2–3の長い刺状突起を有するが，まれに平滑，径14.3–21.3 μ。卵胞子は蔵卵器内に通常充満する。雄精器は異株生，まれに同株生，1蔵卵器に1–2個側着する。本新種は三重県下でビート苗立枯病菌として分離された。

Explanation of plates

Plate 1, A *Pythium aphanidermatum* (Edson) Fitzpatrick
1–7 Sporangia
8–9 Sporangia with vesicle containing zoospores
10–20 Oogonia with antheridia

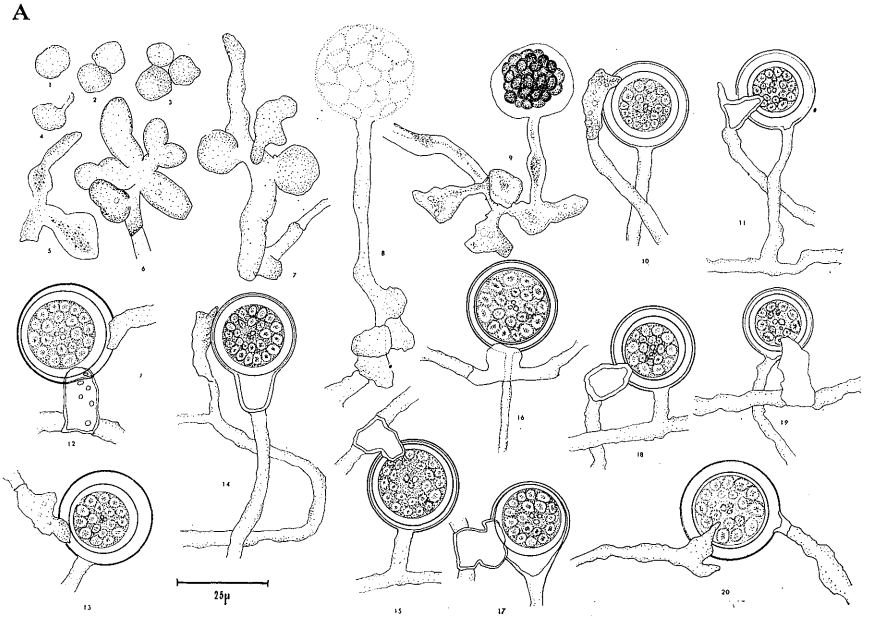
B *Pythium spinosum* Sawada
1–7 Different types of sporangia
8–17 Oogonia with antheridia

Plate 2, C *Pythium* spp.
1–8 Different types of sporangia
9 Sporangia with vesicle containing zoospores
10–22 Oogonia with many spines and antheridia

D *Pythium betae* M. Takahashi n. sp.
1–7 Different types of sporangia
8 Sporangia with vesicle containing zoospores
9 Empty sporangia left after release zoospores from vesicle
10–19 Oogonia and antheridia

Plate I

A



B

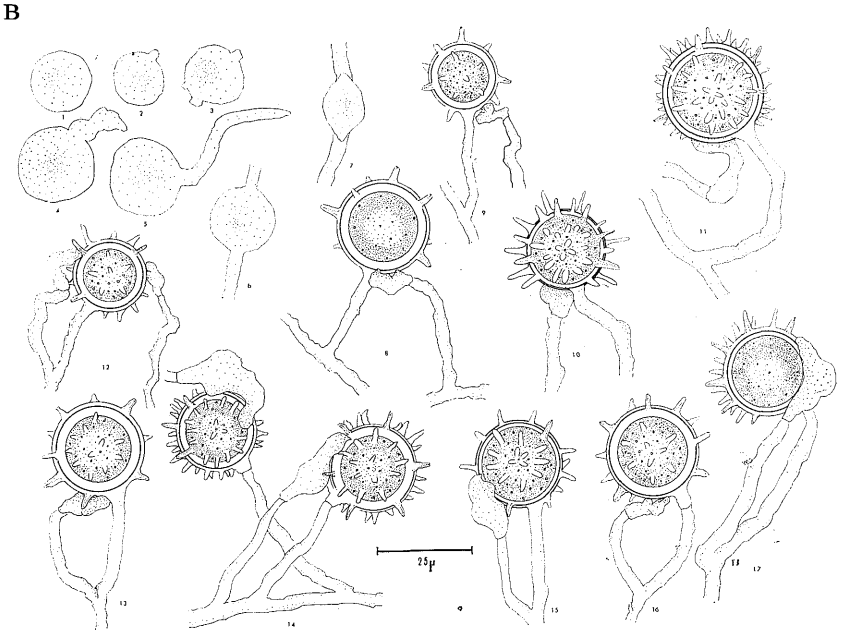
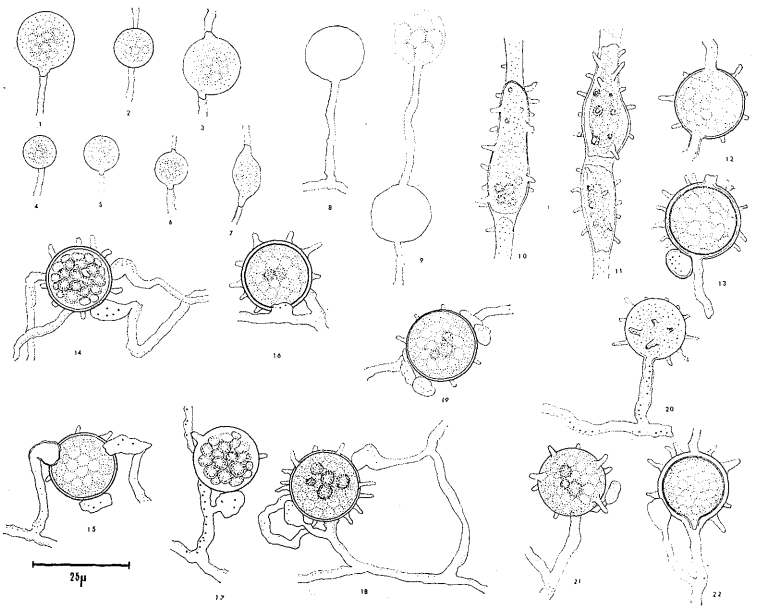


Plate II

C



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