Detection and Quantitative Estimations of *Pythium aphanidermatum* in Soil with Cucumber Seeds as a Baiting Substrate

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ABSTRACT

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Pythium aphanidermatum was detected in soils directly on 2% plain water agar plates, using cucumber seeds as a baiting substrate and Petri's salt solution for inducing sporulation. Sporangia, vesicles, zoospores, and zoospore discharge were observed on plates by 24–48 hr and sexual organs by 48–96 hr (at 30–35 C) after the trapping experiment was started. This method permitted detection and estimation of populations of *P. aphanidermatum* in naturally infested soils in Japan.

Pythium aphanidermatum (Edson) Fitz. is a parasite of more than 74 species of 59 genera of higher plants (3). In Japan, this fungus parasitizes at least 14 species of higher plants in nature and is present in field soils in various districts of the country (6).

The existence and distribution of the fungus is important from both ecological and plant pathological points of view. Several techniques have been described to isolate or detect this fungus in soils. Among them, Hine and Luna's potato-antibiotic technique (1) is simple; however, further culture work may be necessary for positive identification by observing sporangia, zoospore discharge, and sexual organs. In addition, preparation of freshly diced potato cubes treated with an aqueous solution of 100 μg/ml of pimaricin and 100 μg/ml of streptomycin sulfate, and water agar plates supplemented with these antibiotics, is tedious and not economical. Furthermore, potatoes used as a baiting substrate disintegrate during the isolation procedure in some soils.

This study reports a method to detect *P. aphanidermatum* and to estimate its populations in soils directly on 2% plain water agar plates with cucumber seeds as a baiting substrate for trapping and Petri's salt solution for inducing sporulation.

MATERIALS AND METHODS

Cucumber (*Cucumis sativus* L. 'Tokiwa-zibai Kairyo') seeds and soil samples were mixed with sufficient water to keep petri dishes in a flooded condition. Any soil sample weighing more than 1 g was mixed with water equal to 60% of fresh soil weight for qualitative work. As a standard, 10 seeds, 10 g of fresh soil, and 6 ml of water were mixed per 9-cm petri dish. For quantitative work, 10 ml of water was always used to mix any soil sample weighing less than 1 g.

Because *P. aphanidermatum* needs higher temperatures of 28–35 C for optimal mycelial growth in potato-dextrose agar (5) and soil (2), the assay of the fungus by this method was conducted at 30, 32.5, and 35 C, using 1-, 5-, 10-, 20-, and 50-g samples from soil incubated with cucumber seeds for 1, 3, 6, 9, 12, and 24 hr, respectively. After incubation for a given period at 30–35 C, seeds were removed, washed under running tap water for 30 min, air-dried to remove surface water (less than 30 min), and placed on 12 ml of 2% plain water agar plates (usually two seeds per plate).

Petri's salt solution (PS) (150 mg KH_2PO_4, 150 mg $MgSO_4$, 60 mg KCl, and 400 mg $Ca(NO_3)_2$ in 1,000 ml of distilled water, pH 4.9) (4) was used to induce sporulation. Four milliliters of PS was poured aseptically over water agar plates with the recovered cucumber seeds (mostly with germ tubes) and incubated for more than 12 hr at 30–35 C.

The fungus on the plates was observed directly under a dissecting microscope at ×30 or more and further confirmed under a compound microscope at ×200.

Naturally infested field soil (pH 5.9) cropped to wheat in winter and soybeans in summer at the National Institute of Agroenvironmental Sciences in Tsukuba, Japan, was used for development of this method.

Samples A and B, each a composite of the respective five subsamples from the five 150-cm^3 samples of surface soil at the same location, were collected on 5 January and 8 August 1983, respectively, and assayed within 7 days of collection.

Twenty-eight soil samples collected in Kinki district in September 1982 and stored for nearly 5 mo at 4 C were also assayed for the fungus by this method.

RESULTS AND DISCUSSION

P. aphanidermatum forms lobulate sporangia, intercalary antheridia, and aplerotic oospores (3). Using this method, lobulate sporangia, vesicles, zoospores, and zoospore discharge were observed by 24–48 hr (Fig. 1A) and sexual organs by 48–96 hr (Fig. 1B) after the trapping experiment was started. Experiments were conducted several times in January and August 1983

Table 1. Frequency of detection of *Pythium aphanidermatum* in Tsukuba soils using a combination of trapping with cucumber seeds as a baiting substrate and soaking water agar culture with Petri's salt solution at 30 or 35 C

Incubation period (hr)	Detection frequency[a]									
	Sample A[b]				Sample B					
	Exp. 1 (10 g)				Exp. 2 (1 g)		Exp. 3 (10 g)			
	30 C		35 C		30 C		30 C		35 C	
	1	2	1	2	1	2	1	2	1	2
1	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
3	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10
6	0/10	0/10	0/10	0/10	1/10	3/10	3/10	2/10	2/10	2/10
9	...[c]	...	...	...	...	2/10	2/10	4/10	3/10	4/10
12	3/10	2/10	1/10	1/10	2/10	3/10	3/10	4/10	2/10	5/10

[a] No. of seeds yielding *P. aphanidermatum*/no. of seeds tested. Data were summarized from three experiments, two trials per experiment.

[b] Samples A and B were collected in January and August 1983, respectively, and assayed within 7 days of collection.

[c] Not tested.

Part of this work was conducted at National Institute of Agricultural Sciences (presently National Institute of Agroenvironmental Sciences), Tsukuba, Japan.

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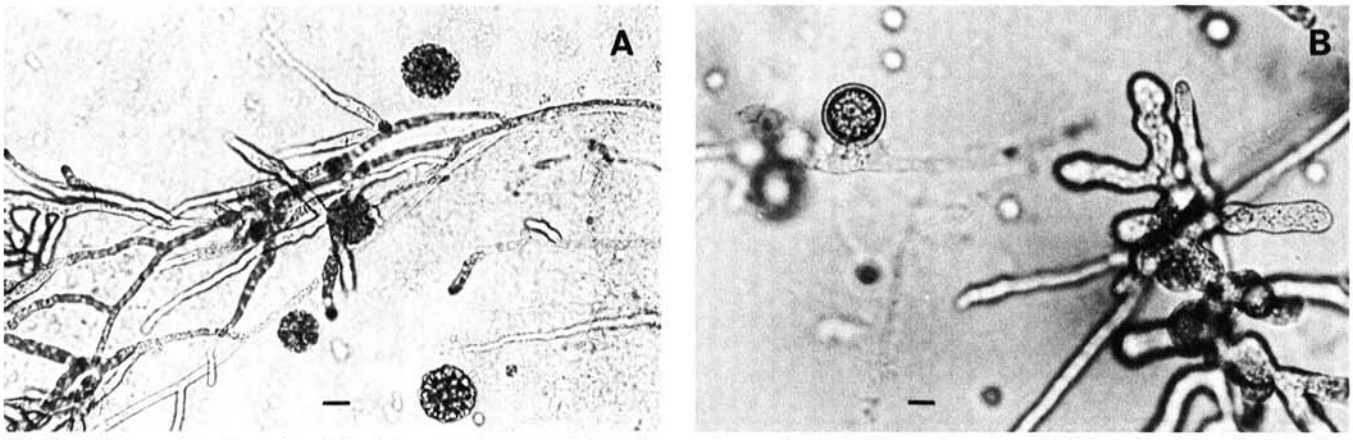

Fig. 1. *Pythium aphanidermatum* detected on a water agar plate with cucumber seeds as a baiting substrate and Petri's salt solution for inducing sporulation. **(A)** Lobulate sporangia, four vesicles and partially lysed hyphae. **(B)** Lobulate sporangia and sexual organ together with scattered encysted zoospores. Photographs were taken (A) 24 hr and (B) 72 hr after the trapping experiment was started. Scale bars = 10 μm.

Table 2. Quantitative estimations of *Pythium aphanidermatum* in Tsukuba soil using a combination of trapping with cucumber seeds as a baiting substrate and soaking water agar culture with Petri's salt solution at 35 C

Soil weight (mg)		Detection frequency[a]						Detection rate[b]
		Exp. 1			Exp. 2			
Fresh	Dry	1	2	3	1	2	3	
2	1.4	0/10	0/10	0/10	...[c]	...	...	0/3
8	5.8	0/10	0/10	0/10	...	...	...	0/3
32	23.1	1/10	0/10	1/10	0/10	1/10	2/10	4/6
64	46.1	2/10	1/10	1/10	1/10	1/10	0/10	5/6
128	92.2	3/10	5/10	3/10	...	...	...	3/3
512	368.6	3/10	7/10	4/10	...	...	...	3/3

[a] No. of seeds yielding *P. aphanidermatum*/no. of seeds tested. Data were summarized in two experiments, three trials per experiment.
[b] No. of trials positive fungus detection/total no. of trials (replicates).
[c] Not tested.

immediately after sample collection; the representative results are summarized in Table 1.

Detection frequency was similar at 30, 32.5, and 35 C, using samples from 1 to 50 g. The fungus was trapped more frequently and in shorter incubation periods in sample B than in sample A. The populations must have been higher in sample B than in sample A, because samples A and B were collected at the same location in January and August 1983, respectively.

This fungus was rarely trapped during 1–6 hr of incubation but was always trapped within 12 hr in sample A, whereas in sample B, it was occasionally trapped even within 3 hr and was always trapped within 6–12 hr (Table 1).

With this method, this fungus was also detected in seven of 28 samples from Kinki district, even after 5 mo of storage at 4 C. The detection frequency was similar to the present assay when isolation by trapping was done immediately after sample collection (T. Watanabe, *unpublished*).

P. spinosum Sawada and unidentified sterile *Pythium* spp. were occasionally encountered during the experiments, but *P. aphanidermatum* was rather easily differentiated from these fungi on the basis of morphology.

To demonstrate the quantitative potential for the method described, populations of the fungus in Tsukuba soil (sample B) was estimated quantitatively at 35 C using small individual fresh samples weighing 2–512 mg (dry weight 1.4–368.9 mg) (Table 2). The fungus was not detected in any sample weighing 2–8 mg. It was frequently detected in samples weighing 32 and 64 mg and always detected in samples weighing more than 128 mg.

If the estimated value for population was calculated on the basis of an assumption that at least one propagule must be present in any sample from which the fungus was detected, the population in 32 mg of fresh soil (equal to 23.1 mg of dry soil), for example, was calculated as 43.3 propagules per gram of dry soil. However, the fungus was detected in four of six samples in two separate experiments for this sample (detection rate of 0.67). The average population was therefore calculated as 29/g by multiplying the estimate value of 43.3 by the detection rate of 0.67.

This quantitative approach may help to estimate the fungus population in soil by a trapping technique.

The detection method in this study was a combination and modification of two techniques, ie, a trapping technique with cucumber seeds as a baiting substrate (6) and a technique for inducing sporulation by soaking plain water agar culture with PS (7). This was a simple, economical, and reproducible means for detecting *P. aphanidermatum* in small samples of soil within a very short time. Also, no additional culture work is necessary for positive identification. Other baiting substrates such as lupine and corn seeds and potato cubes can be used in place of cucumber seeds for trapping (T. Watanabe, *unpublished*).

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