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

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**ABSTRACT**


*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 Kairoy') 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, 35, and 37 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 nin, 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 KH₂PO₄, 150 mg MgSO₄, 60 mg KCl, and 400 mg Ca(NO₃)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 X30 or more and further confirmed under a compound microscope at X400.

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-cm³ 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.

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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

<table>
<thead>
<tr>
<th>Incubation period (hr)</th>
<th>Sample A</th>
<th>Sample B</th>
<th>Sample B</th>
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<tbody>
<tr>
<td></td>
<td>Exp. 1 (10 g)</td>
<td>Exp. 2 (1 g)</td>
<td>Exp. 3 (10 g)</td>
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<td></td>
<td>30 C</td>
<td>35 C</td>
<td>30 C</td>
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<td>1</td>
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<td>0/10</td>
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</table>

**Detection frequency**

No. of seeds yielding *P. aphanidermatum*; no. of seeds tested. Data were summarized from three experiments, two trials per experiment. Samples A and B were collected in January and August 1983, respectively, and assayed within 7 days of collection.

Not tested.

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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, i.e., 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).

LITERATURE CITED