Morphological and molecular identification of *Pythium aphanidermatum* isolates from a root-rotted hydroponic spinach culture in Hiroshima Prefecture

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**Abstract**
Isolates of *Pythium aphanidermatum* from the rotted roots of spinach grown in hydroponic culture in the Hiroshima Prefecture, Japan, were characterized based on morphology, hyphal growth temperature, and internal transcribed spacer (ITS)-rDNA sequences. **Key words:** *Pythium aphanidermatum*, spinach, root rot, hydroponic culture, morphological and molecular identification

Root rot, caused by the pathogen *Pythium aphanidermatum* (Edson) Fitzpatrick, results in significant problems for commercial hydroponic spinach (*Spinacia oleracea* L.) production (Bates and Stanghellini, 1984; Kusakari et al., 1996; Sutton et al., 2006). In Japan, four species of *Pythium* have been identified as the causal agents of root rot of hydroponically grown spinach: *P. aphanidermatum* (Kusakari et al., 1996), *P. myriotyrum* (Tojo et al., 1995), *Pythium* 'group F' (Tojo et al., 1995), and *P. ultimum* (Kusakari et al., 1996). *P. aphanidermatum* is the most prevalent causal agent of root rot in spinach during the warm summer production months and causes severe damage to spinach as compared to the other *Pythium* species (Bates and Stanghellini, 1984; Kusakari et al., 1996). Despite the economic loss incurred by this pathogen, there is no public information (The Phytopathological Society of Japan, 2020) and materials (NARO Genebank, 2019) regarding hydroponically grown spinach infected with this pathogen. In August 2018, the spinach plants grown in a hydroponic culture in the Hiroshima Prefecture, Japan, became stunted with rotted roots. *P. aphanidermatum* was frequently isolated from the rotted roots. Here, we describe the morphological and molecular characteristics of the *P. aphanidermatum* isolates recovered from these spinach plants in order to provide public information and materials for the advanced study of this pathogen. This is also the first report on the causal agent of root rot of hydroponically grown spinach in the Hiroshima Prefecture.

**Materials and Methods**
Root-rotted spinach plants were collected from a commercial hydroponic culture in the Hiroshima Prefecture in August 2018. Approximately 600 plants, one third of the culture system, were damaged by the disease. Black and rotted lesions were observed on the roots of the diseased plants (Fig. 1A). Sections of rotted roots were washed in tap water, air dried, and incubated on NARM medium (Morita and Tojo, 2007). A total of seven *Pythium* isolates were obtained, which were morphologically identical. Therefore, we selected two representative isolates, which were cultured on potato dextrose agar (PDA) and corn meal agar (CMA) prepared according to Tojo et al. (2012) until use. These two isolates were used for further studies. Their pathogenicity was evaluated as follows. Mycelium disks (5mm diameter) of each isolate were obtained from their CMA cultures grown for 5 days at 25°C. The disks were placed on primary roots of 7 days-old spinach seedlings (cv. Active, Sakata Seed Co. Ltd.) which were cultured on rock wool cube moistened with tap water in a 50 mL-plastic cup. The inoculated plants were cultured at 28°C for 0 to 7 days with a 12-h photoperiod in a growth chamber. The *Pythium* isolates were grown in a...
grass blade culture prepared according to Martin (1992) for morphological observation. Morphological identification was based on the van der Plaats-Niterink (1981) keys. Hyphal growth rates at different temperatures were determined on potato carrot agar (PCA) according to the method described by Tojo et al. (2012). For molecular identification of the two isolates, DNA was extracted from mycelium grown on PDA using PrepMan Ultra Sample Preparation Reagent (Applied Biosystems, Foster City, CA). Amplification and sequencing of the internal transcribed spacer (ITS) region of rRNA gene of the isolates were performed according to the method described by Uzuhashi et al. (2008).

Results and Discussion

The two representative Pythium isolates, HSP-1 (MAFF 247162) and HSP-2 (MAFF 247163), which were recovered from the rotted roots of spinach grown in a hydroponic culture, showed similar pathogenicity symptoms to spinach, morphology, and growth temperatures.

The inoculated spinach seedlings with the respective Pythium isolates became stunted, and their roots rotted in a few days and completely wilted after 7 days of inoculation. The inoculated Pythium isolates were reisolated from the root lesions of the respective spinach plants.

The taxonomic features of the isolate HSP-1 were observed by microscopy. Primary hyphae were up to 10 μm wide. Sporangia were mostly terminal, but occasionally intercalary, consisting of inflated filamentous structures or complexes of swollen hyphal branches (Fig. 1B). Zoospores were formed in a vesicle produced at the tip of discharge tube (Fig. 1C). The diameter of the encysted zoospores ranged from 8.9–14.4 μm (Fig. 1D and E). Oogonia were terminal and 20.3–25.8 (average 20.3) μm in diameter (Fig. 1F). Antheridia were terminal or intercalary, one per oogonium, monoclinous or diclinous, and bell-shaped (Fig. 1F). The breadth of antheridial cells ranged from 8.1–12.2 μm. Oospores were aplerotic and 17.2–21.4 (average 19.3) μm in diameter (Fig. 1F). The thickness of the oospore wall ranged from 1.1–1.7 (average 1.4) μm. The cardinal temperatures for hyphal growth on PCA were a minimum of 13°C, an optimum of 40°C, and a maximum of 43°C. The daily growth rate at 25°C was 34.1 mm per day. Based on the morphology and hyphal growth temperature, the isolates were identified as P. aphanidermatum.

Both the isolates HSP-1 and HSP-2 had the identical ITS sequences. These sequences shared a 100% identity with the ITS sequences of P. aphanidermatum isolate CBS118.80, which was used for the species description by van der Plaats-
Niterink (1981). As a result, morphological identification was confirmed by the ITS sequences. The sequences of the isolates HSP-1 and HSP-2 have been deposited in the DDBJ/EMBL/GenBank database under accession numbers LC512456 and LC512457, respectively.

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