First Report of *Pythium dissotocum* Causing Root Rot on Hydroponically Grown Spinach in China

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In July 2016, more than 60% of hydroponic commercially grown spinach (*Spinacia oleracea*) suddenly wilted in commercial greenhouse production in Songming (25°N, 103°E), Yunnan province. The infected plants were stunted, chlorotic, and wilted, with root of the plant appearing necrotic black-brown and rotting with a stench smell. Typical root rot samples were collected to isolate the possible pathogen. Symptomatic roots were washed with tap water, surface sterilized in 70% alcohol for 30 s, rinsed three times with sterile water, cut into small slices, placed on both potato dextrose agar and rye agar medium amended with 25 mg/liter of rifampin and 50 mg/liter of kanamycin, and incubated at 25°C in the dark for 4 to 5 days. Colonies of white mycelia growth were observed from the edge of the diseased tissue and were further identified as *Pythium* spp. based on morphological characteristics of asexual and sexual structures (van der Plaats-Niterink 1981). The hyphae were aseptate, and colonies had filamentous sporangia with a dendroid structure. The oogonia were subglobose, smooth, on average 25.4 µm in diameter, often including a small part of the subtending hypha. They developed on unbranched stalks and declinuous, antheridia barrel shaped, and oospores aplerotic or nearly plerotic, globose, on average 21.2 µm. For pathogenicity tests, four- to five-leaf-old healthy spinach seedlings were inoculated with a suspension

(5×10^5 zoospores/ml) of the *Pythium* isolate that was induced in V8-juice liquid medium, and control plants were inoculated with sterile distilled water. The treated plants were incubated at 25°C, under a 12-h light/12-h dark photoperiod. After 5 days, spinach seedlings inoculated with zoospore suspension showed the symptoms of stunting, chlorotic leaves, and necrotic roots, which were similar to those observed in commercial greenhouses. In contrast, no symptoms were observed in the control plants. *Pythium* spp. was reisolated from infected root tissues, which had similar morphological characteristics with the inoculated isolate. DNA of *Pythium* isolate was extracted according to the modified CTAB protocol (Griffith and Shaw 1998). Two molecular targets, the internal transcribed spacer (ITS) region of ribosomal DNA and cytochrome c oxidase subunit II (CoxII), were amplified and sequenced using universal primers ITS1/ITS4 and FM58/FM66 (Villa et al. 2006). The nucleotide sequences were assigned the GenBank accession numbers MG719858.1 and MG719859.1, respectively. From a GenBank BLAST search, the ITS sequence showed 99.76% identity with *Pythium dissotocum* (KM061660.1 and KM061654.1) and *Pythium coloratum* (KU211454.1). The CoxII sequence showed 100% identity with *P. dissotocum* (AB468893.1). Based on these results, this *Pythium* pathogen was classified as *P. dissotocum* (Martin 2000). Recently, this pathogen was also reported causing root rot on hydroponic lettuce in the United States (McGehee et al. 2018). To our knowledge, this is the first report of *P. dissotocum* causing root rot on spinach in China. This new finding is important to develop an efficient disease management strategy to manage this *Pythium* root rot disease under a hydroponic culture system.

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