Pathogenicity and Mycological Characteristics of *Pythium myriotylum* Causing Rhizome Rot of Ginger

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생강뿌리썩음병균 *Pythium myriotylum*의 병원성 및 균학적 특성

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**ABSTRACT**: Six pathogenic *Pythium* isolates obtained from diseased ginger rhizomes were identified as *Pythium myriotylum* Drechsler based on various morphological and physiological characteristics. The isolates showed strong virulence on underground parts of buds, crowns, rhizomes, roots and aerial parts of leaves and stems as well. The isolates caused rot of germinated seeds of 10 different crops and weeds including cucumber and pepper, and markedly inhibited seedling growth of 3 crops tested, including corn and barley. Maximum, optimum and minimum growth temperatures for *P. myriotylum* were 39–45°C, 33–37°C and 5–7°C, respectively. Optimum pH for the growth was 6–7. Mycelial linear growth was most rapid on V-8 juice agar, but aerial mycelia were most abundant on PDA and corn meal agar. Zoosporangial and oogonial formation was greatest on V-8 juice agar. Optimum temperatures for the production of zoosporangia and oogonia were 20–35°C and 15°C, respectively.

**Key words**: ginger, *Zingiber officinale*, rhizome rot, *Pythium myriotylum*, pathogenicity, physiology, mycological characteristics.

Rhizome rot of ginger has been a serious problem in major ginger production areas in Korea (9). It occurs almost every year and often devastates ginger fields, particularly in the year with hot and wet summer. Average incidence of the disease was recorded 18.1% in 1995 in Choongnam province (9). In diseased plants, underground parts of the stems enlarging from each buds of ginger rhizomes were first rotten and caused yellowing of the lower leaves that gradually extended to whole plants until blighted to death. Underground rhizomes became rotten and mummified at the end of season. Several species of *Pythium*, such as *P. myriotylum*, *P. zingiberum*, *P. voluitum*, and *P. aphanidermatum* have been described as causal organisms of the disease by many researchers (3,5,13,14,16). However, taxonomic distinction among these species has not been fully clarified(3,6,13). In Korea and Japan, *Pythium zingiberum* has been listed as a causal pathogen of rhizome rot (1,15), but mycological properties of the species were not fully examined.

This study was conducted to identify the causal pathogen of ginger rhizome rot, to test its pathogenicity on ginger as well as on other crops, and to examine some environmental factors affecting growth and reproduction of the pathogen. Parts of the research results have been published elsewhere (8).

**MATERIALS AND METHODS**

Isolation and identification of the pathogen. Diseased ginger plants were collected and portions of their rotted crowns, stems and rhizomes were plated on water agar after surface sterilization with chlorox. Hyphal tips of emerging colonies were transfered to PDA. The medium selective to *Pythium* was also used to isolate the pathogen (10). Species identification of *Pythium* from ginger plants was generally followed the key suggested by Waterhouse (13), and referred to the descriptions of CMI (14), Ichitani and Chikuo (5), Ichitani

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and Shinsu (6), Chattopadhyay (3) and Shinsu (11) for comparisons among *P. myriotylum*, *P. zingiberum* and the pathogenic *Pythium* isolates obtained in this study.

**Pathogenicity tests on ginger and other plants.** Healthy young ginger plants grown in the greenhouse, approximately 22–25 cm long were chosen for pathogenicity tests. The plants were removed from the pots, washed thoroughly under tap water and put into plastic bags, one in each, containing wet paper towel to maintain moisture. The inoculation was done by placing a 0.5 cm diam. PDA culture disk of each *Pythium* isolate on the crown of each plant. The plastic bags were sealed roughly with rubber bands, and put at room temperature. Disease development on whole parts of the inoculated plants including leaves was examined 11 days after inoculation. A total of 23 *Pythium* isolates were tested with 2 replications.

In another set of pathogenicity test, 4 isolates of *Pythium* were also examined by a soil inoculation method. Ginger plants, 22–27 cm high were transplanted into beakers (15 cm diam.) containing artificially infested soil (peat : vermiculite : perlite = 3 : 1 : 1) with each *Pythium* isolate at the concentration of 15–20 cfu/g soil. The inoculated plants were placed in the greenhouse at 21–42°C. Disease development was examined 10 days after transplanting.

Fifteen different plant species including vegetables, cereals and weeds were examined for their susceptibility to 3 pathogenic isolates obtained from gingers. Germinated seeds of each plant species were plated on 3-day-old water agar (WA) culture of each isolate, and incubated at 28°C under a cool-white fluorescent lamp with 12 hr light/dark regime. Root development on the germinated seeds was examined 5 days after inoculation. A total of 12 to 25 seedlings were tested in each crop. To examine the effects of the pathogenic *Pythium* isolates on seedling growth, surface-disinfect ed seeds of corn, barley, and radish were plated on 3-day-old culture of each isolate, and were incubated at 30°C under 12 hr light/dark condition. Height and root length of the seedlings were measured and compared to the uninoculated check 5 days after inoculation. Fifteen to 20 seedlings were examined in each crop.

**Effects of temperature, pH and nutrient sources on mycelial growth and production of zoosporangium and oogonium.** In order to determine maximum, optimum and minimum temperature for mycelial growth, a disk of 0.5 cm diam. PDA culture of 6 isolates of the pathogenic *Pythium* isolates were inoculated by placing the disk at the center of petri dishes containing PDA. The dishes were incubated at 29°C to 49°C at 2°C intervals to determine maximum and optimum temperatures, and 3°C to 11°C at 2°C intervals to determine minimum temperature. Rate of linear growth per day was calculated by total growth/no. days. Each treatment was replicated 5 times.

To examine pH effect on mycelial growth, each isolate was inoculated similarly on PDA at pH 5 to 9 at 1 intervals that was adjusted with 1 N HCl. The dishes were incubated at 28°C and colony diameter was measured 24 hr after inoculation. The treatment was replicated five times.

Five media, WA, PDA, corn meal agar (CMA, corn meal 17 g, agar 20 g, distilled water 1 l), Czapek-dox agar (Difco), and V-8 juice agar (V-8 juice 200 ml, agar 20 g, distilled water 1 l) were prepared following the standard methods (4) and compared to examine nutrient effects on vegetative growth and zoosporangial and oogonial production of two isolates 4-4 and 9-3.

The isolates were inoculated as described above, and incubated at 30°C. Linear growth and degree of aerial mycelia were examined 2 days after inoculation. Rate of linear growth was obtained by total growth/hours of incubation. Sporangial and oogonial formation on each medium was examined under a microscope 7 days after inoculation. The treatment was replicated three times.

In a separate experiment to examine temperature effects on zoosporangial and oogonial formation, ginger leaves were detached, and cut into 1 cm² pieces. The leaf pieces were placed 3 each on a WA petri dish culture of 3 pathogenic *Pythium* isolates 4-3, 9-3 and 960318. The dishes were incubated at 15°C to 40°C at 5°C intervals. Number of zoosporangia and oogonia formed on leaf portions in a 100x field of a microscope was counted 4 days after inoculation, based on 3 observations each in 3 replications.

**RESULTS**

**Morphological and physiological characteristics of the pathogen.** Morphology of *Pythium* isolates was examined on V-8 juice agar or broth. Mycelium is up to 8.0 μm wide in diameter, and it forms numerous clavate appressoria, 20–25×18 μm in size (Table 1). Zoosporangia are lobulate, terminal or intercalary, 70–90×80–100 μm in size, and they arise from the hyphal branches. Oogonia are abundant, globose, terminal or intercalary, wall smooth, 29–33 μm in di-