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

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생강뿌리썩음병균 *Pythium myriotyrum*의 병원성 및 균학적 특성

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ABSTRACT : Six pathogenic *Pythium* isolates obtained from diseased ginger rhizomes were identified as *Pythium myriotyrum* 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℃, 33~37℃ and 5~7℃, 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℃ and 15℃, respectively.

***Key words*: ginger, *Zingiber officinale*, rhizome rot, *Pythium myriotyrum*, 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, particulary 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. myriotyrum, P. zingiberum, P. volutum*, 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 clorox. 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 thorougly 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 developement 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℃. 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℃ under a cool-white fluorescent lamp with 12 hr light/dark regime. Rot 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-disinfected seeds of corn, barley, and radish were plated on 3-day-old culture of each isolate, and were incubated at 30℃ 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℃ to 49℃ at 2℃ intervals to determine maximum and optimum temperatures, and 3℃ to 11℃ at 2℃ 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℃ 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 m*l*, 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℃. 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 cm^2 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℃ to 40℃ at 5℃ 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-

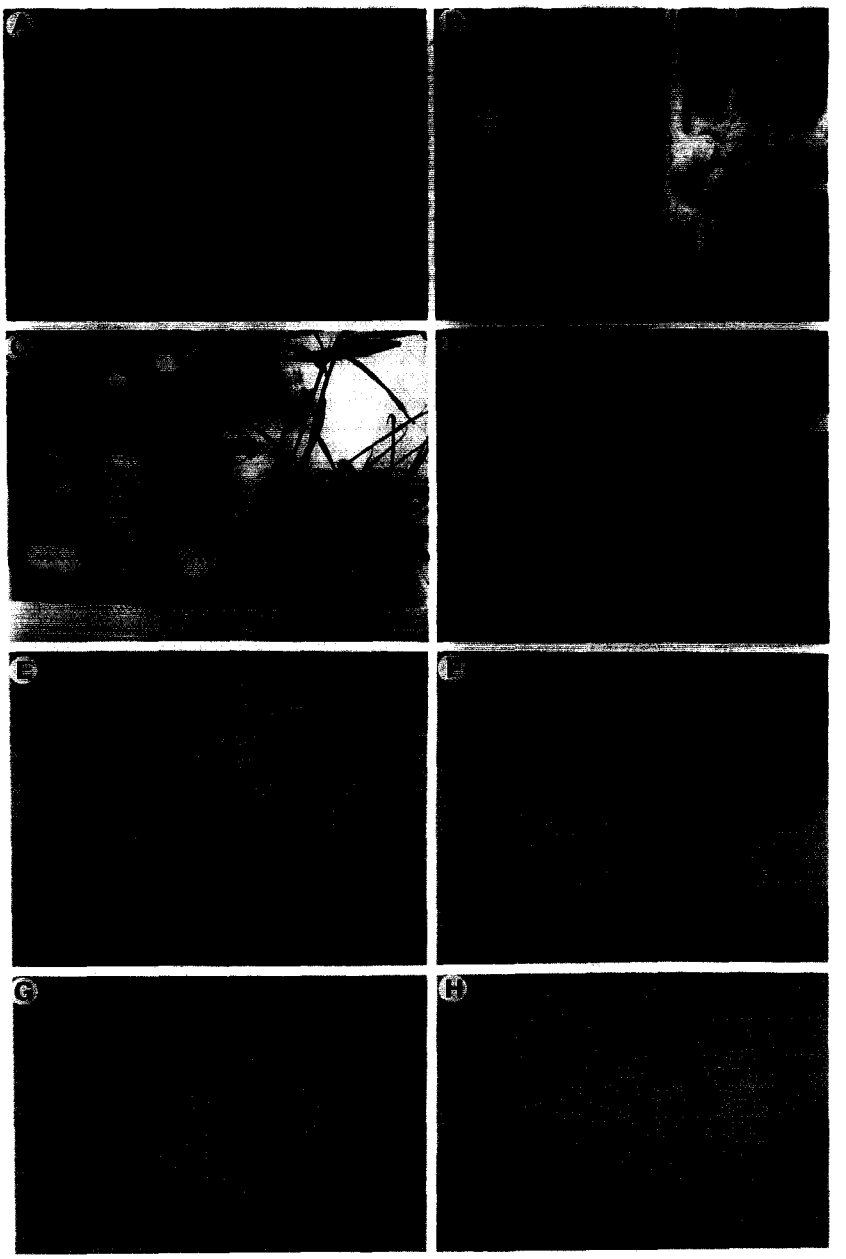

Fig. 1. A: Typical symptoms of rhizome rot of ginger at the mid-growth stage in field, showing severe yellows and blight of aerial parts of the plants (left), B: Underground symptoms of ginger rhizome rot (left), compared to healthy rhizomes (right) at the harvesting stage, C: Pathogenicity tests of *Pythium* isolates obtained from diseased rhizomes, showing that the isolates 960318 and 4-3 of *Pythium myriotylum* (rhigt row) caused severe symptoms 10 days after inoculation (CK: uninoculated check, others: isolates used as negative control), D: Reduction in seed germination and seedling growth of cucumber, and seed colonization caused by inoculation of the isolates 960318, 9-3 and 4-3 of *Pythium myriotylum* and the isolate 2-1 of *Pythium* sp. (upper: inoculated, bottom: uninoculated check), E: Lobulate zoosporangia(zs) of *Pythium myriotylum* (bar: 16 μm), F: a globose ogonium(oo) with declinous antheridia (bar: 16 μm), G: An oogonium(oo) attached with a crook-necked antheridium(an) (bar: 32 μm), H: Aplerotic oospores(os) (bar: 16 μm).

Table 1. Comparison of mycological characteristics of *Pythium zingiberum*, *P. myriotylum* and *Pythium* species from this study

Mycological characteristics	*P. zingiberum*[a]	*P. myriotylum*[b]	This study
Hyphae width (μm)	3.5~8.0	~8.5	~8.0
Appressoria			
Shape	clavate	clavate, knob-like sickle shape	clavate
Size(μm)	26-34	~60×~11	20~25×18
Oogonium			
Shape	globose	globose	globose
Size(μm)	-	20~26×32~35	29×33 (avr.)
Formed	terminal	terminal or intercallary	terminal or intercallary
Growth rate at 25℃ on PDA (mm/day)	-	28	22~24
Growth temperature(℃)			
Maximum	40~43	40	43~45
Optimum	34	37	33~37
Minimum	8	5	5~7
Oospore			
Shape	globose/ aplerotic	globose/ aplerotic	globose/ aplerotic
Size(μm)	19~34	18×20~27×29	22~24
Wall thickness (μm)	1~2	2	1
Projection	present	present	present
Antheridia			
Shape	clavate, crook necked	clavate, crook necked	clavate, crook necked
Number	1~3	3~6	2~8
Attachment	diclinous	diclinous, occationally monoclinous	diclinous, occationally monoclinous
Zoosporangium			
Shape	lobulate	lobulate	lobulate
Size(μm)	-	-	70~90× 80~100

[a] References 7, 8, 11.
[b] Reference 14.

ameter. Antheridia are clavate or crook necked, paragynous, 2~8 per oogonium, typically declinous, but occationally monoclinous. Antheridial branch that entwins oogonial stalks was occasionally found. Oospores are formed singly in each oogonium, globose, occationally aplerotic, 22~24 μm in diameter, wall 1 μ thick, smooth small round projections present. Maximum, optimum, and minimum growth temperatures are 43-45℃, 33~37℃, and 5~7℃, respectively. Growth rate at 25℃ on PDA is 22~24 mm/day. Growth is very rapid particularly at high temperature with upto 40 mm/day. Based on morphological and physiological characteristics observed, pathogenic *Pythium* isolates on ginger obtained in this study were identified as *P. myriotylum* Drechsler (13).

Table 2. Pathogenicity of 23 *Pythium* isolates obtained from diseased rhizomes and crowns on various parts of ginger plants in the laboratory inoculation test

Isolate	Pathogenicity[a]				
	Leaf	Stem	Crown	Rhizome	Root
1-1, 2-3, 2-6, 4-6, 7-2, 8-1, 8-4, 100	-	-	-	-	-
2-1, 2-2, 6-1, 8-2, 9-4, G-Ti	-	-	+	+	-
4-3, 4-4, 9-1, 9-2, 9-3, 960318	++	++	++	++	++
5-2	±	-	++	++	-
6-2	+	+	-	-	-
6-4	-	-	++	++	++

[a] -: no symptom, ±: surface-discolured lesions, but not rotten, +: water-soaked lesions with mild rot, ++: completely rotten.

Pathogenicity on ginger. Twenty three isolates obtained from diseased rhizomes varied in pathogenicity on various parts of ginger plants (Table 2). Six isolates including 4-3, 9-3, and 960318 had strong virulence to all parts of ginger plants. Other 9 isolates such as 5-2 and 6-4 showed virulence depending on parts of ginger plants inoculated. Remaining 8 isolates were not pathogenic. In a soil inoculation test, isolates 4-3 and 960318 were strongly virulent, causing severe infections on all parts of ginger plants including leaf, stem, crown, rhizome, root and bud (Table 3). Whereas, the non-pathogenic isolates 2-1 and PSD-19 that were used as a negative control did not infect any part of ginger plants.

Pathogenicity on other plants. Three isolates, 4-3, 4-4 and 9-3 caused severe seedling rot of cucumber, watermelon, chinese melon, squash, pepper, wheat, tomato, egg plant, and radish, although their virulence

Table 3. Pathogenicity of *Pythium* isolates to various parts of ginger plants grown in pot soil infested with each isolate in the greenhouse

Isolate	Pathogenicity (no. diseased/no. inoculated)					
	Leaf	Stem	Crown	Rhizome	Bud	Root
4-3	34/45	6/7	3/6	3/6	3/5	4/4
960318	45/46	7/7	5/6	5/6	6/6	5/6
2-1	0/28	0/5	0/5	0/5	0/5	0/7
Uninoculated	0/41	0/6	0/8	0/8	0/5	0/6

[a] Data were obtained 10 days after inoculation.

differed with isolate (Table 4). Isolate 4-4 also caused seedling rot of westworld grasses. However, all isolates tested did not infect germinated seeds of barley, corn, crown daisy and barnyard millet.

The three isolates also reduced seedling growth significantly. Degree of growth reduction on 3 plant species in terms of height and root length ranged 0 to 99%, depending on the isolates and plant species tested (Table 5). Among the five plant species, growth reduction was largest on barley.

Table 4. Pathogenicity of *Pythium myriotylum* isolates 9-3 and 4-4 causing ginger rhizome rot as examined by rot development on germinated seeds of 15 different plant species

Plant species	No. germinated seeds inoculated	% germinated-seeds rotten[a]			
		4-3	4-4	9-3	Uninoulated
Cucumber, Water melon, Chinese melon, Squash, Pepper, Wheat	12~16	100	100	100	0
Tomato	12	82	83	100	0
Eggplant	16	33	67	25	0
Radish	20	19	67	100	0
Westworld grass	16	-	100	-[b]	0
Barley, corn, Crown daisy, Barnyard millet	12~25	0	0	0	0
Italian ryegrass	20	-	-	0	0

[a] Germinated seeds were plated on water agar culture plates of each isolate. Colonization of germinated seeds was examined 5 days after inoculation.

[b] Not tested.

Table 5. Growth reduction of seedlings of 3 different crops caused by three *Pythium myriotylum* isolates, 9-3, 4-3 and 960318, the pathogen of ginger rhizome rot

Crop	Growth reduction (%)[a]					
	Seedling height			Root length		
	4-3	9-3	960318	4-3	9-3	960318
Corn	9	13	0	0	29	69
Barley	80	88	42	99	93	81
Radish	68	-	37	64	71	13

[a] % reduction as campared to the uninoculated check.

Effect of temperature on *in vitro* growth, zoosporangial and oogonial formation. Maximum temperature for mycelial growth was 43~45℃ for all isolates except the isolate 960318 (Table 6). The minimum growth temperature was 5℃ for most isolates. Optimum growth temperature was mostly 35~37℃, but was 33℃ for the isolate 9-2. Isolate 960318 grew much slowly and had lower growth temperature compared to other 5 isolates, where maximum, optimum and minimum temperatures were 39℃, 31~33℃ and 7 ℃, respectively.

Zoosporangial formation on ginger leaf portions varied with isolates and was greatest at 30~35℃ for the isolate 4-3, 25℃ for the isolate 9-3 and 20℃ for the

Table 6. Maximum, optimum and minimum growth temperatures as judged by vegetative growth rate on PDA of 6 isolates *Pythium myriotylum*

Temperature (℃)	Linear growth rate (mm/day)[a]					
	4-3	4-4	9-1	9-2	9-3	960318
49	0	0	0	0	0	0
47	0	0	0	0	0	0
45	0	1.0	0	0	0	0
43	0.7	1.7	0.7	0.3	0.3	0
41	19.0	21.7	5.7	10.7	12.7	0
39	36.8	26.1	23.9	21.3	28.5	3.1
37	38.0	31.7	33.3	25.7	34.0	5.7
35	38.0	36.0	32.7	28.3	33.8	13.3
33	35.0	34.7	32.3	30.5	32.7	19.7
31	31.7	33.5	28.2	29.3	29.7	19.7
29	28.0	27.3	23.3	25.8	25.3	17.3
11	18.0	29.0	29.7	22.3	28.3	14.3
9	10.7	17.7	9.7	12.7	10.3	2.2
7	4.5	7.7	2.7	3.3	2.3	0.3
5	0.7	1.0	0.2	0.2	0.2	0
3	0	0	0	0	0	0

[a] Values are means of 3 replications.

Table 7. Effects of temperature on zoosporangial and oogonial formation of 3 isolates of *Pythium myriotylum* on ginger leaf portions placed on water agar cultures[a]

Temperature (℃)	No. zoosporangia/100× field			No. oogonia/100× field		
	4-3	9-3	960318	4-3	9-3	960318
40	17	9	0	2	0	0
35	125	21	17	2	25	0
30	120	29	54	3	16	0
25	57	86	54	16	1	0
20	21	38	90	81	55	0
15	2	3	12	100	64	105

[a] Data were obtained 4 days after inoculation and based on 5 observations each in 3 replications.

isolate 960318 (Table 7). Zoosporangial formation was greatly inhibited at 15℃ as well as at 40℃. In contrast, oogonial formation was highest at 15℃, regardless of the isolates tested, and tended to reduce at higher temperature. The isolate 960318 did not produce oogonium at above 15℃.

Optimum pH for mycelial growth was pH 6 for isolates 4-3 and 960318, and pH 7 for the isolate 9-3 (Table 8).

Effect of nutrient source on *in vitro* growth, and zoosporangial and oogonial formation. Mycelial linear growth was most rapid on V-8 juice agar, followed by CMA and WA (Table 9). However, aerial mycelia was greatest on PDA followed by CMA. Zoosporangial formation was highest on V-8 juice agar, intermediate on PDA, and least on CMA. Oogonia were formed most abundantly on V-8 juice agar, but was not formed on PDA and CMA.

DISCUSSION

Table 8. Effect of pH on vegetative growth of 3 isolates of *Pythium myriotylum* on PDA at 28℃

pH	Colony diam. (mm) after 24 hr		
	4-3	9-3	960318
5	37	14	36
6	53	28	37
7	48	34	31
8	43	26	32
9	36	23	24

[a] Values are means of 5 replications.

Six *Pythium* species, *P. myriotylum, P. aphanidermatum, P. zingiberum, P. vexans, P. volutum*, and *P. graminicola* have been reported as pathogenic species on ginger causing soft rot of rhizomes (3, 6, 7, 11, 13, 14, 16). Among these species, *P. vexans* has been reported at high altitude areas beyond 1,000 m under lower soil moisture conditions (3). Taxonomically, it could be distinguished from other five species, in the respects that it hardly produces zoospores, and its hyphal width is much narrower than other species (13). *P. graminicola* has been reported to be the causal organism of the rhizome rot of ginger in India (3), but its pathogenicity on ginger has been questioned by other researchers (3), and it requires to be investigated. *P. aphanidermatum* has been reported in India as the major pathogen of rhizome rot along with *P. myriotylum* (3). This species differs morphologically from other three species in the respects that oogonia are smaller, and monoclinous antheridia are often formed intercalary (13). In Japan, *P. aphanidermatum* was reported to be isolated often from rotten rhizomes, but was found to be not pathogenic on ginger (6, 12). In the present study, several species of *Pythium* were iso-

Table 9. Effects of nutrient source on vegetative growth and formation of zoosporangium and oogonium of *Pythium myriotylum* 4-4 and 9-3 at 30 ℃[a]

Nutrient source[b]	Linear growth (mm/hr)		Aerial mycelia[c]		No. zoosporangia/100× field		No. oogonia/100× field	
	4-4	9-3	4-4	9-3	4-4	9-3	4-4	9-3
WA	2.5	2.4	-	-	4.3	5.3	19.3	2.3
PDA	1.7	2.1	++	++	7.7	5.7	0	0
CMA	2.6	2.7	+	+	0.3	2.0	0	0
CZ	2.0	1.6	-	-	4.0	6.3	4.3	2.7
V-8	3.5	3.2	-	-	15.0	21.0	77.7	59.0

[a] Data are based on 3 replications.
[b] WA: Water agar, PDA: Potato dextrose agar, CMA: Corn meal agar, CZ: Czapek-dox agar, V-8: V-8 juice agar.
[c] ++: dense, +: sparse, -: none.

lated from rotten rhizomes, but were neither *P. aphanidermatum* nor *P. vexans*.

P. zingiberum, a new species of *Pythium*, has been reported as the pathogen of ginger rhizome rot in Japan (5, 6, 11, 12). However, several questions in taxonomical distinction of this species has arisen by some researchers (6), and it remains to be clarified. Waterhouse (13) did not consider *P. zingiberum* as a new species of *Pythium*, and recognized it as a synonym of *P. volutum* in the respects that antheridial branch ocassionally entwins oogonial stalk. However, growth habit of the two species is very different, where mycelial growth of *P. volutum* is slow and its maximum temperature for mycelial growth is around 32℃ (13). Whereas *P. zingiberum* grows very rapidly and maximum temperature is over 40℃ (6, 11). For this reason, Ichitani (6) separated *P. zingiberum* from *P. volutum* in spite of Waterhouse's observation (13), and has been used so far in Japan, as the only pathogen of the ginger rhizome rot (11, 12).

P. myriotylum has been reported early in many countries as the major pathogen of soft rot of rhizomes (3, 14), and its taxonomical distintion has been recognized (3, 13, 14). It is interesting to see that mycological characteristics of *P. zingiberum* is very similar to those of *P. myriotylum*, including growth habit. One of the major differences between the two species is that number of antheridia attached on an oogonium is 1-3 or 2-4 for *P. zingiberum* (5, 6), and <10 (avr. 3-6) for *P. myriotylum* (14). Another difference is that oogonia are terminal for *P. zingiberum* (5), but terminal or intercalary (less frequently) for *P. myriotylum* (14). Ichitani and Shinsu (6) insisted that oogonial size is different between the two species, but it was found to be not true (3, 14). Since the major mycological characteristics are similar except the minor differences mentioned above, *P. zingiberum* Takahasi (6) could be considered as a synonym of *P. myriotylum* Drechsler(14).

In this study, our isolates had 2-8 antheridia per oogonium. Most oogonia were terminal, but rarely intercalary. Based on these observations, we identified these isolates as *P. myriotylum* Drechsler. In Korea the causal pathogen of ginger rhizome rot has been used as *P. zingiberum* (1) without significant evaluation on its taxonomic status. The detailed descritption of the type species are not available to date. In this study, pathogenic *Pythium* isolates obtained from major ginger growing areas belonged to all *P. myriotylum*, although the minor variations existed between isolates, such as in mycelial growth rate, temperature reaction, and amounts of production of oospore, appressoria, zoosporangia, and entwinning antheridia. Based on these results, *P. zingiberum*, described as the pathogen of the ginger rhizome rot in Korea, should be reconsidered in taxonomically, and corrected to *P. myriotylum* until its taxonomic clarification.

Host range of *P. zingiberum* has been reported to be very limited to ginger or its closely related species (2). In contrast, *Pythium* isolates obtained in this study showed broad range of pathogenicity on various crops, including vegetable and cereals. The isolates caused severe rot of the germinated seeds of host plants or, if not, inhibited markedly growth of the seedlings. *P. myriotylum*, soft rot pathogen of ginger, has been reported to cause also seedling damping-off of tobacco, black locust and watermelon, seedling root rot of lucerne, papaya and tomato, and fruit rot of watermelon, cucumber and eggplant (14). This species was also reported to be pathogenic to pineapple, peanut, rice and kidney bean (14). Broad host range of the isolates obtained in this study agrees well with that reported on *P. myriotylum*, not on *P. zingiberum*. Further studies on determining host range of the isolates are needed to establish a proper cropping system for the control of ginger rhizome rot.

요 약

병든 생강으로부터 분리한 6개 *Pythium* 균주들은 여러 가지 형태적, 생리적 특성에서 *Pythium myriotylum* Drechsler로 동정되었다. 이 균들은 생강 지하부의 싹, 근경, 뿌리, 땅가줄기 뿐만 아니라 지상부 잎, 줄기 전부분에 걸쳐 병원성이 강하였다. 이 균들은 오이, 수박 등 10개 작물 혹은 잡초의 어린 묘를 부패시켰으며 보리, 옥수수 등 공시한 3개 작물 유묘의 지상부 생장이나 뿌리 생장을 현저히 억제하였다. 이 균들의 생육최고, 최적, 최저온도는 각각 39~45℃, 33~37℃, 5~7℃였으며 생육최적 pH는 6~7이었다. 이 균들의 균사생장은 V-8 juice 배지에서 가장 빨랐으나 기중균사량은 PDA와 옥수수전즙배지에서 가장 많았다. 유주자낭과 장난기는 V-8 juice 배지에서 가장 잘 형성되었으며 유주자낭 형성 최적온도는 균주에 따라 20~35℃, 장난기 형성은 15℃의 저온에서 가장 양호하였다.

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