Kinds, Distribution, and Pathogenicity of *Pythium* Species Isolated from Soils of Kyushu Island in Japan*

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Abstract

In assaying soil samples from 25 locations (four~nine samples/Prefecture) in Kyushu Island for *Pythium* species by a trapping isolation method with cucumber and lupine seeds as baiting substrates, one~seven taxa/sample were isolated from 24 samples, but no *Pythium* was isolated from one sample. A total of 433 isolates were identified into 16 taxa including H-Zs, a provisional *Pythium* group with zoospore formation from hypha-like sporangia, but without sexual organs. Among these fungi, 201 isolates of *P. sylvaticum* obtained from 22 locations were the most dominant, followed by *P. ultimum* and *P. aphanidermatum*. In inoculation experiments of 36 isolates of 16 taxa by the soil-over-agar culture inoculation method, 10 isolates including three each of *P. sylvaticum* and *P. ultimum* caused less than 60% of cucumber seeds unemerged, and after emergence, nine including two each of *P. aphanidermatum*, *P. sylvaticum*, and *P. ultimum* caused more than 40% of the seedlings collapsed or diseased. Emergence rates of komatsuna seeds appeared not to be influenced by any *Pythium* species tested, but after emergence, four isolates of *P. sylvaticum*, three of *P. ultimum* and one of *P. irregulare* caused more than 40% of the seedlings collapsed or diseased. Others were mildly pathogenic or nonpathogenic to both seedlings, but damage rates were below 30%.

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Key words: *Pythium* spp., kinds, distribution, taxon, Kyushu.

INTRODUCTION

Soilborne diseases of various plants due to *Pythium* and other pathogens have been serious recently, and therefore, ecology and distribution of these fungi have been studied in relation to etiological studies^{1,4-8,11,13-17,19}) or just for scientific interest³⁾. Kinds, and distribution of *Pythium* species in some Japanese soils have been reported, including piedmont natural forest at Mt. Fuji¹⁶⁾, and Shikoku Island¹⁹⁾, and *Pythium* is present in almost all Japanese soils¹⁷⁾.

This is an additional report on kinds and distribution of *Pythium* species isolated from soils of Kyushu Island in relation to the previous works¹³⁻²⁰⁾. Pathogenicity of 36 isolates to cucumber and komatsuna seeds and seedlings was also tested

MATERIALS AND METHODS

Samples and sampling. Soil samples from 25 different locations of Kyushu Island (nine, seven, five, and four locations from Fukuoka, Kumamoto, Miyazaki and Kagoshima Pref., respectively) with various habitats, and soil pH values ranged from 5.6 to 7.2 were collected in August, 1980 (Table 1). The respective soil samples consisted of five random cores from the

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top 15-cm surface soil were mixed thoroughly in polyethylene bags. These samples were usually assayed within 7 days after collection.

Isolations. *Pythium* was isolated by trapping methods using seeds of cucumber (*Cucumis sativus* L., cv. Tokiwa-jibai Kairyou) and lupine (*Lupinus albus* L., cv. Shirobana) as trapping substrates following the previous works^{13,17}.

Ten seeds each embedded in each 20~50 g soil sample in a 9-cm petri dish incubated at 26 C for 1~2 days or 10 C for 5~10 days, respectively, were removed from soil, washed under running tap water for 1 hr, air-dried, and placed on water agar (WA, 2 seeds/plate). After a further incubation at 28 C for 1~2 days, pure isolates of *Pythium* spp. grown out of cucumber seeds on WA were obtained by single hyphal tipplings.

Cultures and identifications. The fungi were cultured on various media including Difco corn meal agar for mycological study. Several soaking solutions including Petri salt solution were used to study asexual reproduction. All of fungi were identified following the descriptions by the previous workers^{9,10}.

Inoculations. Pathogenicity of *Pythium* isolates to seedlings of cucumber (cv. Shintokiwa), and komatsuna (*Brassica campestris* L., rapifera group, cv. Misugi) was tested in the soil-over-agar culture inoculation method¹⁸, in which 5~10 seeds were sown in a 50 ml soil-over-agar culture (usually 7-day-old PDA culture) in a 9-cm plastic petri dish after watering (12 ml/plate), and kept moist usually by placing in large petri dishes (17.5 cm in diam, 18 cm high) one by one. The seeds sown in uninoculated soils on sterile PDA in plastic petri dishes served as controls.

Soils used for these experiments were collected from the forest nursery at the Forestry and Forest Products Research Institute at Tsukuba, Ibaraki, air-dried, and sieved through 18-mesh screen and stored in a 18 cm large petri dish for more than 15 days before use.

Inoculation experiments were conducted once for cucumber and komatsuna in continuous cultures in the same infested soils with two replications of a total of 10~20 plants/isolate in a growth chamber regulated at 25 C for 12 hr of light and 19 C for 12 hr of darkness.

Pathogenicity was evaluated by rates of emergence, and damaged (collapsed or diseased) seedlings/total number of seeds tested 7 days after sowing.

RESULTS AND DISCUSSION

Isolations, distribution and notes of Pythium isolates

A total of 433 *Pythium* isolates obtained from soils from 25 locations in Kyushu Island included 234 and 199 isolates trapped by cucumber and lupine seeds, respectively. By cucumber and lupine seeds, 13 species each were isolated, totalling at least 16 taxa including H-Zs (a provisional *Pythium* group with zoospore formation from hypha-like sporangia, but without sexual organ)¹⁹ (Figs. 9~11) and two unidentified isolates. *Pythium* species in Kyushu Island with numbers of samples yielding each taxon, and of the isolates obtained in each taxon are listed, together with representative isolates and their origin in Table 2. Kinds of *Pythium* species and number of isolates obtained from each sample (location) are tabulated in Table 3.

Of the 16 taxa, 11 were commonly isolated by both seeds. However, one isolate each of *P. elongatum* Matthews (Figs. 1~3) and *P. dissotocum* Drechsler were isolated only by cucumber seeds, and two of *P. inflatum* Matthews (Figs. 4~8) and one of *P. myriotylum* Drechsler, only by lupine seeds.

Influence of temperatures for isolation of *Pythium* species was previously discussed¹³. However, of the 205 isolates including 115 trapped by cucumber seeds and 90 by lupine seeds at 26 C and 228 including 119 by cucumber seeds and 109 by lupine seeds at 10 C, 11 taxa were obtained at 26 C by both seeds, whereas eight or six obtained at 10 C by cucumber and lupine seeds, respectively. Especially, five *Pythium* species, i.e., *P. aphanidermatum* (Edson) Fitz., *P. elongatum*, *P. hydnosporum* (Mont.) Schroeter, *P. inflatum*, and *P. torulosum* Coker & Patterson

were isolated only at 26 C, but not at 10 C. The rest of at least 11 taxa were isolated at either temperatures. In addition, 11 isolates of *P. graminicola* Subramaniam and 12 of *P. splendens* Braun were isolated at 26 C, but one isolate each of both species at 10 C, whereas 64 isolates of *P. ultimum* Trow were isolated at 10 C, but only 27 isolates at 26 C.

The respective of 24 samples yielded one to seven species, but no *Pythium* was isolated from one sample, i.e., cucumber field soil at Munakata with sample no. 64-s in Fukuoka Pref., although *Phytophthora* sp. was trapped (Table 3). A total of 66 isolates with lobate sporangia included 51 of *P. aphanidermatum* collected in 13 locations, 12 of *P. graminicola* from three locations, two of *P. torulosum* from one location and an unidentified isolate (Isolate 80-379). Both *P. aphanidermatum* and *P. torulosum* discharged zoospores easily, but *P. graminicola* and an unidentified isolate were not easy for discharging zoospores.

Of 13 isolates with zoospore formation from hypha-like sporangia, one isolate was *P. disotocum*, and 12 from two locations were H-Zs. This H-Zs may be or close to *P. flevoense* Van der Plaats-Niterink, a heterothallic species with hypha-like sporangia¹⁰⁾.

Three isolates collected in cucumber field at Fukuoka Pref. formed sphaerosporangia with zoospore discharge and they were all identified as *P. vexans* de Bary. Another unidentified isolate (Isolate 80-269) with sphaerosporangia with zoospore discharge is close to *P. intermedium* de Bary because of formation of short chains of basipetally-developed sporangia, but different from it because of nondesiduous nature of sporangia.

Of a total of 41 isolates with echinulate oogonia and spherical hyphal swellings (conidia), five isolates were tentatively identified as *P. hydnosporum*, although one isolate from cabbage field at Kagoshima Pref., was slightly different from four others with rather inconspicuous echinulation and half-sized globoid structure. Nine isolates from four locations were *P. irregulare* Buisman, and 27 from eight locations were *P. spinosum* Sawada.

Of 304 isolates forming spherical hyphal swellings without zoospore formation, 13 isolates were *P. splendens* with terminal large hyphal swellings (usually more than 30 μ m in diam) and rarely oogonia with declinous 1~2 antheridia and apierotic oospores, although conidia of Isolate 80-212 were more frequently intercalary than terminal. A total of 91 isolates from 17 locations were *P. ultimum* and included three isolates (Isolates 80-289; -366; -615) with predominantly hypogynous antheridia.

Table 1. Soil samples obtained from 25 different locations with various habitats and soil pH values in Kyushu Island

Sample No.	Location	Habitat	pH	Sample No.	Location	Habitat	pH
Fukuoka Pref.				Kumamoto Pref.			
53	Munakata	Cabbage	5.6	66	Kumamoto	Corn	6.9
55	Munakata	Watermelon	6.5	68	Ueki	Eggplant	7.2
56	Munakata	Watermelon	6.4	70	Ueki	Cucumber	6.6
57	Munakata	Watermelon	6.2	73	Ueki	Cucumber	5.8
59	Munakata	Spinach	6.7	74	Ueki	Cabbage	6.2
62	Munakata	Cucumber	7.1	76	Ueki	Cucumber	6.7
63	Munakata	Watermelon	6.7	77	Ueki	Cabbage	6.8
64-s	Munakata	Cucumber	5.8				
65	Munakata	Cucumber	7.1				
Miyazaki Pref.				Kagoshima Pref.			
78	Miyakonojyo	Taro	6.0	81	Sueyoshi	Cabbage	6.7
79	Miyakonojyo	Peanut	6.6	82	Kouyama	Sweet potato	6.8
80	Miyakonojyo	Soybean	6.3	83	Kaimon	Watermelon	5.8
M-1	Kiyotake	Cucumber	6.0	84	Kagoshima	Sweet potato	6.9
M-2	Kiyotake	Cucumber	6.8				

Of 201 isolates with spherical hyphal swellings (conidia) identified as *P. sylvaticum* Campbell & Hendrix²⁾ in this study, 33 isolates occasionally formed sexual organs with aplerotic oospores and 2~3 or more diclinous antheridia per oogonium (homothallic isolates), but others never formed sexual organs (heterothallic isolates)^{3,4)}. Of the 33 homothallic isolates, 24 (nearly 73%) were isolated at 10 C.

Pythium sylvaticum occurred most commonly and it was isolated from 22 locations. *Pythium aphanidermatum* and *P. ultimum* were isolated from 13 and 17 locations, *P. spinosum* and *P. splendens* from eight and six locations, respectively, but others were isolated from less than five locations (Tables 2 and 3).

At least 11 taxa out of 16 obtained in this study were recorded in the previous report from Shikoku Island¹⁹⁾, but four taxa, i.e., *P. elongatum*, *P. inflatum*, *P. splendens* and *P. myriotylum* were not recorded. However, most of these species were also recorded in soils of different parts of Japan^{13,17)} and other countries^{3,5,11)}.

Pathogenicity

Although pathogenicity of *Pythium* species isolated from soils was demonstrated in England¹²⁾

Table 2. List of *Pythium* species isolated from soils of Kyushu Island, and representative isolates

<i>Pythium</i> species	Sample yielding No.	Isolates No.	Representative isolates and their origin
<i>P. aphanidermatum</i> (Edson) Fltz.	13	51	80-140 (Sample 53) 80-265 (Sample M-2)
<i>P. dissotocum</i> Drechsler	1	1	80-571 (Sample 83)
<i>P. graminicola</i> Subramaniam	3	12	80-214 (Sample 80)=ATCC64150 80-299 (Sample 66) 80-302 (Sample 66)=ATCC64151
<i>P. elongatum</i> Matthews	1	1	80-222 (Sample 80)
<i>P. hydnosporum</i> (Mont.) Schroeter	4	5	80-228 (Sample 81) 80-266 (Sample M-2) 80-295 (Sample 66)
<i>P. inflatum</i> Matthews	1	2	80-150;-152 (Sample 55)
<i>P. irregulare</i> Buisman	4	9	80-243 (Sample 83) 80-348 (Sample 76)
<i>P. myriotylum</i> Drechsler	1	1	80-248 (Sample 84)
<i>P. spinosum</i> Sawada	8	27	80-203 (Sample 78)
<i>P. splendens</i> Braun	6	13	80-212 (Sample 79) 80-311 (Sample 68)
<i>P. sylvaticum</i> Campbell & Hendrix	22	201	80-164 (Sample 56) 80-225 (Sample 81) 80-272 (Sample M-1) 80-620 (Sample 81)
<i>P. torulosum</i> Coker & Patterson	1	2	80-149;-155 (Sample 55)
<i>P. ultimum</i> Trow	17	91	80-186 (Sample 62) 80-247;-255 (Sample 84) 80-289 (Sample 65)
<i>P. vexans</i> de Bary	1	3	80-274 (Sample 65)
H-Zs ^{a)}	2	12	80-151 (Sample 55) 80-239;-240;-241;-570;-576 (Sample 83)
<i>Pythium</i> spp.	2	2	80-269 (Sample M-2) 80-379 (Sample 55)

a) A provisional *Pythium* group with zoospore formation from hypha-like sporangia, but without sexual organs.

Table 3. Kinds of *Pythium* species and number of isolates obtained from soils of 25 different locations in Kyushu Island

Sample No.	Kind of <i>Pythium</i> ^{a)} and no. of isolates									
	aph	gra	hyd	irr	spi	spl	syl	ult	H-Zs	Miscellaneous ^{b)}
Fukuoka Pref.										
53	1						12	8		
55	2						3	5	1	tor, 2; inf, 2; Pyth. sp., 1
56	4				1		3	10		
57	2				3		10	5		
59	7						9	4		
62	7						4	10		
63	1						11	5		
64-s										
65						1	3	10		vex, 3
Kumamoto Pref.										
66	2	6	1				4	10		
68	3			1	3	2	10			
70	11				1	1		5		
73							19	1		
74							23	2		
76				1			16			
77							22	1		
Miyazaki Pref.										
78					7		6			
79					1	3	9			
80		5			2	1	4			elo, 1
M-1	1		1				12			
M-2	9		2				9	3		Pyth. sp., 1
Kagoshima Pref.										
81	1		1				10	1		
82					9	5	1			
83				1			1	3	11	dis, 1
84		1		6				8		myr, 1
Total isolates	51	12	5	9	27	13	201	91	12	12

a) Abbreviated as follows: aph=*P. aphanidermatum*, dis=*P. dissotocum*, elo=*P. elongatum*, gra=*P. graminicola*, hyd=*P. hydnosporum*, inf=*P. inflatum*, irr=*P. irregulare*, spi=*P. spinosum*, spl=*P. splendens*, syl=*P. sylvaticum*, tor=*P. torulosum*, ult=*P. ultimum*, vex=*P. vexans*, H-Zs=a provisional *Pythium* group with zoospore formation from hypha-like sporangia, but without sexual organs.

b) Isolated only from one location (sample), and unidentified *Pythium* spp.

and in the United States⁴⁾, only a few reports have been published on Japanese soil isolates. These reports include data on pathogenicity of two Kyushu isolates¹⁵⁾ of *P. aphanidermatum* and 23 Shikoku isolates²⁰⁾ of *Pythium* spp. to cucumber, and/or komatsuna and Japanese black pine seedlings. To obtain further information, 36 isolates of 16 taxa from Kyushu Island were tested for their pathogenicity to komatsuna and cucumber seedlings by the soil-over-agar culture inoculation method (Table 4). Of these isolates, 10 including *P. splendens* (Isolate 80-311) and *P. ultimum* (80-247) caused emergence rates of cucumber to 0~60%, and the respective test fungi were recovered 100% from each sample. Rest of 20 isolates did not influence emergence rates of cucumber seeds, but after emergence, nine isolates including *P. ultimum* (80-186) were most pathogenic, causing more than 40% damage rates, seven isolates including two of *P. graminicola* were mildly pathogenic, causing less than 10% damage rates, and rest of the isolates were non-pathogenic.

Table 4. Pathogenicity^{a)} to seedlings of cucumber (cv. Shintokiwa), and *Brassica campestris* L. (rapifera group), (komatsuna, cv. Misugi) of 36 *Pythium* isolates from soils of Shikoku Island by the soil-over-agar culture inoculation method in a growth chamber

Fungus	Cucumber			Komatsuna		
	Emerg. (%)	Damage (%)	Recov. (%)	Emerg. (%)	Damage (%)	Recov. (%)
<i>P. aphanidermatum</i> 80-140	90	70	100	100	10	0
<i>P. aphanidermatum</i> 80-265	90	40	75	100	0	0
<i>P. dissotocum</i> 80-571	90	10	25	95	0	25
<i>P. elongatum</i> 80-222	100	0	0	100	0	0
<i>P. graminicola</i> 80-214	100	10	25	90	0	0
<i>P. graminicola</i> 80-299	100	10	25	95	5	50
<i>P. graminicola</i> 80-302	100	0	0	90	0	25
<i>P. hydnosporum</i> 80-228	90	0	0	90	0	0
<i>P. hydnosporum</i> 80-266	100	0	0	95	0	0
<i>P. hydnosporum</i> 80-295	100	0	0	100	0	0
<i>P. inflatum</i> 80-150	90	0	25	100	0	0
<i>P. inflatum</i> 80-152	100	10	25	90	0	0
<i>P. irregulare</i> 80-243	60	40	100	95	40	75
<i>P. irregulare</i> 80-348	30	10	100	100	0	0
<i>P. spinosum</i> 80-203	40	40	100	90	25	100
<i>P. splendens</i> 80-212	80	70	100	95	5	25
<i>P. splendens</i> 80-311	0	—	100	95	10	50
<i>P. sylvaticum</i> 80-164	60	50	100	95	50	100
<i>P. sylvaticum</i> 80-225	20	20	100	95	75	100
<i>P. sylvaticum</i> 80-272	70	60	100	100	65	100
<i>P. sylvaticum</i> 80-620	30	30	75	85	65	100
<i>P. torulosum</i> 80-149	100	0	0	85	0	0
<i>P. torulosum</i> 80-155	100	10	25	100	0	0
<i>P. ultimum</i> 80-186	90	90	100	100	5	50
<i>P. ultimum</i> 80-247	0	—	100	100	100	100
<i>P. ultimum</i> 80-255	60	30	100	100	90	100
<i>P. ultimum</i> 80-289	50	40	100	95	40	100
<i>P. vexans</i> 80-274	80	0	75	90	0	0
H-Zs ^{b)} 80-151	100	0	0	100	0	0
H-Zs 80-239	100	0	0	95	0	25
H-Zs 80-240	90	10	100	95	5	75
H-Zs 80-241	100	0	0	90	0	0
H-Zs 80-570	90	10	25	100	0	0
H-Zs 80-576	100	0	0	100	0	25
<i>Pythium</i> sp. 80-269	90	0	0	85	0	0
<i>Pythium</i> sp. 80-379	100	0	25	90	0	0
Control	90	0	0	90	0	0

a) Evaluated on the basis of rates of emergence and damaged (collapsed or diseased) seedlings/total number of seeds tested 7 days after sowing. Average of 10~20 plants/isolate/test. The respective test fungi were recovered by plating 1-cm basal hypocotyl segments of four most damaged seedlings in each test.

b) Provisionally-named *Pythium* group, with zoospore discharge from hypha-like sporangia, but without sexual organs.

Emergence of komatsuna seeds appeared not to be influenced by any *Pythium* species, but after emergence, eight isolates including two of *P. ultimum* (80-255; -247) were most pathogenic, causing more than 40% of the seedlings collapsed or diseased, seven including *P. spinosum* (80-

283) were mildly pathogenic, causing 5~25% damage rates, and rest of isolates were nonpathogenic.

It is interesting to note that two isolates each of *P. aphanidermatum* and *P. splendens* were mildly pathogenic or nonpathogenic to komatsuna, but most pathogenic to cucumber seedlings. Results of these experiments were more or less identical with the previous report²⁰⁾.

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和 文 摘 要

渡辺恒雄：九州地方の土壌から分離した *Pythium* 菌の種と分布および病原性

九州地方の土壌に生息する *Pythium* 菌の種名と分布を明らかにするため、キュウリとルービン種子による捕捉法を用いて菌を分離し、調査した。供試した 25 カ所の土壌 (4~9/県) 中、24 試料から 1 試料当り 1~7 種の *Pythium* 菌が分離されたが、福岡県の 1 試料からはまったく分離できなかった。分離した合計 433 菌株は、H-Zs (糸状胞子嚢から遊走子を形成するが、生殖器官は未形成の一群) を含む 16 種に分類・同定できた。最も一般的に分布していたのは、*P. sylvaticum* で、22 カ所から 201 菌株が分離された。次いで、*P. ultimum* と *P. aphanidermatum* の分布が広く、13~17 カ所から 51~91 菌株が分離された。寒天培養覆土接種法を用いて 16 種の 36 菌株 (未同定菌を含む) の接種試験を行ったところ、*P. sylvaticum* と *P. ultimum* の各 3 菌株を含む合計 10 菌株はキュウリの萌芽率を 60% 以下に低下させ、また、*P. aphanidermatum*、*P. sylvaticum* および *P. ultimum* の各 2 菌株を含む 9 菌株は萌芽後の子苗の 40% 以上を枯死または罹病させた。コマツナでは、いずれの処理区でも 85% 以上の萌芽率を示し、萌芽前の被害は認められなかった。しかし、*P. sylvaticum*、*P. ultimum*、*P. irregulare* など 8 菌株はいずれも萌芽後に 40% 以上の被害率を引き起こした。他の供試菌の病原性は、かなり強いものからまったく認められないものまで、さまざまであった。

Explanation of plate**Plate I**

Figs. 1~11. Morphology of *Pythium* spp. from soils of Kyushu Island.

1~3. *P. elongatum* (Isolate 80-222)

Vesicle formation from long exit tubes or hypha-like sporangia (1, 2) and irregular (1), globose (2) and elliptical (1, 3) hyphal swellings.

4~8. *P. inflatum* (Isolate 80-150)

Lobate sporangia (4, 5), oogonia with typical declinuous antheridia (6, 7), and a plerotic oospore (8).

9~11. H-Zs (Isolate 80-570)

Vesicle formation (9, 10) from hypha-like sporangia, and filamentous dendroid hyphae (11).

Scale bar (Fig. 3)=20 μ m in Figs. 1~6, 9, 11; bar=10 μ m in Figs. 7, 8, 10.

Plate I