Kinds and Distribution of *Pythium* Species Isolated from Soils in South Kinki District*

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Abstract

Soil samples from 28 locations (4-10 samples/Pref.) in South Kinki district were assayed for *Pythium* taxa by a trapping isolation method with cucumber seeds as a baiting substrate. One to seven taxa/sample (=location) were isolated or detected from 26 samples, but no *Pythium* was isolated from two samples from Wakayama prefecture. A total of 253 isolates were identified into 18 taxa including H-Zs, a provisional *Pythium* group with zoospore formation from hypha-like sporangia, but without sexual organs. Among these fungi, H-Zs was predominantly isolated from 20 samples, followed by *P. sylvaticum*, *P. irregulare*, *P. ultimum*, and *P. aphanidermatum*, all of which were isolated from 10-15 samples.

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Key words: *Pythium* spp., kinds, distribution, soil, South Kinki district.

INTRODUCTION

Pythium is widely distributed all over the world⁶⁾. In Japan, a series of papers concerned with kinds and distribution of *Pythium* species have been published, including Hokkaido Island²⁴⁾, Tohoku district²³⁾, Shikoku Island²⁰⁾, Kyusyu Island²¹⁾, the Ryukyu Islands²²⁾, and the piedmont natural forest at Mt. Fuji¹⁸⁾ during the past six years since 1985.

In this report, *Pythium* species from soils of Mie, Osaka, Wakayama, and Nara prefectures in South Kinki district were assayed, and the results are summarized.

MATERIALS AND METHODS

Samples and sampling. Soil samples were collected from 28 different locations of Mie, Osaka, Wakayama, and Nara prefectures in South Kinki district in September, 1982 (Table 1). They consisted of six samples from Mie, four from Osaka, ten from Wakayama, and eight from Nara with various habitats including cabbage (*Brassica oleracea* L.), chrysanthemum (*Chrysanthemum morifolium* Ramat.), cucumber (*Cucumis sativus* L.), eggplant (*Solanum melongena* L.), fig (*Ficus carica* L.), garland chrysanthemum (*Chrysanthemum coronarium* L.), ginger (*Zingiber officinale* Rosc.), iris (*Iris* sp.), kidney bean (*Phaseolus vulgaris* L.), muskmelon (*Cucumis melo* L.), onion (*Allium cepa* L.), pea (*Pisum sativum* L.), pumpkin (*Cucurbita maxima* Duch.), radish (*Raphanus sativus* L.), red pepper (*Capsicum annuum* L.), spinach (*Spinacia oleracea* L.), strawberry (*Fragaria* × *ananassa* Duch.), tomato (*Lycopersicon esculentum* Mill.), umbrella-pine (*Sciadopitys verticillata* (Thunb.) Sieb. et Zucc.), Welsh onion (*Allium fistulosum* L.) and Zanthoxylum (*Zanthoxylum piperitum* DC.), and soil pH values ranged from 4.7 to 7.5.

The respective soil samples were collected from five random cores from the top 15-cm surface soil, and mixed thoroughly in polyethylene bags. These samples were usually assayed within 10 days after

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Table 1. Soil samples obtained from 28 different locations of South Kinki district with various habitats and soil pH values

Sample Location no.	Habitat	pH	Sample Location no.	Habitat	pH
MIE Pref.			OSAKA Pref.		
134 Ureshino	Cucumber	6.6	140 Sayama	Tomato	7.5
135 Ureshino	Cabbage, Spinach	6.1	141 Sayama	Radish	5.3
136 Mikumo	Fig	5.9	142 Sayama	Eggplant	6.5
137 Misono	Chrysanthemum	6.1	143 Habikino	Onion	6.5
138 Misono	Chrysanthemum	4.7	WAKAYAMA Pref.		
139 Misono	Chrysanthemum	6.6	145 Wakayama	Garland chrysanthemum	6.1
NARA Pref.			146 Wakayama	Spinach	6.8
155 Kashihara	Welsh onion	6.7	147 Wakayama	Radish	6.5
156 Kashihara	Muskmelon	6.3	148 Wakayama	Iris	5.3
157 Gojo	Zanthoxylum	6.1	149 Wakayama	Ginger	6.9
158 Gojo	Eggplant	7.0	150 Wakayama	Red pepper	6.2
159 Kouryou	Eggplant	6.3	151 Wakayama	Radish	5.7
160 Tenri	Spinach	5.6	152 Wakayama	Pumpkin	6.6
161 Tenri	Kidney bean	6.7	153 Kouya	Umbrella-pine	5.0
162 Tenri	Strawberry	6.4	154 Gobou	Pea	6.4

collection.

Isolations. *Pythium* was isolated by trapping methods using seeds of cucumber (cv. Tokiwa-jibai) as a trapping substrate following the previous works^{16,17,19)} in four separate experiments.

Ten seeds embedded in each 20–50 g soil sample in a 9-cm Petri dish incubated at 26°C for 1 day 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 26°C for 1–3 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 (CMA) for mycological study. Several soaking solutions including Petri's salt solution were used to study asexual reproduction. All isolates were identified following the descriptions by the previous workers^{8–10,25)}.

Most of the isolates in this study were stocked by T. Watanabe at the Forestry & Forest Products Research Institute, or in the National Institute of Biological Resources, Ministry of Agriculture, Forestry & Fisheries.

RESULTS AND DISCUSSION

Distribution and notes of the fungi isolated

A total of 253 *Pythium* isolates obtained from soils from 28 locations in Kinki district consisted of 142 isolates from cucumber seeds incubated at 26°C, and 111 at 10°C, and they were identified into 18 taxa including H-Zs (a provisional *Pythium* group with zoospore formation from hypha-like sporangia, but without sexual organ) and 15 unidentified isolates (Tables 1–3). The respective of 26 samples yielded one to seven taxa, but no *Pythium* was isolated or detected from two samples, i.e., garland chrysanthemum field soil (Sample 145), and red pepper field soil (Sample 150) at Wakayama (Tables 1 and 2). It is interesting to note that although *Pythium* was not isolated from Sample 150, three *Phytophthora* isolates were obtained from its sample. In two other samples (Samples 140 and 157), four *Phytophthora* isolates were also obtained together with *Pythium* spp.

Pythium isolates in each taxon, and samples yielding each taxon are shown in Table 3. *Pythium*

Table 2. Kinds of *Pythium* species and number of isolates obtained from soils of 28 different locations in South Kinki district

Sample no.	Kind of <i>Pythium</i> ^{a)} and no. of isolates													Miscellaneous ^{b)}
	aca	aph	car	ech	hyd	irr	oed	spi	syl	tor	ult	vex	H-Zs	
MIE Pref.														
134		2				1		1	3	5			1	
135		3				1			2		1		1	P.sp.,1
136							2		2	1		2		P.sp.,2
137		1				1				1	2	2	4	
138								+ ^{c)}						
139		4							1		1		6	P.sp.,1
OSAKA Pref.														
140													2	
141								4			1		6	
142		2	2	2				1					2	
143		1							2	2			4	P.sp.,2
WAKAYAMA Pref.														
145														
146													1	
147											2			
148						1		1	1				1	P.sp.,3
149									1	2	1	1	2	Zin,6
150														
151	1					6			1					
152	1					1			1	1	1		2	
153			1			1	2		4					
154		5				1					4	2	2	gra,1
NARA Pref.														
155		3	1		1				1		4		3	
156				2		3			5		2			
157	2			1		1			4				1	dis,2; inf,1
158		3			2					1	3		3	
159						3		2	1				4	P.sp.,3
160			1			1	3		1	1			1	spl,1
161						8	1				1	1	4	P.sp.,1
162		3	3				7						2	P.sp.,2
Total isol.	4	27	8	5	3	29	15	9	30	14	23	8	52	26

a) Abbreviated as follows: aca=*P. acanthophoron*, aph=*P. aphanidermatum*, car=*P. carolinianum*, dis=*P. disotocum*, ech=*P. echinulatum*, gra=*P. graminicola*, hyd=*P. hydonosporum*, inf=*P. inflatum*, irr=*P. irregulare*, oed=*P. oedocephalum*, spi=*P. spinosum*, spl=*P. splendens*, syl=*P. sylvaticum*, tor=*P. torulosum*, ult=*P. ultimum*, vex=*P. vexans*, zin=*P. zinjiberum*, H-Zs=a provisional *Pythium* group with zoospore formation from hypha-like sporangia, but without sexual organs.

b) Isolated only from one location (sample), or unidentified species (=P.sp.).

c) Detected on the isolation plates, but not studied further.

aphanidermatum (Edson) Fitz. and *P. zinjiberum* Takahashi were isolated from cucumber seeds incubated only at 26°C, but others at both 26 and 10°C.

Of 42 isolates with lobate sporangia, 27 of *P. aphanidermatum* collected in 10 locations and 14 of *P. torulosum* Coker & Patterson from eight locations discharged zoospores during this study, but one isolate of *P. inflatum* Matthews never discharged zoospores.

Six isolates each obtained from the ginger field at Wakayama (Sample 149) and the rotted rhizome of ginger concomitantly studied were all identical. These fungi formed lobate sporangia (nearly 10 µm wide) with long exit tubes (nearly 200 µm long), and oogonia often with one to two papillae, and two to

Table 3. List of *Pythium* isolates obtained from soils from South Kinki district, and samples yielding each taxon, indicated by isolate numbers and parenthesized numbers, respectively

<i>P. acanthophoron</i> (four isolates):	82-744(151);-750(152);-785,-792(157)
<i>P. aphanidermatum</i> (27 isolates):	82-657,-658,-659(135);-672(137);-685(139);-700,-701(142);-711(143);-761,-765(154);-768,-769(155);-795,-796,-803(158);-982,-983(134);-984,-985,-986(139);-987,-988,-989(154);-990,-991,-992(162);-996(155)
<i>P. carolinianum</i> (eight isolates):	82-702,-703(142);-758(153);-774(155);-813(160);-976,-977,-981(162)
<i>P. dissotocum</i> (two isolates):	82-945,-946(157)
<i>P. echinulatum</i> (five isolates):	82-780,-781(156);-786(157);-878,-879(142)
<i>P. graminicola</i> (one isolate):	82-762(154)
<i>P. hydnosporum</i> (three isolates):	82-771(155);-798,-799(158)
<i>P. inflatum</i> (one isolate):	82-787(157)
<i>P. irregulare</i> (29 isolates):	82-670(137);-722(148);-739,-740,-741(151);-748(152);-777,-779(156);-808,-810(159);-819,-820,-821,-825(161);-838(134);-847(135);-907,-908,-909(151);-923(153);-927(154);-942(156);-948(157);-959(159);-963(160);-967,-968,-970,-972(161)
<i>P. oedochilum</i> (15 isolates):	82-751,-752(153);-815,-818(160);-822(161);-828,-829,-830,-831,-832,-834(162);-853,-854(136);-964(160);-980(162)
<i>P. spinosum</i> (nine isolates):	82-694,-695(141);-806,-807(159);-839(134);-874,-875(141);-880(142);-890(148)
<i>P. splendens</i> (one isolate):	82-997(160)
<i>P. sylvaticum</i> (30 isolates):	82-653(134);-665,-669(136);-713(143);-742(151);-754(153);-784(156);-814(160);-836,-840(134);-845,-846(135);-870(139);-884(143);-891(148);-901(149);-913(152);-920,-921,-922(153);-935(155);-938,-939,-940,-941(156);-947,-949,-950,-951(157);-960(159)
<i>P. torulosum</i> (14 isolates):	82-648,-649,-650,-651(134);-666(136);-707(143);-802(158);-842(134);-863(137);-883(143);-897,-898(149);-916(152);-966(160)
<i>P. ultimum</i> (23 isolates):	82-671(137);-687(139);-717(147);-759,-760(154);-767,-773(155);-778(156);-862(137);-873(141);-888(147);-902(149);-912(152);-925,-926(154);-933,-934(155);-937(156);-954,-955,-956(158);-969(161);-995(135)
<i>P. vexans</i> (eight isolates):	82-664,-668(136);-675,-678(137);-763,-764(154);-903(149);-973(161)
<i>P. zingiberum</i> (six isolates):	82-724,-725,-726,-727,-730,-731(149)
H-Zs (52 isolates):	82-652(134);-673,-674(137);-682,-683,-684(139);-688,-689(140);-692,-693,-697,-698(141);-705,-709(143);-770(155);-791(157);-797(158);-804,-805(159);-823,-824(161);-848(135);-859,-860(137);-867,-868,-869(139);-871,-872(141);-876,-877(142);-881,-882(143);-886(146);-892(148);-899,-900(149);-914,-915(152);-928,-929(154);-931,-932(155);-952,-953(158);-957,-958(159);-965(160);-971(161);-978,-979(162);-994(161)
Unidentified <i>Pythium</i> spp. (15 isolates):	82-667(136);-686(139);-706,-710(143);-720,-721,-723(148);-811,-812(159);-844(135);-855(136);-961(159);-974,-975(162);-993(161)

three declinous antheridia (per oogonium) which occasionally coiled around the oogonial stalk and oogonia, bearing single aplerotic oospores. Its oogonia were often aborted. In addition, they were often directly connected to lobate sporangia. This fungus was tentatively identified as *P. zingiberum*^{7,12)}.

Of 54 isolates with zoospore formation from hypha-like sporangia, two from one location were *P. dissotocum* Drechsler and the rest of 52 from 20 locations were H-Zs. Predominance of H-Zs is interesting to note, because in four other areas²⁰⁻²⁴⁾, this taxon was not so common, and one to five samples in each district yielded this taxon.

Of all of 31 isolates with zoospore formation from sphaerosporangia, eight each were identified as *P. carolinianum* Matthews, and *P. vexans* de Bary, and 15 tentatively identified as *P. oedochilum* Drechsler, although its internal proliferation of sporangia was not observed in this study.

Of a total of 48 isolates with echinulate oogonia, 29 from 13 locations were *P. irregulare* Buisman with 1-3 protuberances on oogonia, nine from five locations, *P. spinosum* Sawada with numerous digitate protuberances, all over the oogonia, four from three locations, *P. acanthophoron* Sideris with short protuberances (less than 3 μ m long) on oogonia bearing single plerotic oospores and inflated clavate, or papillate antheridia, often with one to two constrictions, making broad oogonial contact, in which spherical hyphal swellings (conidia) were not formed, five from three locations, *P. echinulatum* Matthews with sharp protuberances (up to 7.5 μ m long) on oogonia, together with hypogynous, monoclinal or declinous antheridia, single plerotic oospores, and spherical hyphal swellings which were discrete, and occasionally catenulate, and three from two locations, *P. hydnosporum* (Mont.) Schroeter with sharp protuberances (up to 5 μ m long) on oogonia, together with single aplerotic oospores, and inconspicuous-

ly hypogynous antheridia, in which hyphal swellings were not formed. Isolates of *P. echinulatum* were tentatively identified, because they never discharged zoospores. This fungus was not recorded in the previous works¹⁶⁻²⁴⁾. In addition, *P. spinosum* was detected from Sample 138 on the isolation plates, but no further work was conducted.

Twenty three isolates from 12 locations formed smooth oogonia with hypogynous or sessile antheridia, aplerotic oospores, and spherical hyphal swellings without zoospore formation, and they were identified as *P. ultimum* Trow.

Of 31 isolates with spherical hyphal swellings (conidia), 30, the conidia of which were 20-25 μm in diam, were tentatively identified as *P. sylvaticum* Campbell & Hendrix²⁾, and one, the conidia of which exceeds 35 μm in diam as *P. splendens* Braun. Of 30 *P. sylvaticum* isolates, five occasionally formed sexual organs with aplerotic oospores and two to three or more diclinous antheridia per oogonium (homothallic isolates^{2,4)}), but others did not form sexual organs (heterothallic isolates).

Predominance of *Pythium* taxon, H-Zs, and isolation of as many as 18 taxa in Kinki district were characteristic in comparison with *Pythium* studied in five other districts¹⁸⁻²⁴⁾ in Japan (Tables 2 and 3).

Of the 15 unidentified isolates, 11 formed lobate hyphal swellings, and among these, seven discharged zoospores, but others did not. The rest of four isolates just formed aseptate hyphae, but never discharged zoospores.

Most of the 18 taxa identified in this study were recorded previously¹⁶⁻²⁴⁾ in Japan, and also in other countries^{1,3,5,8-11,13-15,26)}.

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和 文 摘 要

渡辺恒雄：南近畿地方の土壌から分離した*Pythium*菌の種と分布

南近畿地方の土壌に生息する*Pythium*菌の種と分布を明らかにするため、キュウリ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28試料中26試料から1試料当たり1〜7種の*Pythium*菌が分離または検出されたが、2試料からは全く分離できなかった。分離した合計253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18種に分類・同定できた。最も一般的に分布していたH-Zsは、20カ所から52菌株が分離された。次いで、*P. sylvaticum*, *P. irregulare*, *P. ultimum*, *P. aphanidermatum*の分布が広く、10〜15カ所から23〜30菌株が分離された。