アルファルファ圃場の土壌から分離された宿主非特異的Phytophthora megasperma

誌名	北海道農業試験場研究報告 = Research bulletin of the Hokkaido National Agricultural Experiment Station
ISSN	03675955
著者名	松本,直幸 佐藤,徹
発行元	北海道農業試験場
巻/号	143号
巻号補足	
掲載ページ	p. 75-84
発行年月	1985年3月

Res. Bull. Hokkaido Natl Agric. Exp. Stn, 143, 75−84 (1985)

Host Non-specific *Phytophthora megasperma* with Small Oogonia from Alfalfa-field Soil

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Summary

During the course of investigation of *Phytophthora* root rot of alfalfa, host non-specific *P. megasperma* isolates were obtained from alfalfa-field soil. Their morphological characteristics, growth temperature relations, and growth rate were identical to those of alfalfa-specific isolates. They were pathogenic to various plants including alfalfa. Their mycelia showed petaloid patterns on potato-dextrose agar and malt extract agar. Electrophoretic protein patterns of these isolates were most similar to alfalfa-specific isolates, and these two groups of isolates resembled soybean-specific isolates in band patterns. A host non-specific *P. megasperma* isolate with large oogonia showed a considerable different band pattern from that of other groups of isolates. Relationships between these host non-specific isolates and alfalfa-specific isolates are discussed, and taxonomic confusion in the *P. megasperma* complex is briefly reviewed.

Introduction

Isolates of *Phytophthora megasperma* Drechsler vary in the size of oogonia and host range, causing taxonomic controversy. WATERHOUSE[17] divided the *P. megasperma* complex into var. *megasperma* and var. *sojae* Hildebrand based on the diameter of oogonia. Var. *megasperma* has large oogonia (>45 μm diam) and var. *sojae* small oogonia (<45 μm diam). KUAN & ERWIN[9] demonstrated a continuous variation in oogonium diameters of 29 isolates of *P. megasperma* from various hosts including alfalfa (*Medicago sativa* L.), soybean [*Glycine max* (L.) Merr.], trees, etc., and established two formae speciales, i.e. f.sp. *medicaginis* specific to alfalfa and f. sp. *glycinea* specific to soybean. This view is supported by other workers[7,12]; however, HAMM & HANSEN[6] did not designate a new forma specialis for their isolates specific

Received November 5, 1984.

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to Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] and emphasized that the broadest possible range of isolates and hosts should be used to establish taxonomic grouping based on morphology or host specificity. WILCOX & MIRCETICH[18] suggested the inappropriateness for the use of formae speciales designation due to a lack of host specificity among various *P. megasperma* isolates in their inoculation experiments.

Thus, the confusion in the *P. megasperma* complex still remains. During the course of investigation of *Phytophthora* root rot of alfalfa, we obtained host non-specific isolates of *P. megasperma* with small oogonia, and their description and taxonomic implication are presented here in the hope that the findings on these isolates contribute to clarification of the complication of the *P. megasperma* complex.

We wish to thank Dr. D. C. Erwin, University of California, and Drs. F. Kodama and S. Tsuchiya, Hokkaido Prefectural Agricultural Experiment Station, for providing us with *P. megasperma* isolates.

Materials and methods

Ten *P. megasperma* isolates consisting of one host non-specific isolate with large oogonia (HNS-LO), 2 soybean-specific isolates with small oogonia (SS-SO), and 4 alfalfa-specific isolates with small oogonia (AS-SO), and 3 host non-specific isolates with small oogonia (HNS-SO) were used (Table 1). They were maintained on corn meal agar (CMA) at 22 ℃. Colony morphology on potato-dextrose agar (PDA), malt extract agar (MEA), CMA, oatmeal agar, and V-8 juice agar (V-8 A) was compared after 12 days' incubation at 25 ℃. Diameters of 50 oogonia and of 50 oospores on V-8 A were determined for each isolate. V-8 A cultures were flooded with the basic salt solution[13] for sporangium production. Cardinal tempratures for mycelium growth were determined on CMA after 4 days' incubation. Twenty plant species belonging to 9 families were used for host range studies. Host range of Japanese isolates were determined using onemonthold seedlings grown in sterilized soil under glasshouse

Table 1. *Phytophthora megasperma* isolates examined

Isolate	Abbreviation	Oogonium	Host specificity	Source
IMI56348	HNS-LO	large	non-specific	D. C. Erwin
Pm-1	SS-SO	small	soybean	F. Kodama & S. Tsuchiva
Pms-21	SS-SO	small	soybean	F. Kodama & S. Tsuchiva
E-1	AS-SO	small	alfalfa	authors
51	AS-SO	small	alfalfa	authors
IFO 31623	AS-SO	small	alfalfa	authors
P 1057	AS-SO	small	alfalfa	D. C. Erwin
IFO 31624	HNS-SO	small	non-specific	authors
G-1-1	HNS-SO	small	non-specific	authors
KS	HNS-SO	small	non-specific	authors

conditions. Five seedlings grown in a pot were inoculated with each of the isolates. A small agar block with mycelia was placed on the hypocotyl in the soil, and the plants were heavily watered for 5 days. One to 2 months later, plants were pulled up for reisolation of the isolates. Roots were cut to pieces, surface-sterilized, and placed on CMA plates acidified with lactic acid at 25 ℃. Experiments were repeated, and plant species from which the isolate had been reisolated at least once were regarded as " + ". Disc electrophoresis of buffer-soluble protein of mycelia was conducted as previously described[10].

Results

P. megasperma isolates generally grew well on every medium used and produced fluffy mycelia, but on PDA and MEA, the SS-SO isolates grew poorly and their mycelia on them were dense (Table 2). The AS-SO isolates showed similar culture morphology each other. Cultures of the HNS-SO isolates were characterized by petaloid patterns on PDA and MEA

Table 2. Culture morphology of *Phytophthora megasperma* isolates on various media[a]

Isolate	potato dextrose agar	malt extract agar	corn meal agar	oatmeal agar	V-8 juice agar
IMI 56348 HNS-LO	+ powdery	±	− radiate	± radiate	+
Pm-1 SS-SO	+ growth poor, mycelium dense	+ growth poor, mycelium dense	+	++ somewhat powdery	±
IFO 31623 AS-SO	++ powdery	++	++	++	++
P 1057 AS-SO	++ powdery	++	++	±	++
IFO 31624 HNS-SO	++ staroid to petaloid	++ finely petaloid	++ very fluffy	±	++
G-1-1 HNS-SO	++ petaloid	++ finely petaloid	− slightly radiate	±	++
K S HNS-SO	++ staroid to petaloid but obscure	++ finely petaloid but obscure	− slightly radiate	±	±

a) Cultures were grown for 12 days at 25 ℃.
++ : aerial mycelium abundant.
+ : aerial mycelium moderate.
± : aerial mycelium poor.
− : no aerail mycelium.

(Plate 1). There were no differences in morphology of oogonia or oospores. Oogonia were spherical to rarely ellipsoidal and smooth. Oospores were globose, smooth, and yellowish and did not fill the oogonium. Mean diameters of oogonia and oospores of the isolates with small oogonia ranged 30.0-37.2 μm (mean 34.0 μm) and 25.0-31.3 μm (mean 28.8 μm), respectively

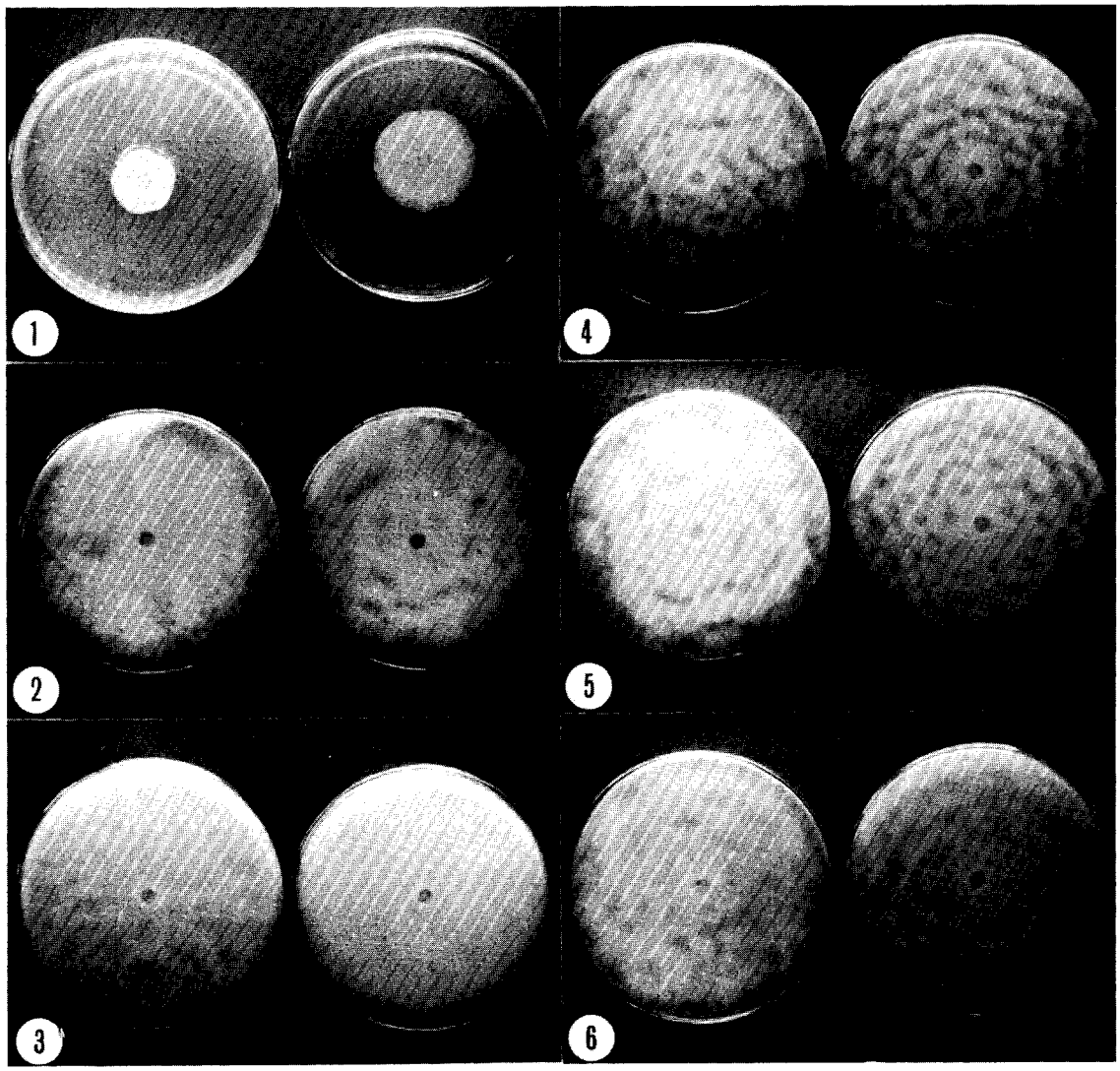

Plate 1 Colony characteristics of *Phytophthora megasperma* isolates with small oogonia on potato-dextrose agar (left) and malt extract agar (right) incubated at 25 ℃ for 12 days. 1: Pm-1; 2: IFO 31623; 3: P 1057; 4: IFO 31624; 5: G-1-1; and 6: KS.

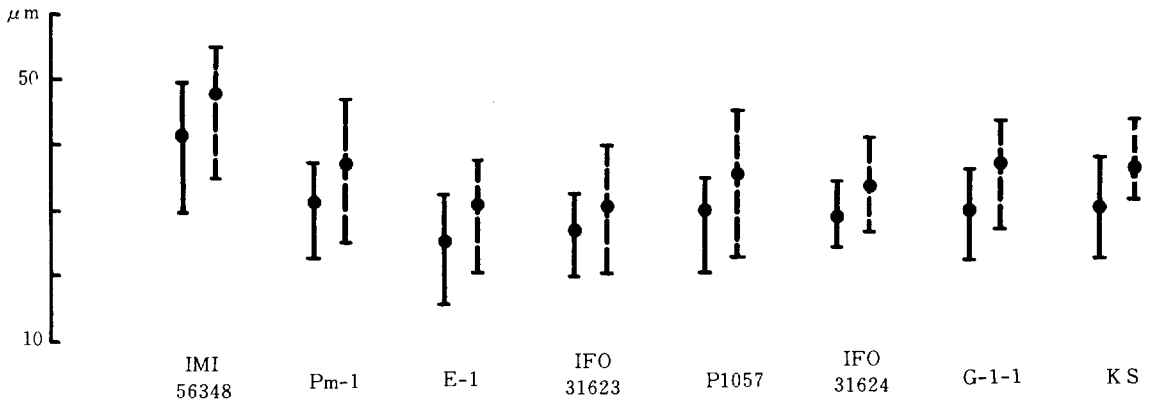

Fig.1 Comparison of diameters of oospores (solid line) and oogonia (broken line) of *Phytophthora megasperma* isolates

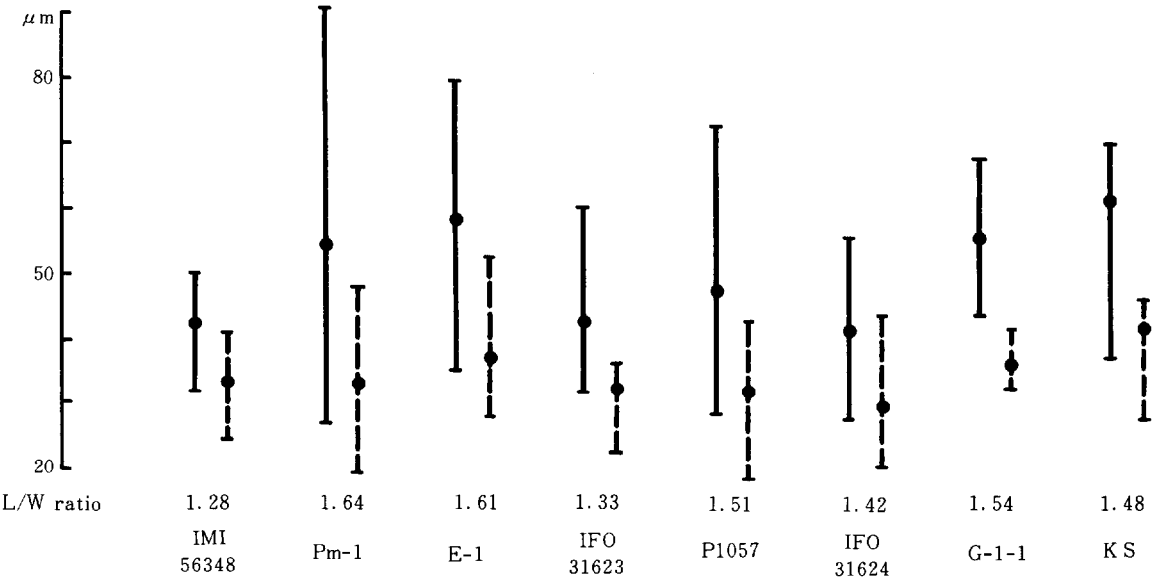

Fig. 2 Comparison of dimension (solid line: length; broken line: width) and L/W ratio of sporangia of *Phytophthora megasperma* isolates

(Fig. 1). The HNS-LO isolate had larger oogonia (mean diam 47.2 μm, range 34.6-54.8 μm) and larger oospores (mean diam 41.4 μm, range 29.6-49.4 μm), but small diameters of both organs merged with those of the isolates with small oogonia. There was no distinction among the isolates in sporangial characteristics, either. Sporangia were non-papillate or inconspicuously papillate, ovoid to obpyriform, and with no or little apical thickening, but those of the HNS-SO isolates showed a slight variation in shape. Sporangia of the HNS-LO isolate were especially uniform in size and shape, and were somewhat short and thick with an L/W ratio of 1.28 (Fig. 2). The isolates with small oogonia had longer sporangia (L/W ratio 1.33-1.64). *P. me-*

gasperma isolates used grew best at 25 or 28 ℃ (Table 3). The HNS-LO isolate (IMI 56348) failed to grow at 30 ℃, and the SS-SO isolate (Pm-1) grew even at 35 ℃.

The host-specific isolates were reisolated only from the leguminous plants (Table 4). The SS-SO and AS-SO isolates produced symptoms only on their respective host. The HNS-SO isolates showed a wide host range, and plants inoculated with them occasionally died. They were not virulent to alfalfa as the AS-SO isolates.

Table 3. Growth temperature relations of *Phytophthora megasperma* isolates[a)]

Isolate	Growth rate (mm/day) at temperatures (℃)							
	5	10	15	20	25	28	30	35
IMI 56348	0.63	1.67	4.63	5.54	5.92	5.54	0.00	0.00
Pm-1	0.04	0.83	2.25	2.92	4.33	3.06	2.44	0.29
IFO 31623	0.25	1.58	3.08	4.42	6.21	5.96	5.63	0.00
P 1057	0.29	2.00	4.83	6.75	8.54	8.42	6.83	0.00
IFO 31624	0.29	2.06	4.25	6.13	7.79	7.25	6.54	0.00
G-1-1	0.50	2.38	4.21	6.21	7.17	7.21	7.08	0.00
K S	0.46	2.06	4.67	6.17	8.50	7.94	6.17	0.00

a) Three replicates for each isolate were made on corn meal agar for 4 days.

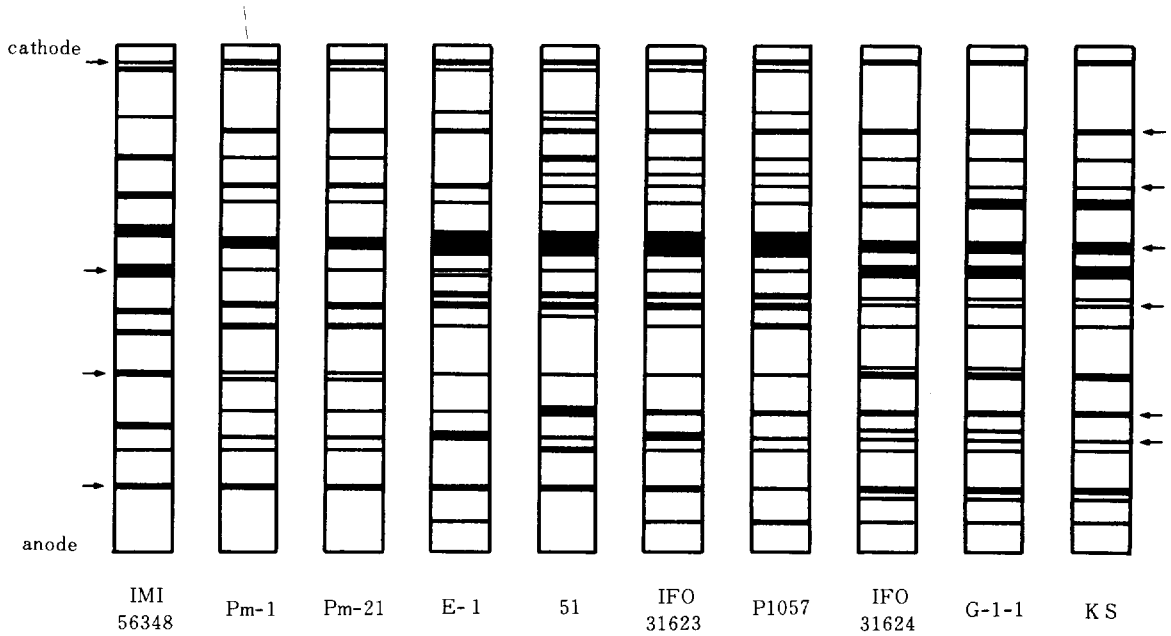

Fig. 3 Diagramatic presentation of the electrophoretic patterns of buffer-soluble protein extracts of *Phytophthora megasperma* isolates. Arrows on the left side indicate the bands common to all of the isolates examined. Arrows on the right side indicate the bands common to the isolates with small oogonia (i.e. except for IMI56348).

Table 4. Pathogenicity of *Phytophthora megasperma* isolates with small oogonia to various plants

Plant inoculated	*P. megasperma* isolate					
	Pm1	E-1	IFO 31623	IFO 31624	G-1-1	KS
Leguminocae						
Glycine max	+	−	−	−	+	−
Phaseolus angularis	−	−	−		+	−
P. aureus	−	−	−	−	−	+
Medicago sativa	−	+	+	+	+	+
Trifolium pratense	−	−	+	−	−	−
Vicia amoena	−	+	−	−	−	+
V. cracca	−	+	−	−	+	−
Pueraria lobata	−	−	−	−	−	−
Lespedeza bicolor	+	−	−	+	+	+
Chenopodiaceae						
Spinacia oleracea	−	−	−	⊕	−	−
Chenopodium album	−	−	−	−	−	+
Solanaceae						
Lycopersicon esculentum	−	−	−	+	+	⊕
Solanum nigrum	−	−	−	⊕	−	+
Polygonaceae						
Rumex obtusifolius	−	−	−	−	−	+
Polypogonum longisetum	−		−		−	−
Compositae						
Callistephus chinensis	−	−	−	⊕	⊕	⊕
Geranaceae						
Geranium thunbergii	−	−	−	−	⊕	⊕
Cucurbitaceae						
Cucumis sativus	−	−	−	−	−	−
Pedaliaceae						
Sesamum indicum	−	−	−	−	+	+
Labiatae						
Elsholtzia ciliata	−	−	−	−	−	+

+ : fungus reisolated.
− : not reisolated.
○ : plants occasionally killed.

Comparisons of the electrophoretic patterns of buffer-soluble protein indicated close relationships among the isolates with small oogonia and less close relationships between these and the HNS-LO (IMI 56348) isolate (Fig. 3). The isolates examined shared 4 bands, and 10 bands were common to the isolates with small oogonia. Six bands were unique to them.

Discussion

The HNS-SO isolates from alfalfa-field soil were most similar to the AS-SO isolates. These two groups were identical in morphology of spores, growth temperature relations, and growth rate. Their electophoretic patterns of buffer-soluble protein resembled as well. The most distinguishing characteristics were their host ranges; the HNS-SO isolates were less virulent to alfalfa than the AS-SO isolates but had a wider host range. Though we have never tried to isolate HNS-SO population from fields planted with other crops than alfalfa or from uncultivated land, it may exist there as a weak parasite of various crops, weeds, and indigenous plants. HNS-SO and AS-SO populations are unlikely to coexist in alfalfa fields where AS-SO population was found, and vice versa. AS-SO population may have selective advantage over HNS-SO population in alfalfa fields due to its specific pathogenicity. SATO recently isolated *P. megasperma* from diseased indigenous legume, *Lotus corniculatus* L. var. *japonicus* Regel. These isolates had 41.5 μm diam oogonia and seemed to be host-specific, and bird's-foot trefoil (*L. corniculatus*) or alfalfa was not diseased (SATO, unpublished). They should be further examined.

Despite of diferences in conditions of inoculation and interpretation of host range, host-specific isolates of *P. megasperma* often cause damage on more than two plant species[1,6,7,9,11,12], blurring the concept of host specificity. PRATT[12] proposed a system of primary and secondary hosts. Use of formae speciales without varietal designation emphasizes pathogenic differences between isolates of *P. megasperma* and leaves morphological questions unanswered[6]. KUAN & ERWIN[9] emphasized the invalidity of variety separation by oogonium diameter; however, in their figure there were no host-specific isolates with large oogonia. This is true of other host-specific isolates[1,16] except for isolates[6] from Douglas fir with oogonia of variable diameters (39-59 μm).

Thus, forma specialis designation may distinguish some of host-specific populations, but the vast variation in the *P. megasperma* complex is not fully characterized. Neither morphology nor pathogenicity is the sole criterion to establish taxa. Other criteria should be reinforced in combination with these criteria. SANSOME & BRASIER[14] revealed that the chromosome number of var. *megasperma* is 22-27, twice as many as that of var. *sojae* with chromosome number of 10-13. According to STEPHENSON et al.[15], var. *sojae* has 9 chromosomes. Electrophoresis of protein is now commonly conducted as an aid for identification of species[3,4,5,10]. IRWIN & DALE[7] showed significant differences in protein band patterns between isolates from soybean and isolates from alfalfa or chickpea (*Cicer arietinum* L.). In the present studies, however, the alfalfa isolates showed similar patterns to those of the soybean isolates. This discrepancy may be ascribed to that they used SDS and 2-mercaptoethanol to dissociate proteins. Electro phoretic comparisons on a large scale between LO and SO isolates or between host-specific and host non-specific isolates is lacking and should be made. Serological studies[2] should also provide findings on inter-taxon relationships.

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北海道農試研報
143,75－84(1985)

アルファルファ圃場の土壌から分離された宿主非特異的 *Phytophthora megasperma*

松本直幸* 佐藤 徹**

摘 要

宿主特異性のない *Phytophthora megasperma* が，アルファルファ圃場の土壌から分離された．本菌株の形態的特徴，生育温度反応，及び生育速度は，アルファルファを特異的に侵す菌株（アルファルファ菌株）と同じであった．本菌株の可溶性タンパクの電気泳動パターンは，アルファルファ菌株のパターンと似ていた．本菌株は，ジャガイモ煎汁寒天や麦芽エキス寒天上で花弁状の菌そうを示すことと，アルファルファ以外の植物にも寄生性があることで，アルファルファ菌株と異なった．*P. megasperma* は，形態と病原性を異にするさまざまな菌株を含むが，本菌株はアルファルファ菌株に最も近縁であり，分類学的に重要であると考えられる．

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