Separation by Protein Electrophoresis of Six Species of *Phytophthora* Associated with Deciduous Fruit Crops

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ABSTRACT

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Polyacrylamide gel electrophoresis was employed to compare the patterns of native and sodium dodecyl sulfate dissociated proteins obtained from mycelia of six species of *Phytophthora* isolated primarily from deciduous fruit crops grown in the Great Lakes states. The intraspecific variation in banding patterns among isolates identified as *P. cactorum*, *P. cambivora*, and *P. syringae* was less than that among isolates identified as *P. megasperma*, *P. cryptogea*, and *P. drechsleri*. When native proteins were analyzed, the number of distinct subgroups distinguished were two in *P. cactorum*, two in *P. syringae*, one in *P. cambivora*, two in *P. drechsleri*, three in *P. cryptogea*, and six in *P. megasperma*. When dissociated proteins were analyzed, *P. cactorum*, *P. syringae*, and *P. cambivora* each formed single, distinct groups; *P. cryptogea* and *P. drechsleri* each formed two subgroups, one of which was common to isolates of both species. Most isolates of *P. megasperma* from deciduous fruit crops, when compared with isolates representing the six protein subgroups previously established for isolates of *P. megasperma*, had protein patterns belonging to the "broad host range" group, whereas the remaining few belonged to the "apple, cherry, apricot" group. The results obtained with electrophoresis support the use of this approach as an aid in distinguishing the species and subgroups within species of *Phytophthora* encountered on deciduous fruit crops.

Additional keywords: Phytophthora crown rot of apple, Phytophthora root and crown rot of cherry.

Phytophthora species cause root and crown rots of various deciduous fruit crops including apple, cherry, peach, apricot, and raspberry in the Great Lakes states of Michigan, New York, Ohio, and Wisconsin (1,19,20,28,29,32, and S. N. Jeffers, *unpublished*). *Phytophthora megasperma* Drechsler, *P. cactorum* (Lebert & Cohn) Schroeter, *P. cryptogea* Pethybridge and Lafferty, and *P. cambivora* (Petri) Buisman have been the species most commonly associated with fruit crops in these states. In addition, *P. syringae* (Klebahn) Klebahn was found to be infrequently associated with apple rootstocks in New York (19) and was recovered from a few declining cherry trees in Michigan (1) and California (30). *P. drechsleri* Tucker has been a problem on cherry trees in California (25,30) but has not been recovered from symptomatic fruit crops in the Great Lakes region.

The identification of *Phytophthora* species is based presently on the morphology of sexual and asexual reproductive bodies and on cultural characteristics (1,10,19,20,25,30). Although the species of *Phytophthora* most commonly recovered from deciduous fruit crops often can be identified using these criteria, problems of identification remain. For example, individual isolates may exhibit atypical morphological characteristics or may fail to form a sufficient number of reproductive structures for proper identification. Isolates exhibiting characteristics not conforming to the currently described species have been reported (25,29,30).

Techniques that aid in identification could be important to improving our understanding of the ecology of *Phytophthora* and the epidemiology of crown and root rots on deciduous fruit crops.

Electrophoresis of soluble proteins from mycelia has been useful (reviewed in 8,10,21) and is increasing in importance (7,9,15,16,18,24) as an aid in the identification and classification of numerous species of *Phytophthora*. Gallegly (10) critiqued the application of several physiological methods for the identification and classification of species of *Phytophthora* and concluded that electrophoretic analysis of soluble proteins was the most promising method. This approach has not been employed previously in the identification of isolates of *Phytophthora* species associated with the roots and crowns of deciduous fruit crops. In this study, we compare protein patterns among isolates of four species of *Phytophthora* (*P. cactorum*, *P. megasperma*, *P. cryptogea*, and *P. cambivora*) commonly recovered from fruit crops in the Great Lakes states, as well as *P. syringae* and *P. drechsleri*, and examine the utility of two electrophoretic procedures as aids in their identification.

MATERIALS AND METHODS

Isolates. In all, 184 isolates of six species of *Phytophthora* were assayed. The species and number of isolates of each were as follows: *P. cactorum*, 64; *P. megasperma*, 62; *P. cryptogea*, 32; *P. cambivora*, 14; *P. syringae*, 8; and *P. drechsleri*, 4. Many isolates had been in culture for less than 1 yr, whereas some had been in culture for many years (for example, 67 yr for *P. cactorum* P715). Isolates of *P. citricola* Sawada, a species occasionally associated with deciduous fruit crops (19,30) and morphologically similar to *P. cactorum* and *P. syringae*, also were used for comparison in some experiments.

The original culture designation, host or source, and geographical origin of the isolates used in this study are given in

TABLE 1. Variation in protein patterns among isolates of six species of *Phytophthora* recovered primarily from deciduous fruit crops worldwide and compared by polyacrylamide gel electrophoresis of native protein extracts from mycelia before and after treatment with sodium dodecyl sulfate (SDS)

Species	Isolate	Host or source	Origin[a]	Electrophoretic group		Isolate	Host or source	Origin[a]	Electrophoretic group	
				Native	SDS[b]				Native	SDS[b]
P. cactorum										
	AFG3	Pear	Aus.	1		NY279	Soil/native flora	NY	1	
	AL3A3	Peach	MS	1		NY295	Rootwash/apple	GA	1	
	AP45	Pear	Aus.	1		NY307	Soil/apple	NY	1	
	AP55	Apple	Aus.	1		NY308	Soil/apple	NY	1	
	Ap-62	Apple	NY	1		NY310	Soil/apple	NY	1	
	EM154	Strawberry	Eng.	1	A	NY323	Raspberry	NY	1	
	EM314	Strawberry	Eng.	1		NY327	Raspberry	NY	1	
	EM315	Strawberry	Eng.	1		NY349	Cherry	NY	1	
	M3	Douglas fir	MI	1		NY359	Apple	NY	1	
	M117	Cherry	MI	1	A	NY411	Peach	OH	1	
	M212	Cherry	MI	1		P1	Apple	Pol.	1	A
	M272	Apple	MI	1		P9	Apple	Pol.	1	
	M277	Apple	MI	1		P15	Apple	Pol.	1	
	M353	Cherry	MI	1		P37	Apple	Pol.	1	
	M354	Cherry	MI	1		P38	Apple	Pol.	1	
	M358	Cherry	MI	1	A	P44	Apple	Pol.	1	
	M413	Apple	MI	1		P235	Rhododendron	OH	1	
	NC570	Apple	NC	1		P274	Pear	Aus.	1	
	NY007	Apple	NY	1		P285	Apple	Aus.	1	
	NY020	Apple	NY	1	A	P472	Soil/pear	CA	1	
	NY066	Walnut	CA	1		P512	Apple	Mex.	1	
	NY159	Rhododendron	NY	1		P714	Lilac	...[c]	1	
	NY170	Strawberry	NY	1	A	P715	... (IMI 21168)[d]	Eng.	1	
	NY195	Apple	Can.	1		P985	Apple	Aus.	1	
	NY230	Ginseng	WI	1		P1012	Christmas berry	CA	1	
	NY238	Rootwash/apple[e]	NY	1		P1034	Apple	Ger.	1	
	NY252	Rootwash/apple	Net.	1		NY193	Strawberry	OH	2	A
	NY254	Rootwash/apple	WA	1		P1013	Strawberry	CA	2	A
	NY262	Rootwash/apple	NY	1		R31	Strawberry	USA	2	A
	NY270	Rootwash/apple	MI	1		R32	Strawberry	USA	2	A
	NY275	Soil/native flora[f]	NY	1		St-18	Strawberry	NY	2	A
	NY277	Soil/corn	NY	1		St-19	Strawberry	NY	2	A
P. syringae										
	AP81	Almond	Aus.	3		NY218	Rootwash/apple	WA	3	
	EM120	Soil	Eng.	3	B	NY219	Apple	OR	3	B
	EM144	Soil	Eng.	3		NY257	Apple	OR	3	
	M446	Cherry	MI	3	B	M119	Cherry	MI	4	B
P. cambivora										
	5-4-3 (NY151)	Cherry	CA	5		NY187	Cherry	NY	5	C
	C-45	Soil/cherry	MI	5		NY196	Soil/apple	NY	5	C
	M72	Cherry	MI	5		NY216	Cherry	OR	5	C
	M431	Apple	MI	5		NY235	Apple	WA	5	
	M433	Cherry	MI	5		NY268	Rootwash/apple	WA	5	C
	M436	Cherry	MI	5		NY350	Cherry	NY	5	
	NY113	Apple	NY	5	C	R-43	...	...	5	

(continued next page)

TABLE 1. (cont'd)

P. cryptogea										
	13-4-9	Cherry	CA	6	D	NY413	Peach	NY	7	
	14-2-5	Apple	CA	6	D	NY414	Peach	NY	7	
	AP4	Almond	Aus.	6	D	NY415	Peach	NY	7	E
	AP8	···	Aus.	6	D	NY416	Peach	NY	7	E
	0-1	Gerbera	Pol.	6	D	NY417	Peach	OH	7	E
	0-2	Gerbera	Pol.	6		M440	Cherry	MI	8	E
	0-3	Gloxima	Pol.	6		NY082	Apple	NY	8	E
	P1088 (CBS 290.35)	Aster	USA	6	D	NY220	Apple	KY	8	E
	C-13	Soil/cherry	MI	7	E	NY298	Apple	NY	8	E
	M172	Cherry	MI	7		NY315	Raspberry	NY	8	E
	M417	Cherry	MI	7	E	NY316	Raspberry	NY	8	
	M455	Cherry	MI	7	E	NY317	Raspberry	NY	8	
	NY001	Apple	NY	7	E	NY320	Raspberry	NY	8	E
	NY154	Cherry	NY	7	E	NY353	Apple	NY	8	E
	NY155	Cherry	NY	7		NY361	Peach	NY	8	
	NY221	Cherry	NY	7	E	W011	Cherry	WI	8	E
P. drechsleri										
	5-2-7 (NY152)	Cherry	CA	9	E	9-1-5	Safflower	CA	10	F
						P1087	Potato	···	10	F
	AFG4	Apple	Aus.	9	E	(CBS 292.35)				
P. megasperma[g]										
	2	Alfalfa	WA	11	ALF	M373	Cherry	MI	13	
	MDTM	Alfalfa		11		M375	Cherry	MI	13a	
	NY128	Alfalfa	NY	11		M400	Cherry	MI	13	
	WisA	Alfalfa		11		M410	Apple	MI	13a	BHR
						M411	Apple	MI	13a	BHR
	20	Douglas fir	OR	12	DF	M419	Cherry	MI	13	
	72	White Cockle	NY	12	DF	M429	Cherry	MI	13	
						M444	Cherry	MI	13	
	39	Noble fir	OR	13a	BHR	M448	Cherry	MI	13	BHR
	71	Alfalfa	OR	13	BHR	M449	Cherry	MI	13	
	5-4-5 (NY150)	Cherry	CA	13a		NY011	Apple	NY	13a	BHR
						NY054	Apple	NY	13a	
	24-4-7	Apple	CA	13a	BHR	NY088	Apple	NY	13a	
	C-23	Soil/cherry	MI	13a		NY153	Cherry	NY	13a	
	M68	Cherry	MI	13a		NY178	Apple	NY	13a	
	M99b	Cherry	MI	13a		NY185	Cherry	NY	13	BHR
	M111	Cherry	MI	13a	BHR	NY186	Cherry	NY	13a	
	M137	Cherry	MI	13	BHR	NY222	Apricot	NY	13a	BHR
	M151	Cherry	MI	13		NY341	Cherry	NY	13a	
	M187	Cherry	MI	13	BHR	NY344	Cherry	NY	13	BHR
	M220	Cherry	MI	13		NY346	Cherry	NY	13a	BHR
	M224	Cherry	MI	13a						
	M251	Cherry	MI	13		62	Cherry	CA	14	AC
	M269	Cherry	MI	13	BHR	65	Apple	CA	14	AC
	M278	Apple	MI	13		M424	Cherry	MI	14	AC
	M308	Cherry	MI	13a		NY190	Soil/cherry	NY	14	AC
	M317	Cherry	MI	13		NY412	Peach	OH	14	
	M321	Cherry	MI	13	BHR					
	M325	Cherry	MI	13	BHR	95	Clover	MS	15	CLO
	M328	Cherry	MI	13a		96	Clover	MS	15	CLO
	M332	Cherry	MI	13a	BHR					
	M346	Cherry	MI	13	BHR	NY168	Soybean	MI	16	SOY
	M347	Cherry	MI	13a	BHR	NY169	Soybean	MI	16	
	M359	Cherry	MI	13	BHR		(ATCC 44032)			
						PMG18	Soybean		16	SOY

[a] Countries and their abbreviations are as follows: Australia (Aus.), Canada (Can,), England (Eng.), Germany (Ger.), Mexico (Mex.), The Netherlands (Net.), Poland (Pol.), and the United States of America (USA). States in the United States are identified by their two-letter postal abbreviations: California (CA), Georgia (GA), Kentucky (KY), Michigan (MI), Mississippi (MS), Missouri (MO), New York (NY), North Carolina (NC), Ohio, (OH), Oregon (OR), Washington (WA), and Wisconsin (WI).

[b] Only selected isolates were subjected to SDS treatment before electrophoresis.

[c] Information unknown or not available.

[d] Alternative designation.

[e] Rootwashings collected from nursery-grown apple rootstocks (19).

[f] Native flora consisted primarily of hardwood and shrubby species. Soil samples were collected in locations not subjected to agricultural practice (19).

[g] *P. megasperma* subgroups according to the terminology of Hansen et al (16).

Table 1. The cultures were isolated and identified by the authors or were supplied by colleagues in various institutions around the world. These latter sources include the following: the *Phytophthora* collection in the Department of Plant Pathology, University of California, Riverside; S. M. Mircetich, Department of Plant Pathology, University of California, Davis; T. J. Wicks, Department of Agriculture, Adelaide, South Australia; D. C. Harris, East Malling Research Station, Maidstone, Kent, England; R. A. Haygood, Department of Plant Pathology and Physiology, Clemson University, Clemson, SC; and T. B. Sutton,

Department of Plant Pathology, North Carolina State University, Raleigh. Representative isolates of the subgroups of *P. megasperma* as defined by protein pattern (16) were supplied by P. B. Hamm and E. M. Hansen, Department of Botany and Plant Pathology, Oregon State University, Corvallis. Isolates were maintained on Difco lima bean agar.

Soluble protein extracts. The procedures for culturing and extracting buffer-soluble (native) proteins were a modification of those described previously (12). Isolates initially were grown in 50 ml of 20% clarified V-8 juice broth, adjusted to pH 6.5, for 10 days at 20 C in the dark. Mycelial mats from three 125-ml flasks per isolate were collected by filtration onto Miracloth, washed with phosphate buffer (pH 7.0), damp dried, and frozen overnight at −20 C. Buffer-soluble proteins were extracted by grinding mycelia with a pestle in a mortar containing acid-washed sand and 0.5 ml of phosphate buffer per gram of mycelium. The mixture was centrifuged at 47,800 *g* for 30 min, and the supernatant containing the soluble proteins was dispensed into small tubes for storage at −20 C.

Separation of native protein. Our methods employed extensive modifications of those described by Ornstein (26) and Davis (6). Electrophoresis of native protein preparations was carried out on a discontinuous system (Model V16, Bethesda Research Laboratories, Inc., Gaithersburg, MD) using a 4.6% polyacrylamide stacking gel and a 7.7% separating gel in a vertical slab mold (7.75 × 7.5 × 1.5 cm). Electrophoresis buffer was a Tris-glycine buffer at pH 8.3. Sucrose and tracking bromphenol blue dye were added to soluble protein preparations, and aliquots containing 40–60 μg of fungus protein were placed into wells in the gel. Protein concentration was determined by the method of Bradford (3). Electrophoresis was performed for 6–7 hr at 20 mA for the stacking gel and at 25 mA for the separating gel in a cold room at 4 C. Protein patterns were visualized by staining overnight with Coomassie brilliant blue G250 in water:methanol:perchloric acid (15:1:4), destaining for 10 min with a similar mixture of water:methanol:acetic acid, restaining if necessary for 30 min with Coomassie brilliant blue R250 in water:methanol:acetic acid, and destaining with several changes of water:methanol:acetic acid (7:2:1) (2). Before drying on white filter paper, gels were impregnated with water:methanol:glycerol (27:70:3).

Separation of dissociated protein. After isolates were grouped based on patterns derived from the electrophoretic separation of native proteins, selected isolates were subjected to a sodium dodecyl sulfate (SDS) discontinuous system of electrophoresis (23). Soluble-protein extracts were mixed 1:3 with disruption buffer (0.5 M Tris-HCl at pH 6.8, glycerol, 4% SDS, and 10% 2-mercaptoethanol) and boiled in a water bath for 2–3 min. Subsequent electrophoresis of dissociated proteins (10–20 μg per well) was at 60–70 V for the stacking gel and 100–120 V for the separating gel at room temperature. The amperage was dictated by the voltage. Gels were stained by immersion in Coomassie brilliant blue G250 (2), restained, if necessary, in Coomassie brilliant blue R250 in acetic acid:methanol:water (1:5:4), and then destained and impregnated as described for native proteins.

RESULTS

When the profiles of native proteins from several isolates were compared on a single gel, isolates of the same species or subgroup within a species were readily identified, both qualitatively by the visual similarity in banding patterns and quantitatively by calculating similarity coefficients (16). Isolates of different species or those identified incorrectly were recognized easily when included in a series of otherwise similar isolates. By regrouping isolates with similar patterns and repeating side-by-side comparisons, it was possible to collect our isolates into 16 groups, each with an identical or nearly identical native protein pattern (Table 1). These groups were assigned arbitrarily numeric designations. Within the 16 groups, 12 major groups were distinguished by the electrophoretic patterns of protein subunits obtained by dissociation of native proteins with SDS (Table 1). Groups within all species except *P. megasperma* were designated arbitrarily with the letters A to F; groups within *P. megasperma* were designated according to the protein groups previously determined by Hansen et al (16).

When isolates of *P. cactorum*, *P. cryptogea*, *P. megasperma*, or

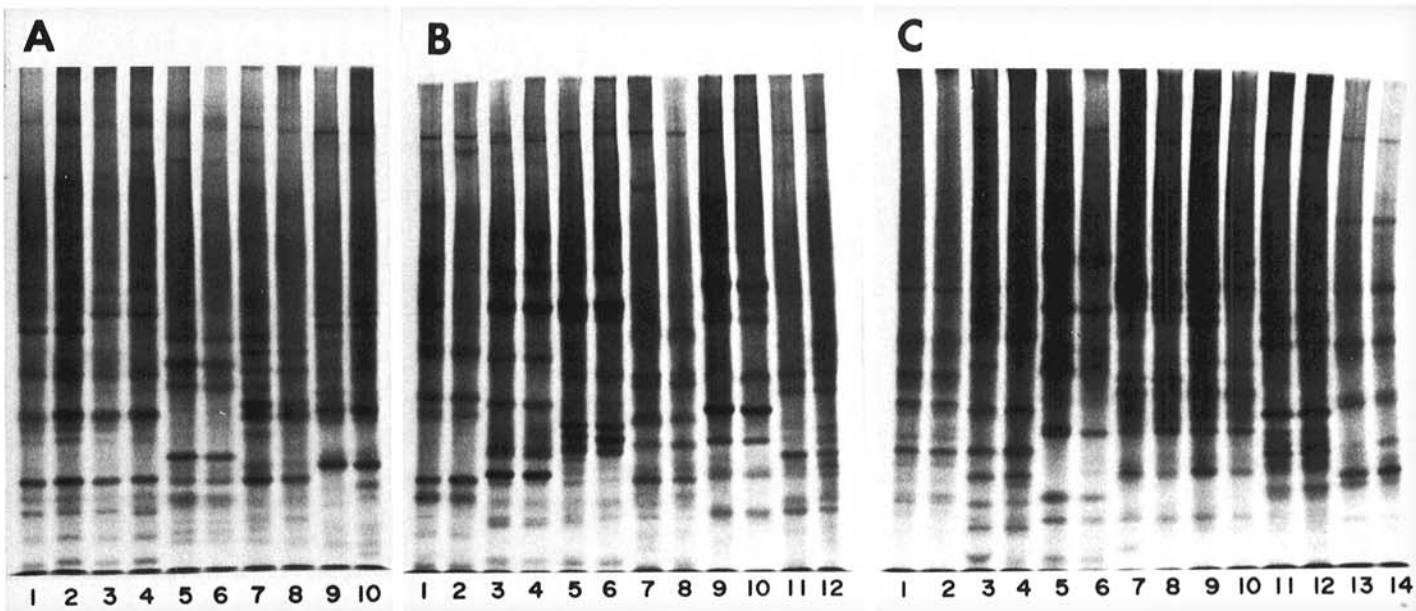

Fig. 1. Representative protein profiles for seven species of *Phytophthora* as differentiated by electrophoresis of native proteins. **A,** Profiles for isolates of *P. cactorum* (groups 1 and 2), *P. syringae* (groups 3 and 4), and *P. citricola*. Lanes 1 and 2 are group 2, *P. cactorum* isolates ST-19 and P1013; lanes 3 and 4 are group 1, *P. cactorum* isolates NY327 and EM154; lanes 5 and 6 are group 4, *P. syringae* isolates M119 and M120; lanes 7 and 8 are group 3, *P. syringae* isolates NY218 and EM120; and lanes 9 and 10 are *P. citricola* isolates M454 and ATCC 42885. **B,** Profiles for isolates of *P. cambivora*, *P. drechsleri* (groups 9 and 10), and *P. cryptogea* (groups 6–8). Lanes 1 and 2 are *P. cambivora* isolates NY187 and NY113; lanes 3 and 4 are group 10, *P. drechsleri* isolates AFG4 and 5-2-7 (NY152); lanes 5 and 6 are group 9, *P. drechsleri* isolates 9-1-5 and P1087; lanes 7 and 8 are group 8, *P. cryptogea* isolates NY315 and NY220; lanes 9 and 10 are group 7, *P. cryptogea* isolates C-13 and NY154; and lanes 11 and 12 are group 6, *P. cryptogea* isolates P1088 and 0-1. **C,** Profiles for isolates of the six protein groups of *P. megasperma*. Similar groups were established previously by electrophoresis of SDS-dissociated proteins (16). Lanes, group, and isolate numbers are as follows: 1 and 2, 16, PMG65 and PMG18; 3 and 4, 15, 95 and 96; 5 and 6, 14, NY190 and 62; 7 and 8, 13a, M332 and 39; 9 and 10, 13, M400 and 71; 11 and 12, 12, 72 and 20; and 13 and 14, 11, NY128 and 2.

P. syringae recovered from single apple or cherry trees in Michigan were compared electrophoretically, isolates of the same species recovered from the same tree always produced identical native or SDS-dissociated protein banding patterns. In fact, such isolates may be from a common thallus or may represent single zoospore isolates from a common source of inoculum. These data were not included in Table 1 but substantiate the reliability of our procedures.

The 64 isolates of *P. cactorum* were divided into two groups (groups 1 and 2) on the basis of banding patterns of native proteins (Table 1). The banding pattern for the six isolates comprising group 2 differed by only one distinct band from the pattern for isolates in group 1 (Fig. 1A). Similarity coefficients (sc) between the two groups were 91%. Each of the six isolates in group 2 was recovered from strawberry fruit or crowns in different geographic areas within the United States, although other fruit and crown isolates from strawberry had banding patterns identical to isolates of *P. cactorum* from other deciduous fruit crops (group 1, Table 1). However, the banding patterns for SDS-dissociated proteins from isolates in groups 1 and 2 were identical (group A, Table 1).

Six isolates of *P. syringae* from England, Australia, Washington, and Oregon had a native protein pattern identical (sc = 100%) to that of isolate M446 recovered from a cherry tree in Michigan (group 3) but different (sc = 64%) from that of isolate M119 recovered from a cherry tree in a second Michigan orchard (group 4) (Table 1, Fig. 1A). Previously, maximum temperatures for growth of these isolates were found to be similar, but the colony type of isolate M119 was atypical for *P. syringae* (1). However, the profiles for SDS-dissociated proteins from isolates in groups 3 and 4 were identical (group B) (Fig. 2). Native and SDS-dissociated protein patterns for the morphologically related species *P. syringae*, *P. cactorum*, and *P. citricola* were similar but distinguishable (Figs. 1A, 2).

The 14 isolates of *P. cambivora* from apple and cherry trees in California, Michigan, New York, and Washington and from soil in Michigan and New York produced a single pattern for native proteins (group 5) (Table 1, Fig. 1B). The profiles obtained with SDS-dissociated proteins also were identical (group C) (Table 1, Fig. 3).

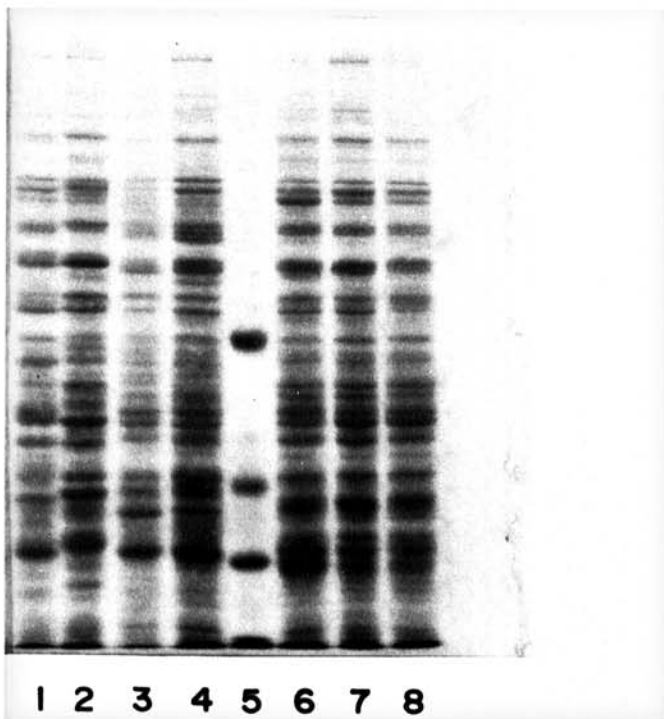

Fig. 2. Representative protein profiles for isolates of *Phytophthora syringae* (lanes 1 and 2 are isolates EM120 and M119), *P. citricola* (lanes 3 and 4 are isolates ATCC 42885 and P713), and *P. cactorum* (lanes 6–8 are isolates P1013, ST-18, and M117) as differentiated by electrophoresis of SDS-dissociated proteins.

Thirty-two isolates identified as *P. cryptogea* were divided into three groups (groups 6, 7, and 8) on the basis of distinctly different banding patterns for native proteins (Table 1, Fig. 1B). All 23 isolates of *P. cryptogea* recovered from deciduous fruit crops in the Great Lakes states were subdivided into groups 7 and 8; however, three isolates from fruit trees in California and Australia were placed in group 6 with four isolates from herbaceous hosts in Poland and the United States and one isolate from an unknown host in Australia. Similarity coefficients were generally much higher between isolates within a group (72–100%) then between isolates in different groups (27–66%) (Table 2). When isolates from native protein groups 6, 7, and 8 were compared on the same gel alongside a single isolate of *P. drechsleri* (group 9), similarity coefficients were low for all comparisons between isolates of *P. cryptogea* and isolate AFG4 of *P. drechsleri* (19–42%). On the basis of patterns for SDS-dissociated proteins, isolates in native protein group 6 fell into one group (group D), whereas isolates in native protein groups 7 and 8 fell into a second group (group E) that also contained two isolates of *P. drechsleri* (Fig. 3).

The four isolates of *P. drechsleri* were subdivided into two groups (groups 9 and 10) on the basis of banding patterns of native proteins (Table 1). These groups were distinctly different from the three groups in *P. cryptogea* (Fig. 1B). However, the banding pattern for SDS-dissociated proteins from isolates AFG4 and 5-2-7 (NY152) of *P. drechsleri* was identical to that of group E of *P. cryptogea*. The banding pattern for SDS-dissociated proteins from the two remaining isolates of *P. drechsleri* varied sufficiently from groups E and D to justify a separate group (group F) (Table 1, Fig. 3).

Nine isolates of *P. megasperma* obtained from P. B. Hamm and E. M. Hansen (isolates 2, 20, 39, 62, 65, 71, 72, 95, and 96), which had previously been separated into five SDS-dissociated protein groups (ALF, DF, BHR, AC, and CLO) by Hansen et al (16), were separated similarly on the basis of electrophoretic patterns of both native (groups 11–15) and SDS-dissociated proteins in our tests (Table 1, Fig. 1C). Three isolates of *P. megasperma* from soybean

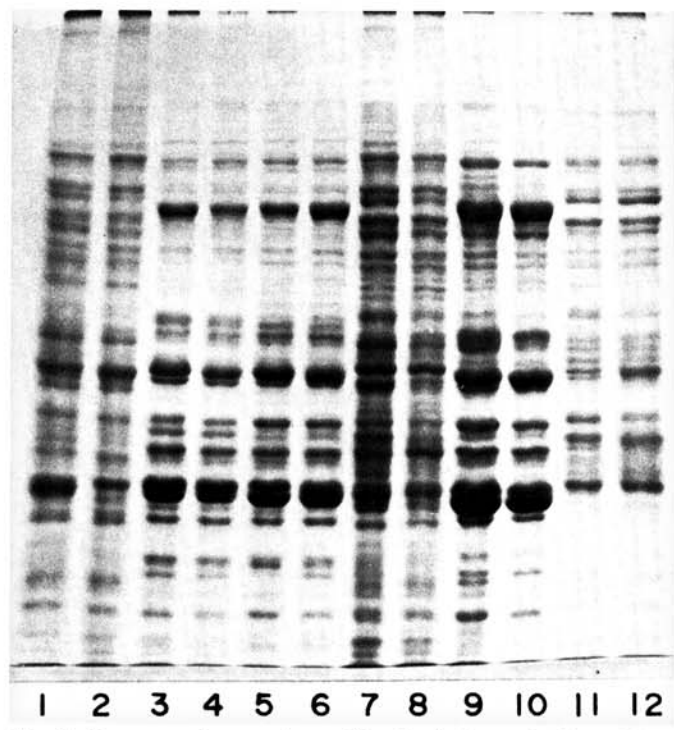

Fig. 3. Representative protein profiles for isolates of *Phytophthora cryptogea*, *P. drechsleri*, and *P. cambivora* as differentiated by electrophoresis of SDS-dissociated proteins. Lanes 1 and 2 are group D, *P. cryptogea* isolates 0-1 and P1088; lanes 3–6 are group E, *P. cryptogea* isolates NY154, C-13, NY220, and NY315; lanes 7 and 8 are group F, *P. drechsleri* isolates P1087 and 9-1-5; lanes 9 and 10 are group E, *P. drechsleri* isolates 5-2-7 (NY152) and AFG4; and lanes 11 and 12 are group C, *P. cambivora* isolates NY113 and NY187.

formed a sixth, distinctive protein group (native protein group 16; SDS-dissociated protein group SOY [16]) (Table 1, Fig. 1C). In group 13, which included isolates previously assigned to the BHR group by Hansen et al (16), it was possible to distinguish a minor subgroup, group 13a, based on a slight difference in banding pattern. However, some group 13a isolates did not give repetitive results from gel to gel; furthermore, the profiles of SDS-dissociated proteins among isolates in group 13 and 13a were identical. Forty-two of 45 isolates of *P. megasperma* associated with fruit crops in the Great Lakes states were placed in groups 13 and 13a; the other three isolates were placed in group 14 (Table 1), which corresponded to the AC grouping established by Hansen et al (16). Similarity coefficients were low between groups 13-13a and group 14 (sc from 42 to 44%).

DISCUSSION

Our results using polyacrylamide gel electrophoresis of soluble mycelial proteins confirmed the identification of isolates recovered from deciduous fruit crops that previously were identified as *P. cactorum*, *P. syringae*, and *P. cambivora* on the basis of the morphology of reproductive structures, colony type, and cardinal temperatures for vegetative growth (1,19–21,28–30,32). Isolates in different species could be visually distinguished on the basis of contrasting SDS-dissociated protein banding patterns, whereas isolates within a single species produced largely homogeneous banding patterns. However, two recognizable banding patterns emerged for undissociated, native proteins of both *P. syringae* and *P. cactorum*. Previously, isolates of *P. cactorum* have produced a single, distinct pattern when undissociated proteins were analyzed by disk electrophoresis (11–13) or by isoelectric focusing (8). However, variation in protein banding patterns was observed when SDS-dissociated proteins were analyzed by polyacrylamide gel electrophoresis (15). The discrepancy between these data and our data may be attributed to specific protocol differences or to the isolates used. In no previous study have so many isolates of *P. cactorum* been compared at one time.

Based on our procedures and the isolates analyzed, it appears that electrophoretic banding patterns of SDS-dissociated proteins provide a more conservative, less fragmentary taxonomic criterion for indicating differences among these three *Phytophthora* spp. than do the patterns of native proteins. However, electrophoresis of native proteins also provided a very useful means of taxonomically separating and grouping isolates of *P. cactorum*, *P. syringae*, and *P. cambivora*, once the subgroups of protein banding patterns within *P. cactorum* and *P. syringae* were recognized. In addition, native proteins were easier to prepare and the resulting gels could be compared more readily because fewer bands developed on these than on SDS-dissociated protein gels.

Electrophoresis of both native and SDS-dissociated proteins from isolates of *P. megasperma* confirmed the distinction of six major subgroups proposed previously by Hansen et al (16). Most isolates of *P. megasperma* recovered from deciduous fruit crops in the Great Lakes states produced protein patterns indistinguishable from those of isolates placed into the BHR group by Hansen et al (16), whereas the few remaining isolates produced protein patterns identical to those of isolates previously placed into the AC group (16). Isolates within these two protein groups also could be distinguished by colony morphology, cardinal growth temperatures, and oospore size, as noted by Wilcox and Mircetich (31) in their examination of isolates of *P. megasperma* recovered from fruit crops and woody ornamentals in California. Hansen et al (16) also recognized differences in colony morphology and oogonium size between isolates in the BHR and AC groups. In addition, others recently have recognized discrete intraspecific variation among isolates of *P. megasperma* from legume hosts based on electrophoretic patterns of SDS-dissociated proteins and have correlated this variation to differences in oogonium size, cardinal temperatures, and virulence (9,18). Collectively, these data suggest that morphological and cultural characteristics may be used to distinguish the subgroups of *P. megasperma* determined by electrophoresis of soluble proteins.

The fact that the 32 isolates previously identified as *P. cryptogea* were separated into three native and two SDS-dissociated protein subgroups, one of which included some isolates previously identified as *P. drechsleri* (that is, type E pattern), provides additional evidence that the present criteria for distinguishing these species are inadequate. Two independent investigations, using different groups of isolates of *P. cryptogea* and *P. drechsleri*, concluded that there was no reliable taxonomic character to differentiate the two species (5,17). In contrast, Krober (22) emended the description of *P. cryptogea* and proposed that the two species be kept separate. Previously, electrophoresis of undissociated proteins of isolates of these two species produced only a single banding pattern (24), and various serological techniques also failed to reliably distinguish *P. cryptogea* from *P. drechsleri* (14). Therefore, it may be appropriate to merge these taxa, as suggested by several authors (5,17,24).

However, it is notable that authentic cultures of *P. cryptogea* (P1088) and *P. drechsleri* (P1087) produced distinctly different protein patterns, whereas the majority of fruit crop isolates identified as belonging to one of these two taxa produced protein patterns distinctly different from either of the authentic cultures (Table 1). Whether such differences might be representative of

TABLE 2. Comparisons between similarity coefficients for native protein patterns of 18 isolates of *Phytophthora cryptogea* (groups 6, 7, and 8) and one isolate of *P. drechsleri* (group 9) as determined on a single polyacrylamide gel

			Group 7								Group 8							Group 9
	Group 6																	
Isolate	AP8	P1088	NY154	NY155	NY221	NY413	NY414	NY415	NY416	NY417	NY082	NY315	NY316	NY317	NY320	NY353	NY361	AFG4
AP4	100[a]	72	40	40	40	40	40	40	40	27	38	38	38	38	38	38	38	27
AP8		72	40	40	40	40	40	40	40	27	38	38	38	38	38	38	38	27
P1088			44	44	44	44	44	44	44	28	33	33	33	33	33	33	33	19
NY154				100	100	100	100	100	100	77	63	63	63	63	63	63	63	42
NY155					100	100	100	100	100	77	63	63	63	63	63	63	63	42
NY221						100	100	100	100	77	63	63	63	63	63	63	63	42
NY413							100	100	100	77	63	63	63	63	63	63	66	42
NY414								100	100	77	63	63	63	63	63	63	63	42
NY415									100	77	63	63	63	63	63	63	63	42
NY416										77	63	63	63	63	63	63	63	42
NY417											66	66	66	66	66	66	66	28
NY082												100	100	100	100	100	100	33
NY315													100	100	100	100	100	33
NY316														100	100	100	100	33
NY317															100	100	100	33
NY320																100	100	33
NY353																	100	33
NY361																		33

[a] Similarity coefficients were the bands in common divided by the total number of bands for the two isolates being compared times 200 (16).

natural variation among a single heterogeneous species, a product of hybridization between *P. cryptogea* and *P. drechsleri*, or even an undefined third taxon is not clear but should be resolved in any future attempt to redescribe *P. cryptogea* (17). It is unfortunate that only four isolates identified as *P. drechsleri* were available in the present study; clearly, many more such isolates should be examined in further studies of this kind. Ideally, future studies also would include nonpapillate, heterothallic isolates from fruit crops that have been left unidentified by some authors (29,30,32) but that might fit within a broadened species concept of *P. cryptogea* as recently suggested (17).

Confusion regarding the *P. cryptogea-P. drechsleri* "complex" is but one example supporting the contention that a morphological approach to taxonomy is no longer adequate for the genus *Phytophthora* (4,27). Several criteria, including electrophoretic protein banding patterns, have been proposed (4,10) to supplement the morphological characters that currently serve as the sole determinants for identifying and classifying species of *Phytophthora*. Evidence confirming the validity of protein banding patterns as a reliable taxonomic criterion has been reviewed (8,10,21) and was dramatized recently by the ability to use this criterion as a major determinant for distinguishing subgroups of *P. megasperma* (16), a species that for some time has been the subject of considerable taxonomic confusion. Also in support of electrophoretic techniques as functional taxonomic criteria, protein banding patterns recently were employed as one criterion to describe a new species of *Phytophthora*, *P. pseudotsugae* (15).

It is important, however, to maintain a proper perspective on a morphological approach to taxonomy with respect to the utility of protein electrophoresis. For instance, most taxonomic successes with protein electrophoresis, including our ability to readily distinguish isolates of *P. cactorum*, *P. syringae*, and *P. cambivora* in the present study, confirms that morphological features, in most cases, have provided an adequate basis to correctly identify isolates of *Phytophthora*. In fact, work with *P. megasperma*, a group that exhibits considerable morphological variability, has suggested that most subgroups within *P. megasperma* that were identified on the basis of protein patterns also could be distinguished morphologically (9,16,18). As a taxonomic tool applicable to the genus *Phytophthora*, electrophoretic patterns of soluble protein may be most valuable as a guide to distinguish or delimit isolates whose variability in morphological characters might otherwise appear to provide an indeterminate, overlapping continuum of types.

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