Comparative Protein Electrophoretic and Isoenzymic Patterns of *Phytophthora cryptogea* Isolates from Chilean Kiwifruit and North American Deciduous Fruits

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ABSTRACT

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Isolates of *Phytophthora* from fruit crops, previously identified as *P. cryptogea* on the basis of standard morphological criteria, were compared using electrophoretic patterns of total soluble mycelial proteins and isozyme analysis. According to these criteria, all 33 isolates recovered from kiwifruit in Chile were similar or identical to isolates from California recovered from kiwifruit, apple, and cherry. In contrast, the Chilean and California isolates appeared no more related to New York isolates of *P. cryptogea* than they were to isolates of *P. cactorum, P. cinnamomi,* and *P. megasperma* that were similarly examined. Isolates identified as *P. cryptogea* from deciduous fruit crops in New York appear to belong to a different taxon than isolates of *P. cryptogea* from fruit crops in Chile and California.

Additional keyword: taxonomy

Phytophthora root rot is commonly found when kiwifruit vines (*Actinidia chinensis* Planch.) are cultivated on poorly drained soils in Chile. Although root rot is caused by both *P. citrophthora* (R. E. Sm. & E. H. Sm.) Leonian and *P. cryptogea* Pethybr. & Lafferty in the Central Valley of Chile, the latter species is isolated more frequently (17,31). *Phytophthora cryptogea* also causes root rot of kiwifruit in California (7).

Phytophthora cryptogea is a broadly defined species within the Group VI of Waterhouse (25) and is delimited largely on a negative basis, i.e., it lacks corroloid mycelium, chlamydospores, ornamented oospores, homothallism, or other unique characters used to define most remaining species within the group (21,25–27). Consequently, it has been suggested (28) that researchers may have assigned significantly different organisms to this single taxon. Indeed, Mills et al. (20) used isozyme analysis to delimit seven groups of isolates identified previously as *P. cryptogea* by different researchers in North America, Oceania, and South Africa. Similarly, Bielenin et al. (1) separated a smaller collection of isolates, obtained primarily from deciduous fruit crops, into three or two groups based on electrophoretic profiles of undissociated or dissociated total mycelial proteins, respectively. Although kiwifruit isolates from California were examined in the former study, Chilean kiwifruit isolates were not examined in either. In fact, there appears to be no published study in which *P. cryptogea* isolates from any host in South America have been compared with those from other parts of the world.

The purposes of this study were to (i) more completely characterize a large collection of isolates identified as *P. cryptogea* from kiwifruit in Chile, using electrophoretic profiles of total soluble mycelial proteins, isozyme phenotype analysis, and standard morphological features, and (ii) use these electrophoretic characters to determine the relationship between the Chilean isolates and isolates identified as *P. cryptogea* from various deciduous fruit crops in the United States, including kiwifruit from California.

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MATERIALS AND METHODS

Isolates. Thirty-three *Phytophthora* isolates were obtained from roots of diseased kiwifruit vines in Chile and identified as *P. cryptogea* on the basis of standard morphological features (17,21,25–27). These isolates were compared with nine isolates obtained from fruit crops in the United States, which had been identified previously as *P. cryptogea* by their original sources. Also included for comparison were 13 additional isolates: a single isolate of an unidentified *Phytophthora* sp. in Waterhouse's Group VI (25) recovered from cherry in California and morphologically similar to that described by Wilcox and Mircetich (28,29); three isolates of *P. citricola* Sawada; two isolates each of *P. cactorum* (Lebert & Cohn) J. Schröt., *P. citrophthora, P. megasperma* Drechs., and *P. cinnamomi* Rands; and a single isolate of *Pythium ultimum* Trow. (Table 1).

Preparation of protein extracts. Individual isolates were grown for 72 h at 20 to 23°C in 250-ml flasks containing 150 ml of carrot juice broth (prepared by boiling 500 g of fresh carrots in 1 liter of distilled water for 10 to 15 min, followed by filtering and autoclaving). The mycelium was harvested by filtration onto Whatman No. 1 filter paper in a funnel, washed three times with distilled water, and blotted dry. Soluble proteins were extracted by grinding 2 to 5 g of the mycelium with a pestle in a mortar containing about 1 g of acid-washed sand per 4 g of mycelium and 5 ml of buffer solution (0.1M Tris-HCl, pH 6.8) at 0°C. The mixture was centrifuged for 4 min at 14,000 × *g,* the supernatant was collected and the protein content measured by the Bio-Rad Protein Assay (Bio-Rad Laboratories, Richmond, Calif.) (3). Then, the supernatant was stored at −12°C until usage.

Protein electrophoresis. Thawed protein-extract supernatant was mixed with an equal volume of a solution containing 20% glycerol (vol/vol) and 0.1% bromphenol blue (vol/vol) in 0.15M Tris-HCl, pH 6.8. Twenty microliters of the resulting suspension (40 to 60 µg of protein) was subjected to electrophoresis in 25 mM Tris buffer containing 192 mM glycine at pH 8.3. Electrophoresis was conducted at room temperature (approximately 20 to 25°C) on an 8% polyacrylamide gel with a 4.5% stacking gel, at constant voltages of 200 and 50V, respectively, until the dye reached the bottom of the separating gel. Gels were stained for ≥4 h in 0.1% Coomassie Brilliant Blue R-250 (Sigma Chemical Co., St. Louis, Mo.) prepared in a 1:4:6 mixture of 90% acetic acid, 79% methanol, and water, then destained in a 20:7:80 mixture of 96% ethanol, 90% acetic acid, and water (16).

For electrophoresis of dissociated proteins, each supernatant was mixed with an equal volume of a solution consisting of (by volume) 64% buffer (0.15M Tris-HCl, pH 6.8); 20% glycerol; 6% sodium dodecyl sulfate (SDS); 10% 2-6-mercaptoethanol; and 0.1% bromphenol blue, before boiling in a water bath for 3 min. Twenty-microliter samples (40 µg of protein) were subjected to electrophoresis in 8% polyacrylamide prepared in 0.1% SDS. The electrophoresis, staining, and destaining

Table 1. Host, origin, and isozyme phenotypes of *Phytophthora* spp. isolates examined

Species and isolate number	Alternative designations	Host	Geographic origin	Isozyme phenotypes[u] EST	MDH	SOD
P. cryptogea			Chile			
A17-3N (08)	IMI 358635[v]	Kiwifruit	San Clemente	A	a	H
A17-3N (13)		Kiwifruit	San Clemente	A	a	H
A17-3N (30)		Kiwifruit	San Clemente	A	a	H
A17-4N (21)		Kiwifruit	San Clemente	A	a	H
A17-1S (23)		Kiwifruit	San Clemente	A	a	H
A17-2S (20)		Kiwifruit	San Clemente	A	a	H
A17-2S (32)		Kiwifruit	San Clemente	A	a	H
A14-1N (05)		Kiwifruit	Romeral	A	a	H
A14-2N (02)		Kiwifruit	Romeral	A	a	H
A14-1S (07)		Kiwifruit	Romeral	A	a	H
A18-1E (04)		Kiwifruit	Lontué	A	a	H
A18-1E (05)		Kiwifruit	Lontué	A	a	H
A18-2N (05)		Kiwifruit	Lontué	A	a	H
A18-2N (06)		Kiwifruit	Lontué	A	a	H
A18-2S (03)		Kiwifruit	Lontué	A	a	H
A18-10 (03)		Kiwifruit	Lontué	A	a	H
A28-1N (01)		Kiwifruit	Pirque	A	a	H
A28-1N (02)		Kiwifruit	Pirque	A	a	H
A28-1N (06)		Kiwifruit	Pirque	A	a	H
A28-2N (03)		Kiwifruit	Pirque	A	a	H
K-13-4	IMI 330745[v]	Kiwifruit	St. Ana de Chena	A	a	H
K-13-5		Kiwifruit	St. Ana de Chena	A	ND	H
K-16-0		Kiwifruit	Curicó	A	b	H
K-15-2	IMI 332631[v]	Kiwifruit	Curicó	A	ND	H
K-17-1		Kiwifruit	Lontué	A	ND	H
K-17-3		Kiwifruit	Lontué	A	ND	H
K-17-4		Kiwifruit	Lontué	A	ND	H
K-17-5		Kiwifruit	Lontué	A	ND	H
A15-1S (10)	IMI 358634[v]	Kiwifruit	Tutuquén	A′	a	H
A17-1S (11)		Kiwifruit	San Clemente	A′	a	H
A18-3E (04)	IMI 357969[v]	Kiwifruit	Lontué	A′	a	H
A18-2S (02)		Kiwifruit	Lontué	A′	a	H
A18-2S (101)		Kiwifruit	Lontué	A′	a	H
			United States			
13-4-9[w,x]	NY508[y], P3587[z]	Cherry	California	A	d	H
14-2-5[w,x]	NY140[y], P3586[z]	Apple	California	A′	e	H
22-4-2[w]		Kiwifruit	California	A	c	H
NY001[y]	P3649[z]	Apple	New York	*	f	*
NY155[y]		Cherry	New York	*	f	*
NY312[y]		Raspberry	New York	*	f	*
NY413[x,y]	P3317[z]	Peach	New York	*	f	*
NY414[x,y]	P3320[z]	Peach	New York	*	f	*
NY415[x,y]		Peach	New York	*	f	*
Phytophthora sp.						
18-3-1[w]	NY518[y]	Cherry	California	*	*	I
P. cactorum						
NY568[y]		Apple	New York	B	h	J
			Chile			
M-12-0		Apple	Lontué	B	h	J
P. cinnamomi			United States			
14-3-3[w]		Apple	California	C	i	*
22-1-3[w]		Almond	California	C	i	*
P. citricola						
18-1-1[w]		Raspberry	California	ND	ND	ND
			Chile			
F-11-0	IMI334961[v]	Raspberry	Curicó	D	j	H
F-12-1		Raspberry	Curicó	D	k	H
P. citrophthora						
K-14-1	IMI 332632[v]	Kiwifruit	Requinoa	E	l	H
F-16-12	IMI 334962[v]	Raspberry	Padre Hurtado	E	j	H
P. megasperma						
E-1-0		Asparagus	Linares	F	m	*
E-2-0	IMI 332460[v]	Asparagus	Chimbarongo	F	m	*
Pythium ultimum			United States			
Py-10-0			New York	G	n	*

[u] EST = esterase, MDH = malate dehydrogenase, SOD = superoxidismutase. * = null isozymic activity; ND = not determined.
[v] International Mycological Institute (IMI), CAB International, Egham, Surrey, UK.
[w] Collection of S. M. Mircetich, University of California, Davis.
[x] Also examined by Bielenin, et al. (1).
[y] Collection of New York State Agricultural Experiment Station, Cornell University.
[z] Collection of University of California, Riverside. Also examined by Mills et al. (20).

were conducted as described for undissociated proteins.

Similarity coefficients (SC) were calculated for native protein profiles as the percentage of the common bands relative to the total number of bands observed between two isolates (13). The following isolates were compared based on their SC values: (i) three representative Chilean isolates of *P. cryptogea* from kiwifruit were compared with the California isolates of *P. cryptogea* from kiwifruit, five other *Phytophthora* spp., and *Pythium ultimum*; and (ii) a larger representative sample of nine Chilean isolates of *P. cryptogea* from kiwifruit were compared with four isolates of this species from fruit trees in New York. The SC calculations included all protein band positions without consideration of band densities.

Isozyme analysis. Undissociated proteins were separated electrophoretically as described above. The activity of nonspecific alpha-esterase (EST, Enzyme Commission [EC] number 3.1.1.1) (13,14) was detected as described by Esbenshade and Triantaphyllou (10). Gels were immersed in a staining solution (0.1M $KHPO_4$ at pH 7.2, 1 mM EDTA, 2.15 mM alpha-naphthyl acetate prepared in 2 ml of 79% acetone, and 1.55 mM Fast Blue RR [Sigma]) for 1 h at 25 to 27 °C, then fixed in 10% glycerol plus 10% acetic acid and dried (15). Malate dehydrogenase (MDH, EC 1.1.1.37) (13) and super oxidismutase (SOD, EC 1.15.1.1) (13) activities were detected as described for alpha-esterase activity, except that gels were immersed in a different staining solution (75 mM Tris-HCl at pH 7.9, 10 mM L-malic acid, 1 M sodium carbonate, 0.37 mM nitro blue tetrazolium [NBT], 0.065 mM fenazine metasulfate, and 750 mM ß-nicotinamide adenin dinucleotide [NAD] as a cofactor) (20). Isozyme activity was recorded based on the relative mobility of the banding patterns and each band was interpreted as an allele of a specific locus (19,23,24).

Morphological and physiological characters. All Chilean *P. cryptogea* isolates were examined for (i) colony morphology on V8-juice agar after 4 to 5 days incubation at 20 C, (ii) morphology and dimensions of sporangia produced in nonsterile soil extract solution, and (iii) the production of gametangia in single culture or in the presence of known A_1 and A_2 mating types of *P. cinnamomi*, using techniques described previously (17,18). Temperature responses were determined for the same isolates by transferring small pieces of agar cut from the edge of actively growing colonies onto duplicate dishes of Difco corn meal agar and measuring radial growth after 4 days incubation at 5, 20, 30, and 35°C.

Ability to induce symptoms of pink rot on potato tubers was evaluated for all Chilean isolates of *P. cryptogea* and compared with two representative *P. cryptogea*

isolates from New York fruit trees (NY155 and NY414). Mycelial mats of each isolate were produced on carrot juice broth for 4 days at 20 to 23°C as described above, and washed mats were macerated in sterile distilled water for 3 to 5 min at high speed in a blender. The inoculum suspension was adjusted to 1.4×10^6 fragments per ml with the aid of a hemacytometer, and 0.5 ml was delivered into each of two wells produced on a surface-disinfested potato tuber using a 5-mm-diameter cork borer. Wounds were sealed with Parafilm and tubers were incubated in a sealed plastic box for 4 days at 23°C. Then tubers were split with a knife and exposed to the air for 30 or 60 min at room temperature, and rated for the presence or absence of typical pink rot symptoms (20).

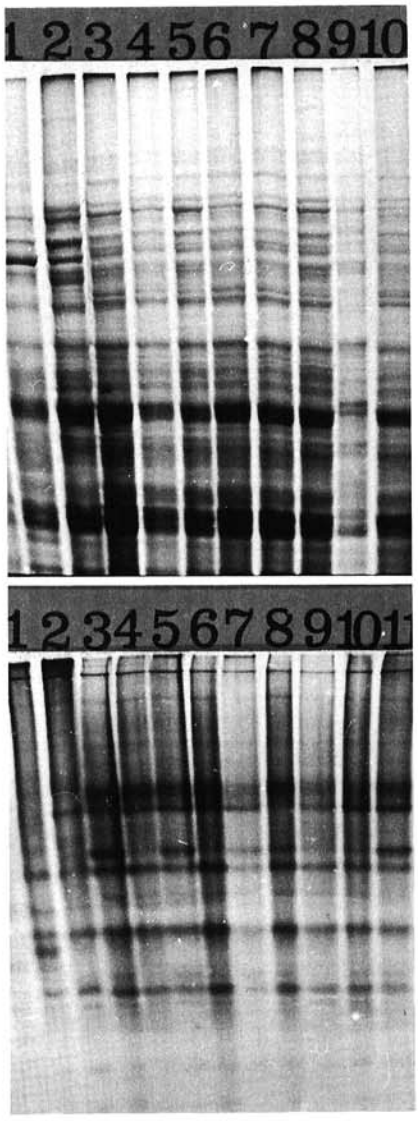

Fig. 1. Representative mycelial protein profiles of *Phytophthora cryptogea* isolates from kiwifruit, characterized using polyacrylamide gel electrophoresis of sodium dodecyl sulfate–dissociated proteins (above) and undissociated native proteins (below). In the upper photo, lane 1 was isolate 22-4-2 from California; in lanes 2 through 10 were isolates from Chile: 2 = K-13-4; 3 = A17-2S(20); 4 = A17-4N(21); 5 = A14-2N(02); 6 = A14-1N(05); 7 = A18-2N(05); 8 = A18-3E(04); 9 = A28-2N(02); and 10 = A15-1S(10). In the lower photo, lane 1 was isolate 22-4-2 from California; in lanes 2 through 11 were isolates from Chile: 2 = K-13-4; 3 = A17-2S(20); 4 = A17-4N(21); 5 = A14-2N(02); 6 = A14-1N(05); 7 = A14-1S(07); 8 = A18-2N(05); 9 = A18.2S(03); 10 = A18-3E(04); and 11 = A28-1N(01).

RESULTS

Electrophoretic protein profiles. All 33 *P. cryptogea* isolates from kiwifruit in Chile and the single kiwifruit isolate from California formed a homogeneous group based on electrophoretic patterns of undissociated proteins, although a few unique bands were present in Chilean isolate A28-2N(02) (Fig. 1). Protein profiles of Chilean kiwifruit isolates were similar to those of California *P. cryptogea* isolates 13-4-9 and 14-2-5 from cherry and apple, respectively; however, they differed from four *P. cryptogea* isolates from fruit crops in New York, which formed a separate homogenous group (Fig. 2). SCs ranged from 51 to 80% in comparisons between the two California fruit tree isolates of *P. cryptogea* and selected Chilean kiwifruit isolates of the species. The SC was 62% when these two California isolates were compared with each other and ranged from 80 to 97% for comparisons between the individual Chilean isolates (Table 2). In contrast, comparisons between Chilean kiwifruit isolates and New York fruit tree isolates of *P. cryptogea* yielded SC values of 19 to 25%, which were within the range obtained for interspecific comparisons between the kiwifruit isolates and isolates of *P. cactorum*, *P. cinnamomi*, and *P. megasperma* (SC = 17 to 33%) (Table 2).

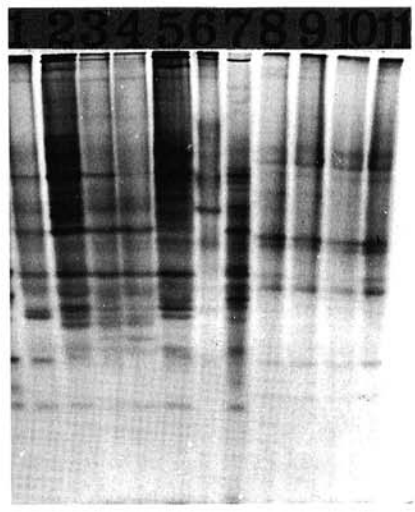

Fig. 2. Mycelial protein profiles of 10 *Phytophthora cryptogea* isolates and an unidentified *Phytophthora* sp., as differentiated by polyacrylamide gel electrophoresis of undissociated proteins. In lanes 1 through 4 were isolates obtained from kiwifruit in Chile (K-13-4, K-16-0, A18-3E(04), and A15-1S(10), respectively); in lanes 5 through 7 were isolates obtained from apple and cherry in California (14-2-5, 18-3-1 = unidentified *Phytophthora* sp., and 13-4-9, respectively); in lanes 8 through11 were isolates obtained from cherry and peach in New York (NY415, NY155, NY413, and NY414, respectively).

When a second group of isolates were examined, kiwifruit isolates of *P. cryptogea* from Chile and California were similar (SC = 56 to 59% in comparisons among three Chilean isolates and the single California isolate, SC = 68 to 78% for comparisons among the Chilean isolates themselves). However, all four of these kiwifruit isolates were pronouncedly different from the two isolates of *P. citrophthora* (SC = 15 to 25%), three isolates of *P. citricola* (SC = 15 to 36%), one isolate of *P. cactorum* (SC = 15 to 42%), two isolates of *P. megasperma* (SC = 15 to 33%), and two isolates of *P. cinnamomi* (SC = 9 to 27%) with which they were compared (Table 3). Intergeneric comparisons between the isolate of *Pythium ultimum* and the 14 isolates of *Phytophthora* spp. yielded SC values of 8 to 11% (Table 3).

Although conforming to the description of *P. cryptogea sensu lato* (26,27), the unidentified *Phytophthora* sp. isolate 18-3-1 from cherry in California appeared unrelated to any of the *P. cryptogea* isolates, with SC values of 23 to 36, 32 to 39, and 37 to 40% when compared with the two California fruit tree isolates, four Chilean kiwifruit isolates, and four New York fruit tree isolates, respectively (Table 2).

Separation of dissociated proteins. A similar banding pattern was obtained for SDS-dissociated proteins from each of the 33 Chilean kiwifruit isolates of *P. cryptogea* and the single California kiwifruit isolate of the species (Fig. 1). Four bands (Rf: 55 to 56, 67 to 72, 84 to 88, and 87 to 90), corresponding to molecular weights of 30,000 to 45,000, characterized the protein profiles of these isolates. The SDS banding patterns of *P. cryptogea* were different from those obtained for *P. cactorum*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. megasperma*, and *Pythium ultimum* (Fig. 1).

Isozyme analysis. Based upon EST activity, two distinctive phenotypes were present among the *P. cryptogea* isolates from Chile and California (Fig. 3). Twenty-nine of the 33 Chilean kiwifruit isolates, the single California kiwifruit isolate, and one of the two California fruit tree isolates belonged to phenotype A, which consisted of one distinct band (Rf: 46). The remaining four Chilean kiwifruit isolates and one California fruit tree isolate belonged to phenotype A′, which consisted of this band plus two additional distinct bands (Rf: 43 and 50) (Fig. 3). Additional faint bands occurred at relative migration distances outside the range considered for analysis. Isolates of *P. cryptogea* from New York gave a very weak or diffuse banding pattern, which was impossible to characterize as a distinct phenotype. Isolates of *P. cactorum*, *P. cinna-*

momi, and *P. megasperma* each produced a single, unique esterase band (Rf: 22, 25, and 37, respectively).

Six distinct MDH phenotypes were determined for the *P. cryptogea* isolates examined. These six phenotypes corresponded to: 26/27 Chilean kiwifruit isolates (Rf: 11, 31, 34, and 39); 1/27 Chilean kiwifruit isolates (Rf: 11, 24, 31, 32, and 37); the single California kiwifruit isolate (Rf: 34); the single California cherry isolate (Rf: 35 and 49); the single California apple isolate (Rf: 34 and 36); and 6/6 isolates from fruit crops in New York (Table 1). Each of these MDH phenotypes was distinct from the MDH phenotypes of all other *Phytophthora* spp. included in the study (Table 1).

All kiwifruit isolates of *P. cryptogea* showed a single SOD phenotype, which also was shared by the two *P. cryptogea* isolates from fruit trees in California and by the two isolates each of *P. citricola* and *P. citrophthora*. Two additional phenotypes were represented by the two isolates of *P. cactorum* and the single isolate of the unidentified (Group VI) *Phytophthora* sp., respectively (Table 1). No detectable SOD activity was found among the six isolates of *P. cryptogea* from fruit trees in New York, nor among isolates of *P. cinnamomi*, *P. megasperma*, or *Pythium ultimum*.

Morphological and physiological characters. All Chilean isolates were similar to those described previously from kiwifruit (7,17) and fit the description of *P. cryptogea* (21,25,26). Sporangia were ovoid to ellipsoid or obpyriform with a length/breadth ratio <1.6, nonpapillate, and internally proliferating. Globose hyphal swellings were produced by some isolates in aqueous soil extract. Gametangia were produced most frequently in the presence of an A_2 mating type of *P. cinnamomi*. No isolate grew at 35°C, whereas 9 out of 33 grew at 5°C. A radially stellate growth pattern was produced by Chilean isolates on V8-juice agar, whereas the New York isolates of *P. cryptogea* produced a distinctive petalloid and cottony pattern (Fig. 4). All Chilean isolates and the two New York isolates of *P. cryptogea* produced typical pink rot lesions on inoculated potato tubers.

Table 2. Similarity coefficients from comparisons of representative isolates of *Phytophthora cryptogea* from Chile, California, and New York plus three additional *Phytophthora* spp., based upon electrophoretic banding patterns of native mycelial proteins

		P. cryptogea										Other *Phytophthora* spp.[y]			
		Chile				California		New York							
		Kiwifruit				Apple	Cherry	Cherry/peach				sp.	cac	meg	cin
Isolate		A1	A2	A3	A4	B1	B2	C1	C2	C3	C4	D1	E1	F1	G1
A1	K-13-4	100[z]	80	88	57	57	66	20	20	20	20	34	17	23	28
A2	K-16-0		100	91	92	80	68	25	19	19	19	38	31	21	33
A3	A18-3E (04)			100	97	73	51	25	25	19	25	39	28	23	25
A4	A15-1S (10)				100	59	51	19	20	20	20	32	28	23	25
B1	14-2-5					100	62	35	37	37	37	36	21	16	28
B2	13-4-9						100	22	17	17	22	23	20	20	22
C1	NY415							100	92	92	92	37	19	19	11
C2	NY155								100	100	92	40	19	19	11
C3	NY413									100	92	37	19	19	11
C4	NY414										100	37	19	19	11
D1	18-3-1											100	31	26	17
E1	M-12-0												100	28	19
F1	E-2-0													100	15
G1	22-1-3														100

[y] sp. = unidentified *Phytophthora* sp. (Waterhouse Group VI) from cherry in California; cac = *P. cactorum* from apple in Chile; meg = *P. megasperma* from asparagus in Chile; and cin = *P. cinnamomi* from almond in California.

[z] Similarity coefficient for each pair of compared isolates was calculated as (number of bands in common) ÷ (total number of bands for the two isolates) x 200.

Table 3. Similarity coefficients from comparisons of representative isolates of*Phytophthora cryptogea* from kiwifruit vines in Chile and California plus five additional *Phytophthora* spp. and *Pythium ultimum*, based upon electrophoretic banding patterns of native mycelial proteins

		Phytophthora spp.														*Pythium*
		cryptogea				*citrophthora*		*citricola*			*cactorum*	*megasperma*		*cinnamomi*		*ultimum*
Isolate[y]		A1	A2	A3	A4	B1	B2	C1	C2	C3	D1	E1	E2	F1	F2	G1
A1	K-13-5	100[z]	68	70	59	17	20	19	22	15	15	20	20	27	27	8
A2	K-17-4		100	78	59	25	19	21	24	25	33	15	16	9	9	8
A3	K-17-3			100	56	22	18	21	24	25	33	16	16	9	9	8
A4	22-4-2				100	15	19	21	35	36	42	33	33	25	25	11
B1	F-16-12					100	73	13	17	25	17	32	32	16	16	8
B2	K-14-1						100	13	17	25	33	46	46	27	27	8
C1	F-12-1							100	82	75	28	10	10	38	38	10
C2	F-11-0								100	74	41	42	42	30	31	8
C3	18-1-1									100	33	25	25	17	17	10
D1	M-12-0										100	40	38	23	32	10
E1	E-1-0											100	85	26	26	9
E2	E-2-0												100	29	22	10
F1	14-3-3													100	78	8
F2	22-1-3														100	10
G1	Py-10-0															100

[y] Host and geographical origin of individual isolates is as follows: A1 through A3 = kiwifruit, Chile; A4 = kiwifruit, California; B1 = raspberry, Chile; B2 = kiwifruit, Chile; C1 and C2 = raspberry, Chile; C3 = raspberry, California; D1 = apple, Chile; E1 and E2 = asparagus, Chile; F1 = apple, California; F2 = almond, California; G1 = unknown host, New York.

[z] Similarity coefficient for each pair of compared isolates was calculated as (number of bands in common) ÷ (total number of bands for the two isolates) x 200.

DISCUSSION

All 33 isolates of *P. cryptogea* obtained from kiwifruit vines in eight geographical districts within the Central Valley of Chile composed a homogeneous group based upon their morphological and physiological characters, electrophoretic profiles of dissociated and undissociated soluble mycelial proteins, and their MDH and SOD enzymatic phenotypes. These isolates clearly fit the description of *P. cryptogea sensu lato* (21,25,26), and our identification was confirmed by IMI, CAB International (Egham, Surrey, UK) for five representative isolates (Table 1).

The Chilean kiwifruit isolates were similar to two California isolates of *P. cryptogea* from deciduous fruit trees with respect to electrophoretic profiles of total mycelial proteins and were indistinguishable with respect to EST and SOD isozymic phenotypes. The sole California isolate of *P. cryptogea* from kiwifruit also exhibited a similar protein electrophoretic profile and the same enzyme phenotypes. However, the Chilean kiwifruit isolates, the California kiwifruit isolate, and the California fruit tree isolates exhibited five different MDH phenotypes.

Mills et al. (20) examined these same two California fruit tree isolates and placed them into their Group C based on analyses of isozyme systems and mitochondrial DNA restriction fragment length polymorphisms (mtDNA). They concluded that isolates in this group were related closely to those in their Group E, which included kiwifruit isolates from California, but maintained the groups separately because the isolates within each were considered identical according to isozyme and mtDNA analysis. We also found that these two "Group C" isolates were related closely to kiwifruit isolates from Chile and California, but found them to differ as much from each other as they did from the kiwifruit isolates with which they were compared using our analytical criteria. These data indicate that isolates identified as *P. cryptogea* from kiwifruit in Chile and from at least some deciduous fruit plants in California represent the same or an extremely similar organism.

In contrast, isolates identified as *P. cryptogea* from deciduous fruit crops in New York showed virtually no relatedness to either the Chilean or California isolates with respect to electrophoresis of total mycelial proteins or isozyme phenotypes. Bielenin et al. (1) also found a similar group of fruit tree isolates from the Great Lakes region (including four of the New York isolates used in our study) to differ distinctly from other isolates of *P. cryptogea*, including those from fruit trees in California. Similarly, Mills et al. (20) found such a group to represent a distinct genetic entity with no apparent relatedness to any of the other currently identified groups of *P. cryptogea*. Collectively, these three studies support the suggestion that fruit tree isolates from the Great Lakes region previously identified as *P. cryptogea* may actually belong to an as yet unidentified taxon (1). The unidentified *Phytophthora* sp. isolate 18-3-1 examined in

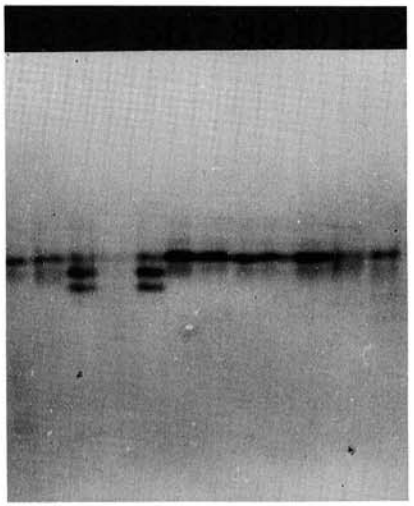

Fig. 3. Esterase phenotypes of *Phytophthora cryptogea* isolates from kiwifruit from Chile (lanes 1 through 11) and California (lane 12). The isolates in lanes 3 and 5 are phenotype A′, whereas all others are phenotype A. The specific isolates in each lane: 1 = K-17-1; 2 = K-17-4; 3 = A15-1S(10); 4 = A28-2N(03); 5 = A18-3E(04); 6 = A18-2N(05); 7 = A14-1N(05); 8 = A14-2N(02); 9 = A17-4N(21); 10 = A17-2S(20); 11 = K-13-4; and 12 = 22-4-2.

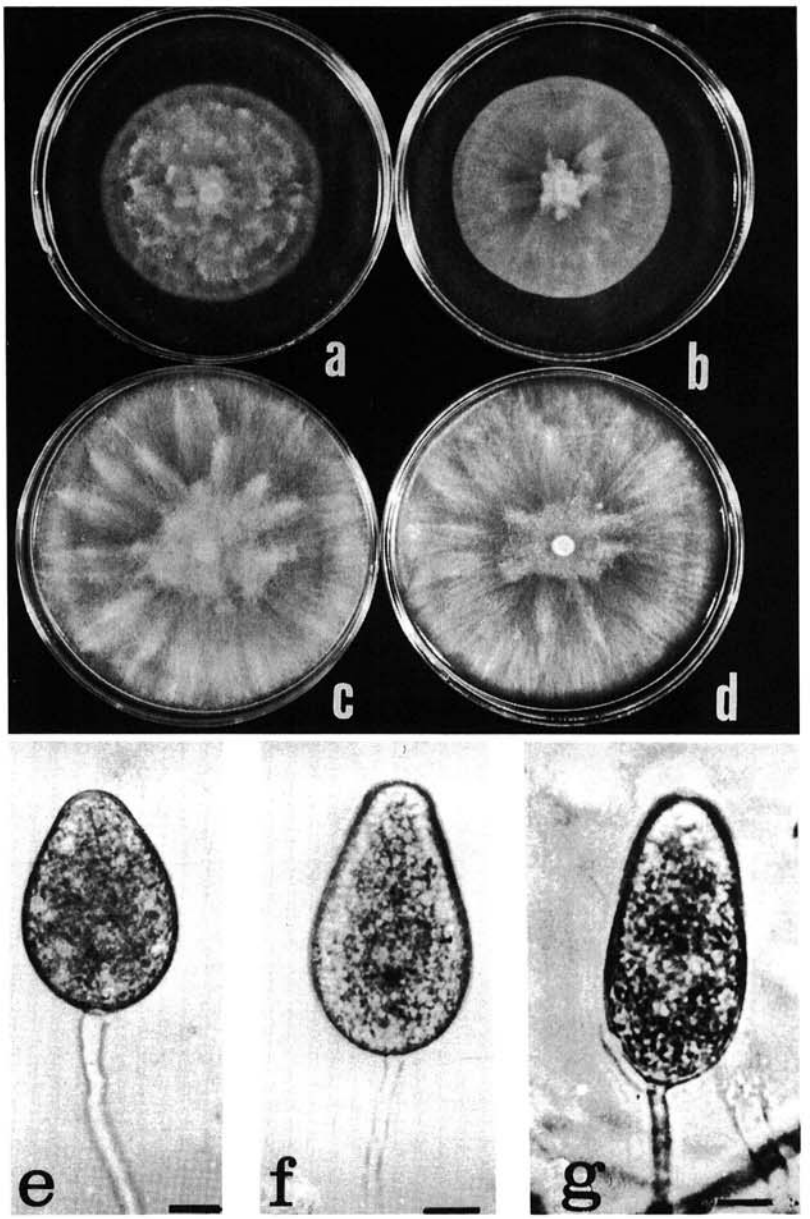

Fig. 4. Morphological features of isolates of *Phytophthora cryptogea*. (**A** through **D**) Colony morphology on V8-juice agar after 5 days incubation at 20 C. **A** and **B**, isolates NY415 and NY155 from peach and cherry in New York, respectively; **C**, isolate 14-2-5 from apple in California; and **D**, isolate A18-2S(101) from kiwifruit in Chile. (**E, F, G**) Range in sporangial morphology found among Chilean isolates from kiwifruit.

the present study was also electrophoretically and isozymically distinct from all *P. cryptogea* isolates, although it fit the description of the species. Thus, the current broad definition of *P. cryptogea* (21,25,26) is clearly inadequate for distinguishing among unrelated organisms that may share the general characters upon which it is based (1,20,28).

Our study confirms the value of using electrophoretic protein profiles and isozyme analysis to characterize fungi and identify species within the genus *Phytophthora* (1,2,4–6,8,9,11–13,15,19,20,22–24, 30), and to differentiate groups within *P. cryptogea* as the species is currently defined (1,20). We found that undissociated proteins provided superior results to those obtained using SDS-dissociated proteins, since they were easier to prepare and resulted in fewer bands that were easier to interpret. These same advantages were noted by Bielenin et al. (1), although they also noted advantages of SDS-dissociated profiles that we did not encounter. Of the three enzyme systems that were evaluated, a high activity was found for esterase, which provided a high resolution and reproducible results. Except for *P. cactorum*, *P. capsici*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. nicotianae*, and *P. palmivora* (12,13), other *Phytophthora* spp. have not been widely characterized for this trait. The genetic interpretation of EST banding fits the pattern suggested for a dimeric enzyme controlled by three electrophoretically distinct alleles (19).

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