Genetics of *Phytophthora infestans*: Determination of Recombination, Segregation, and Selfing by Isozyme Analysis

R. C. Shattock, P. W. Tooley, and W. E. Fry

Visiting fellow, research associate, and professor, respectively, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.
Permanent address of senior author: School of Plant Biology, University College of North Wales, Bangor, Gwynedd, LL57 2UW, U.K.
We acknowledge support from the NATO Science Programme, Ciba-Geigy Corporation, the U.S. Department of Agriculture CRGO Grant 0034157 and the technical advice and support of J. A. Sweigard.
Accepted for publication 25 September 1985.

ABSTRACT

Shattock, R. C., Tooley, P. W., and Fry, W. E. 1986. Genetics of *Phytophthora infestans*: Determination of recombination, segregation, and selfing by isozyme analysis. Phytopathology 76:410-413.

High levels of oospore germination (greater than 50%) were observed in some matings between A1 and A2 compatibility types of *Phytophthora infestans*. The parental isolates were predominantly of recent Mexican origin. The electrophoretic patterns at two enzyme loci, glucosephosphate isomerase and peptidase were identified in 685 single-oospore progeny from seven crosses. Progeny from parents homozygous for different alleles were heterozygous. Progeny from parents heterozygous for these alleles segregated 1:2:1 (homozygous fast:heterozygous:homozygous slow), as expected for a diploid organism. The ratio of A1:A2 compatibility type in the progeny was generally 1:1. A large number of self-fertile progeny were recorded from several crosses.

Knowledge of the genetics of the genus *Phytophthora* has developed slowly and fitfully during the last 30 yr. Results of early studies with *Phytophthora infestans* demonstrated that oospores were common in central Mexico, where both A1 and A2 compatibility types existed. The fungus was primarily heterothallic, though some isolates appeared to be self-fertile and selfing appeared to be a possible phenomenon in nature (5,7,21). Following the discovery of the sexual stage, numerous genetic analyses were attempted (6). Although single-oospore progeny showed recombination for virulence and sexual compatibility type in all these studies, progress in understanding the patterns of inheritance was severely hampered by difficulties in germinating oospores and establishing single-oospore cultures. For example, Romero and Erwin (13) detected high levels of oospore germination in some crosses, but established only 34 single-oospore cultures. In another study with siblings, only 4 of 620 germinated oospores were established in pure culture, thus preventing a study of inheritance in F2 progeny (2).

Despite these difficulties with crosses of *P. infestans*, knowledge of the genetics of *Phytophthora* has been advanced by using other techniques and other species (4,8,9). Cytological investigations of chromosomal divisions indicated that *P. infestans* and other heterothallic *Phytophthora* species have gametangial meiosis and are, therefore, diploid (1,16). These studies have been reviewed by Shaw (19,20).

The need for genetic analysis of *P. infestans* remains. The genetic control of pathogenicity and virulence is not yet defined and the mechanisms of variation are still ambiguous. However, progress in this area requires fertile parents, methods for establishing single oospore cultures, and easily scored genetic markers. To this end, we have recently collected isolates from the sexual population in Mexico and developed isozymes as genetic markers in *P. infestans* (22).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

We report here the successful production and germination of oospores and establishment of single-oospore cultures of *P. infestans* and the use of isozyme banding patterns to distinguish selfs from hybrid progeny.

MATERIALS AND METHODS

Cultures. Parental isolates were selected on the basis of their isozyme banding patterns at two enzyme loci, glucosephosphate isomerase (GPI-1) and peptidase (PEP) (22) (Table 1). Nine of the 10 parental isolates were of recent Mexican origin and the other was obtained from Wales (22). All manipulations with Mexican isolates were done under containment conditions. All 10 isolates were single-zoospore lines obtained by using the method of Caten and Jinks (3).

Oospores were produced when isolates of opposite mating type were cultured together. Three strips (50 × 5 mm) of parental isolates growing on 20% clarified V-8 juice agar (C-V8) (17) were arranged on C-V8 agar containing β-sito-sterol (40 μg/ml), $CaCO_3$ (0.2%), and Difco Bacto-Agar (0.6%). The mycelial strips were arranged on 9-cm-diameter petri dishes so that the isolate in the center was of one compatibility type, and the isolate on each side was of the other compatibility type. Hyphae from these strips made contact with each other within 3–4 days when incubated in sealed petri dishes at 20 C in darkness.

Snails were used to extract oospores from the mycelia as described elsewhere (18). Oospores were fed to snails within 10–28 days after matings were established. After surface disinfestation, oospores were spread on distilled water agar (0.6%). Oospores were incubated under continuous blue light (430–490 nm) with background white light at 18 C, and observed daily for germination. Once germination was detected (usually after 3–5 days) a thin overlay of particle-free rye A broth (3) was added. Germlings and small single-oospore colonies were transferred to rye B (3) or V-8/lima bean agar supplemented with vancomycin and rifampicin as described previously (18).

The compatibility type of single-oospore cultures was determined by pairing each culture against a single A1 isolate (isolate 515 [22]) and the presence or absence of oospores was recorded. In addition, between 15 and 20% of single-oospore cultures from each cross were checked against a single A2 isolate (isolate 503 [22]). Each single-oospore culture growing on 10% C-V8 or V-8/lima bean agar was examined for oospores after 14–21 days of growth. The presence of oospores indicated self-fertility.

TABLE 1. Compatibility type, and presumed genotypes at the glucose-phosphate isomerase (GPI-1) and peptidase (PEP) loci in parental isolates of *Phytophthora infestans*

Isolate	Compatibility type	Presumed genotype[a]	
		GPI-1	PEP
503	A2	100/122[b]	
511	A2	100/100	92/100
515	A1	100/100	
519	A2	86/100	
525	A2	100/100	
529	A1	86/100	
533	A1	122/122	92/100
536	A2	100/100	
550	A2	86/86	
40/34	A1	100/100	

[a] The allele coding for the most commonly occurring isozyme at a locus is designated 100. Other alleles at that locus are designated in reference to the most common allele. When an allele coded for an isozyme that migrated 22% further than the most common (reference) one, it was designated 122. When an allele coded for an isozyme which migrated 92% as far as the reference allele, it was designated 92.

[b] Both GPI and PEP are dimeric enzymes. Each allele produces a subunit of the dimeric enzyme. Homozygotes (e.g., 100/100) produce single-banded patterns made up of homodimers of a particular mobility. Heterozygotes (e.g., 86/100) produce three-banded patterns because in addition to fast and slow homodimers, heterodimeric forms with intermediate mobility, are also present.

Gel electrophoresis and allelic nomenclature. Electrophoresis of mycelial homogenates of parental and progeny cultures was accomplished as previously described (22). Gels were stained to detect glucosephosphate isomerase (GPI) and peptidase (PEP). Bands are described in terms of their relative mobilities. The mobility of the most commonly occurring band is designated as 100, and the mobilities of other bands are described in relation to the most common one. Thus, a value of 92/100 for isozymes of peptidase means that in addition to the most commonly occurring band, another band occurred which migrated 92% as far as did the common isozyme. Since both enzymes are dimeric, heterozygotes also have a third heterodimeric band which migrates approximately halfway between the two homodimeric bands (Table 1).

RESULTS

Oospores were produced in all crosses between A1 and A2 compatibility types (Table 2). The manner of germination was similar for oospores from all crosses, but the level of germination differed dramatically among crosses. In almost all cases, germinated oospores produced from one to three germ sporangia (Fig. 1A). Germination was first observed after 3 days of incubation at 18 C on water agar. The contents of oospores became granular prior to germination. The cytoplasmic contents then flowed from the oospore into the germ tube and finally into the germ sporangium (Fig. 1C). The addition of rye broth encouraged direct germination of the germ sporangia and rapid development of branching hyphae. Germination of oospores from some crosses

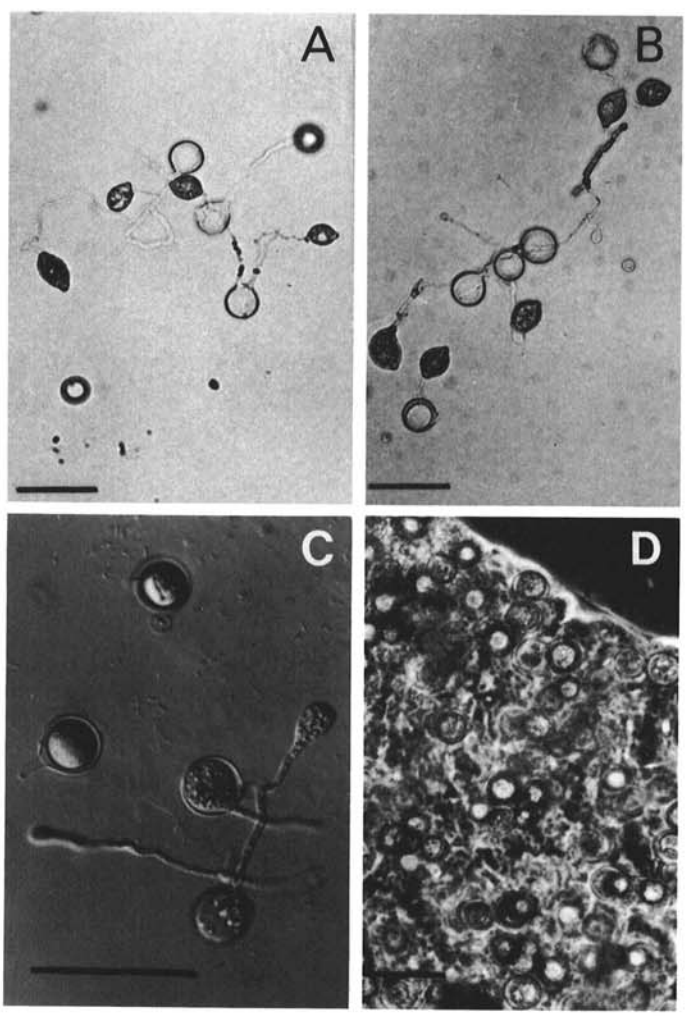

Fig. 1. Oospores of *Phytophthora infestans*. **A,** Germinating oospores showing multiple germ sporangia; **B,** clusters of germinating oospores; **C,** granular appearance of two germinating oospores, and **D,** densely packed oospores in fecal pellet of snail. Scale bar = 100 μm.

was greater than 50% (533 × 550, 533 × 511, 533 × 525, and 529 × 519). In other crosses, however, the germination was less than 5% (515 × 550, 529 × 536, and 40/34 × 503). In crosses with high levels of oospore germination, it was common to observe whole clusters of germinated oospores (Fig. 1B).

In most cases, there were similar numbers of A1 and A2 types in the progeny of a given cross (Table 2). However, in the progeny of one cross from which high numbers of progeny were established (533 × 525) the ratio of A1:A2 was approximately 2:1. In this cross, and in others involving isolate 533, a large number of the progeny were self-fertile.

Electrophoretic phenotypes at the GPI-1 locus were determined for 685 single-oospore progeny from seven crosses (Table 1). From the cross between 533 × 550, 329 of 332 progeny produced banding patterns consistent with hybridization between two diploid parents. Isolate 550 (A2) is a single banded phenotype for GPI and produces a slow-migrating homodimer (designated 86); isolate 533 (A1) is also a single banded phenotype, but it produces a faster-migrating homodimer (designated 122). The 329 hybrid progeny produced three-banded patterns (Fig. 2A). The faster and slower bands corresponded to the parental homodimers, and the middle band represented the heterodimer. The genotype of these 329 progeny is presumably 86/122. The remaining three single-oospore cultures gave single-banded patterns (genotype 122/122) and were also compatibility type A1. Therefore, these three cultures were identical to the parent, isolate 533.

Isolate 533 (A1) (GPI-1 presumed genotype = 122/122) was involved in two other crosses which generated large numbers of progeny whose banding patterns were consistent with hybridization in a diploid organism. Both of the additional A2 parents (isolates 525 and 511) had a single-banded GPI-1 genotype (100/100). All 158 progeny of cross 533 × 525 produced three-banded patterns consistent with heterozygotes of genotype 100/122 (Table 2). For cross 533 × 511, 107 of 109 progeny produced three-banded patterns consistent with hybrids with genotype 100/122. However, two progeny produced single-banded patterns (presumed genotype = 122/122).

The banding patterns observed at the loci coding for GPI and PEP in progeny from crosses in which both parents were heterozygous were also consistent with segregation in a diploid organism. In crosses 533 × 511 and 529 × 519, both parents were heterozygous (producing three-banded phenotypes) at one of the two enzyme loci. The progeny from each cross segregated 1:2:1 (homozygous fast:heterozygous:homozygous slow). At the PEP locus, isolates 533 and 511 both produced three-banded phenotypes consistent with a 92/100 genotype. The progeny segregated 1:2:1 (100/100:92/100:92/92, $\chi^2 = 1.74$). At the GPI-1 locus, isolates 529 and 519 each produced three-banded phenotypes consistent with a genotype of 86/100. The progeny from cross 529 × 519 segregated 1:2:1 (100/100:86/100:86/86, $\chi^2 = 0.65$) (Table 2).

Crosses 529 × 536, 40/34 × 503, and 515 × 550 produced limited additional information. Only banding patterns of the parental types were expected and were observed in 529 × 536 (Table 2). Similarly, only parental types were expected in progeny of cross 40/34 × 503, but recombination was observed for compatibility type and isozyme genotype at the GPI-1 locus. However, of the six progeny obtained from this cross, one of them appeared to have arisen via selfing and segregation in the A2 (isolate 503) parent (Tables 1 and 2). In cross 515 × 550, all progeny produced the expected hybrid genotype at the GPI-1 locus. Isolate 515 was homozygous for the most common allele (100/100) and isolate 550 was homozygous for an allele producing a slower isozyme (86/86).

TABLE 2. Compatibility type, and presumed genotypes at the glucosephosphate isomerase (GPI-1) and peptidase (PEP) loci in single oospore cultures of *Phytophthora infestans*

	Progeny (no.) of compatibility type			GPI-1		PEP	
Parents	A1	A2	Self-fertile	Genotype	Progeny (no.)	Genotype	Progeny (no.)
533 × 550	150	140	71	86/122	329		
				122/122	3		
515 × 550	4	12	3	86/100	22		
529 × 536	3	4	0	86/100	3		
				100/100	4		
40/34 × 503	4	2	0	100/100	2		
				100/122	3		
				122/122	1		
533 × 511	41	44	28	100/122	107	100/100	28[a]
				122/122	2	92/100	61
						92/92	22
533 × 525	63	33	38	100/122	158		
529 × 519	26	22	7	100/100	13[b]		
				86/100	23		
				86/86	15		

[a]The χ^2 value for an expected ratio of 1:2:1 with two degrees of freedom is 1.74.
[b]The χ^2 value for an expected ratio of 1:2:1 with two degrees of freedom is 0.65.

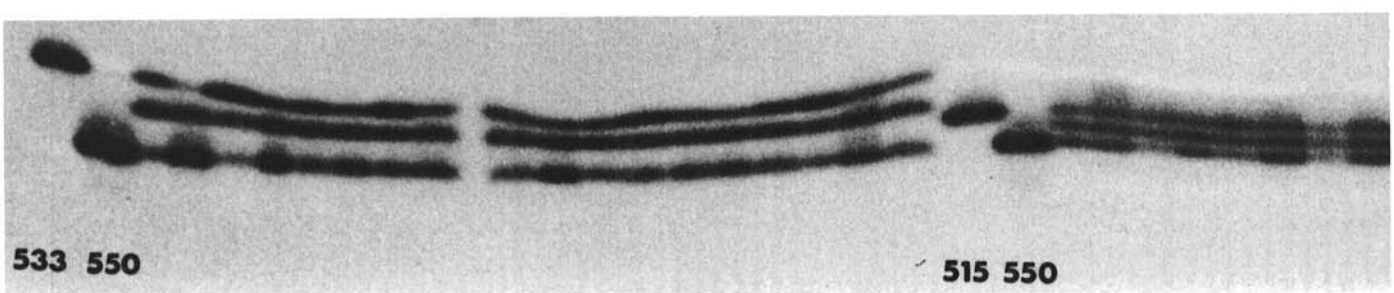

Fig. 2. Electrophoretic phenotypes at the glucosephosphate isomerase-1 locus of parental and single-oospore cultures of *Phytophthora infestans*. Isolates 533 (122/122) and 550 (86/86) are parental types followed by 18 hybrid progeny (86/122). Isolates 515 (100/100) and 550 (86/86) are parents followed by eight hybrid progeny (86/100).

Progeny were three banded, which is consistent with the heterozygous genotype (86/100) (Table 2).

DISCUSSION

The application of isozyme analysis has enabled positive identification of hybrids in single-oospore progeny from mating cultures of *P. infestans*. When the parental isolates were homozygous for different alleles at the GPI-1 locus, progeny had the heterozygous three-banded pattern. Some of the progeny identified in this study had banding patterns and presumed genotypes which we had not seen previously in our collections of *P. infestans* (22). The three-banded pattern of 329 of 332 single-oospore progeny from cross 533 × 550 had not been detected previously.

Isozyme analysis enabled the detection of progeny which must have arisen from selfing rather than hybridization. A very few progeny studied in these crosses were identical to a parental isolate at the GPI-1 locus. Although the bisexual nature of *P. infestans* (5,18) permits selfing, its occurrence was limited. Of 685 progeny, only six were apparent selfs. Three came from cross 533 × 550, two came from cross 533 × 511, and one came from cross 40/34 × 503. The apparent self (A1) from cross 40/34 × 503 must have arisen from segregation at the compatibility type locus. The individual was homozygous (122/122) for GPI-1. Since the A2 parent (503) has GPI-1 genotype 100/122, and since A2 compatibility types are thought to be heterozygous (12,15), segregation must have occurred due to selfing of the A2 parent. The A1 parent (40/34) was homozygous (100/100) at the GPI-1 locus.

Our data strongly support the diploid nature of the *P. infestans* thallus. When parents were heterozygous for alleles at the GPI-1 and PEP loci, the progeny had expected ratios of 1:2:1 for homozygous fast:heterozygous:homozygous slow. The large numbers of progeny from crosses 533 × 511 and 529 × 519 enabled calculation of χ^2 values which indicated no significant differences between the observed and the expected ratios.

Further experiments to optimize conditions for oospore germination should be initiated. We did not determine whether or not oospore germination was enhanced via passage through snails. In some preliminary experiments, we detected oospore germination after agar cultures had been fragmented in a blender. The snails, however, effectively digest hyphae and sporangia which could contaminate single-oospore cultures (Fig. 1D). The occurrence of clusters of germinating oospores in crosses 533 × 550, 533 × 511, and 533 × 525 may indicate mutual stimulation of germination.

The occurrence of self-fertile individuals in progeny from all but two of the crosses described here is consistent with earlier observations with *P. infestans*, other heterothallic species of *Phytophthora*, and *Bremia lactucae* Regel (2,6,8,10,11).

The genetics of *P. infestans* can now be fully investigated. With the proper parental isolates, high levels of oospore germination and establishment of single-oospore cultures can be achieved. Our results support the suggestions of previous workers that in certain crosses the high level of germination is indeed from oospores and not from oogonia (6,14). The application of isozyme electrophoresis enables one to distinguish hybrids from selfs. Further analysis of hybrids produced in these studies should answer important questions concerning the genetics of *P. infestans*. For example, the inheritance of host-specific pathogenicity (virulence), compatibility type, and insensitivity to fungicides can now be investigated.

LITERATURE CITED

1. Brasier, C. M., and Sansome, E. 1975. Diploidy and gametangial meiosis in *Phytophthora cinnamomi*, *P. infestans* and *P. drechsleri*. Trans. Br. Mycol. Soc. 65:49-65.
2. Castro, J., and Zentmyer, G. A. 1969. Mortality of germinated F_2 oospores from crosses of F_1 single oospore cultures of *Phytophthora infestans*. (Abstr.) Phytopathology 59:10.
3. Caten, C. E., and Jinks, J. L. 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. Can. J. Bot. 46:329-348.
4. Elliott, C. G., and MacIntyre, D. 1973. Genetical evidence on the life-history of *Phytophthora*. Trans. Br. Mycol. Soc. 60:311-316.
5. Galindo, J., and Gallegly, M. E. 1960. The nature of sexuality in *Phytophthora infestans*. Phytopathology 50:123-128.
6. Gallegly, M. E. 1968. Genetics of pathogenicity of *Phytophthora infestans*. Annu. Rev. Phytopathol. 6:375-396.
7. Gallegly, M. E., and Galindo, J. 1958. Mating types and oospores of *Phytophthora infestans* in nature in Mexico. Phytopathology 48:274-277.
8. Khaki, I. A., and Shaw, D. S. 1974. The inheritance of drug resistance and compatibility type in *Phytophthora drechsleri*. Genet. Res. 23:75-86.
9. Long, M., and Keen, N. T. 1977. Genetic evidence of diploidy in *Phytophthora megasperma* var. *sojae*. Phytopathology 67:675-677.
10. Michelmore, R. W., and Ingram, D. S. 1980. Heterothallism in *Bremia lactucae*. Trans. Br. Mycol. Soc. 75:47-56.
11. Michelmore, R. W., and Ingram, D. S. 1981. Recovery of progeny following sexual reproduction of *Bremia lactucae*. Trans. Br. Mycol. Soc. 77:131-137.
12. Mortimer, A. M., Shaw, D. S., and Sansome, E. R. 1977. Genetical studies of secondary homothallism in *Phytophthora drechsleri*. Arch. Microbiol. 111:255-259.
13. Romero, S., and Erwin, D. C. 1969. Variation in pathogenicity among single-oospore cultures of *Phytophthora infestans*. Phytopathology 59:1310-1317.
14. Romero, S., and Gallegly, M. E. 1963. Oogonium germination in *Phytophthora infestans*. Phytopathology 53:899-903.
15. Sansome, E. 1980. Reciprocal translocation heterozygosity in heterothallic species of *Phytophthora* and its significance. Trans. Br. Mycol. Soc. 74:175-185.
16. Sansome, E., and Brasier, C. M. 1973. Diploidy and chromosomal structural hybridity in *Phytophthora infestans*. Nature 241:344-345.
17. Shen, C. Y., Bower, L. A., Erwin, D. C., and Tsao, P. H. 1983. Formation of sex organs in the A1 mating type of *Phytophthora infestans* induced chemically by A2 isolates of other species of *Phytophthora*. Can. J. Bot. 61:1462-1466.
18. Shattock, R. C., Tooley, P. W., and Fry, W. E. 1986. Genetics of *Phytophthora infestans*: Characterization of single-oospore cultures from A1 isolates induced to self by intraspecific stimulation. Phytopathology 76:000-000.
19. Shaw, D. S. 1983. The cytogenetics and genetics of *Phytophthora*. Pages 81-94 in: *Phytophthora*: Its Biology, Taxonomy, Ecology, and Pathology. D. C. Erwin, S. Bartnicki-Garcia, and P. H. Tsao, eds. American Phytopathological Society, St. Paul, MN.
20. Shaw, D. S. 1983. The Peronosporales: A fungal geneticist's nightmare. Pages 85-121 in: Zoosporic Plant Pathogens: A Modern Perspective. S. T. Buczacki, ed. Academic Press, London.
21. Smoot, J. J., Gough, F. J., Lamey, H. A., Eichenmuller, J. J., and Gallegly, M. E. 1958. Production and germination of oospores of *Phytophthora infestans*. Phytopathology 48:165-171.
22. Tooley, P. W., Fry, W. E., and Villarreal Gonzalez, M. J. 1985. Isozyme characterization of sexual and asexual *Phytophthora infestans* populations. J. Hered. 76:431-435.