Neth. J. Pl. Path. 98 (1992) 277 – 289

A comparison of morphology, pathogenicity and restriction fragment patterns of mitochondrial DNA among isolates of Phytophthora porri Foister

ARTHUR W.A.M. DE COCK[1]*, AFRA NEUVEL[2], GÜNTHER BAHNWEG[1]**, JOHANNA C.J.M. DE COCK[1]* and HERMANN H. PRELL[1]

[1] Gesellschaft für Strahlen- und Umweltforschung München, Abteilung für Molekulare Genetik, Grisebachstraße 6, D-3400 Göttingen, Germany.
[2] Zaadunie B.V., P.O.Box 26, 1600 AA Enkhuizen, the Netherlands.

Accepted 4 August 1992

Abstract

Thirteen strains of *Phytophthora porri* from five different hosts were compared with respect to their morphology, cardinal temperatures for growth, pathogenicity to leek and cabbage and restriction fragment patterns of mitochondrial DNA. Morphology of vegetative growth was rather similar in most isolates. Those characters which differed among isolates showed overlapping variability and could not be used to distinguish groups, with the exception of production of oogonia and sporangia and the antheridium type. Considerable differences were found in restriction patterns of mitochondrial DNA, isolates from the same host mostly showing identical patterns. Isolates from different *Allium* species showed relatively similar restriction patterns if compared to the other isolates. Isolates from *Brassica oleracea* proved to be a homogeneous group, quite different from the others with respect to restriction patterns, production of sporangia, production of oogonia, antheridium type and pathogenicity. One isolate, CBS 366.59, isolated from and pathogenic to *A. porrum*, deviated in many characters from the other isolates. It showed the restriction patterns of *Phytophthora nicotianae* and also the high cardinal temperatures for growth typical for this species. The sporangia, however, were distinctly non-papillate and the majority of antheridia was of the paragynous type.

Additional keywords: restriction fragment length polymorphism, RFLP, *Allium porrum, Brassica oleracea*.

Introduction

Phytophthora porri Foister was originally described as a pathogen of leek (*Allium porrum*), causing waterlogged areas followed by a whitening of the tips of leaves and other affected parts of this plant (Foister, 1931). Later it has been isolated principally from this host and other members of the Liliaceae (Van Hoof, 1959; Taylor, 1965; Tiche-

* On leave of absence from Centraalbureau voor Schimmelcultures, Baarn, the Netherlands. Present address: C.B.S. Yeast Division, Julianalaan 67, 2628 BC Delft, the Netherlands.
** Present address: G.S.F. Forschungszentrum für Umwelt und Gesundheit, Institut für biochemische Pflanzenpathologie, W-8042 Neuherberg, Germany.

laar & Van Kesteren, 1967; Katsura et al., 1969; Katsura, 1970; Griffin & Jones, 1977). *P. porri* has also been isolated from dicotyledonous hosts: from *Campanula persicifolia* (Legge, 1951), *Dianthus caryophyllus* (Kouyeas, 1977), *Chrysanthemum* sp. (Commonwealth Mycological Institute, 1983), *Daucus carota* (Stelfox & Henry, 1978; Ho, 1983) and several times from stored cabbages (Geeson, 1976, 1978; Semb, 1971).

Identification of these isolates was (probably) based on the following characters, diagnostic for *P. porri*: coiling growth of hyphae, semi-papillate sporangia, both amphigynous and paragynous antheridia, slow growth and low cardinal temperatures for growth (although the latter character is a point of discussion: Kouyeas, 1977).

From a phytopathological point of view, there is doubt whether isolates from *Brassica oleracea* are identical with those from *A. porrum* (Van Kesteren, personal communication). New approaches to taxonomy are available nowadays, and in particular restriction fragment analysis of mitochondrial DNA (mtDNA) has proven its value for taxonomy of *Phytophthora* (Förster et al., 1988, 1989, 1990; Hwang et al., 1991; De Cock et al., in preparation). We decided to apply this method to analyze 13 isolates from leek, cabbage and some other hosts. Four isolates from stored cabbage (*B. oleracea*) were received by the Centraalbureau voor Schimmelcultures in recent years, where they were identified as *P. porri* and included in the CBS collection. The strain Barr 369, isolated from *D. carota*, examined by Ho (1983), was also present in the CBS collection (CBS 688.79), as were four isolates from *A. porrum*. This number was extended by two isolates from *A. cepa* and one from *A. grayi*, which were received from the collection of the Institute for Fermentation in Osaka, Japan.

In addition to restriction-pattern analysis, an extensive study was made of the morphology of the isolates, their minimum, optimum and maximum temperatures for growth and their pathogenicity to *A. porrum* and *B. oleracaea*.

Material and methods

Fungal isolates. The isolates used in this study, their origin and accession numbers in some major fungal collections are listed in Table 1.

Cultivation of isolates. Stock cultures of isolates were maintained on cornmeal agar. Morphology was studied in cultures on cornmeal agar in petri dishes at several temperatures near and at the optimum in the dark, and in water cultures at temperatures between 15 and 20 °C in daylight. Water cultures were prepared by placing autoclaved hemp seeds on young, growing colonies for 1–2 days, followed by transfer of the colonized seeds to soil extract (sterilized extract of 200 g sandy soil in 1 l of distilled water).

Minimum, optimum and maximum temperatures for growth were determined in cornmeal agar cultures at temperatures between 0 and 39 °C at 3 °C intervals.

For DNA isolation small blocks of actively growing cultures on cornmeal agar were used to inoculate petri dishes containing pea broth: filtered and autoclaved decoction of 200 g frozen peas boiled for 20 min in 1 l of deionized water, to which 5 g of glucose was added.

Pathogenicity tests. Before inoculation of plants, the fungi were grown on hempseed agar (100 g of hemp seeds, boiled in 1 l of distilled water, filtered through one layer of cheese cloth, and autoclaved after adding 20 g of Difco agar) for 7 days, at

Table 1. Isolates used in this study, their host, geographic origin and collection accession numbers.

No.	Species	Host	Country	Accession numbers[a]
1	*P. porri*	*Allium porrum*	Netherlands	CBS 567.86
2	*P. porri*	*Allium porrum*	Netherlands	CBS 141.87
3	*P. porri*	*Allium porrum*	Belgium	CBS 142.87
4	*P. porri*	*Allium porrum*	Netherlands	CBS 181.87
5	*P. porri*	*Allium cepa*	Japan	CBS 138.87, IFO 30416
6	*P. porri*	*Allium grayi*	Japan	CBS 139.87, IFO 30417
7	*P. porri*	*Allium cepa*	Japan	CBS 140.87, IFO 30418
8	*P. porri*	*Brassica oleracea*	Netherlands	CBS 212.82
9	*P. porri*	*Brassica oleracea*	Germany	CBS 178.87
10	*P. porri*	*Brassica oleracea*	Netherlands	CBS 179.87
11	*P. porri*	*Brassica oleracea*	Netherlands	CBS 180.87
12	*P. porri*	*Daucus carota*	Canada	CBS 688.79
13	*P. porri*	*Allium porrum*	Netherlands	CBS 366.59, IMI 180617

[a] CBS = Centraalbureau voor Schimmelcultures, Baarn, the Netherlands; IMI = CAB International Mycological Institute, Kew, England; IFO = Institute for Fermentation, Osaka, Japan.

21 °C for 12 h in the dark and 12 h under UV light (360–370 nm).

Inoculation of leek plants: a wetted cotton plug, together with a small piece (1 cm^2) of an agar culture was put on a leaf, which was scarified with a knife. The leaf was subsequently folded and stuck together with plastic tape. Incubation was at 18–20 °C, 90% relative humidity and 14 h of light per day. Leek plants of three cultivars, Wintinia, Tillina and Carina, and of two different ages (2 and 4 months old) were treated this way with each of the isolates. These conditions for incubation were chosen according to the method described by Van Hoof (1959).

Inoculation of white cabbages: a hole 2 cm deep was punched with a cork-borer (0.5 cm diameter) in the stem of the cabbages. A piece of an agar culture was placed in this hole and the cabbages were incubated at 10–15 °C and 60% humidity in the dark. These conditions for incubation were chosen according to the method described by Geeson (1976). Each isolate was tested in two cabbages.

Disease development caused by the fungi was recorded 1 and 2 weeks after inoculation.

Isolation of mitochondrial DNA (mtDNA). Isolation of mtDNA from freeze-dried mycelium from pea broth cultures was performed as described before (Hwang et al., 1991).

Restriction-fragment analysis. mtDNAs were digested with the restriction endonucleases *Ava*II, *Hae*III, *Scr*FI, *Hpa*II, *Bst*NI and *Hin*dII following the instructions of the manufacturer (Boehringer Mannheim). These enzymes were selected because they produced numbers of fragments which were low enough to allow separation and detection by agarose gel electrophoresis, but high enough to permit calculation of

meaningful similarity coefficients. Digested DNA was analyzed by electrophoresis in 1% agarose gels in TAE buffer (40 mM Tris-acetate, 20 mM EDTA, pH 8.0) at 1.5 V/cm. Gels were stained with ethidium bromide (0.5 μg/ml) and photographed with incident UV light. The resulting restriction patterns were compared pairwise: the number of common bands as well as the total number of bands was determined for each pair. *Hae*III and *Hpa*II only generated fragments which were either unique for groups with identical patterns or common to all strains. They could thus not contribute to the determination of relationships and are therefore not included in the analysis. Similarity coefficients were calculated for the remaining four enzymes using the following formula:

$$F = 2\, n_{xy} / (n_x + n_y)$$

in which n_x and n_y are the numbers of bands in isolate x and y respectively, and n_{xy} is the number of bands shared by the two isolates (Nei and Li, 1979). This resulted in four similarity coefficients for each pair: one for each of the enzymes used. The average of these four values (Table 6) was used in group average cluster analysis (Sneath and Sokal, 1973), to construct a dendrogram showing the relationships of the isolates.

Results

Temperature–growth relationships (Table 2). All isolates except CBS 688.79 and 366.59 showed rather similar temperature–growth relationships: they all grew well at 3 °C (radial growth: 0.6–1.8 mm per day), suggesting that the minimum temperature for growth may be even lower. Their optimum temperature for growth was 18–21 °C.

Table 2. Temperature–growth relationships of isolates examined. For detailed explanation see text.

No.	Strain CBS no.	Temperatures for growth (°C)			Radial growth (mm/day)	
		minimum	optimum	maximum	at optimum	at 24 °C
1	567.86	3	18	24	3.9	1.0
2	141.87	3	21	27	5.2	2.4
3	142.87	3	21	27	4.1	1.3–2.7
4	181.87	3	18	21	2.7	0
5	138.87	3	21	27	5.0	1.0–2.0
6	139.87	3	18/21	27	5.8	4.3
7	140.87	3	21	27	6.2	4.0
8	212.82	3	21	24	5.2	5.0
9	178.87	3	21	24	6.2	5.1
10	179.87	3	21	27	6.4	2.8
11	180.87	3	21	27	6.5	5.4
12	688.79	3	24	30	3.7	3.7
13	366.59	9	30	36	8.8	8.5

Above the optimum the daily growth rate sharply declined; the maximum temperature was generally only 3–6 °C above the optimum. At this upper limit, growth often decreased slowly and ceased within a few days but resumed after transfer to room temperature. Temperatures above the maximum were lethal after 6 days. Isolate CBS 366.59 grew at very high minimum, optimum and maximum temperatures; at both extremes (9 and 36 °C) growth rate decreased to zero. These temperatures, however, were not lethal after 6 days.

The cardinal temperatures for growth and growth rates at the optimum and at 24 °C are presented in Table 2.

Morphology

Colony growth and mycelium. All isolates showed a similar colony development. Colonies grew submerged in agar but tended to produce some low, condensed aerial mycelium at higher temperatures. Mycelium growth was not visible macroscopically; however, sometimes a very vague chrysanthemum pattern was present. Hyphae were often irregular in width, with a diameter mostly between 2.5 and 7 μm. Coiled hyphae were sparse to abundant, except in CBS 366.59 where they were absent.

Sporangia (Table 3). All isolates developed sporangia in hempseed/water cultures; on agar they were produced abundantly by isolates from *Brassica* but only sparsely and/or occasionally by the others. Sporangia were either single or up to five on a sympodially elongating hypha. In CBS 366.59 rare internal proliferation of empty sporangia was observed. Morphology of sporangia was rather uniform within and

Table 3. Production and morphology of sporangia.

No.	Strain CBS no.	Sporangia produced[a]		Papilla type[b]	Length[c] (μm)	Width[c] (μm)	l : b[c] ratio
		on agar	in water				
1	567.86	±	+ +	S	53 – 74	41 – 48	1.4
2	141.87	–	+	N/S	38 – 79(– 122)	33 – 48(– 53)	1.5
3	142.87	±	±	N/S	36 – 48(– 53)	29 – 38(– 43)	1.3
4	181.87	–	±	N	53 – 67(– 81)	36 – 43(– 45)	1.5
5	138.87	–	–				
6	139.87	±	+ +	N/S	48 – 71	41 – 55	1.3
7	140.87	±	±	N/S	36 – 180	24 – 34(– 37)	dist.
8	212.82	+	+ +	N/S	40 – 55(– 80)	27 – 40(– 45)	1.5
9	178.87	+ +	+ +	N/S	37 – 55(– 80)	25 – 40(– 45)	1.4
10	179.87	+ +	+ +	S	45 – 69(– 74)	38 – 48	1.4
11	180.87	+ +	+ +	S	48 – 69	36 – 50	1.3
12	688.79	±	+	S	65 – 79(– 91)	48 – 57	1.3
13	366.59	±	+ +	N	32 – 48(– 60)	19 – 30(– 33)	1.8

[a] – = absent, ± = sparse, + = moderate number, + + = abundant.
[b] N = non-papillate, S = semi-papillate. Measurements from water cultures only.
[c] Range of common sizes; occasionally occurring extreme sizes at the upper limit are given in parentheses; l:b ratio = average length to breadth ratio; dist. = distorted shapes.

Table 4. Production and morphology of sexual structures and chlamydospores. Data for sexual structures from cornmeal agar cultures, for chlamydospores from water cultures. Data for CBS 688.79 were taken from Ho (1983).

No.	Strain CBS no.	Oogonia[a]		Oogonium wall[b]					Antheridia[c]		Chlamydospores[d]	
		production	size (μm)	thckn (μm)	und	cll	py	dy	para	amphi	production	size (μm)
1	567.86	+	(27–)38–48	4	+	+	+	+	++	±	+	48
2	141.87	+	(29–)36–55	3	+	+	–	+	++	±	±	50
3	142.87	+	31–43(–48)	2.5	+	+	+	+	++	–	±	53
4	181.87	+	(24–)29–43(–48)	2.5	+	–	+	+	++	±	–	
5	138.87	+	(36–)43–53	3	+	+	+	+	++	±	–	
6	139.87	+	(24–)29–41(–45)	2.5	+	+	–	+	+	±	++	50
7	140.87	+	(31–)38–48	2.5	+	–	+	+	++	–	–	
8	212.82	i	42–65	6	+	+	–	+	+	+	±	35
9	178.87	i	29–36	4	+	+	–	–	±	++	+	30
10	179.87	i	30–41	2	+	+	+	–	±	++	+	53
11	180.87	–									+	50
12	688.79	i	28–37	2.5	+	+	–	–	±	++	++	65
13	366.59	i	21–32	1	–	+	–	–	++	±	±*	30*

[a] Production: + = moderate to abundant, i = occasionally developed, – = never observed. Size: range of common sizes; occasionally occurring extreme sizes are put between brackets.

[b] thckn = maximum thickness; und = undulate inner contour; cll, py, dy = colourless, pale yellow and dark yellow respectively; – = absent, + = present.

[c] Para = paragynous, Amphi = amphigynous; – = absent, ± = up to 20%, + = about 50%, ++ = over 80%.

[d] Production: – = absent, ± = sparse, + = moderate number, ++ = abundant. Size = maximum size.

* CBS 366.59 produced more and larger chlamydospores on agar.

among isolates. They were generally broadly ovoid in shape, except in CBS 366.59, in which the sporangia were narrow. Distorted, extremely long sporangia were often observed in CBS 140.87 and sometimes in CBS 366.59. The most common ranges of size of sporangia in young, developing water cultures are listed in Table 3. Extremely small sizes (falling outside the most common range) are not included because sporangia that are formed later in the development of the sympodium tend to be smaller, and, moreover, small sporangia are produced under unfavourable conditions (e.g. in older cultures). Uncommon extreme sizes at the upper limit are given in parentheses.

Sporangia were with or without a more or less pronounced, broad papilla. This is indicated in Table 3 by S or N respectively. In all isolates the apical thickening at the tip of the sporangia was limited to the apex of the papilla and shallow (up to 1 μm), if present at all. All sporangia showed a broad opening after release of zoospores. A basal plug was often present in the subtending hypha; in some isolates a significant number of sporangia broke off below this plug during preparation of a microscopic slide, resulting in 'deciduous' sporangia with a short pedicel. However, spontaneous shedding of sporangia was never observed.

Sexual structures (Table 4). Oogonia and antheridia were consistently produced by isolates from *Allium* spp. (except CBS 366.59) only. CBS 180.87 from *Brassica* never developed oogonia. The remaining isolates from *Brassica* and CBS 366.59 formed oogonia when they were freshly isolated, but oogonium production rapidly decreased during subculturing. Incidentally they produced oogonia under special conditions, but this could not be reproduced (e.g. CBS 212.82 after transfer of a young colony from 24 to 21 °C and CBS 366.59 after putting a piece of hemp-seed agar in water). CBS 688.79 did not develop oogonia during the period of investigation; data for this isolate were taken from Ho (1983).

A general feature of all isolates was that oogonia were often abortive, i.e. no oospore was produced. If an oospore was produced, it was mostly abortive, containing a contracted clump of protoplasma. Healthy appearing oospores with fine granular contents were rare.

In general, oogonia were rather large, (sub-)globose, thick-walled with an undulate inner contour, colourless to dark yellow (often brownish). The most distinctive exception was CBS 366.59, which had small, colourless, thin-walled oogonia. One or, occasionally, two antheridia were present. Antheridia were chiefly paragynous in the isolates from *Allium* but amphigynous in the other isolates. Some isolates, however, showed a fifty-fifty ratio of both types. The shapes of the paragynous antheridia in all isolates was club-shaped, tubular or irregular, often rather large (up to 33 μm).

Chlamydospores and hyphal swellings. The distinction between chlamydospores and 'hyphal swellings' was not clear in many cases. According to its definition, a chlamydospore should have a thick wall and be separated from the hypha by a septum (Blackwell, 1949). It is often impossible to see septa and, in fact, a chlamydospore normally starts its development as a thin-walled hyphal swelling.

In water cultures two types of structures were developed (besides the sporangia): (1) globose structures, which were often very large and sometimes thick-walled, relative to the hyphal wall. They are referred to as chlamydospores in this paper (produc-

tion and sizes are presented in Table 4). They were either single or in short chains. Occasionally they gave rise to some radiating hyphae. (2) Ellipsoid structures with tapering ends, up to 20 μm in diameter, not delimited by cross septa and mostly in chains or in network configurations. They were found in all isolates except CBS 140.87 and are referred to here as hyphal swellings.

In agar cultures, thick-walled chlamydospores (up to 45 μm diameter) were observed only in CBS 366.59. Thick-walled (sub-)globose structures were also observed in several other isolates, but they showed an undulate inner wall contour and contracted protoplasma and were presumably abortive oogonia without antheridia. Agar cultures in which sporangia were present also contained a number of empty, thin-walled subglobose hyphal swellings.

Pathogenicity tests (Table 5). Only some of the isolates proved to be pathogenic. None of the isolates derived from *Allium* was highly pathogenic to cabbage, nor was any of the isolates from *Brassica* highly pathogenic to leek. CBS 142.87 (from *A. porrum*) only weakly infected cabbage and CBS 178.87 (from *B. oleracea*) only weakly infected leek. Young leek plants were slightly more susceptible to fungal attack than older plants, except by CBS 139.87. In general, a distinct reaction was observed after 1 week; the reaction only occasionally increased in the second week of incubation (from none to weak or from weak to strong). The results after 2 weeks are summarized in Table 5.

Table 5. Pathogenicity of isolates examined on three cultivars of leek (one young and one old plant each) and white cabbages (duplicate results). Infection of plants was recorded 2 weeks after wound inoculation.

No.	Isolate CBS no.	Leek cultivars			White cabbage
		Wintina	Tillina	Carina	
1	567.86	+	+/+	+/+	−/−
2	141.87	−/−	−/−	−/−	−/−
3	142.87	−/−	−/−	−/−	w/w
4	181.87	+/+	+/+	+/+	−/−
5	138.87	+/+	+/−	−/−	−/−
6	139.87	−/w	w/+	−/w	w/w
7	140.87	−/−	−/−	−/−	−/−
8	212.82	−/−	−/−	−/−	+/+
9	178.87	w/−	w/w	w/−	−/−
10	179.87	−/−	−/−	−/−	+/+
11	180.87	−/−	w/−	−/−	+/+
12	688.79	w	w/w	w	−/−
13	366.59	−/−	+/−	+/+	w/−

− = no reaction, w = weak reaction (up to 5 mm around inoculum), + = strong reaction (more than 3 cm around inoculum).

Restriction-fragment patterns of mtDNA (Fig. 1).
Isolates from *Brassica* showed identical restriction-fragment patterns with any of the enzymes used. The same was true for four of the five isolates from *A. porrum*; CBS 366.59, however, was quite different. One of the Japanese isolates from *Allium* spp. (CBS 140.87) was somewhat different from the other two (CBS 138.87 and 139.87), which were identical. The isolate from *D. carota* was quite different from all the others.

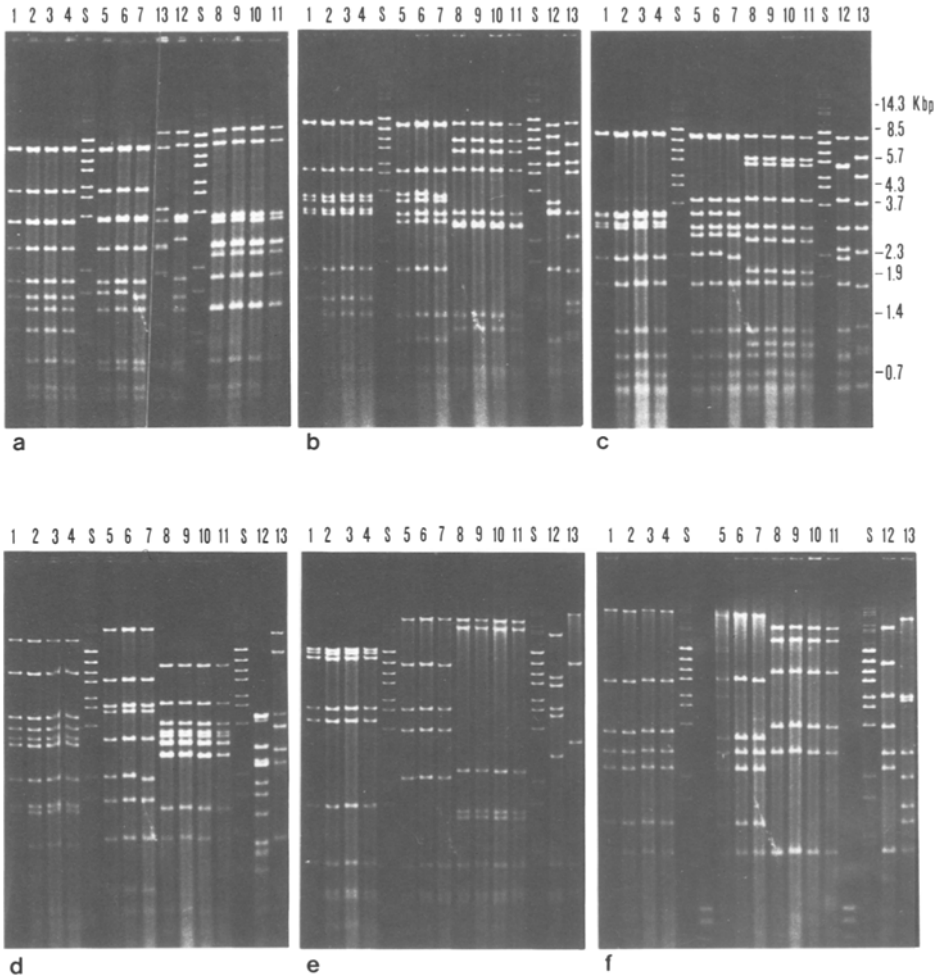

Fig. 1. Restriction patterns of mitochondrial DNA of isolates examined, digested with (a) *Ava*II, (b) *Bst*NI, (c) *Scr*FI, (d) *Hin*dII, (e) *Hae*III and (f) *Hpa*II. Isolate 1 = CBS 567.86, 2 = CBS141.87, 3 = CBS 142.87, 4 = CBS 181.87, 5 = CBS 138.87, 6 = CBS 139.87, 7 = CBS 140.87, 8 = CBS 212.82, 9 = CBS 178.87, 10 = CBS 179.87, 11 = 180.87, 12 = 688.79, 13 = 366.59. S = size marker: combinations of lambda DNA digested with *Bst*EII and lambda DNA partially digested with *Pvu*I. Lane 6 and 14 are size markers not used in this study.

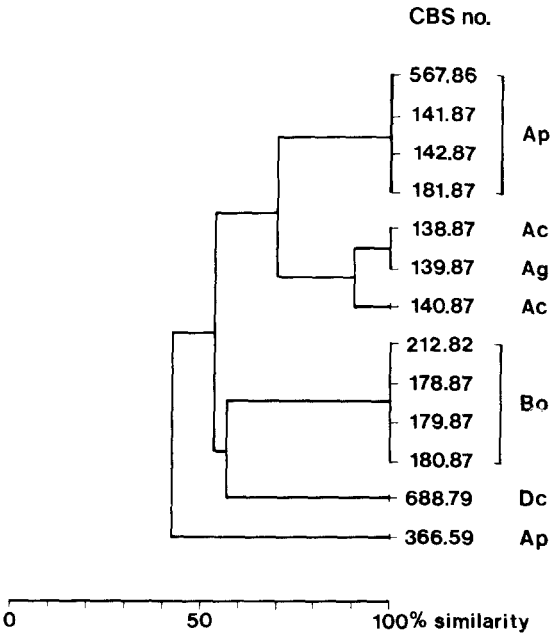

Fig. 2. Dendrogram from group-average cluster analysis based on similarity coefficients of restriction patterns of mitochondrial DNA of 13 isolates of *Phytophthora porri*, from different hosts. Isolates are indicated by their CBS accession numbers and their respective hosts. Abbreviations of hosts: A.p. = *Allium porrum*, A.c. = *A. cepa*, A.g.= *A. grayi*, B.o. = *Brassica oleracea*, D.c. = *Daucus carota*.

Table 6. Matrix of the average similarity coefficients between restriction-pattern groups. Groups: Ap = CBS 567.86, 141.87, 142.87, 181.87; Acg = 138.87, 139.87; Ac = CBS 140.87; Bo = CBS 212.82, 178.87, 179.87, 180.87; Dc = 688.79 and Apn = CBS 366.59.

	Acg	Ac	Bo	Dc	Apn
Ap	0.66	0.74	0.50	0.53	0.48
Acg		0.91	0.56	0.56	0.44
Ac			0.56	0.60	0.42
Bo				0.57	0.41
Dc					0.41

The result of group average cluster analysis, based on restriction pattern similarities (Table 6) and showing the relationships among the isolates, is presented in Fig. 2.

Discussion

The results presented in this study indicate that the isolates examined, which had been identified as *P. porri* on morphological grounds, do not constitute a homogeneous

group. Differences were found in morphology, cardinal temperatures for growth, pathogenicity and restriction-fragment patterns of mtDNA.

Restriction patterns of mtDNA gave the most distinct grouping. Moreover, relationships of groups could be quantified by cluster analysis of similarities of the patterns (Table 6; Fig. 2). Restriction patterns correlated very well with the host species from which the strains had been isolated. Isolates from different *Allium* species are more mutually related than to the isolates of *B. oleracea* and *D. carota*. The only exception is CBS 366.59 from *A. porrum*, which is an exceptional isolate in many respects (see discussion below). The isolates from *Brassica* and *Daucus* and CBS 366.59 differ from the *Allium* isolates at a similarity level of around 50%. A representative study of mtDNA restriction- fragment patterns of almost all species of *Phytophthora* using the restriction enzymes *Ava*II, *Scr*FI, *Hae*III and *Hin*dII (De Cock et al., in prep.) revealed a similarity level of 50% to be indicative of different species of this genus. We therefore conclude that the isolates of *Brassica* and *Daucus* might represent different species. On the other hand, the isolates from different *Allium* species might possibly be assigned the status of formae speciales; however, it can not be concluded from the results whether the differences in restriction patterns are due to the difference in host or geographic origin.

Grouping according to restriction patterns agreed quite well with temperature-growth relationships, pathogenicity and some morphological characters:

The optimum temperatures of all isolates is low (18–21 °C) except the isolate from *Daucus carota* and CBS 366.59 (24 and 30 °C respectively). The maximum temperature for growth was also higher in the latter two isolates. There is some confusion about the cardinal growth temperatures for *P. porri* (Kouyeas, 1977). The present study provides evidence that *P. porri* sensu stricto has an optimum temperature for growth between 18 and 21 °C and a maximum of (21–)24–27 °C.

Isolates from *Allium* and *Brassica* proved to be relatively host-specific, as far as pathogenicity could be confirmed (pathogenic to the host from which they were isolated or to a related host). Except for CBS 366.59, patterns of pathogenicity concur here with restriction patterns.

Morphology of the isolates is markedly similar. Colony appearance, hyphal growth (coiling), hyphal swellings, papillation and length to breadth ratio of sporangia, thickness and colour of oogonium walls and shape and size of antheridia are the same in many isolates and if they are different, they do not or hardly correlate with the clustering found with restriction pattern analysis. Sizes of oogonia and sporangia can vary within and among isolates but they overlap and do not correlate with restriction groups. There are only three morphological characters which coincide rather well with the restriction-fragment pattern groups. The strongest correlation is observed in oogonium and sporangium production. All isolates belonging to the *Allium* restriction pattern cluster produced oogonia consistently, whereas all the other isolates developed oogonia only shortly after isolation and occasionally afterwards. On the other hand, only the isolates from *Brassica* produced large numbers of sporangia, even on agar. Finally, the antheridium type was in agreement with restriction patterns: all isolates from *Allium* (except CBS 139.87) produced predominantly paragynous antheridia, the isolates from *Brassica* (except CBS 212.82) and *Daucus* produced mainly amphigynous antheridia. Both mentioned exceptions formed paragynous and amphigynous antheridia in a fifty-fifty ratio.

CBS 366.59 differed from the other isolates in many respects. When restriction patterns of this isolate were compared with those of other species of *Phytophthora* (De Cock et al, in prep.) they turned out to be identical with the patterns of *P. nicotianae*. *P. nicotianae* is a heterothallic species; however, oogonium production in single culture (as in CBS 366.59) has been reported many times for heterothallic species (Elliott, 1983). *P. nicotianae* is one of the very few *Phytophthora* species able to grow at or above 35 °C (Stamps et al., 1990), a property also observed in CBS 366.59. Also its chlamydospore production and oogonium morphology are in agreement with those of *P. nicotianae*. It is therefore concluded that CBS 366.59, despite its non-papillate sporangia and paragynous antheridia, is a representative of this species.

Summary of conclusions. Isolates of *P. porri* from *Allium* species are closely related, showing similar morphology, pathogenicity and restriction patterns of mtDNA and low cardinal growth temperatures.

Isolates from *B. oleracea* are morphologically similar to the isolates from *Allium* but they show rather different mtDNA restriction-fragment patterns; they might be considered to form a distinct species and can be distinguished morphologically from *P. porri* by their abundant sporangium production on agar, lack of oogonium production after subculturing and mainly amphigynous antheridia.

The isolate from *D. carota* is probably also a distinct species: although it shares many morphological characters with isolates from *Allium* as well as from *Brassica*, it has very different restriction patterns, higher optimum and maximum temperatures for growth, and colourless oogonia.

CBS 366.59 is shown to be an atypical representative of *P. nicotianae*, as proven by the restriction patterns of mtDNA, the extremely high optimum and maximum growth temperatures and some morphological characters.

Restriction pattern analysis has provided stable characters, correlated with the host of the isolates and some other characters, and thus this work confirms the value of this method in taxonomy of *Phytophthora*.

Acknowledgements

We thank D. Yarrow for correcting the English text. This work was supported by a fellowship of the Alexander von Humboldt Foundation to the first author.

References

Blackwell, E., 1949. Terminology in *Phytophthora*. Mycological Papers 30: 24 pp.

Commonwealth Mycological Institute, 1983. Catalogue of the culture collection. Eight edition 1982 & 1983 supplement. CAB-Commonwealth Mycological Institute, Kew, 198 + 30 pp.

Elliott, C.G., 1983. Physiology of sexual reproduction in *Phytophthora*. In: D.C. Erwin, S. Bartnicki-Garcia & P.H. Tsao (Eds), Phytophthora, its biology, taxonomy, ecology, and pathology. The American Phytopathological Society, St. Paul, MN, 392 pp.

Foister, C.E., 1931. The white tip disease of leeks and its causal fungus, *Phytophthora porri* n.sp. Transactions of the Botanical Society of Edinburgh 30: 257–281.

Förster, H., Kinscherf, T.G., Leong, S.A. & Maxwell, D.P., 1988. Estimation of relatedness between *Phytophthora* species by analysis of mitochondrial DNA. Mycologia 80: 466–478.

Förster, H., Kinscherf, T.G., Leong, S.A. & Maxwell, D.P., 1989. Restriction fragment length

polymorphisms of the mitochondrial DNA of *Phytophthora megasperma* isolated from soybean, alfalfa, and fruit trees. Canadian Journal of Botany 67: 529–537.
Förster, H., Oudemans, P. & Coffey, M.D., 1990. Mitochondrial and nuclear DNA diversity within six species of *Phytophthora*. Experimental Mycology 14: 18–31.
Geeson, J.D., 1976. Storage rot of white cabbage caused by *Phytophthora porri*. Plant Pathology 25: 115–116.
Geeson, J.D., 1978. Careful harvest is vital for white cabbage storage success. The Grower 89: 27, 29–31.
Griffin, M.J. & Jones, O.W., 1977. *Phytophthora porri* on autumn-sown salad onions. Plant Pathology 26: 149–150.
Ho, H.H., 1983. *Phytophthora porri* from stored carrots in Alberta. Mycologia 75: 747–751.
Hwang, B.K., De Cock, A.W.A.M., Bahnweg, G., Prell, H.H. & Heitefuss, R., 1991. Restriction fragment length polymorphisms of mitochondrial DNA among *Phytophthora capsici* isolates from Pepper (*Capsicum annuum*). Systematic and Applied Microbiology 14: 111–116.
Katsura, K., 1970. Studies on the blight and rot of tulip caused by *Phytophthora porri* Foister. Kyoto University Science Reports 22: 9–16.
Katsura, K., Isaka, M. & Miyagishi, M., 1969. *Phytophthora porri* Foister, the causal fungus of the leaf blight and bulb rot of scallion, *Allium bakeri* Regel. Annals of the Phytopathological Society of Japan 35: 55–61.
Kouyeas, H., 1977. Two new hosts of *Phytophthora porri* Foister. Annals of the Institute for Phytopathology of Benaki (N.S.) 11: 357–360.
Legge, B.J., 1951. A *Phytophthora* crown rot of *Campanula*. Transactions of the British Mycological Society 34: 293–303.
Nei, M. & Li, W.H., 1979. Mathematical models for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences U.S.A. 76: 5269–5273.
Semb, L., 1971. A rot of stored cabbage caused by a *Phytophthora* sp. Acta Horticulturae 20: 32–35.
Sneath, P.H.A. & Sokal, R.R., 1973. Numerical taxonomy. Freeman and Company, San Francisco, CA.
Stamps, D.J., Waterhouse, G.M., Newhook, F.J. & Hall, G.S., 1990. Revised tabular key to the species of *Phytophthora*. Mycological Papers 162: 28 pp.
Stelfox, D. & Henry, A.W., 1978. Occurrence of the rubbery brown rot of stored carrots in Alberta. Canadian Plant Disease Survey 58: 87–91.
Taylor, J.C., 1965. White tip of leeks (*Phytophthora porri* Foister). Plant Pathology 14: 189–190.
Tichelaar, G.M. & Van Kesteren, H.A. 1967. Aantasting van ui door *Phytophthora porri*. Netherlands Journal of Plant Pathology 73: 103–104.
Van Hoof, H.A., 1959. Oorzaak en bestrijding van de papiervlekkenziekte bij prei. Tijdschrift over Plantenziekten 65: 37–43.