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Extrachromosomal plasmid-like DNA in the obligate parasitic fungus *Erysiphe graminis* f. sp. *hordei*

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Summary. The obligate parasitic fungus, *Erysiphe graminis* f. sp. *hordei*, was found to harbour plasmid-like extrachromosomal DNA. A 1.35-kb fragment of this 9-kb plasmid was cloned into the pUC12 vector. No homology was detected to nuclear or mitochondrial DNA. As only about half of the 27 isolates examined contained plasmid-like DNA, this appears to be inessential for fungal survival. The plasmid is frequent in European isolates and is found in both newly collected isolates and in isolates kept under laboratory conditions for many years. No correlation between presence of plasmid and specific avirulence/virulence genes was found. The plasmid appear to be located in the mitochondria.

Key words: Barley powdery mildew – Gene-for-gene hypothesis

Introduction

Barley powdery mildew is an obligate pathogen dependent upon living cells as substrate. This fungus/plant interaction conforms to the gene-for-gene hypothesis described by Flor (1955), and the existence of avirulence/virulence genes in the mildew corresponding to resistance genes in barley has long been established (Moseman 1959). The gene products involved in this interaction are unknown.

Several fungi are now reported to contain plasmid-like DNAs (Tudzynski and Esser 1985), including the phytopathogenic fungi *Rhizoctonia* (Hashiba 1987), *Fusarium* (Kistler and Leong 1986), *Claviceps* (Tudzynshi et al. 1983), *Gaeumannomyces* (Honeyman and Currier 1986), and *Ceratocystis* (Giasson and Lalende 1987). These plasmids, whose function is still unresolved, resemble the mitochondrial plasmids of the higher plants, *Zea mays* (Zabala and Walbot 1988), *Sorghum* (Dixon and Leaver 1982), and *Brassica* (Turpen et al. 1987), which have been associated with male sterility. The presence of plasmid-like DNA in phytopathogenic fungi opens the possibility that these are involved in determining avirulence/virulence.

In the present study we established the presence of plasmid-like DNA in certain isolates of barley powdery mildew. To examine whether this DNA is correlated to particular virulence genes or characteristics such as mating type, origin, and age, a large number of isolates were analysed for presence or absence of plasmids. Transmission of the plasmid was studied in progeny derived from crosses between mildew isolates differing in virulence and plasmid characters.

Materials and methods

Fungal material

Isolates of barley powdery mildew (*Erysiphe graminis* DC. FR. f. sp. *hordei*) were grown on susceptible barley until maximum conidia production; the infected leaves were then harvested in liquid nitrogen and stored at −20 °C. Pure conidia of the isolates EmA30, Race IX, NIIS, C15, and A6 were harvested by suction from infected leaf sections kept on 0.5% agar with 40 mg l^{-1} benzimidazol. The conidia were frozen in liquid nitrogen and lyophilysed.

Crosses between the isolates Race IX × NIIS and Race IX × C15 were carried out in isolated greenhouse cabinets in the spring. Leaves of 3-weeks-old barley plants were inoculated with the two appropriate cultures. After about 3 months, cleistothecia could be harvested on leaf sections and stored under dry conditions.

Cleistothecia were placed on wet filter paper above barley plants grown in a glass chimney (8 × 29 cm). As soon as mildew colonies could be detected, they were isolated individually and propagated on fresh barley plants. The virulence spectra of each progeny isolate were determined by inoculating leaf sections

(kept on 0.5% agar, 40 mg l^{-2} benzimidazol) from 'Pallas' isogenic barley lines (Kølster et al. 1986) carrying different resistance genes. For DNA analysis, leaves infected with each progeny isolate were harvested in liquid nitrogen and stored at −20 °C.

Purification of mildew DNA and detection of extranuclear DNA

Conidia were ground in liquid nitrogen using a mortar and pestle. This preparation was suspended in 0.025 *M* TRIS-Cl, 25% w/v buffer containing 1 mg ml^{-1} lysozyme, and 10 mg ml^{-1} RNase, and loaded onto a 0.7% agarose gel essentially as described by Eckhardt (1978). DNA from the high-molecular-weight band containing nuclear and mitochondrial DNA and DNA from the low-molecular-weight band were electroeluted according to Maniatis et al. (1982).

Cloning of mildew DNA

Purified extrachromosomal mildew DNA from isolate EmA30 was digested to completion with the restriction endonuclease EcoRI or HindIII and ligated, using T4 ligase, Bethesda Research Laboratory (B.R.L.), into EcoRI and HindIII-cut pUC12 vector, respectively (Maniatis et al. 1982; Messing 1983). *Escherichia coli* JM83 was transformed using the ligation mix and Amp^r, Lac^- transformants were analysed by alkaline lysis mini-preparation (Maniatis et al. 1982).

DNA preparations

High-molecular-weight DNA from mildew-infected leaves was extracted by the method described by Sharp et al. (1988). Plasmid DNA was prepared essentially as described by Birmboim and Doly (1979). Mitochondrial DNA from barley leaves infected with the isolates C15 and A6 was isolated according to the method described by Kemble (1987).

Restriction enzyme digestion, electrophoresis, and Southern blotting

Restriction endonuclease digestions were carried out using enzymes and buffers from B.R.L. Horizontal gel electrophoresis in TRIS-borate buffer and Southern blotting were performed as described by Maniatis et al. (1982). Nitrocellulose (Schleicher & Schuell) and Zeta Probe (Bio-Rad) membranes were used.

Nuclease treatments

Mitochondrial DNA preparations were treated with DNase I, RNase A (Boeringer Mannheim), respectively, according to the manufacturers' instructions.

Hybridisation methods

Blotted membranes were prehybridised in plastic boxes at 42 °C for 2–16 h in 45% formamide, 4 × SSPE (1 × SSPE = 180 m*M* NaCl, 10 m*M* sodium phosphate, 1 m*M* EDTA, pH 7.5), 6 × Denhard's solution (0.02% BSA, PVP 360 and Ficoll 400, w/v) 200 µg/ml salmon sperm DNA and 0.1% SDS. The hybridisation was carried out in identical buffer with the addition of 10% dextran sulphate for 16–48 h at 42 °C.

Plasmid DNA was nick-translated (B.R.L. Kit) with either 32P-labelled deoxynucleotides (Amersham International) or biotinylated dUTP (B.R.L.). The DNA probes were denatured by boiling for 5 min and were cooled on ice.

Radioactive blots were washed twice with 2 × SSC (1 × SSC is 150 m*M* NaCl, 15 m*M* sodium citrate, pH 7.0), 0.1% SDS for 1 h at 67 °C and twice with 0.5 SSC, 0.1% SDS for 0.5 h at 67 °C. Filters were exposed at −70 °C to Hyperfilm-MP film from Amersham. Biotin blots were washed four times with 2 × SSC, 0.1% SDS for 0.5 h at 42 °C, then subjected to the detection procedure described by Chan et al. (1985).

Results

When a crude extract of powdery mildew conidia, isolate EmA30, was loaded directly onto a gel and separated by standard DNA electrophoresis, two bands appeared (Fig. 1 Aa) – an upper band representing nuclear and mitochondrial DNA, and a lower band of approximately 9 kb in size. We presume the lower band to be extrachromosomal plasmid-like DNA.

Only with HindIII-digested plasmid-like DNA did we succeed in obtaining a clone which gave a signal when hybridising with labelled total plasmid-like DNA. This clone, pPEH-7, contains a 1.35-kb fragment of the plasmid. Using this clone as a probe it became clear that the 1.35-kb HindIII fragment specifically recognised the mildew plasmid DNA and showed no homology to high-molecular-weight DNA (Fig. 1 Ac). As reference, a mildew ribosomal clone, pGEH-45 [showing strong ho-

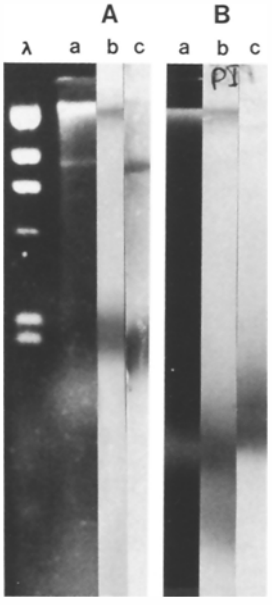

Fig. 1. **A** Southern hybridization analysis of DNA from mildew isolate EmA30. *Lane a:* ethidium bromide stained gel section; *lane b:* the signal obtained using a biotin-labelled ribosomal pGEH-45 probe; *lane c:* the hybridisation signal obtained using pPEH-7 as probe. *Hin*dIII-digested λ DNA was used as marker, 23130, 9416, 6557, 4361, 2322, 2027 bp. **B** Gel electrophoresis and Southern hybridisation analysis of powdery mildew isolate A6. *Lane a:* ethidium bromide-stained gel section; *lane b:* hybridisation with (biotin-labelled) (pGEH-45) ribosomal probe; *lane c:* hybridisation using pPEH-7 as probe

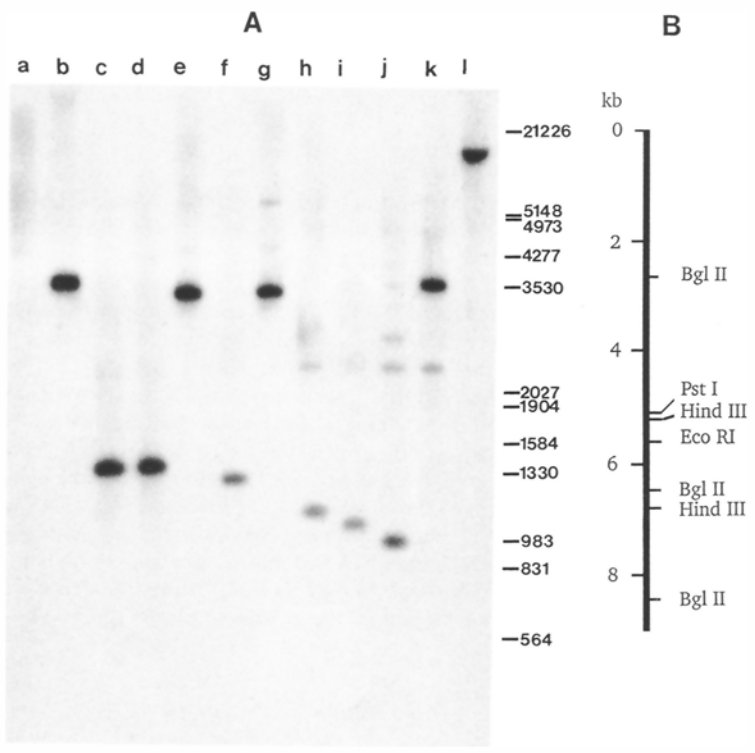

Fig. 2. **A** Autoradiogram of DNA from uninfected barley (*lane a*) and barley infected with mildew isolate EmA30 digested with restriction endonucleases and analysed by Southern hybridisation analysis, using pPEH-7 as probe. The following restriction digests were carried out: *lane b, Pst*I; *lane c, Pst*I/*Hin*dIII; *lane d, Hin*dIII; *lane e, Eco*RI/*Pst*I; *lane f, Eco*RI/*Hin*dIII; *lane g, Eco*RI; *lane h, Bgl*II/*Pst*I; *lane i, Bgl*II/*Hin*dIII; *lane j, Bgl*II/*Eco*RI; *lane k, Bgl*II; *lane l, Bam*H1. *Hin*dIII and *Eco*RI double-digested λ DNA was used as size marker. **B** Tentative restriction map of barley powdery mildew plasmid-like DNA

mology to wheat ribosomal clone pTA71 (Gerlach and Bedbrook 1979)], was used as a probe, which only showed homology to high-molecular-weight DNA (Fig. 1 Ab).

As only very small amounts of mildew plasmid-like DNA could be obtained, restriction analysis was carried out on restriction digests of total barley leaf and mildew DNA. Southern blots and hybridisation with pPEH-7 then revealed the restriction map of the mildew plasmid to the extent that the fragments obtained carry sequences homologous to the HindIII fragment (Fig. 2 A). No hybridisation signal was obtained to restriction-digested pure barley DNA, supporting the validity of the results obtained from this analysis. A tentative map of the mildew plasmid was constructed on the basis of single and double digests (Fig. 2 B).

An attempt was made to use the entire purified mildew plasmid as a probe. However, too many unrelated sequences were present in the preparation, resulting in a banding pattern that was too complex to interpret with certainty.

Results of hybridisation with the clone pPEH-7 to EcoRI restriction digest of 27 different powdery mildew isolates are shown in Fig. 3. The signals obtained indicate that different amounts of plasmid DNA may be present in different isolates, but the results could also reflect the proportion of mildew to barley DNA in the different preparations. In 15 of the examined isolates, the cloned fragment recognised homologous sequences, while the remaining 12 isolates gave no signal. All isolates giving signals had the same banding pattern, which was also the case when the same experiment was carried out using the restriction enzyme BglII (data not shown). Plasmids in different isolates thus appear to be closely related.

To check that these results reflect the presence or absence of plasmids in the respective isolates, pure mildew DNA from the isolates C15 and NIIS, with signals, and A6 and Race IX, without signals, were analysed. The results showed that C15 and NIIS does carry a plasmid, and hybridisation with pPEH-7 and a genomic probe, pGEH-45, gave a pattern identical to that observed for EmA30 (Fig. 1 A). The isolate A6 did

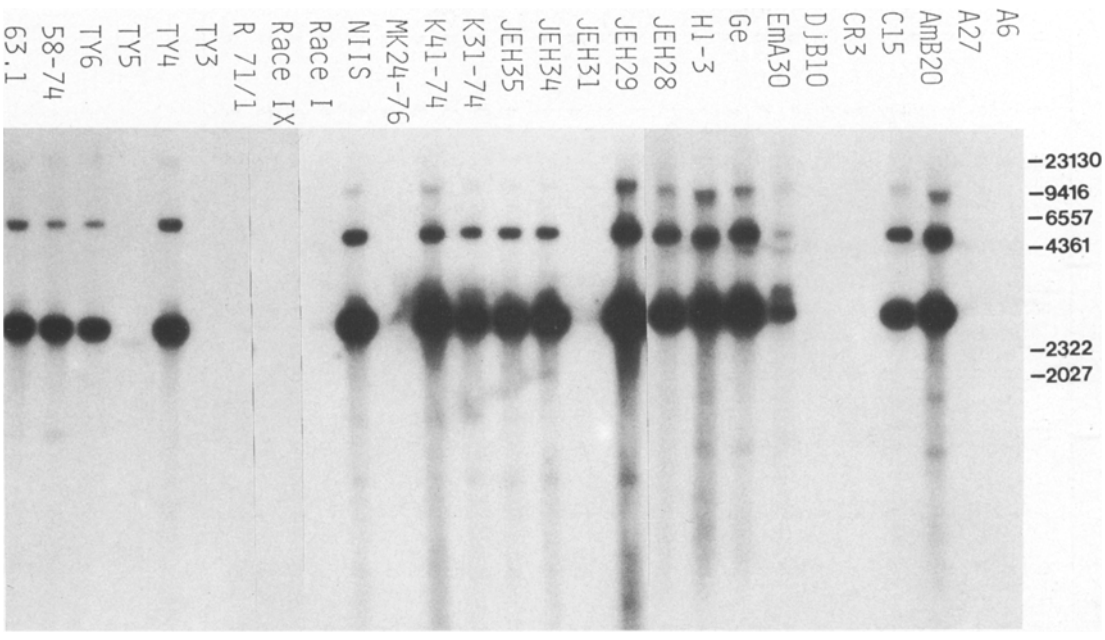

Fig. 3. Autoradiogram from Southern hybridisation analysis of *Eco*RI-digested DNA from the mildew isolates listed in Table 1 probed with pPEH-7. *Hin*dIII-digested λ DNA was used as size marker

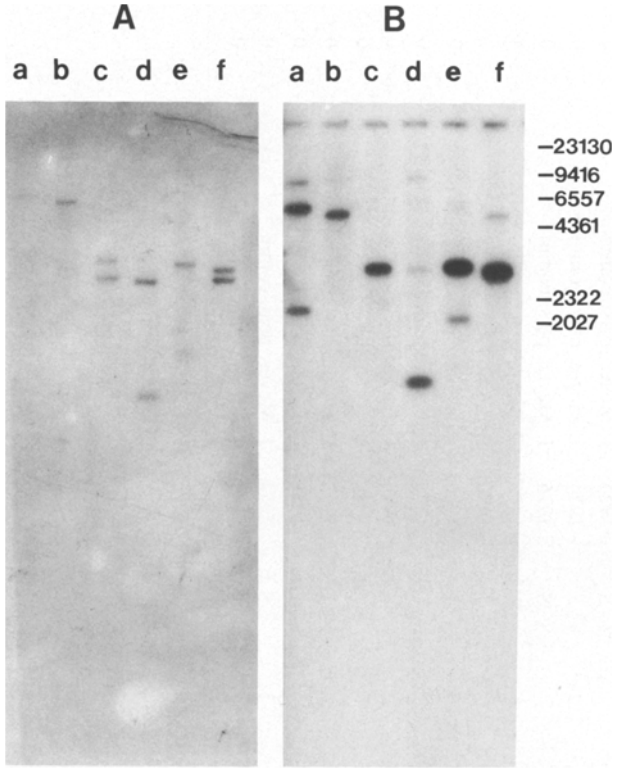

Fig. 4 A and B. Southern analysis of endonuclease-digested DNA from barley and mildew isolates MH9, **A**, and C15, **B**, using pPEH-7 as probe. The following restriction digests were carried out: *lane a, Eco*RV; *lane b, Xho; lane c, Pst*I; *lane d, Hin*dIII; *lane e, Bgl*II; *lane f, Eco*RI. *Hin*dIII-digested λ DNA was used as size marker

not contain a plasmid (Fig. 1 Ba) and hybridisation with pPEH-7 showed that no homologous sequences were present in the high-molecular-weight nuclear or mitochondrial DNA (Fig. 1 Bc). The same results were obtained for Race IX.

Mildew isolates collected in the field, Zealand 1986 and Jutland 1987, were tested for the presence of plasmid-like DNA. Five of the six isolates from Jutland harboured plasmid, whereas the six isolates from Zealand did not (data not shown). Different isolates of wheat mildew from Zealand were tested for presence of the plasmid sequence, pPEH-7, but no homology was detected.

A more detailed restriction digest analysis was carried out on the Scandinavian isolate, C15 (which has been kept under laboratory conditions for 25 years), the Israeli isolate, NIIS, and three of the newly collected isolates from Jutland, MH12, MH9, and MH4. NIIS, C15, MH12, and MH4 all had the restriction pattern shown in Fig. 4 B, whereas MH9 showed a different restriction pattern (Fig. 4 A).

All available data on the examined barley powdery mildew isolates are collected in Table 1. From this it is apparent that the plasmid is not related to a specific mating type. Mildew isolates originating from Europe and Israel are found both with and without plasmid DNA. The isolate CR3 from the USA and both the Japanese isolates, Race I and Race IX, lack plasmids. Plasmids are present in isolates which have been kept under laboratory conditions for up to 25 years, C15

Table 1. Presence of plasmid, mating type, origin, age, and infection type (virulence spectrum) of different powdery mildew isolates

Isolate	Presence of plasmid	Mating type	Country and year of sampling		Reference	Infection type 0–4 (4 = virulent) of powdery mildew towards different barley resistance genes													
						Ml-a1 +?	-a3	-a6 +a14	-a7 +k	-a9 +k	-a10	-a12	-a13 +(Ru3)	-at +?	-g	-h	-k	-(La) +?	-ra
A6	–	1	S	1958	Wiberg 1974	4	1	0	1	0	1	0	0	4	0	1	2	3	0
A27	–	2	S	1972	Wiberg 1974	0	1	2	1	0	1	0	0	2	0	1	4	3	4
AmB20	+		S	1973	Wiberg 1974	0	1	0	4	4	4	0	0	2	4	1	4	3	4
C15	+	1	S/DK	1964	Wiberg 1974	0	4	0	1	0	1	0	0	2	4	4	2	3	4
CR3	–	2	USA	1951	Moseman 1968	0	1	0	1	0	1	0	0	4	0	1	2	3	4
DjB10	–		NL	(1978)	A. Balkema-Boomstra, pers. comm.	0	1	0	2	0	1	0	0	4	4	1	2	4	4
EmA30	+	2	S	1972	Wiberg 1974	0	1	2	1	0	1	4	0	2	0	4	2	3	0
GE	+		D	1979	Swarzbach 1979	0	1	4	2	0	1	0	0	2	4	4	2	3	4
HL-3	+	2	D	1979	Swarzbach 1979	0	1	4	2	0	1	0	0	2	4	4	2	3	4
JEH28	+		DK	1975	Hermansen 1980	0	1	4	4	0	4	0	0	2	0	4	4	3	4
JEH29	+		DK	1976	Hermansen 1980	0	1	4	4	0	4	0	0	2	4	4	4	3	4
JEH31	–		DK	1972	J. E. Hermansen, pers. comm.	0	1	4	4	0	4	0	0	2	4	4	4	3	4
JEH34	+		DK	1977	J. E. Hermansen, pers. comm.	0	1	4	4	0	0	0	0	2	4	1	4	3	4
JEH35	+		DK	1975	J. E. Hermansen, pers. comm.	0	1	0	4	0	4	0	0	2	4	4	4	3	4
K31–74	+		S	1974	J. Meyer, pers, comm.	0	1	4	4	0	4	0	0	2	4	4	4	3	4
K41–74	+	2	S	1974	J. Meyer, pers. comm.	0	1	0	2	0	4	0	0	4	0	1	2	4	0
MK24–76	–		S	1976	J. Meyer, pers. comm.	0	1	0	1	4	4	0	4	2	4	4	4	3	4
NIIS	+	1	IL	(1979)	E. Swarzbach, pers. comm.	4	4	4	1	0	1	4	2	4	4	4	4	4	4
Race I	–	1	J	(1980)	Hiura and Heta 1955	2	1	0	1	0	1	0	0	0	0	4	2	0	0
Race IX	–	2	J	(1980)	Hiura and Heta 1955	0	1	0	1	0	1	0	0	4	0	1	2	3	4
R 71/1	–		DK	1976	Jensen and Jørgensen 1981	0	1	0	2	0	4	4	0	2	0	4	2	3	4
TY3	–	2	DK	(1978)	C. H. Nielsen, pers. comm.	0	1	4	2	0	1	0	0	2	4	4	2	4	4
TY4	+	2	DK	(1978)	C. H. Nielsen, pers. comm.	0	1	0	1	0	4	4	0	2	4	1	2	4	4
TY5	–		DK	(1978)	C. H. Nielsen, pers. comm.	0	1	4	4	0	1	0	0	2	0	4	2	4	4
TY6	+		DK	(1981)	C. H. Nielsen, pers. comm.	0	1	0	4	0	4	0	0	4	4	1	4	3	4
58–74	+	1	S	1974	J. Meyer, pers. comm.	0	1	0	4	4	4	0	4	2	4	4	4	3	4
63.1	+	1	IRL	1962	Moseman 1968	0	1	0	4	0	1	0	0	2	4	4	2	3	4

( ) = year received at Risø

Table 2. Segregation for plasmid type (+ and −) and infection type (0–4) of powdery mildew progeny isolates

		Plasmid	Ml-a1	-a3	-a6	-a12	-g	-k	-h	-(La)
Parental isolates:	NIIS	+	4	4	4	4	4	4	4	4
	Race IX	−	0	1	0	1	0	2	1	2
Progeny isolates:	A1	−	0	4	4	1	0	2	1	4
	2	−	0	4	4	1	0	2	1	4
	3	−	4	4	4	1	0	2	4	4
	4	−	0	4	4	4	0	2	4	4
	5	+	0	4	4	1	0	2	1	4
	6	−	4	4	4	4	0	2	4	4
	B1	+	0	1	4	4	0	4	1	4
	2	+	4	1	4	1	0	2	4	4
	4	+	4	1	0	1	0	2	1	4
	C1	+	4	1	4	1	0	4	1	4
	2	+	0	1	4	1	0	2	1	2
	3	+	4	1	0	4	0	2	1	2
		Plasmid	Ml-a3	-at	-g	-h				
Parental isolates:	C15	+	4	2	4	4				
	Race IX	−	1	4	0	1				
Progeny isolates:	D1	+	4	4	4	1				
	2	+	1	4	0	4				
	3	+	4	4	4	1				
	4	+	1	2	0	4				

(Table 1), and also in newly sampled isolates. Presence or absence of plasmid shows no correlation to particular avirulence/virulence genes.

To determine the inheritance of the plasmid DNA, progeny was isolated from single cleistothecia of crosses between mildew isolates with and without plasmid. From 100 single cleistothecia from a cross between Race IX and NIIS, only 3 gave progeny. One group consisted of five progeny isolates without plasmid and one with, and two groups of three progeny isolates all carried plasmid DNA. Only one set of progeny was obtained from 100 single cleistothecia from a cross between Race IX and C15. The four progeny in this group all carried plasmid DNA. The plasmid appear to be mostly, but not strictly, maternally inherited. The virulence spectra and presence or absence of plasmid-like DNA in progeny isolates are presented in Table 2. None of the segregations of mildew avirulence/virulence genes tested correspond to that observed for the plasmid. Thus, there is no correlation between a particular virulence character and the presence or absence of plasmid-like DNA.

Mitochondria from mildew-infected barley leaves were isolated to determine whether the plasmid was present in this organelle. Figure 5A shows that, in addition to high-molecular-weight DNA, the mitochondria from isolate C15 also contains the 9-kb band and four additional low-molecular-weight bands. The A6 mitochondria only contain high-molecular-weight DNA. Hybridisation with pPEH-7 confirms the presence of plasmid in mitochondria of C15 (Fig. 5B), and no homology to high-molecular-weight DNA is observed. No signals were obtained to mitochondrial DNA of A6 (Fig. 5B). Hybridisation with pGEH-45 only showed homology to total DNA (Fig. 5C), indicating that the mitochondrial fraction was free of genomic DNA.

To determine whether the low-molecular-weight bands consist of DNA or RNA, the mitochondrial DNA preparations (Fig. 6a) were treated with DNase I and RNase A, respectively. DNase I treatment caused the disappearance of high-molecular-weight DNA and the 9-kb plasmid band (Fig. 6b). The lower bands, however, were resistant to DNase I treatment (Fig. 6b), but susceptible to RNase A treatment (Fig. 6c), indicating that they represent double-stranded RNA.

Discussion

The plasmid reported in this paper is the first to be found in a strictly obligate parasitic fungus. Due to the difficulty of obtaining a sufficient amount of pure plasmid DNA, we have not been able to determine whether the plasmid is circular or linear. The cloned sequence does not show homology to high-molecular-weight DNA but, until we are able to analyse the entire plasmid, we cannot exclude the possibility that it harbours mitochondrial or nuclear sequences. Circular plasmids from *Cochliobolus heterostrophus* (Garber et al. 1984) and *Podospora anseri-*

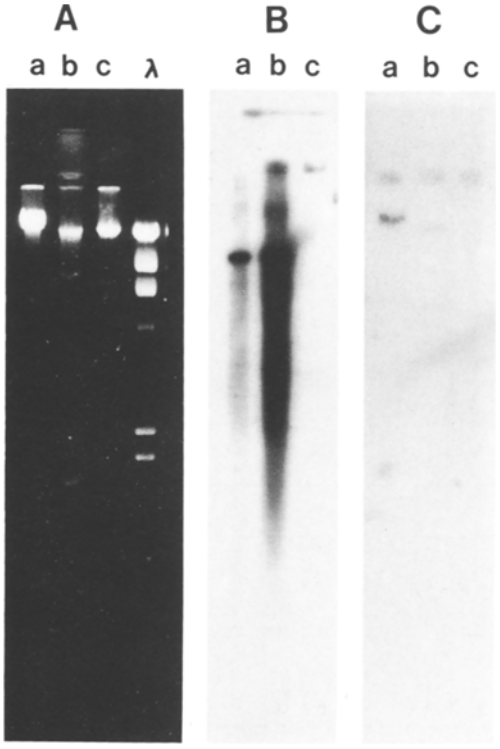

Fig. 5 A–C. Southern analysis of mitochondrial DNA from barley leaves infected with the isolates C15 and A6. **A** Ethidium bromide-stained gel of; *lane a,* total DNA from barley infected with mildew isolate C15; *lane b,* mitochondrial DNA from barley infected with isolate C15; *lane c,* mitochondrial DNA from barley infected with isolate A6. *Hin*dIII-digested λ DNA was used as molecular marker. **B** Autoradiogram showing signals from hybridisation with ^{32}P-labelled pPEH-7. **C** Autoradiogram showing signal from hybridisation with ^{32}P-labelled pGEH-45

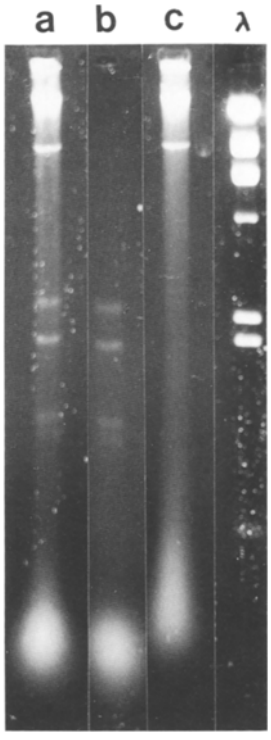

Fig. 6. Ethidium bromide-stained gel of nuclease-treated mitochondrial DNA preparation of barley and mildew isolate C_{15}; *lane a,* untreated sample; *lane b,* DNaseI-treated sample; *lane c,* RNase A-treated sample. *Hin*dIII-digested λ DNA was used as molecular marker

na (Stahl et al. 1978) have been found which show homology to the mitochondrial genomes of these species. In *Claviceps purpurea* and *Fusarium oxysporum* f. sp. *conglutinans,* linear plasmids have been found in mitochondria, but no homology to mitochondrial DNA was detected (Tudzynski et al. 1983; Kistler and Leong 1986).

The tentative restriction map (Fig. 2) shows that if the plasmid is linear, the cloned HindIII fragment is located internally and that only one site for EcoRI is present. Attempts at cloning plasmid DNA using EcoRI have all failed, which could be explained by linearity of the plasmid. Future attempts at cloning should concentrate on the BglII sites.

Restriction analysis of different barley powdery mildew isolates using the cloned sequence indicated strong homology between plasmids (Figs. 3 and 4). It is curious that the only polymorphism detected in the barley powdery mildew plasmids is among the recently collected isolates in Jutland (Fig. 4).

As the clone only covers a part of the whole plasmid, pure mildew DNA from a few isolates which did not show homology to the cloned sequence was analysed by preparative gel electrophoresis (Fig. 1 B). No plasmid bands were observed in these isolates, which indicated that plasmid DNA is inessential for fungal survival, as also reported for plasmids of *Claviceps purpurea* (Düvell et al. 1988) and *Fusarium oxysporum* (Kistler and Leong 1986).

In an attempt to assign a function to the plasmid, all available data on 27 different mildew isolates were collected (Table 1). There appears to be no correlation between the presence of plasmid DNA and mating type. Geographically, the plasmid occurs frequently in European isolates, but the American isolate, CR3, and the Japanese isolates, Race I and Race IX, do not harbour plasmids. Accordingly, we cannot conclude anything about the presence of plasmids in mildew from these regions. Our one isolate from Israel, NIIS, does contain plasmid DNA and the restriction digest pattern is identical to that of C15 isolated in Sweden (Fig. 4).

Geographical separation, NIIS and C15, and the 23-year time span between the collection of C15 and HM12 have otherwise shown the plasmid to be stable and with

limited polymorphism. The plasmid in the barley mildew thus appears to be less diverse than plasmids in *Fusarium solani* (Samac and Leong 1988), *Rhizoctonia solani* (Hashiba 1987), and *Claviceps purpurea* (Düvell et al. 1988), where differences are found in restriction sites and cross homology. Caution must be taken in interpreting our results, as only an internal 1.35-kb clone of the 9-kb mildew plasmid has been used in the analysis. It is possible that a study of the entire plasmid would reveal greater polymorphism than we have been able to detect.

By analysing Table 1 it is clear that none of the known avirulence/virulence genes towards particular resistance genes are correlated to the presence or absence of the plasmid DNA. This is further supported by our results on the transmission of plasmid DNA and the virulence spectra of the progeny from the crosses between isolates NIIS and Race IX, and C15 and Race IX (Table 2). From this it is clear that the segregation of the specific avirulence/virulence genes analysed is independent of the transmission of plasmid DNA.

The segregation ratios of particular avirulence/virulence genes in the progeny isolates are not as expected for random segregation (Table 2), e.g., there are no segregations for five of the eight genes tested in the progeny lines A 1–6. Since the progeny segregate for the correct genes as known from the parental types, we presume the cross to be correct. This is also supported by analysis of segregation from progeny lines derived from a large number of cleistothecia from the same cross which showed the expected ratios. As these are the first results from analysis of progeny from single cleistothecia, we do not know whether this may cause some bias.

The powdery mildew fungus is heterothallic and the determination of which isolate acts as the maternal or paternal parent is presumably random. To establish the inheritance of the plasmid, progeny from single cleistothecia was analysed. This revealed that the plasmid was predominantly maternally inherited (Table 2), agreeing with the mitochondrial localisation of the plasmid (Figs. 5 and 6). However, it appears that the mildew mitochondria are not strictly maternally transmitted, as one progeny isolate diverged from its sibling lines with regard to the presence of plasmid. Maternal inheritance has been reported for linear plasmids in *Fusarium* (Samac and Leong 1988) and *Ascobolus* (Francon 1981), and for the circular mitochondrial plasmids in *Neurospora* (Stohl et al. 1982).

Only in the plasmid containing mitochondria from the isolate C15 did we observe the presence of double-stranded RNAs (Figs. 5 and 6). Whether there is a connection between the plasmid and the RNAs, and what the function of these molecules is in the pathogen, cannot be determined by the present results.

In conclusion, the plasmid-like DNA in barley powdery mildew is found in the mitochondria and does not carry any of the specific avirulence/virulence genes examined. The plasmid is frequently found in European isolates and is present both in newly collected field isolates and in isolates which have been kept under laboratory conditions for many years. Maternal inheritance opens the possibility of using the presence of plasmid as a cytoplasmic marker.

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