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A novel virus of the late blight pathogen, *Phytophthora infestans*, with two RNA segments and a supergroup 1 RNA-dependent RNA polymerase

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ABSTRACT

Double-stranded RNA representing four distinct electrophoretic patterns was found in a screen of *Phytophthora infestans* isolates. Two dsRNAs that always appeared together were sequenced. RNA 1, which was 3160 nt plus a poly (A) tail, contained a single deduced ORF with the potential to encode a polyprotein of 977 aa with motifs characteristic of supergroup I viral RdRps. The 2776 nt, polyadenylated RNA2 contained an ORF with a potential to encode a polyprotein of 847 aa including a possible trypsin-like serine protease, and a second putative ORF of unknown function. An alternative form of RNA2, in which a 19-nt stretch was replaced by a 9-nt sequence, was detected in 4 of 17 clones sequenced. Based on genome structure and phylogenetic analysis, this virus did not fit into any known virus family and we tentatively named it *Phytophthora infestans* RNA virus 1 (PiRV-1).

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Phytophthora infestans causes potato late blight, the disease that led to the Irish potato famine in the 1840s and continues to devastate potatoes and tomatoes worldwide with annual losses in the billions of dollars (Fry and Goodwin, 1997). *Phytophthora infestans* belongs to the oomycetes, a group that includes many pathogens of plants, animals, and insects, as well as saprophytes. Most of the approximately 60 species of *Phytophthora* are pathogenic on plants (Erwin and Ribeiro, 1996).

Morphologically and ecologically, oomycetes are similar to filamentous fungi and were traditionally classified as fungi. However, based on molecular and biochemical analyses, these two groups have little taxonomic affinity. Oomycetes are more closely related to brown algae and diatoms and fall in the major group Stramenopila (Baldauf et al., 2000; Cavalier-Smith, 2000; Sogin and Silberman, 1998), part of the super group Chromalveolata, while the fungi belong to a different super group, the Ophisthokonta (Adl et al., 2005).

Despite their economic importance and unique evolutionary affinities, oomycetes are chronically understudied at the molecular level. Furthermore, while viruses and their effects on many fungal hosts have been examined in some depth, there have been only a very few studies on extrachromosomal genetic elements in *P. infestans* and other oomycetes. Double-stranded RNAs (dsRNAs) presumably associated with viral infections were first reported in 1989 in some isolates of *P. infestans* but these were not characterized in molecular detail (Newhouse et al., 1992; Tooley et al., 1989). An autonomous linear, single-stranded (ss) RNA was found in a different *P. infestans* isolate and was sequenced (Judelson and Fabritius, 2000), but it lacked obvious open reading frames (ORFs), had no apparent capacity to encode an enzyme for its own replication, and remains enigmatic. More recently, a large 14 kilobase (kb) dsRNA was extracted from an unnamed *Phytophthora* isolate from Douglas fir (Hacker et al., 2005). Cloning and sequencing of that dsRNA revealed a single deduced ORF with the potential to encode a large polyprotein of 4548 amino acids (aa) that contained motifs characteristic of an RNA-dependent RNA polymerase (RdRp), an RNA helicase, and a UDP glycosyltransferase. Phylogenetic analysis revealed that the dsRNA represented the genome of a virus in the recently described genus *Endornavirus* and was given the tentative name *Phytophthora* endornavirus 1 (PEV1). Other viruses identified in oomycetes include two positive-sense ssRNA viruses in the downy mildew pathogen, *Sclerophthora macrospora* (Honkura et al., 1983; Shirako and Ehara, 1985; Yokoi et al., 2003) and virus-like particles encapsidating electrophoretically distinct dsRNAs in *Pythium irregulare* (Gillings et al., 1993).

Two aspects of our long-term studies with *P. infestans* brought us to examine our collection of isolates for viruses. First, we sought to look for extrachromosomal elements that could be engineered into efficient functional genetic tools for gene expression/silencing in *P. infestans*, as the lack of such a tool is the major bottleneck in molecular studies of the oomycetes. A second objective was to look for viruses

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that might serve as biocontrol agents for the late blight pathogen, a goal that has been achieved for several plant-pathogenic fungi including the well-known example of hypovirulence in the chestnut blight fungus, *Cryphonectria parasitica*, with its resident viruses (Nuss, 2005). The screen of our collection revealed dsRNAs that appear to constitute the genomes of four independent viruses. The characterization here of two biologically co-segregating dsRNAs reveals a complex and interesting bipartite viral genome with affinities to RdRp supergroup 1 viruses, such as plant-infecting potyviruses and mammal-infecting astroviruses. This represents the first virus genome in *P. infestans* characterized at the molecular level, and likely represents the first member of a new virus family.

Results and discussion

dsRNAs in P. infestans

Twenty-two isolates of *P. infestans* and one isolate of *Py. aphanidermatum* currently being used in our lab for various studies were screened for dsRNAs. dsRNAs were detected in nine *P. infestans* isolates (Table 1). dsRNAs representing five different mobility classes were consistently identified in four distinct patterns (I–IV in Fig. 1) with a putative sixth dsRNA of ~5.15 kb detected in some but not all extractions of isolate MX980317. dsRNA was not found in the *Py. aphanidermatum* isolate. The chemical identity of dsRNA was confirmed by resistance to DNase I and S1 nuclease and resistance to RNase A in high but not low ionic strength conditions (not shown).

The *P. infestans* dsRNAs ranged in estimated size from 2.9 kb to 11.2 kb. Accuracy of these estimates was supported by sequence data for the two dsRNAs from isolate MX980400 (pattern I) estimated at 3.3 kb and 2.9 kb, which were close to their actual sizes (see below). Newhouse et al. (1992) reported seven dsRNAs in *P. infestans*. The estimated sizes were 11.10 kb, 3.25 kb, 3.15 kb, 2.80 kb, 1.67 kb, 1.54 kb, and 1.35 kb, and they occurred in nine patterns. They found that the 3.25 kb and 2.80 kb segments always appeared together, as did the 1.67 kb and 1.54 kb segments. Although none of their isolates was available to be included in this study for comparison, similarities are notable: first, the dsRNAs of 3.3 kb and 2.9 kb in our study, similar in size to the 3.25 and 2.80 kb dsRNAs reported by Newhouse et al. (1992), have also been found only as a pair; and second, the 11.2 kb segment in pattern II and 3.0 kb segment in patterns III and IV in our study are similar in size to the 11.10 kb and 3.15 kb segments in the earlier report.

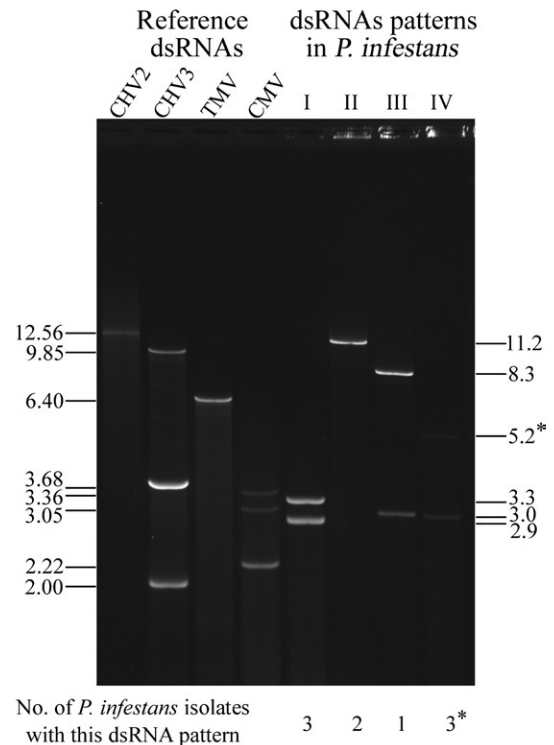

Fig. 1. Agarose gel electrophoresis of double-stranded RNAs (dsRNAs) in *Phytophthora infestans* and dsRNAs of reference viruses: CHV2, *Cryphonectria hypovirus 2* from *C. parasitica* strain NB58 (Hillman et al., 1994); CHV3, *Cryphonectria hypovirus 3* from *C. parasitica* strain GH2 (Smart et al., 1999); TMV, *Tobacco mosaic virus* (Goelet et al., 1982); and CMV, *Cucumber mosaic virus* (Owen et al., 1990; Rizzo and Palukaitis, 1988, 1989). Sizes of reference dsRNAs (in kb) are labeled on the left. CHV2 and CHV3 dsRNAs have poly (A) tails of varying lengths and they were assumed to be 50 nt. Sizes of *P. infestans* dsRNAs (in kb) were estimated using appropriate reference dsRNAs (see Material and methods for detail) and they are labeled on the right. *, all three isolates contained the 3.00 kb dsRNA; the weak 5.15 kb segment was visible only in some but not all extractions of isolate MX980317.

Table 1
Double-stranded RNA patterns in isolates of *Phytophthora infestans*.

Isolate	Mating type	Metalaxyl resistance	Origin	dsRNA pattern
MX980027	A1	nd	Mexico	None
MX980046	A1	Resistant	Mexico	None
MX980129	nd[a]	Sensitive	Mexico	None
MX980137	A2	Sensitive	Mexico	I
MX980211	nd	Resistant	Mexico	IV
MX980221	nd	Resistant	Mexico	None
MX980230	A1	Resistant	Mexico	None
MX980317	A2	Sensitive	Mexico	IV
MX980400	A2	Sensitive	Mexico	I
MX010005	nd	Resistant	Mexico	None
MX010006	A2	Resistant	Mexico	None
MX010007	A1	Resistant	Mexico	I
MX010046	A2	Resistant	Mexico	None
SA960008	A1	Intermediate	South Africa	None
US940480	A2	Sensitive	USA	II
US970001	A1	nd	USA	IV
US040009	A2	Intermediate	USA	II
FLa2005	A2	Sensitive	USA	III
2216	A1	nd	USA	None
Ka28	A2	Sensitive	Estonia	None
La1	A2	Intermediate	Estonia	None
V30	A2	Sensitive	Estonia	None

[a] nd, not determined.

The present study focused only on the two dsRNAs in isolate MX980400 (pattern I). Sequence analysis of these two dsRNAs showed that they were interdependent parts of the same virus genome (see below). We tentatively named this virus *P. infestans* RNA virus 1/MX980400 (PiRV-1/MX980400), following the convention used for viruses of mycelial lower eukaryotes (Fauquet et al., 2005). Throughout this paper, we refer to this virus as PiRV-1 for simplicity. Characterization of the dsRNAs in other *P. infestans* isolates described in this paper is underway. Preliminary sequence analysis indicates that the dsRNA segments of 11.2 kb, 8.3 kb and 3.0 kb belong to three distinct virus families (unpublished data).

Attempts to determine biological effects of PiRV-1

Despite repeated attempts with thermotherapy and chemotherapy using Ribavirin, we have been unable to generate an isogenic isolate of *P. infestans* isolate MX980400 that is free of PiRV-1. Similarly, we have thus far been unable to transfer PiRV-1 to a virus-free isolate by somatic fusion. Therefore, at this time, we cannot ascribe a specific phenotype to PiRV-1 presence in *P. infestans*. We have not observed consistent and distinctive symptoms or differences in pathology, such as those that are often associated with virus-infected strains of *C. parasitica* (Hillman and Suzuki, 2004), in *P. infestans* isolates harboring PiRV-1, but note that they have tended to die more often in storage and between transfers compared to other isolates. We cannot quantify this trait without virus-free, isogenic isolates.

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