Research Note

Presence of Ty1-Copia Group Retrotransposon Sequences in the Potato Late Blight Pathogen Phytophthora infestans

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Multiple copies of retrotransposon reverse transcriptase coding sequences were identified in Phytophthora infestans by polymerase chain reaction (PCR) amplification using degenerate primers. The P. infestans sequences belong to the Ty1-copia superfamily, and putative elements from different P. infestans isolates show restriction site polymorphisms. Some contain complete open reading frames while others do not, indicating the presence of potentially active as well as inactive elements.

Additional keywords: potato late blight, transposable element.

Few genetic mechanisms have been elucidated to explain the high degree of pathogenic variation long noted within Phytophthora infestans (Mont.) de Bary, causal agent of potato late blight. High rates of evolution of new, virulent, fungicide-resistant phenotypes suggest that transposable element activity may play a role. Transposons and retroelements have recently been described in diverse genera of filamentous fungi; to date, most have belonged to the gypsy group of LTR retrotransposons (Oliver 1992; Garfinkel 1992). LTR retro-elements within the Ty1-copia group, although found in a diverse array of organisms (Flavell 1992) have not, to our knowledge, been identified in filamentous fungi. The genus Phytophthora (a member of the class Oomycetes) is placed taxonomically in the same group as slime molds, certain algae, and protozoans, and is not considered a “true” fungus (Margulis et al. 1989). Thus, if Phytophthora species contain transposable elements they might be expected to be different from those described in other filamentous fungi. Indeed, the Tp1 element of the slime mold Phasmarum polycyphalum, whose sequence family constitutes over half of the organism’s repetitive DNA fraction, has been found to belong to the Ty1-copia group (Rothnie et al. 1991).

Reverse transcriptase is required for replication of retroviruses and retroviral-like elements and is present in numerous organisms (Xiong and Eckbush 1990). To determine whether reverse transcriptase sequences and hence retroviral-like transposable elements are present in P. infestans, we employed primers successful in amplifying reverse transcriptase sequences of the Ty1-copia superfamily from plants

![Fig. 1. Polymerase chain reaction (PCR) products obtained from DNA of three isolates of Phytophthora infestans. PCR reactions were carried out in a Perkin-Elmer Cetus (Norwalk, CT) model 9600 thermal cycler programmed for 30 cycles of 94°C for 15 s, 55°C for 15 s, and 72°C for 15 s, followed by a 6 min extension at 72°C. Reactions contained 1 U Taq polymerase (Perkin-Elmer Cetus), 50 μM each primer, 100 μM deoxynucleoside triphosphates, and 1.5 mM MgCl₂ in 25-μl reaction volume with no oil overlay. A, Primers (ACNGCNTYTTCIMG and ARCATRTCRTCNACRTA, where Y = C + T, R = A + G, and N = A + C + G + T) were as described in Flavell et al. (1992b). PCR products obtained from P. infestans were identical in size to products obtained when potato DNA was used as template (first lane). DNA was extracted from mycelium grown on pea broth as well as on synthetic (defined) medium (Xu et al. 1982).](image-url)
Fig. 2. Southern blots of Phytophthora infestans genomic DNA restricted with EcoRI and probed with radiolabeled plasmid DNA containing A, cloned retroviral-like element PIS80-4 and B, cloned element PIS80-1. Element PIS80-4 appears to be present in higher copy number in the P. infestans genome compared with element PIS80-1. With both elements, variation was observed among P. infestans isolates, illustrating potentially useful polymorphisms. Isolate sources are described elsewhere (Deahl et al. 1991; Tooley et al. 1985).

Fig. 3. Amino acid sequence alignment of Ty1-copia element reverse transcriptase sequences from Phytophthora infestans and other organisms. Sequences were analyzed using the UW-Genetic Computer Group package of programs (Devereaux et al. 1984) and aligned using CLUSTAL W (Thompson et al. 1994). Boxes delineate residues present in the consensus sequence, which is shown at the bottom of the figure. Phytophthora infestans sequences are represented by designations P176-4, P180-2, etc. Other Ty1-copia group sequences included in the alignment are as follows: 1731 and copia (Drosophila melanogaster), Tstl (Solanum tuberosum), Ty dependent (Saccharomyces cerevisiae), TaeI (Arabidopsis thaliana), Tn1 (Nicotiana tabacum), Tct1 (Clupea harengus), Volvox and Volvox (Volvox carteri), and Tp1 (Physarum polycephalum) (Camarin and Brisson 1990; Flavell and Smith 1992; Rothnie et al. 1991; Voytas et al. 1992; Xiong and Eickbush 1990).
clones from isolates 176 and 580, respectively. Clones PI580-1 (302 bp product) and PI580-4 (260 bp product) were used to probe Southern blots of total genomic DNA of eight isolates of *P. infestans* restricted with EcoRI (Fig. 2). Clone PI580-4 (Fig. 2A) hybridized with 7 to 10 bands for the eight *P. infestans* isolates chosen, while clone PI580-1 hybridized with 1 to 3 bands (Fig. 2B). Variation in band intensity within isolates and clear polymorphisms among isolates were observed (Fig. 2). There were no obvious correlations noted between banding patterns and mating type or geographic origin of the isolates.

DNA sequences obtained from eight clones from isolate 176 and six clones from isolate 580 were translated into protein sequences using the UW-GCG TRANSLATE program. The correct frame and orientation of each coding sequence was determined by the presence of the primer sequences on each end of the coding sequence. Open reading frames (ORFs) present in each sequence were identified and protein

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**Fig. 4.** Dendrogram produced in PHYLIP using neighbor-joining (Felsenstein 1993; Saitou and Nei 1987), showing relationships among the 13 *Phytophthora infestans* reverse transcriptase sequences (eight from isolate 176 and five from isolate 580). Seven sequences were identical or very similar to one another. Distances are measured on the horizontal axis only, in PAM units (Dayhoff et al. 1978; Felsenstein 1993). Primer sequences were removed for analyses of sequence relatedness.

**Fig. 5.** Dendrogram showing clustering relationships among *Phytophthora infestans*, *Tyl-copia*, and gypsy group reverse transcriptase sequences. See Figure 3 caption for abbreviations of *Tyl-copia* group members. The gypsy elements shown are as follows: gypsy and 412 (*Drosophila melanogaster*), Ty3 (yeast), Foret-1 (*Fusarium oxysporum*), and Dirs (*Dictyostelium discoideum*) (Flavell and Smith 1992; Julien et al. 1992; Xiong and Eickbush 1990). Distances are in PAM units (Dayhoff et al. 1978; Felsenstein 1993).
sequences were scanned for presence of the conserved Tyl-
copia group motif SLYXLKQAXRXW (Flavell et al. 1992b; 
Flavell et al. 1995; Xiong and Eickbush 1990). The single 
clone (PI580-1) that represented the higher molecular weight 
(302 bp) PCR product was found to contain portions of the 
conserved motif. However, it had the same primer sequence 
on each end, indicating that it may be a cloning artifact or the 
result of amplification with only one of the primers; it thus 
was not included in further analysis. Of the remaining 13 
clones (eight from isolate 176 and five from isolate 580), 
eight contained full-length ORFs and five contained short 
ORFs and stop codons.

Multiple sequence alignments were performed using the 
five non-full-length ORF sequences and the eight sequences 
with complete ORFs to determine the location of framehifts. 
Non-full-length ORF sequences were “corrected” by simple 
sequence editing where possible to facilitate multiple align-
ment and construction of dendrograms indicating sequence 
relationships. However, some of the sequences may represent 
inactive transposons and thus would not be expected to con-
tain ORFs or be otherwise functional. For clones PI176-6, 
PI580-2, PI580-4, and PI580-6, an extra base was added or 
subtracted from the sequence to put it in frame and eliminate 
stop codons. For sequence PI176-11, it was not possible to 
produce a full-length ORF by simple sequence editing, and it 
thus contains two stop codons (represented by Xs in the se-
quence).

Amino acid sequences from P. infestans were aligned with 
those of other Tyl-copia group members including the photo-
synthetic protist Volvox carteri and the slime mold Physarum polypephalum (Fig. 3). The P. infestans sequences are clearly 
members of the Tyl-copia retrotransposon group due to the 
presence of the consensus motif SLYXLKQAXRXW in some 
sequences, and very similar motifs in others (Fig. 3).

Sequences PI176-1, PI176-4, PI176-6, PI176-7, PI176-10, 
PI580-2, and PI580-5 were nearly identical (Fig. 4) with per-
cent similarities (measures of exact amino acid matches plus 
conserved substitutions) ranging from 93 to 100%. These se-
quences clustered separately from sequences PI176-5, PI176-
8, PI176-11, PI580-3, PI580-4, and PI580-6, which showed 
substantial variation (Fig. 4). Percent similarities among all of 
the P. infestans sequences ranged from 47 to 100%.

A dendrogram was constructed to show relationships 
among various Tyl-copia group elements including the P. in-
festans elements (Fig. 5). To avoid redundancy, only one se-
quence (PI176-4) was chosen from the large cluster of seven 
very similar P. infestans sequences (Fig. 4), along with the 
additional six less closely related P. infestans sequences. Also 
include were 10 members of the Tyl-copia group as well as 
five members of the gypsy group of retroelements. The gypsy 
sequences clustered separately from the Tyl-copia elements 
(Fig. 5). Some of the P. infestans sequences showed closer relationships with other Tyl-copia group elements than with other P. infestans elements. For two P. infestans sequences (PI176-4 and PI580-6), closest relationships (i.e., minimum or near-minimum distances) were observed with a sequence from the alga Volvox carteri (VOLVOX1), while for other P. infestans sequences (PI176-5, PI176-8, PI176-11, PI580-3, and PI580-4) such relationships were observed with elements Tnt1 from tobacco and/or element TAL from Arabidopsis. No consistent pattern of similarity was observed between P. in-
festans sequences and those of specific other Tyl-copia group 
members, such as the alga Volvox carteri or the slime mold 
Physarum polycephalum.

Flavell (1992) has suggested that horizontal transmission of 
Tyl-copia sequences between different species has occurred 
in the past and contributed to their evolution. Our data may 
support the horizontal transmission hypothesis in that se-
quences from P. infestans and its plant host, Solanum tubero-
sum L. (element Tst1), showed percent similarities ranging 
from 51 to 64%, which exceeds some of the percent similari-
ties observed among P. infestans elements (range 47 to 
100%).

Variation in the intensity of specific bands observed within 
isolates such as 176 and 177 on Southern blots may indicate 
that a portion of a given element may be present in low copy 
number while other portions may be highly repeated. It is also 
possible that multiple Tyl-copia families may exist within P. 
infestans and that multiple bands may reflect different types 
of elements within a given isolate. Additional characterization 
of full-length elements will be required to determine whether 
retroelements contribute to variation in pathogenicity and 
other characters in P. infestans.

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