Linear Mitochondrial Plasmids of *Fusarium oxysporum* Contain Genes with Sequence Similarity to Genes Encoding a Reverse Transcriptase from *Neurospora* spp.

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Two linear mitochondrial plasmids called pFOXC1 and pFOXC2 from the fungus *Fusarium oxysporum* were previously described. DNA sequence comparisons indicated that the derived amino acid sequences of both plasmids exhibit similarity to the reverse transcriptase of the Mauriceville and Varkud plasmids of *Neurospora* spp. The derived amino acid sequence of pFOXC2 has 51% similarity and 32% identity to the *Neurospora* reverse transcriptase; sequence similarity was greatest for seven blocks of amino acids that are conserved in reverse transcriptases from a wide range of biological sources. Northern analysis suggests that full-length RNAs corresponding to the plasmids are found in representative isolates.

Isolates of *Fusarium oxysporum* Schltdl.:Fr. causing disease on crucifers have been categorized on the basis of their relative ability to cause disease in particular host species. Isolates causing the cabbage yellows disease have been defined as *Fusarium oxysporum* f. sp. *conglutinans* (18), while isolates pathogenic to the radish or to the ornamental crucifer stock (*Matthiola incana*) have been called *F. oxysporum* f. sp. *raphani* or f. sp. *matthioli*, respectively (4, 10). Bosland and Williams (4), using isozyme polymorphisms and vegetative compatibility groupings (VCGs), were able to establish that the cabbage, radish, and stock pathogens were genetically distinct.

DNA polymorphisms in mitochondrial DNA also clearly distinguished isolates in these formae speciales and corresponding VCGs (12, 13). No polymorphisms in mitochondrial DNAs were noted for isolates within a forma specialis or VCG. Linear plasmids were associated with the mitochondria in all examined isolates of *F. oxysporum* f. sp. *conglutinans*, f. sp. *raphani*, and f. sp. *matthioli* from a global collection (9, 13, 14). These plasmids have been called pFOXC1, pFOXC2, and pFOXC3, respectively, and each is 1.9 kb in size. The plasmids have different restriction fragment length polymorphism maps, and while pFOXC2 and pFOXC3 cross-hybridize, Southern hybridization analyses suggest that pFOXC1 and pFOXC2 have no sequence similarity (13, 14). The purpose of this study was to further characterize the plasmids of *F. oxysporum* by examining the plasmids found in isolate 777 of *F. oxysporum* f. sp. *conglutinans* and isolate 699 of *F. oxysporum* f. sp. *raphani*. Cultures of these isolates are maintained by H. C. Kistler and by the Crucifer Genetics Cooperative, Department of Plant Pathology, University of Wisconsin, Madison.

The 785-bp internal *Bgl*II fragment of the 1.9-kb plasmid pFOXC1 from isolate 777 was cloned in the *Bam*HI site of pUC118, and the internal 1,726-bp *Bgl*II fragment of the 1.9-kb plasmid pFOXC2 from isolate 699 was cloned in the *Bam*HI site of pUC12 (14). The clone from pFOXC1 was called pBB1, and the clone from pFOXC2 was called pCK2. The DNA sequences of the two inserts were determined. Automated

DNA sequencing was performed with a Perkin-Elmer/Applied Biosystems apparatus (ABI 373 Stretch) at the University of Florida (UF) Interdisciplinary Center for Biotechnology Research (ICBR) DNA Sequencing Core Facility. Oligonucleotide primers were made on an Applied Biosystems ABI 394 synthesizer at the UF ICBR DNA Synthesis Facility. Sequence analyses were done at the UF ICBR Biocomputing Facility with the Genetics Computer Group (GCG) package (7), version 8.1.

Although DNA sequence similarity between the plasmids was not detected by Southern hybridization, 57.8% DNA sequence similarity between the cloned plasmid sequences was determined with the BESTFIT program of GCG. The relatively low level of similarity and the presence of 12 gaps of various sizes probably account for the lack of hybridization in stringent Southern blot assays.

A BLASTX search performed via the National Center for Biotechnology Information BLAST network system (1, 2) revealed significant similarity among the derived amino acid sequences of pFOXC1, pFOXC2, and genes coding for reverse transcriptase (RT) associated with fungal plasmids and group II introns. The sequence most closely related to both pFOXC1 and pFOXC2 was a putative RT from the mitochondrial plasmid Et2.0L of the fungal endophyte *Epichloë typhina* (16). The amino acid sequence derived from the coding region of this 2.0-kb linear plasmid has extensive similarity to the sequence of an authentic RT (19) encoded by the *Neurospora* plasmid Mauriceville. An alignment of putative coding regions from pFOXC1 and pFOXC2 with the Mauriceville RT is shown in Fig. 1. For all sequences, including pFOXC1 and pFOXC2, the codon UGA was presumed to encode tryptophan, as it does for other fungal mitochondrial genes.

The putative pFOXC2 polypeptide consists of 397 amino acids. The Mauriceville RT has 710 amino acids and thus is much longer than the other polypeptides that were compared. However, the complete size of the inferred pFOXC1 polypeptide is unknown since analysis was based only on partial DNA sequence data; the entire inferred polypeptide (261 amino acids) was used for sequence comparison purposes. When pairwise comparison of polypeptides was performed, the pFOXC2 open reading frame (ORF) showed 51.2% amino acid similar-

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FIG. 1. Multiple alignment of coding regions of authentic or presumed RTs from fungal mitochondrial plasmids. The alignment was produced by the GCG PILEUP program (7). The derived amino acid sequences of pFOXC1 (GenBank accession no. AF005240), pFOXC2 (accession no. AF005241), and the *N. crassa* Mauriceville plasmid ("Mauric"; accession no. K03295) were compared. Identical amino acids within the seven conserved regions common to RT sequences (15) are shown in blocks. The comparison begins with amino acid 101 of Mauriceville. Vertical lines indicate the first and last amino acids of the specified regions. Dots indicate the positions of gaps.

ity and 31.8% identity to the Mauriceville RT, 53.4% similarity and 31.5% identity to the Et2.0L ORF, and 57.9% similarity and 39.6% identity to the pFOXC1 ORF.

Further analysis of pFOXC1 and pFOXC2 was carried out by Northern hybridization to total RNA isolated from fungal isolates 777 and 699, respectively, with pBB1 and pCK2, respectively, as probes. RNAs for Northern hybridization were isolated and separated on formaldehyde-1.5% agarose denaturing gels essentially as described previously (6). Plasmid pBB1 hybridized to a major RNA species of approximately 1.95 kb and a minor band of about 1.6 kb (Fig. 2, left). Plasmid pCK2 hybridized to several RNAs ranging in size from 1.95 to 0.56 kb (Fig. 2, right).

In conclusion, DNA sequence determination of portions of the linear mitochondrial plasmids pFOXC1 and pFOXC2 indicated that they are related to mitochondrial plasmids from *Neurospora* spp. The circular Mauriceville plasmid is a retroelement that has been observed in certain isolates of *N. crassa*, *N. intermedia*, and other species of *Neurospora* (3, 8). Both Mauriceville and the closely related and circular Varkud plasmids yield a single major transcript of unit length (11) encoding an 81-kDa RT (15, 19). Thus, despite having different contours (circular or linear), the plasmids from *Neurospora* species and *F. oxysporum* each correspond to a single major RNA of unit size seemingly coding for a RT.

Also, like the *Neurospora* plasmids, the *F. oxysporum* mitochondrial plasmids are variably distributed among isolates of their respective genus or species (3, 8, 9) and can be transmitted independently of the mitochondrial chromosome. The Varkud plasmid has been shown to be transferred during unstable *N. intermedia* pairings and between isolates of *N. crassa* and *N. intermedia* (5). The *F. oxysporum* plasmid pFOXC2 also has been shown to be transmitted from an *F. oxysporum* f. sp.

FIG. 2. Northern hybridization of pBB1 (left) and pCK2 (right) to approximately 10 μ g of total RNA from *F. oxysporum* f. sp. *conglutinans* isolate 777 (left) or *F. oxysporum* f. sp. *raphani* isolate 699 (right). The arrows indicate the positions of RNA size standards of (from top to bottom) 2.3, 1.77, 1.52, 1.28, 0.78, and 0.53 kb.

raphani isolate to an *F. oxysporum* f. sp. *conglutinans* genetic background during transient protoplast fusion (17). The variable distribution of the plasmids and their ability to be transferred independently of nuclear or mitochondrial chromosomes suggest that these plasmids are infectious agents capable of horizontal transmission.

In contrast to the well-characterized *Neurospora* plasmids, the mechanism of replication for linear mitochondrial plasmids in other fungi is poorly characterized (8). While the presence of an RT-like gene on the pFOXC plasmids may imply a similar, RT-based replication strategy, there is no currently available evidence indicating that the plasmid genes encode a functional RT; this possibility is currently being investigated (10a). Plasmids pFOXC1 and pFOXC2 have been determined to be linear elements based on their restriction maps, sensitivity to exonuclease III, and contour when examined by electron microscopy (14). Additionally, DNA elements corresponding to the pFOXC plasmids but twice the unit size (3.8 kb) are found in low abundance in perhaps all host isolates, as determined by Southern hybridization analysis (14). The linear contour and larger related forms of the plasmid perhaps should be taken into consideration when proposing a mechanism of replication. The mechanism proposed for replication of the circular Mauriceville plasmid (11), however, would not preclude a similar replication strategy for linear molecules. The linear mitochondrial plasmids pFOXC1 and pFOXC2, along with plasmid Et2.0L from *E. typhina*, may represent a new class of fungal plasmids with a linear contour and encoding an RT-like polypeptide.

Nucleotide sequence accession numbers. The nucleotide sequences of pBB1 and pCK2 have been submitted to GenBank

and assigned the accession numbers AF005240 and AF005241, respectively.

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