MOLECULAR APPROACHES TO DIFFERENTIATE SUBPOPULATIONS OR Formae specialis OF PHYTOPATHOGENIC FUNGI

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Introduction

Ustilago violacea Microbotryum violaceum) is a heterobasidiomycete plant pathogen that infects over 200 species of Caryophyllaceae (Pinks). Circumstantial evidence has suggested that physiological races or *formae specialis* of *U. violacea* can be defined according to which host species are productively infected, as individual strains of the fungus tend to be only able to infect host species from which they are isolated and such host preference varies among different *U. violacea* isolates. Since development of the dikaryotic and spore forms of the fungus is obligately parasitic, the molecular bases for such "races" (if *bona fide*) and for their host preference are of interest. To date, there has been a paucity of genetic linkage data concerning *U. violacea* races. In the absence of such data, we have investigated several approaches to produce molecular "fingerprints" of *U. violacea* strains isolated from different host species and to further characterize the relatedness of such strains. Eighteen different sporidial strains representing 7 different *formae specials* were examined for electrophoretic karyotype, RAPD-PCR profile, by phylogenetic analysis of intron sequences in the γ-tubulin gene, and analysis of Melting Curves of total genomic DNA (DNA Thermal Profiles).

Methods

Electrophoretic karyotypes of *U. violacea* haploid sporidial cells were produced from cells embedded and treated in agarose (1), using a CHEF DRII or CHEF Mapper System (Bio-Rad, Costa Mesa, CA). RAPDS employed random 10-mers (Operon Technologies), individually in PCR reactions. For *DNA sequence comparisons,* a 392 bp fragment of the γ-tubulin gene (2) was amplified by PCR.

The fragment contained the 6th and 7th introns of the gene. Amplified PCR fragments from each sporidial isolate were cloned into pCR-Script using a kit (Stratagene, La Jolla, CA) and sequenced using T7 DNA polymerase (US Biochemical, Cleveland, OH). *DNA Thermal Profiles* of total genomic DNAs were performed in a thermally-controlled cuvette holder of a Gilford Response II spectrophotometer.

Results and Discussion

Electrophoretic karyotypes and RAPDs. Comparison of electrophoretic karyotypes in conjunction with Southern hybridization with γ-tubulin gene as a probe provided a measure for gauging strain relatedness. The combination of these methods with RAPD profiles identified isolates in a manner consistent with their anecdotal race designations. Polymorphisms in chromosome length and number are apparently common between different *U. violacea* sporidial strains. Moreover, dramatic differences were observed from strains isolated from greatly disparate hosts.

DNA Sequence Analyses of Introns. U. violacea isolates from *Silene latifolia* had identical DNA sequence in the region examined in this study. An isolate from a closely-related host (*S. dioica*) had identical sequence in Intron 6, two changes in Intron 7 and one change in the third position of codons (in Exon 7). An isolate from a more distant host had eight changes in Intron 6, eight changes in Intron 7, and four changes in the third positions of codons (in the coding region, Exon 7). The changes observed preserved the consensus splice signals for these introns.

That so many differences were observed for this more distant *forma specialis* was consistent with the marked difference in electrophoretic karyotype also seen for it relative to that for the isolates for the isolates from *S. latifolia.*

DNA thermal denaturation profiles. DNA Thermal Profiling utilizes the entire genomic DNA of the organism being examined. As such, it has the advantage of detecting and utilizing microheterogeneities throughout the test organisms genomes. To date, this method has been used by us to distinguish Cuban crocodiles from Cuban/American hybrids and to correctly predict familial relationships for alligators and red-winged blackbirds. For *U. violacea* the groupings tested produced one parsimonious tree of relationship between the different isolates. For trees with five members, the consistency index was 0.75; the largest tree had a consistency index of 0.6. Thus, the trees were quite robust. The relationships designated by this new method were very reproducible, even when outgroups were changed or when additional members were included in the tree. In most cases, the cladograms produced from such analyses reflected known population differences (see Figure 1). This suggests that the method provides a quick and simple alternative for characterizing different populations of fungi. In conjunction with the other methods in this study, one may thus produce a reliable DNA fingerprint of different pathotypes of a fungal species.

1. Mc Cluskey K, Russell BW and Mills D. Curr. Genet. 1990;18:385-386.
2. Luo H and MH Perlin. Gene 1993;137:187-194.

Figure 1. ***Cladograms produced using maximum parsimony analysis of DNA Thermal Profile data from sporidial isolates: Six, Nine, 2,8, 22 000 were all from S. latifolia; UWO2, from an S. latifolia/S. dioica hybrid; UWO14, from S. dioica; 26-1 and 26-2, from Dianthus carthusianorum; Yeast, S. cerivisiae, used as outgroup.***