BRIEF NOTE

Phylogenetic Affinities of the Species in Fusarium
Section Sporotrichiella

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Sequence comparisons of selected regions from small (18 S) and large (25 S) subunit ribosomal RNA were used to examine species relationships in Fusarium section Sporotrichiella (F. poae, F. tricinctum, F. sporotrichioides, and F. chlamydosporum). The region 25 S-635 is the most variable of the three primer regions considered. Sequence differences among the species (with a minimum of three different bases) support the morphotaxonomic distinction, confirming them to be distinct taxa. F. sporotrichioides NRRL-3299 and F. poae NRRL-13637 are closely related while the other two species are less so. Phylogenetic affinities of the species correlate with the production of trichothecene mycotoxins by the species. © 1991 Academic Press, Inc.


The species of Fusarium section Sporotrichiella include some serious plant pathogens (Booth. 1971), and some strains are able to produce potent mycotoxins implicated in animal and human diseases (Marasas et al., 1984).

Despite the need for precise identification, this section presents some morphotaxonomic difficulties. Morphological features of strains may degenerate rapidly in culture, making it impossible to identify the isolates. In strains that retain their morphological characteristics, several different taxonomic systems based on the relative importance of these traits exist. Wollenweber and Reinking (1935) and Nelson et al. (1983) distinguish four species, primarily on the basis of microconidium shape (pyriform to globose): F. poae (Peck) Wollenw., F. chlamydosporum Wollenw. & Reinking, F. tricinctum (Corda) Sacc., and F. sporotrichioides Sherb. Booth (1971), on the basis of sporogenous cell morphology, splits the section in half leaving only F. poae and F. tricinctum in Sporotrichiella. Finally, Snyder and Hansen (1945) suggested that, from the phytopathological point of view, all the species of section Sporotrichiella should be grouped under the species F. tricinctum (Corda) Snyd. et Hans.

Attempts have been made to classify the species in section Sporotrichiella using profiles of secondary metabolites, including mycotoxins (Thrane, 1989; Logrieco et al., 1990a). Even in this case, there is some confusion because of misidentification or incomplete taxonomic characterization of the toxin-producing strains.

Recently, molecular biological studies have proven to be helpful in understanding the phylogenetic relationships within the fungal kingdom (Kurtzman, 1985). Ribosomal RNA is among the most highly conserved genes known, and its sequence is a potential source of information for inferring patterns of evolutionary relationships among taxa.

We describe rRNA sequences from three regions of small and large subunit rRNA and evaluate the most useful regions for further molecular genetic studies in Fusarium. Phylogenetic relationships among the
SPECIES AFFINITIES OF *Sporothrichiella*

species belonging to section *Sporothrichiella* derived from the sequence data are presented.

For this study, one representative strain from each of the four species in section *Sporothrichiella* (sensu Nelson et al., 1983) was studied. The fungal strains were provided by Paul E. Nelson (Fusarium Research Center, The Pennsylvania State University) (numbers in parentheses) and are maintained in the Agriculture Research Service Culture Collection (NRRL Peoria, IL). These strains are *F. poae* NRRL-13637 (= T-247); *F. sporotrichioides* NRRL-3299 (= T-424); *F. chlamydosporum* NRRL-13636 (= T-227), and *F. tricinctum* NRRL-13639 (= T-429).

Stock cultures were grown on hay-agar slants (Raper, 1937) at 25°C under alternate light-dark conditions to induce sporulation. These slants were used to inoculate flasks containing 100 ml of YM medium (Wickerham, 1951). The flasks were incubated at 25°C on a rotary shaker (200 rpm) for 16-36 h until the cultures were in log phase growth. Ribosomal RNA was isolated according to the procedure of Chirgwin et al. (1979), except that the cells were harvested by filtration and, after suspension in guanidine thiocyanate reagent (10 ml/g), broken in a Braun cell homogenizer with 0.5-mm glass beads.

The base sequences of the selected regions of the large (25S) and small (18S) subunits of rRNA were determined using specific oligonucleotide primers and the dideoxy nucleotide chain termination method for RNA sequencing as described by Lane et al. (1985). The oligonucleotide primers (Guého et al., 1989) were synthesized with an Applied Biosystems Model 380a DNA synthesizer. The first bases copied from the large subunit primers, based on *Saccharomyces cerevisiae* primary structure (Georgiev et al., 1981), are 1841 (primer 25S-1841) and 635 (primer 25S-635). The first base synthesized with the small subunit primer, 18S-1627, is base 1627 (Rubstov et al., 1980). Sulfur-35-labeled nucleotide fragments generated in the chain extension reactions were separated by electrophoresis on 8% acrylamide–8 M urea denaturing polyacrylamide gels. RNA base sequences (recorded as A, C, G, or U) were read from autoradiographs of the fixed and dried gels and aligned manually. Where the proper base could not be determined, N was assigned. The data were evaluated measuring the similarity of aligned sequences.

Large and small subunit partial rRNA sequences from the species examined are presented in Fig. 1. In primer region 25S-635, sequence differences between the species range from 3 to 9 base substitutions. In region 25S-1841, the number of substitutions ranges from 1-7, and in region 18S-1627, differences are only 0 to 2 bases. The relatively rapid mutations of region 25S-635, lower variability in region 25S-1841, and far lower variability in region 18S-1627 were previously found in *Penicillium* species (Logrieco et al., 1990b) and ascomycetous yeasts (Peterson and Kurtzman, 1991). Relatively large numbers of base substitutions between species in section *Sporothrichiella* are only found in region 25S-635, and the use of this particular region of rRNA is therefore most likely to produce adequate variability for distinguishing other *Fusarium* species.

Studies of rRNA sequences from strains of several ascomycetous yeasts species (Peterson and Kurtzman, 1991) and multiple strains of *F. sambucinum* (Peterson and Logrieco, 1991), as well as other *Fusarium* species (Guadet et al., 1989), showed very little sequence variation (0-1 substitution) among strains of a fungal species. Ribosomal RNA genes occur in fungi as a single linkage group containing many tandemly repeated units [100-140 in *S. cerevisiae* (Fangman and Zakian, 1981)]. Dover (1982) has suggested reasons why tandemly repeated genes often have the same sequence in an individual and why individuals in a species may have the same sequence. Because a variety of fungi have been exam-
Fig. 1. Ribosomal RNA sequence of three primer regions from the species of Sporotrichia section. Dots indicate that the base is the same as that in the reference strain (line 1), and N indicates that the base was not determined.
### SPECIES AFFINITIES OF *Sporotrichella*

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<th>Primer Region (bp)</th>
<th>Species</th>
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<th>F. chlamydosporum</th>
<th>F. tricinctum</th>
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**Fig. 2.** Number of different bases in three primer regions among the species.

... (cited above), and little or no sequence variation has been detected among various strains of a species, and because sequence change of ribosomal RNA is influenced by its multiple-copy tandemly repeated structure in the genome, the sequence from a single fungal strain is a good indicator of the sequence for the species as a whole, both in theory and in the limited sequence data available. Genetic sibling species of yeasts are typically separated by 2–9 sequence differences in region 25 S–635, an approximately 1–4% sequence difference (Peterson and Kurtzman, 1991). The presence of 3 or more base substitutions between the species of section *Sporotrichella* strongly suggests that each is a distinct taxon.

*F. poae* and *F. tricinctum* differ by 7 nucleotide differences in region 25 S–635 and show 0 differences in region 18 S–1627. *F. poae* and *F. sporotrichioides* display 3 and 1 nucleotide difference in the same regions. The relative distances between taxa, measured in different priming regions, are not entirely consistent. The molecular clock is based on discrete steps occurring over time in the form of base substitutions, insertions, deletions, and other rearrangements. When dealing with a small number of nucleotide changes, as in this data set, we believe the time period involved is too short to give an accurate rate of change for the slowly evolving regions. This is especially true of the primer region 18 S–1627, which seems unsuitable for measurements of genetic distance between these taxa. The data from the primer region 25 S–635 reveal more nucleotide substitutions and may be a more accurate reflection of genetic distance. The sequence 25 S–635 is an insertion region (Hassouna et al., 1984), and in human rRNA nonchronometric change has been documented in this insertion region (Hancock and Dover, 1991). However, in our data set there is no indication of repetitive, slippage-generated fragments (Hancock and Dover, 1991) occurring nor other anomalies in the data suggesting nonchronometric changes.

In region 25 S–635, *F. poae* shows 3, 6 and 7 different bases for *F. sporotrichioides*, *F. chlamydosporum*, and *F. tricinctum*, respectively. *F. sporotrichioides* displays eight different bases with both *F. chlamydosporum* and *F. tricinctum*, and the latter two species have 9 different bases. Among these species, *F. sporotrichioides* and *F. poae* are the most closely related phylogenetically, with only three base differences separating them. This relatedness correlates with the secondary metabolite profiles reported in which only these two species of section *Sporotrichella* produce trichothecenes (Thrane, 1989; Lo-
To our knowledge this work is the first report of a positive correlation between phylogeny based on rRNA sequence and secondary metabolite biochemical data in Fusarium species. The number of base substitutions present in the data set (although small) can form part of an argument for the phylogenetic similarity of F. sporotrichioides and F. poae when taken in conjunction with other available data.

The sequence similarity found among multiple isolates of Fusarium and other fungal species (cited above) suggests that rRNA sequence fragments could be very useful in identifying the affinities of morphologically degenerate or atypical strains through comparison with sequences from morphologically typical strains. It will be interesting to determine whether other trichotheccene-producing species of Fusarium also cluster with these species on the basis of the rRNA sequence.

ACKNOWLEDGMENTS

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REFERENCES


sequence of the 18S ribosomal RNA gene from *Saccharomyces cerevisiae*. Nucleic Acids Res. 8: 5779–5794.


