EVOLUTION

Extensive Fungal Diversity in Plant Roots

Philippe Vandenkoornhuyse,[1,2]* Sandra L. Baldauf,[1] Corinne Leyval,[2] Jean Straczek,[3] J. Peter W. Young[1]

Fungi play crucially important roles in the biosphere, mediating many ecological processes. Despite this, the fungal diversity in natural habitats is poorly known. Using newly designed fungal-specific polymerase chain reaction (PCR) primers (*1*), we examined total fungal diversity in a relatively mundane habitat, the roots of a plant.

Total "environmental DNA" was prepared from individual, extensively cleaned roots of the grass *Arrhenatherum elatius* (*2*). After PCR amplification, separate small subunit ribosomal RNA (SSU rRNA) gene libraries were constructed, and 200 clones were sequenced at random (*2*). Forty-nine different sequences (phylotypes) were found and all were fungal. Only seven of the 49 phylotypes are closely similar to known sequences (>99% identity). This diversity from the roots of a single plant species and from a single sampling location is completely unexpected.

Phylogenetic analyses were conducted on the 49 environmental phylotypes together with all 1208 fungal SSU rRNA gene sequences available up to August 2001 (*2*). Phylogenetic analyses were carried out at two levels. First, a global "meta-phylogeny" of all the fungal terminals was computed (*2*, *3*). On the basis of this all-inclusive phylogeny, the data set was then trimmed to be broadly representative of all fungi plus all close neighbors of the environmental phylotypes (Fig. 1) (*2*). Both trees are largely in agreement with published phylogenies [e.g., (*4*)].

The 49 phylotypes are distributed across all fungal phyla (1 Chytridiomycota, 8 Zygomycota, 16 Basidiomycota, and 25 Ascomycota) (Fig. 1). By contrast, culture-based analyses of endophytic fungi have yielded mostly Ascomycetes (*5*). As expected, the phylotypes include relatives of mutualistic arbuscular mycorrhizal fungi (AF202280, AF202299, and AF204217; Fig. 1). However, we can only speculate on the possible roles of the other 94% of the root fungal diversity found here.

In addition to this unexpected breadth of taxa, our study suggests the existence of previously unknown groups of fungi (I, II, III, IV, and V in Fig. 1). The public databases now include representatives of all fungal classes and most known fungal orders and families, so fungal sequences without matches are especially interesting. The most striking of these are two deeply branching lineages (II and IV) without apparent close relatives. These sequences are unlikely to be chimeric because each was found with the roots of three different plants analyzed independently. The placement of AF202282 (II, Fig. 1) at the base of the Basidiomycetes is unaffected by inclusion or exclusion of the long Uredinomycetes and Ustilagomycetes branches. In fact, deleting the latter sequences substantially increases the bootstrap support for both the nodes immediately above [neighbor joining (NJ) 87%, maximum parsimony (MP) 89%] and below AF202282 (NJ 99%, MP 97%). The position of AF202279 between the Pezizomycotina and Saccharomycotina (IV, Fig. 1) is unchanged by including the extremely long-branched sequences of Archaeascomycetes, *Pneumocystis carinii*, and various *Taphrina* species. Likewise, the phylogenetic positions of II and IV do not appear to be due to a long-branch attraction artifact (*6*). Within the Ascomycota, a monophyletic group of 13 phylotypes (V), subdivided into three distinct clusters (Fig. 1), is related to two unidentified fungal sequences and may be a previously unknown group.

Our results raise questions about the possible role and ecological implications of this fungal diversity associated with plant roots, about the lifestyle and functions of these fungi, and about the fungal diversity in other ecological niches.

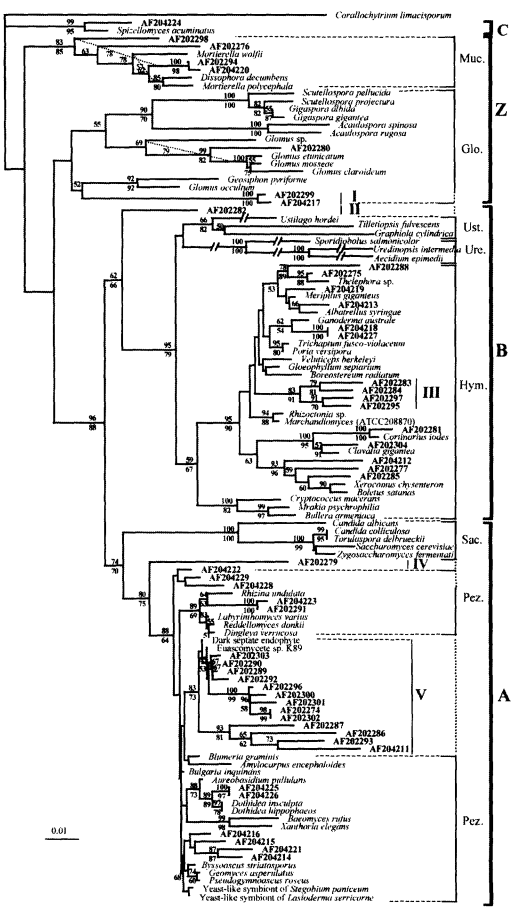

Fig. 1. Phylogenetic affinities of fungal SSU rRNA sequences amplified from *Arrhenatherum elatius* roots. The tree shown was derived by NJ distance analysis of all 49 nonchimeric environmental DNA sequences (rectangles in the figure) along with their highest scoring BLAST hits and a taxonomically representative set of known fungal sequences. Bootstrap values >50% for NJ and MP are shown above and below the lines, respectively, for major clades only; all others >70% are indicated by solid circles. The MP analysis generated 11 equiprobable trees (Rohlf's consistency index = 0.698; retention index = 0.777). The NJ and MP trees differed only in some weakly supported branches, as indicated by the dashed lines. I, II, III, IV, and V are possible previously unknown lineages. Major groups of fungi indicated to right are A, Ascomycota; B, Basidiomycota; C, Chytridiomycota; Z, Zygomycota; Glo, Glomales; Hym, Hymenomycetes; Muc, Mucorales; Pez, Pezizomycotina; Sac, Saccharomycotina; Ure, Uredinomycetes; Ust, Ustilagomycetes. An expanded version of the figure is available on *Science* Online (*2*).

References and Notes

1. The forward primer AU2 (TTTCGATGGTAGGATAGDGG) and reverse primer AU4 (RTCTCACTAAGCCATTC) were designed for the amplification of the fungal SSU rRNA gene (*2*).
2. See supplemental data available on *Science* Online at www.sciencemag.org/cgi/content/full/295/5562/2051/DC1.
3. Available upon request from P.V.
4. A. Tehler, J. S. Farris, D. L. Lipscomb, M. Källersjö, *Mycologia* **93**, 459 (2000).
5. A. E. Arnould, Z. Maynard, G. S. Gilbert, P. D. Coley, T. A. Kursar, *Ecol. Lett.* **3**, 267 (2000).
6. J. Felsenstein, *Syst. Zool.* **27**, 401 (1978).
7. We thank K. Ridgway and T. Helgason for critical comments of the manuscript. This work was funded by a grant from the Bureau des Ressources Génétiques. P.V. is supported by the Natural Environment Research Council "Soil Biodiversity" programme.

[1]Department of Biology, University of York, Post Office Box 373, York, YO10 5YW, UK. [2]Centre de Pédologie Biologique, rue ND des Pauvres, 54500 Vandoeuvre-les-Nancy, France. [3]Centre Hospitalier Universitaire de Nancy, Biochimie B, 54000 Nancy, France.

*To whom correspondence should be addressed. E-mail: psjv1@york.ac.uk or p.vandenkoornhuyse@free.fr