Abstract

Intraspecific and intragenomic variation of *Armillaria ostoyae* were observed through sequencing of ribosomal DNA (rDNA) including nuclear large ribosomal subunit (nLSU), internal transcribed spacer (ITS), 5.8S rDNA, and intergenic spacer (IGS-1). Sequencing of ribosomal DNA (rDNA) including nuclear large ribosomal subunit (nLSU), internal transcribed spacer (ITS), 5.8S rDNA, and intergenic spacer (IGS-1) were sequenced at the University of Idaho, Moscow, ID 83844.

Introduction

The fungal genus *Armillaria* is a group of root-associated basidiomycetes, which includes thirty-nine species worldwide and ten North American biological species (Barbosa 1993). In the western United States, *A. ostoyae* is a serious plant pathogen that causes root rot and shoot necrosis on a variety of woody plant species. It adversely impacts commercial timber production by causing significant tree mortality and a reduction in tree growth. Growth losses due to *A. ostoyae* are often difficult to detect because signs of infection may not be readily observable. Volume loss has been shown as high as 40% over 4-8 years in eighteen-year-old Douglas-fir (Cruckshank 2000). Growth loss and mortality due to *A. ostoyae* in British Columbia cause annual losses between 1.4 and 3.8 million m$^3$ (White and Morrison 1999). Studies have shown that genes of *A. ostoyae* can show varying levels of pathogenicity and virulence (Omalu et al. 1993, Morrison and Pellow 2002). However, little has been done to identify genetic differences among genotypes of *A. ostoyae*. Assessing these differences may be important in understanding varying levels of pathogenicity and virulence within *A. ostoyae*. 2. Phylogenetic relationships among *A. ostoyae* genet, and 3) adaptation to environmental factors.

Objectives

1. Assess intraspecific genetic diversity and intraspecific hybridization within *A. ostoyae*, and examine for possible relationships to ecological function (e.g., pathogenicity, host specificity, habitat type), geographic origin, forest management practices (e.g., fertilization), and interactions among *Armillaria* genotypes.

2. Evaluate the intraspecific and intragenomic variation of *A. ostoyae* genet using direct PCR method (mycelium scraped from pure culture and added directly to PCR reaction mixture). Twenty-six of the 61 genets were: A. *ostoyae* from the western United States. Ten other genets, including two *A. ostoyae* genets from other geographical regions and eight genets from other *Armillaria* species, were used for phylogenetic comparisons (Table 1). PCR products were purified and sequenced at Davis Sequencing, Inc. (Davis, CA). The sequences were edited by hand and Juke and Cantor:

Materials and Methods

PCR products from ribosomal DNA (RDN) including nuclear large ribosomal subunit (nLSU), internal transcribed spacer (ITS), 5.8S rDNA, and intergenic spacer (IGS-1) were obtained from thirty-six genets of *Armillaria* by direct PCR method (mycelium scraped from pure culture was added directly to PCR reaction mixture). Twenty-six of the 61 genets were: *A. ostoyae* from the western United States. Ten other genets, including two *A. ostoyae* genets from other geographical regions and eight genets from other *Armillaria* species, were used for phylogenetic comparisons (Table 1). PCR products were purified and sequenced at Davis Sequencing, Inc. (Davis, CA). The sequences were edited by hand and electronically. When possible, heterogenetic sequences described by one of the methods described in Figure 1 before phylogenetic analysis. The method described in Figure 1b is similar to that of the mismatch model developed by B. Brown.

Phylogenetic analysis was performed for each DNA region using MrBayes v3.0.4 (Huelsenbeck and Ronquist 2001) for Bayesian inference of phylogeny. Indels were treated as a single event and coded using the simple gap coding method (Symon and Ochoterena 2000). To select a model for use in Bayesian inference, we used MrModeltest v2.3 (Nylander et al. 2003). Three thousand generations were run for three million generations generating files with thirty thousand trees, the first six thousand of these trees were discarded as the "burn-in" of the chains. The remaining trees were used to make majority rule consensus trees.

Results and Discussion

Direct PCR has been shown to detect 90% of the heterogenous rDNA products in an individual and the relative peak height seems to reflect relative concentrations (Rauscher et al. 2002). In this study, heterogenous products indicating intraspecific and intragenomic variation were common in all regions analyzed, with the exception of the 3.48 rDNA. Figure 2 shows variation in the IGS-1 region that resulted in the formation of significant clades. Although the intergenic spacer region is frequently used as a non-functional marker, some suggest that variation in IGS DNA spaces has major influences on organism growth rates (Ehler et al. 2004). Table 3 shows that a significant amount of variation exists in the LSU region. A region commonly thought to have limited importance. Figures 3a and 3b show the importance of using non-heterogenous sequences in phylogenetic analysis. When heterogenous sequences are included in phylogenetic analysis, the parental phylogenies become more resolved. Figure 3a includes heterogenous sequences, showing only one clade with a posterior probability of 64 percent. Figure 3b excludes the heterogenous sequences, thereby improving the probability of the single clade to 98% and resolving a second clade with a probability of 98%. Continued work is underway at the USDA Forest Service - RMRS, Forestry Sciences Laboratory in Idaho to analyze a larger number of *A. ostoyae* genotypes.

Literature Cited


