Intraspecific and intragenomic variation of *Armillaria ostoyae* within the western United States

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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). Many of the *A. ostoyae* genets contained heterogeneous sequences, an indication of intragenomic variation/intraspecific hybridization. Intragenomic variation was verified by visual analysis of sequence chromatograms and PCR with specific internal primers. Intraspecific and intragenomic variation was found to exist in all rDNA regions analyzed, with the exception of the 5.8S rDNA. Variation will be further analyzed using Parsimony and Neighbor-Joining methods for phylogenetic analysis. Genetic diversity within *A. ostoyae* can be examined for relationships to ecological function (e.g., pathogenicity, host specificity, habitat type), geographic origin, forest management practices (e.g., fertilization), and interactions among *Armillaria* genotypes.

Introduction

The fungal genus *Armillaria* is a group of root-associated basidiomycetes, which includes thirty-nine species worldwide and ten North American biological species (Burdsall 1993). In the western United States *A. ostoyae* is a serious plant pathogen that causes root and butt rot on a variety of woody plant hosts. It adversely impacts commercial timber production by causing significant tree mortality and a reduction in tree growth. Growth loss due to *A. ostoyae* is 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 (Cruickshank 2000). Growth loss and mortality due to *Armillaria* in British Columbia causes annual losses between 1.4 and 3.8 million m^3 (White and Morrison 1999). Studies have shown that genets of *A. ostoyae* can show varying levels of pathogenicity and virulence (Omdal et al. 1995, Morrison and Pellow 2002). However, little has been done to identify genetic differences among genets of *A. ostoyae*. Assessing these differences may be important in understanding 1) varying levels of pathogenicity and virulence within *A. ostoyae*, 2) phylogenetic relationships among *A. ostoyae* genets, 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.

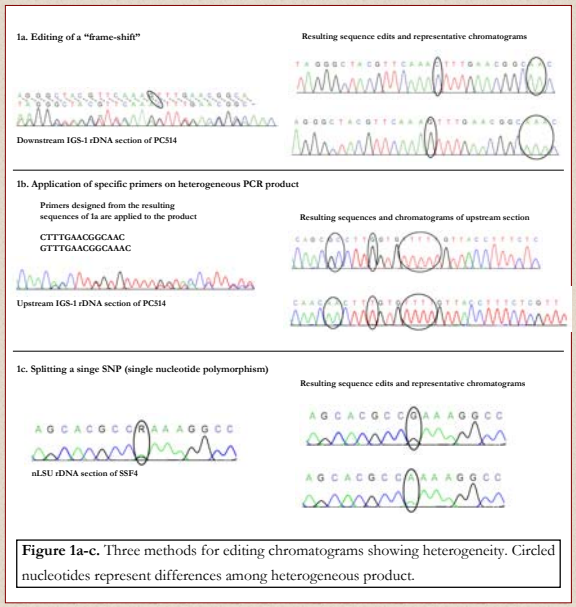

Figure 1a-c. Three methods for editing chromatograms showing heterogeneity. Circled nucleotides represent differences among heterogeneous product.

Table 1. *Armillaria* species and genets

Species	Isolate[a]	Origin
A. ostoyae	BC18F[b]	Washington, USA
	MNF4[c]	Oregon, USA
	NC1070[d]	Idaho, USA
	NC1126[e]	Idaho, USA
	NC540[f]	Idaho, USA
	NC765[g]	Washington, USA
	NC837[g]	Washington, USA
	NC94[g]	Idaho, USA
	NM115[h]	New Mexico, USA
	NM120[h]	New Mexico, USA
	OR1[i]	Oregon, USA
	OR10[i]	Oregon, USA
	P1404[g]	Idaho, USA
	P4352[g]	Idaho, USA
	PC514[g]	Idaho, USA
	R1299[g]	Idaho, USA
	R1329[g]	Montana, USA
	R1330[g]	Montana, USA
	R1348[g]	Montana, USA
	R1374[g]	Montana, USA
	R1389[g]	Montana, USA
	SSF4[g]	Montana, USA
	ST1[k]	New Hampshire, USA
	ST2[k]	Washington, USA
	TS7[c]	Mexico
	U16[c]	Utah, USA
	U5[c]	Utah, USA
	U73[c]	Utah, USA
A. gemina	ST8[c]	New York, USA
	ST15[k]	West Virgina, USA
A. nabsnona	C21[c]	New Hampshire, USA
	M90[l]	British Columbia, Canada
	ST16[k]	Alaska, USA
NABS X[k]	837[m]	Idaho, USA
	D82[n]	Idaho, USA
	POR100[o]	Idaho, USA

[a] Each isolate represents a distinct genet
[b] M.-S. Kim - BOISE™ Corporation
[c] B.A. Ferguson - Genet ID (Ferguson et al. 2003)
[d] Intermountain Forest Tree Nutrition Co-op, Moscow, ID.
[e] G.I. McDonald - USDA Forest Service, RMRS, Moscow, ID.
[f] G.I. McDonald - USDA Forest Service, RMRS, Moscow, ID. (Kim et al. 2000)
[g] B.A. Ferguson - Montana Dept. of Natural Resources, Missoula, MT.
[h] USDA Forest Service Center for Forest Mycology Research, Madison, WI. (Kim et al. 2000)
[i] J.J. Worrall (Kim et al. 2000)
[k] D.J. Morrison (Kim et al. 2000)
[l] NABS = North American Biological Species

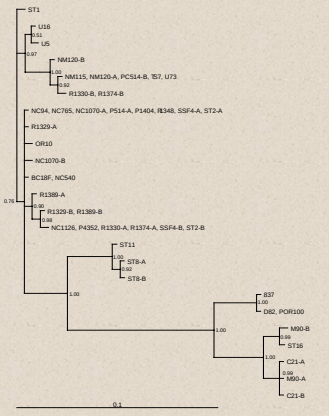

Figure 2. 50% majority rule consensus trees based on 24,000 trees from the Bayesian inference analysis of the IGS-1 spacer region. Scale bar = 0.1 substitutions per site

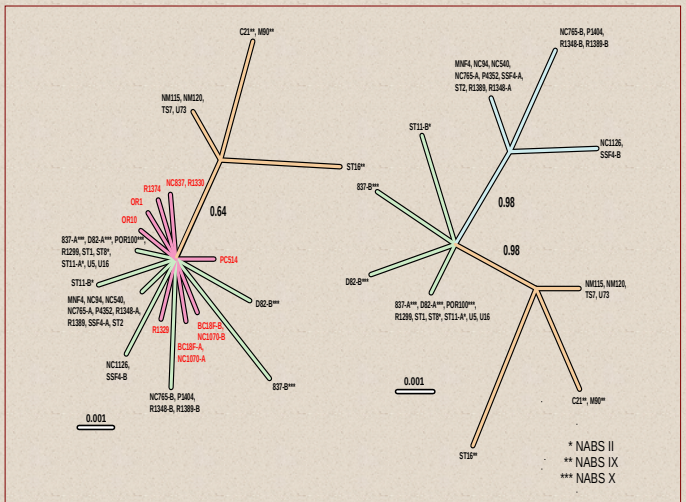

Figure 3a-b. (above)Radial 50% majority rule consensus trees based on 24,000 trees from the Bayesian inference analysis of 850 base-pairs in the nLSU rDNA region. Scale bar = 0.001 substitutions per site. **3a.** (above-left) Heterogeneous sequences (in red text) included. **3b.** (above-right) Heterogeneous sequences excluded. **Table 2.** (below) All variable nucleotide positions of aligned nLSU sequences.

GENET	126bp	129bp	165bp	172-173bp	227bp	407bp	470bp	477bp	481bp	500bp	514bp	518bp	524bp	602bp	656bp	750bp	787bp	812bp
837-A***, D82-A***, POR100***, R1299, ST1, ST8*, ST11-A*, U5, U16	A	T	C	GA	T	C	T	C	C	C	G	A	C	G	A	A	T	T
ST11-B*	A	T	C	GA	T	C	T	C	C	C	G	A	C	G	G	A	T	T
837-B***	A	C	C	GA	T	C	T	C	C	C	G	A	C	G	A	A	T	T
D82-B***	A	T	C	GA	T	C	T	C	C	C	G	G	C	G	A	A	T	T
MNF4, NC94, NC540, NC765-A, P4352, R1348-A, R1389, SSF4-A, ST2	A	T	C	GA	T	C	T	T	C	C	G	A	C	G	A	A	T	T
NC765-B, P1404, R1348-B, R1389-B	A	T	C	GA	T	C	T	T	C	A	G	A	C	G	A	A	T	T
NC1126, SSF4-B	A	T	C	GA	T	C	T	T	C	C	G	A	C	A	A	A	T	T
BC18F-A, NC1070-A	A	T	Y	GA	T	C	T	T	C	C	G	A	C	G	A	A	T	T
BC18F-B, NC1070-B	A	T	Y	--	T	C	T	T	C	C	G	A	C	G	A	A	T	T
R1329	A	T	C	GA	Y	C	T	T	C	C	G	A	C	R	A	A	T	T
OR1	A	T	C	GA	T	C	T	Y	M	C	R	A	C	G	A	A	T	Y
OR10	A	T	C	GA	T	C	T	Y	M	C	R	A	C	G	A	A	T	T
PC514	A	T	C	GA	T	C	T	Y	C	C	G	A	Y	G	A	A	T	T
NC837, R1330	A	T	C	GA	T	Y	Y	Y	C	C	G	A	Y	G	A	A	T	T
R1374	A	T	C	GA	T	Y	Y	Y	C	M	G	A	Y	G	A	A	T	T
NM115, NM120, TS7, U73	A	T	C	GA	T	C	T	C	C	C	G	A	T	G	A	A	T	T
ST16**	G	T	C	GA	T	C	T	C	C	C	G	A	T	G	A	G	T	T
C21**, M90**	A	T	C	GA	T	C	T	C	C	C	G	A	T	G	A	A	C	T

Materials and Methods

PCR products from ribosomal DNA (rDNA) 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 a PCR reaction mixture). Twenty-six of these 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 phylogenic comparisons (Table 1). PCR products were purified and sequenced at Davis Sequencing, Inc. (Davis, CA). The sequences were edited by hand/eye with BioEdit software (Hall 1999) and manually aligned. When possible, heterogeneous sequences were split 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 amplification mutation assay (MAMA) method (Cha et al. 1992, Rauscher et al. 2002).

Phylogenetic analysis was performed for each DNA region using MrBayes v3.0B4 (Huelsenbeck and Ranquist 2001) for Bayesian inference of phylogeny. Indels were treated as a single event and coded using the simple gap coding method (Simmons and Ochoterena 2000). To select a model for use in Bayesian inference, we used MrModeltest 1.0b (Nylander 2003). Four chains were run for three million generations generating files with thirty thousand trees, the first six thousand of these trees were discarded as the "burnin" 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 heterogeneous rDNA products in an individual and the relative peak height seems to reflect relative concentrations (Rauscher et al. 2002). In this study, heterogeneous products indicating intraspecific and intragenomic variations were common in all regions analyzed, with the exception of the 5.8S rDNA. Figure 2 shows variation in the IGS-1 region that resulted in the formation of significant clades. Although the intergenic spacer regions are frequently viewed as non-functional genes, some suggest that variation in rDNA spacers has major influences on organism growth rates (Elser et al. 2000). Table 3 shows that a significant amount of variation exists in the LSU, a region more commonly thought to have functional importance. Figures 3a and 3b show the importance of using non-heterogeneous sequences in phylogenetic analysis. When heterogeneous sequences are included in phylogenetic analysis, the parental phylogenies become more ambiguous. Figure 3a includes heterogeneous sequences showing only one clade with a posterior probability of 64 percent. Figure 3b excludes the heterogeneous 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 Moscow, Idaho to analyze a greater number of *A. ostoyae* genets and examine how these genetic differences may relate to spatial distribution and ecological function.

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