Characterization of North American *Armillaria* species: Phylogenetic relationships from IGS, ITS, and nuclear large subunit ribosomal DNA sequences

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Abstract

Phylogenetic relationships among nine North American *Armillaria* spp. were analyzed using ribosomal DNA (rDNA) sequences from intergenic spacer 1 (IGS-1), internal transcribed spacer (including 5.8S rDNA) (ITS+5.8S), and nuclear large subunit rDNA (nLSU) regions. Phylogenetic trees were generated using Neighbor-Joining analysis. *Armillaria ostoyae* and *A. gemina* were well separated from the other *Armillaria* spp. Several *Armillaria* spp. (*A. calvescens*, *A. sinapina*, *A. gallica*, NABS X, and *A. cepistipes*) clustered together, despite their previous separation based on *in vitro* compatibility and/or morphology. The nLSU sequence data indicate that *A. mellea* is distant from other *Armillaria* spp. A more detailed phylogenetic analysis and an examination of hybridization among *Armillaria* spp. are underway.

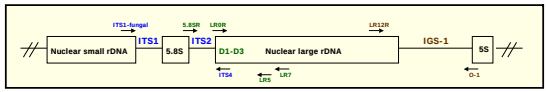


FIG. 1. Diagram of a rDNA repeat. Positions corresponding to the annealing sites for several primers are shown by arrows.

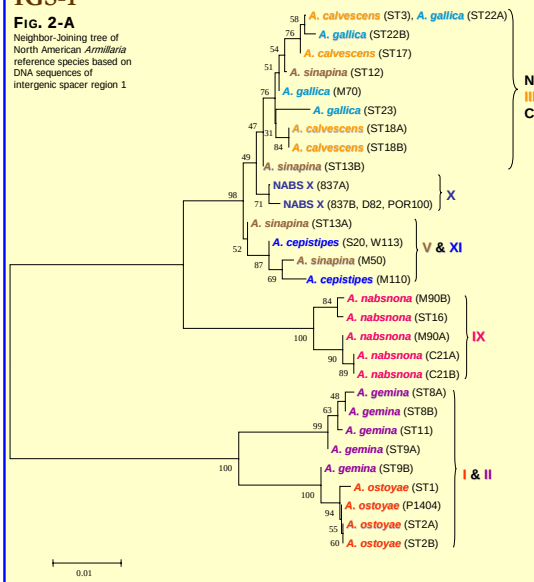
Results and Discussion

The LSU rDNA region is less variable among *Armillaria* spp. than the ITS+5.8S and IGS-1 regions (LSU rDNA<ITS+5.8S<IGS-1). Neighbor-Joining trees separated nine species into three clades: *A. mellea*, *A. ostoyael A. gemina*, and all other *Armillaria* spp. (FIG. 2: A, B, and C). Preliminary analyses indicate that LSU rDNA and ITS+5.8S regions are promising for evaluating evolutional relationships among *Armillaria* spp., while the IGS-1 region is potentially more useful for revealing intra-specific relationships. More detailed phylogenetic analyses (e.g., maximum parsimony and maximum likelihood) and examination of the occurrence of hybridization among *Armillaria* spp. are underway at the USDA Forest Service - RMRS, Forestry Sciences Laboratory, Moscow, ID, USA.

IGS-1

FIG. 2-A

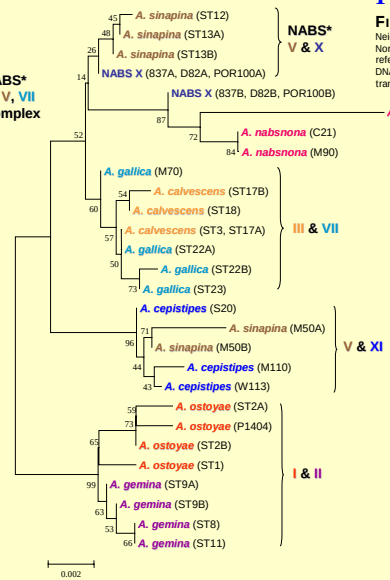
Neighbor-Joining tree of North American *Armillaria* reference species based on DNA sequences of intergenic spacer region 1



ITS + 5.8S

FIG. 2-B

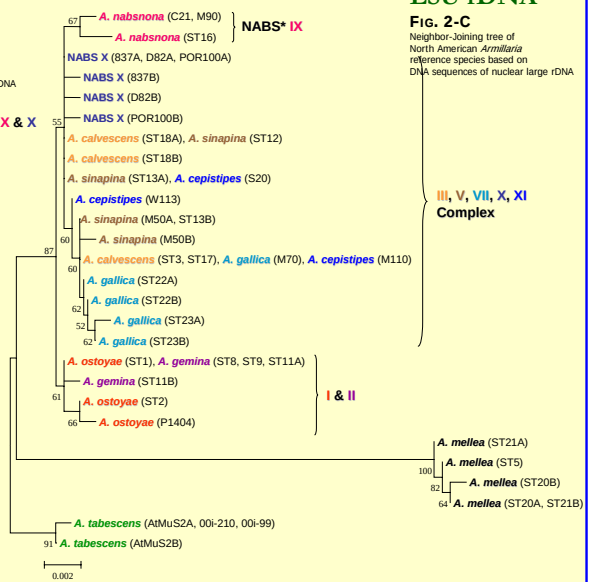
Neighbor-Joining tree of North American *Armillaria* reference species based on DNA sequences of internal transcribed spacer and 5.8S rDNA



LSU rDNA

FIG. 2-C

Neighbor-Joining tree of North American *Armillaria* reference species based on DNA sequences of nuclear large rDNA



*NABS = North American Biological Species

Introduction

Previous studies have reported phylogenetic relationships among *Armillaria* species using intergenic spacer (IGS-1), internal transcribed spacer (ITS), and anonymous nucleotide sequences (Anderson and Stasovski 1992, Coetzee et al 2000, 2001, Piercey-Normore et al 1998). The 5' end of the nuclear large subunit (LSU) rDNA gene, which comprises divergent domains in the D1-D3 region (Michot et al 1984), has been applied to study phylogenetic relationships of agaricaceous fungi (Moncalvo 2000). This region contains the most informative phylogenetic sites in LSU rDNA gene (Hopple and Vilgalys 1999, Kuzoff et al 1998). However, this region has not been previously applied to study phylogenetic relationships among the North American Biological Species (NABS) of *Armillaria*.

Objectives

The objectives of this study were to infer phylogenetic relationships among North American Biological Species of *Armillaria* using IGS-1, ITS (including 5.8S rDNA), and LSU rDNA sequences data. Attaining this objective provides a characterized set of tester strains for use in further biological and taxonomic studies of *Armillaria* species.

Materials and Methods

Tested species included ten North American *Armillaria* species (TABLE 1). The IGS-1, ITS (including 5.8S rDNA gene), and partial LSU rDNA gene (3' and 5' ends) regions were amplified using PCR (FIG. 1). PCR products were purified and sequenced at Davis Sequencing, Inc. (Davis, CA). The sequences were edited with BioEdit software (Hall 1999).

The IGS-1, ITS, and LSU rDNA sequences were aligned manually and analyzed using Mega 2.1 (Kumar et al 2001). Neighbor-Joining with bootstrapping was used to generate phylogenetic trees with distance measured by the Jukes-Cantor algorithm. Alignment gaps and indels (nucleotide insertion/deletion) were treated with pairwise deletion and as one event, respectively. Bootstrap support for branches was based on 1,000 bootstrap replicates.

References

Anderson JB, Stasovski E. 1992. Molecular phylogeny of Northern Hemisphere species of *Armillaria*. Mycology 84: 506-516.

Coetzee MPA, Wingfield BD, Harrington TC, Dalevi D, Coutinho TA, Wingfield MJ. 2000. Geographic diversity of *Armillaria mellea* s.s. based on phylogenetic analysis. Mycologia 92:105-113.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Window 95/98/NT. Nucl. Acids. Symp. Ser. 41: 95-98.

Hopple JS Jr., Vilgalys R. 1999. Phylogenetic relationships in the mushroom genus *Coprinus* and dark-spored allies based on sequence data from the nuclear gene coding for the large ribosomal subunit RNA: divergent domains, outgroups and monophyly. Mol. Phylogenet. Evol. 13: 1-19.

Kuzoff RK, Sweere JA, Soltis DE, Soltis PS, Zimmer EA. 1998. The phylogenetic potential of entire 26S rDNA sequences in plants. Mol. Biol. Evol. 15: 251-263.

Michot B, Hassouna N, Bachellerie J. 1984. Secondary structure of mouse 28S rRNA and a general model for the folding of the large rRNA in eukaryotes. Nucleic. Acids Res. 12: 4259-4279.

Moncalvo JM, Lutzoni FM, Rehner SA, Johnson J, Vilgalys R. 2000. Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. Syst. Biol. 49: 278-305.

Piercey-Normore MD, Egger KN, Bérubé JA. 1998. Molecular phylogeny and evolutionary divergence of North American Biological Species of *Armillaria*. Molecular Phylogenetics and Evolution 10: 49-66.

TABLE 1. *Armillaria* isolates used in phylogenetic analysis.

Species	Collection	Isolate[a]	Origin	Source tissue	Accession #
A. ostoyae	DMR20[c]	ST1	New Hampshire, USA	multisporous	AY213552
	AMM9067[c]	ST2	Washington, USA	basidioma	AY213553
	P1404[d]	P1404	Idaho, USA	basidioma	AY213554
A. gemina	JJW153[e]	ST8	New York, USA	basidioma	AY213555
	JJW64[e]	ST9	New York, USA	basidioma	AY213556 (A), AY213557 (B)
	MIELKE[c]	ST11	West Virginia, USA	unknown	AY213558
A. calvescens	JB56A[f]	ST3	Québéc, Can	basidioma	AY213559
	PR-3[c]	ST17	Michigan, USA	basidioma	AY213560 (A), AY213561 (B)
	FFC-7[c]	ST18	Michigan, USA	basidioma	AY213562
A. sinapina	SP81-1[g]	M50	British Columbia, Can	basidioma	AY213563 (A), AY213564 (B)
	AMM9065[c]	ST12	Washington, USA	basidioma	AY213565
	CF-2[c]	ST13	Michigan, USA	multisporous	AY213566 (A), AY213567 (B)
A. mellea	GB934[c]	ST5	Virginia, USA	multisporous	AY213584 (A), AY213585 (B)
	A3[c]	ST20	Wisconsin, USA	basidioma	AY213586
	TCH-2[c]	ST21	New Hampshire, USA	Multisporous	AY213587
A. gallica	SP81-29[g]	M70	British Columbia, Can	basidioma	AY213568
	EL-1[c]	ST22	Michigan, USA	basidioma	AY213569 (A), AY213570 (B)
	MA-1[c]	ST23	Wisconsin, USA	basidioma	AY213571
A. nabsnona	C21[d]	C21	Idaho, USA	basidioma	AY213572
	M90[g]	M90	British Columbia, Can	basidioma	AY213573
	SHAW,C[c]	ST16	Alaska, USA	multisporous	AY213574
NABS X	837[d]	837	Idaho, USA	basidioma	AY213575 (A), AY213576 (B)
	D82[d]	D82	Idaho, USA	basidioma	AY213577 (A), AY213578 (B)
	POR100[d]	POR100	Idaho, USA	Basidioma	AY213579 (A), AY213580 (B)
A. cepistipes	SP82-14[g]	M110	British Columbia, Can	basidioma	AY213581
	SP83-07[g]	S20	British Columbia, Can	basidioma	AY213582
	HH14867	W113	Washington, USA	basidioma	AY213583
A. tabescens	AT-MU-S2	AT-MU-S2	South Carolina, USA	stalk	AY213588
	OOI-99	OOI-99	South Carolina ??	??	AY213590
	OOI-210	OOI-210	Georgia, USA	basidioma	AY213589