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A PCR-based identification method for species of *Armillaria*

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Abstract: A portion of the Intergenic Spacer (IGS) of the ribosomal RNA operon of 74 isolates of 11 *Armillaria* species from Europe and North America was amplified using the polymerase chain reaction. Amplifications were made from scrapes of living mycelium without DNA extraction. *Alu* I digests of the amplified product were electrophoresed in agarose and stained with ethidium bromide. With few exceptions, each taxon had a unique combination of restriction fragments. Most taxa had a single *Alu* I pattern, but two restriction patterns were seen among isolates of *A. borealis*, *A. cepistipes*, *A. gallica*, *A. tabescens*, and *A. mellea*. *Armillaria ostoyae*, *A. gemina*, one of the *A. borealis* types, and one of the *A. cepistipes* types had identical sizes of *Alu* I fragments, but each of these taxa could be distinguished by their polymorphisms after restriction with the enzymes *Nde* I, *Bsm* I, or *Hind* II. European isolates of *A. gallica* had a distinct *Alu* I restriction pattern, but North American isolates of this species had a restriction pattern identical to *A. calvescens*. IGS amplification products were obtained from 8-year-old spore prints and dried basidiomes, as well as fresh wood decay without DNA extraction. The technique allows for identification from decayed wood, basidiomes or mycelia of these *Armillaria* species in a single day.

Key Words: *Armillaria*, identification, Intergenic Spacer, rDNA

INTRODUCTION

Species of *Armillaria* (Fr. : Fr.) Staude (Agaricales, Tricholomataceae) are among the most important root disease pathogens of trees, but the confused taxonomy of this genus has precluded a clear understanding of the biology of the major species. Until the 1970s, most plant pathologists referred to all Northern Hemisphere species of *Armillaria* as *A. mellea* (Vahl : Fr.) Kummer. Separating the common species and defining their biology has been seriously impeded by difficulties in species identification (Guillaumin et al., 1991; Watling et al., 1991; Harrington et al., 1992; Guillaumin et al., 1993). Characteristics of mycelial fans and rhizomorphs vary among the species only to a limited extent, and basidiomes are seasonal and rare to uncommon in many regions. The fungus may be difficult to isolate from some substrata, it grows slowly in culture, there is little variation among *Armillaria* species in cultural characteristics, and production of basidiomes in culture is unreliable and time-consuming.

Critical identifications of cultures of *Armillaria* rely on pairings with haploid tester strains (Korhonen, 1978). Single basidiospore stains are haploid and are generally fluffy and unpigmented on malt extract agar. Isolates from decay, fans or rhizomorphs are diploid (Hintikka, 1973), generally produce relatively little aerial mycelium, and the mycelium often has a reddish-brown crust. Pairing of single basidiospore strains of the same species will usually result in diploidization, and the fluffy mycelium tends to flatten. Transient clamp connections may also be visible in compatible pairings (Larsen et al., 1992). A haploid tester can be diploidized upon pairing with a diploid isolate of the same species. Subculturing from the pairing plates is recommended (Harrington et al., 1992), but still, the results are often ambiguous, particularly for identification of diploid isolates (Siepmann, 1987; Shaw and Loopstra, 1988; Rizzo and Harrington, 1992). These pairing tests require up to 2 months for identification after isolation in pure culture.

Other techniques have been explored for differentiating species of *Armillaria*. Electrophoresis of isozymes (Morrison et al., 1985), restriction fragments of ribosomal or mitochondrial DNA (Anderson et al., 1987; Anderson et al., 1989; Jahnke et al., 1987; Smith and Anderson, 1989), or DNA-DNA hybridization (Jahnke et al., 1987) can distinguish some of these species. However, none of these techniques have been widely tested or proven to be feasible for routine species identifications.

Anderson and Stasovski (1992) published partial DNA

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sequences for the IGS (Intergenic Spacer) region of the ribosomal RNA (rRNA) operon for most of the Northern Hemisphere species of *Armillaria*. Sequence variation among the limited number of isolates sampled suggested that restriction enzyme digests of this region may discriminate among the species. Using the polymerase chain reaction (PCR) and the primers of Anderson and Stasovski (1992), we amplified the IGS region and screened the PCR products using a number of restriction enzymes for unique restriction fragment length polymorphisms (RFLP) among the European and North American species of *Armillaria*. A 1-day procedure was developed that can identify the 11 taxa examined.

MATERIALS AND METHODS

Isolates.—At least four haploid or diploid isolates of each of the nine described and two nondescribed species of *Armillaria* in Europe and North America were included in the study (TABLE I). These isolates were identified by various investigators using pairing tests. All the isolates were grown on MYEA plates (2% malt extract, 0.2% yeast extract, 1.5% agar) at room temperature prior to amplification.

Template DNA.—DNA was isolated from a limited number of the cultures using the method of Lee and Taylor (1990). However, most of the results presented here were from amplifications done directly from *Armillaria* mycelium on MYEA. Neither the amplifications nor the results from the restriction digests were influenced by the origins of the DNA template.

For direct amplification from mycelium, a pipette tip was scraped approximately 1 cm across the actively growing mycelium at the edge of the colony. The tip was then dipped in the PCR reaction vessel containing the reaction mix and the mineral oil, and the mixture was vigorously stirred with the tip. In this manner fungal material attached to the tip was suspended in the reaction mix.

Polymerase chain reaction (PCR).—The Intergenic Spacer region (IGS) between the 3′ end of the large subunit ribosomal (LSU) RNA (rRNA) gene and the 5′ end of the 5S rRNA gene was amplified using PCR. The primers used were those recommended by Anderson and Stasovski (1992): LR12R, 5′CTGAACGCCTCTAAGTCAGAA3′ (Veldman et al., 1981) and O-1, 5′AGTCCTATGGCCGTGGAT3′ (Duchesne and Anderson, 1990). The PCR reaction mixture included 2.5 units *Taq* polymerase (Promega, Madison, Wisconsin) per reaction, the buffer supplied with the enzyme, 4 mM $MgCl_2$, 200 μM dNTPs, and 0.5 μM of each primer. DNA (10 ng) or fungal mycelium were added as template for the reaction. Mineral oil was overlaid to prevent evaporation of the 100-μl final reaction volume. The thermocycler (MJ Research, Inc., Watertown, Massachusetts) conditions were an initial denaturation at 95 C for 95 sec, followed by 35 cycles of 60 C for 40 sec (annealing), 72 C for 2 min (elongation) and 90 C for 30 sec (denaturation). A final elongation was allowed for 10 min at 72 C to ensure a double stranded amplification product.

DNA restriction.—A number of restriction enzymes were tested for polymorphisms among a select group of *Armillaria* isolates. The enzyme *Alu* I gave the greatest polymorphisms, although some species had identical RFLP with this enzyme. DNA sequence of the IGS region from the end of the LSU to the 5S gene of the rRNA operon had been published for all the tested *Armillaria* species except *A. mellea* and *A. tabescens* (Scop.: Fr.) Emel. (Anderson and Stasovski, 1992), and these sequences were used to identify further diagnostic restriction enzymes. The five enzymes utilized were *Alu* I, *Nde* I or *Hind* II (Promega, Madison, Wisconsin), *Bsm* I (Stratagene, La Jolla, California), and *Tha* I (Gibco BRL, Life Science Technologies, Gaithersburg, Maryland).

The amplified DNA was not purified before restriction enzyme digestion. *Alu* I, *Nde* I or *Hind* II (2–4 units per reaction) was added directly to the PCR reaction mix (20 μl) after amplification and the digestion allowed to proceed for 1–16 h at 37 C. The *Bsm* I and *Tha* I (2–4 units per reaction) digestions were performed at 65 C for 1–16 h. NaCl was added to a final concentration of 50 mM for both the *Nde* I and *Tha* I digestions and to a final concentration of 100 mM for *Bsm* I digestions.

Electrophoresis.—Both the amplified DNA and the restriction enzyme fragments of these products were electrophoresed in agarose gels in a TBE [89 mM Tris, 89 mM boric acid, 2 mM EDTA (pH 8)] buffer system to determine the size of the amplification and restriction products. For routine analysis, 2% agarose gels were run at 100 V for 2 h, but more definite determinations of fragment sizes were based on larger gels of 3% MetaPhor agarose (FMC BioProducts, Rockland, Maine) run at 200 V for 3 h at 10 C. The gels were stained with ethidium bromide and visualized using UV light.

RESULTS

Good amplification of the IGS region was obtained with all isolates using direct amplification from mycelium. At least two amplifications were made of each of the isolates listed in TABLE I. The amplified product from all isolates of *A. mellea* was 875 base pairs (bp), and each isolate of *A. tabescens* yielded a product of

TABLE I. *AluI* restriction fragments of the amplified IGS region of rDNA and origins of the North American and European isolates of *Armillaria*

Species (RFLP group)	Isolate number	Determined by, other isolate number[a]	State, province, or country of origin	Fragment sizes (bp)[b]
A. ostoyae	B481	Anderson, 28-4	Vermont	310, 200, 135
	B482	Anderson, 28-7	Vermont	310, 200, 135
	B483	Anderson, 70-1	Vermont	310, 200, 135
	B484	Anderson, 70-4	Vermont	310, 200, 135
	B577	Mallett, 895	Alberta	310, 200, 135
	B746	Worrall, Renss. no. 3	New York	310, 200, 135
	B747	Worrall, Renss. no. 4	New York	310, 200, 135
	B168	Korhonen, 830920.3.2	Finland	310, 200, 135
	B177	Worrall, AoG2Ld	Germany	310, 200, 135
A. gemina	B485	Anderson, 35-3	Vermont	310, 200, 135
	B486	Anderson, 35-5	Vermont	310, 200, 135
	B487	Anderson, 1608	Vermont	310, 200, 135
	B735	Worrall, Amp4B	New York	310, 200, 135
	B737	Worrall, Utow2A	New York	310, 200, 135
	B744	Worrall, Renss. no. 1	New York	310, 200, 135
	B745	Worrall, Renss. no. 2	New York	310, 200, 135
A. borealis (A)	B373	Korhonen, 841006.2.1/3	Germany	310, 200, 135
	B374	Korhonen, 800921.1.1/4	Germany	310, 200, 135
	B381	Korhonen, 830915.1.1	Finland	310, 200, 135
A. borealis (B)	B370	Korhonen, 840925.1.1/1a	Finland	310, 200, 104
	B371	Korhonen, 740903.4.5/1	Finland	310, 200, 104
	B372	Korhonen, 770919.3.1/1	Finland	310, 200, 104
	B375	Korhonen, 830906.1.3	Finland	310, 200, 104
	B518	Guillaumin, KA2	France	310, 200, 104
A. sinapina	B295	Harrington	New Hampshire	399, 200, 135
	B493	Anderson, 48-3	New York	399, 200, 135
	B494	Anderson, 48-6	New York	399, 200, 135
	B586	Mallett, 985	Alberta	399, 200, 135
A. cepistipes (A)	B165	Gregory, CZ4	Britain	399, 200, 183
	B172	Worrall, A-G7Pa	Germany	399, 200, 183
	B210	Worrall, A-G8Pa	Germany	399, 200, 183
	B212	Gregory, XM2	Britain	399, 200, 183
	B521	Guillaumin, T2.1	France	399, 200, 183
A. cepistipes (B)	B185	Gregory, LLC	Britain	310, 200, 135
	B193	Gregory, BQ6	Britain	310, 200, 135
	B196	Gregory, BSH3	Britain	310, 200, 135
	B218	Gregory, DN1	Britain	310, 200, 135
	B519	Guillaumin, KB2	France	310, 200, 135
	B520	Guillaumin, KB3	France	310, 200, 135
NABS IX	B501	Anderson, 121-1	British Columbia	534, 200
	B502	Anderson, 121-2	British Columbia	534, 200
	B503	Anderson, 139-1	Idaho	534, 200
	B504	Anderson, 139-2	Idaho	534, 200
NABS X	B505	Anderson, 140-5	Idaho	399, 183, 142
	B506	Anderson, 140-6	Idaho	399, 183, 142
	B507	Anderson, 140-7	Idaho	399, 183, 142
	B508	Anderson, 140-9	Idaho	399, 183, 142
A. gallica (European)	B171	Korhonen, 771006.1.2	Britain	399, 240, 183
	B174	Worrall, AbG9	Germany	399, 240, 183
A. gallica (American)	B110	Harrington	New Hampshire	582, 240
	B399	Harrington	New Hampshire	582, 240
	B498	Anderson, 90-4	Vermont	582, 240
	B499	Anderson, 90-10	Vermont	582, 240
	B500	Anderson, 137-1	Michigan	582, 240

TABLE I. *Alu* I restriction fragments of the amplified IGS region of rDNA and origins of the North American and European isolates of *Armillaria* (Cont.)

Species (RFLP group)	Isolate number	Determined by, other isolate number[a]	State, province, or country of origin	Fragment sizes (bp)[b]
A. calvescens	B366	Harrington	New Hampshire	582, 240
	B488	Anderson, 111	Vermont	582, 240
	B489	Anderson, 119	Vermont	582, 240
	B490	Anderson, 212	Vermont	582, 240
	B491	Anderson, 218	Vermont	582, 240
	B679	Harrington	Vermont	582, 240
A. mellea (A)	B495	Anderson, 49-5	Massachusetts	490, 180
	B496	Anderson, 49-8	Massachusetts	490, 180
	B497	Anderson, 97-1	Massachusetts	490, 180
	B784	Harrington	New Hampshire	490, 180
A. mellea (B)	B202	Harrington	California	320, 155
	B173	Rishbeth, M3	Britain	320, 155
	B176	Rishbeth, M1	Britain	320, 155
A. tabescens (A)	B897	Burdsall, Fp-102427-Sp	Ohio	430, 240
	B898	Burdsall, Fp-102423-Sp	Ohio	430, 240
	B900	Burdsall, 12822	Illinois	430, 240
	B531	Guillaumin, Tab2	France	430, 240
	B532	Guillaumin, Tab3	France	430, 240
A. tabescens (B)	B899	Burdsall, HHB-162-Sp	Maryland	320, 240, 100

[a] J. B. Anderson, H. H. Burdsall, S. Gregory, J. J. Guillaumin, K. Korhonen, K. I. Mallett, J. Rishbeth, and J. J. Worrall provided isolates identified through pairing tests.

[b] Fragment sizes determined from the reported sequence data (Anderson and Stasovski, 1992), except for *A. mellea* and *A. tabescens*, which were determined by electrophoresis and staining with ethidium bromide.

845 bp. All other isolates yielded a product of 920 bp.

The product of at least two amplifications of each isolate was digested with *Alu* I and electrophoresed separately with markers to determine the sizes of the restriction fragments (FIG. 1). Only fragments larger than 100 bp were scored because fragments smaller than this were difficult to see clearly and tended to be obscured by the prominent "primer dimer" band produced during amplification. The number and sizes of fragments from the two or more digestions were consistent for each isolate.

One or two *Alu* I digestion patterns were found in each of the 11 taxa tested (TABLE I, FIG. 1). Twelve different patterns were found among the isolates tested. Based on the published sequences of Anderson and Stasovski (1992), it was possible to create a restriction map (FIG. 2) for eight of these patterns, which includes all of the tested species of *Armillaria* except *A. mellea* and *A. tabescens.* Two patterns were seen in *A. borealis* Marxmüller & Korhonen, *A. cepistipes* Velenovsky, and *A. gallica* Marxmüller & Romagnesi. The restriction maps show that the two patterns within each of these species is due to a difference in one or two restriction sites. Two restriction patterns were also seen in *A. mellea* and *A. tabescens,* but the lack of published sequence data for these two species prevented unequivocal determination of the variation in restriction sites.

The IGS amplification products of isolates of *A. ostoyae* (Romagnesi) Herink, *A. gemina* Bérubé & Dessureault, *A. borealis* type A, and *A. cepistipes* type B had the same *Alu* I restriction sites. However, examination of the IGS DNA sequences revealed other diagnostic restriction enzymes for these species. Amplified products of all isolates listed in TABLE I were digested with *Nde* I, but only the isolates of *A. borealis* and *A. ostoyae* were cleaved, yielding products of 550 and 370 bp (FIG. 3). Amplified products of the listed isolates of *A. gemina* and *A. borealis* did not digest with *Bsm* I, but the products of all of the listed *A. ostoyae* isolates except B177 restricted, yielding fragments of 620 and 300 bp (FIG. 3). The amplified product of another *A. ostoyae* isolate, B747, gave inconsistent digestion with *Bsm* I. As predicted from the sequence data, *Hind* II digested *A. cepistipes* isolates, giving fragment sizes of 580 and 340 bp, but did not cleave the IGS amplification products of *A. ostoyae, A. gemina* or *A. borealis.*

North American and European isolates of *A. gallica* differed in their *Alu* I restriction patterns (TABLE I, FIG. 2). The North American isolates had an *Alu* I pattern identical to that of *A. calvescens* Bérubé & Dessureault isolates. Examination of the IGS DNA sequences from North American *A. gallica* and *A. cal-*

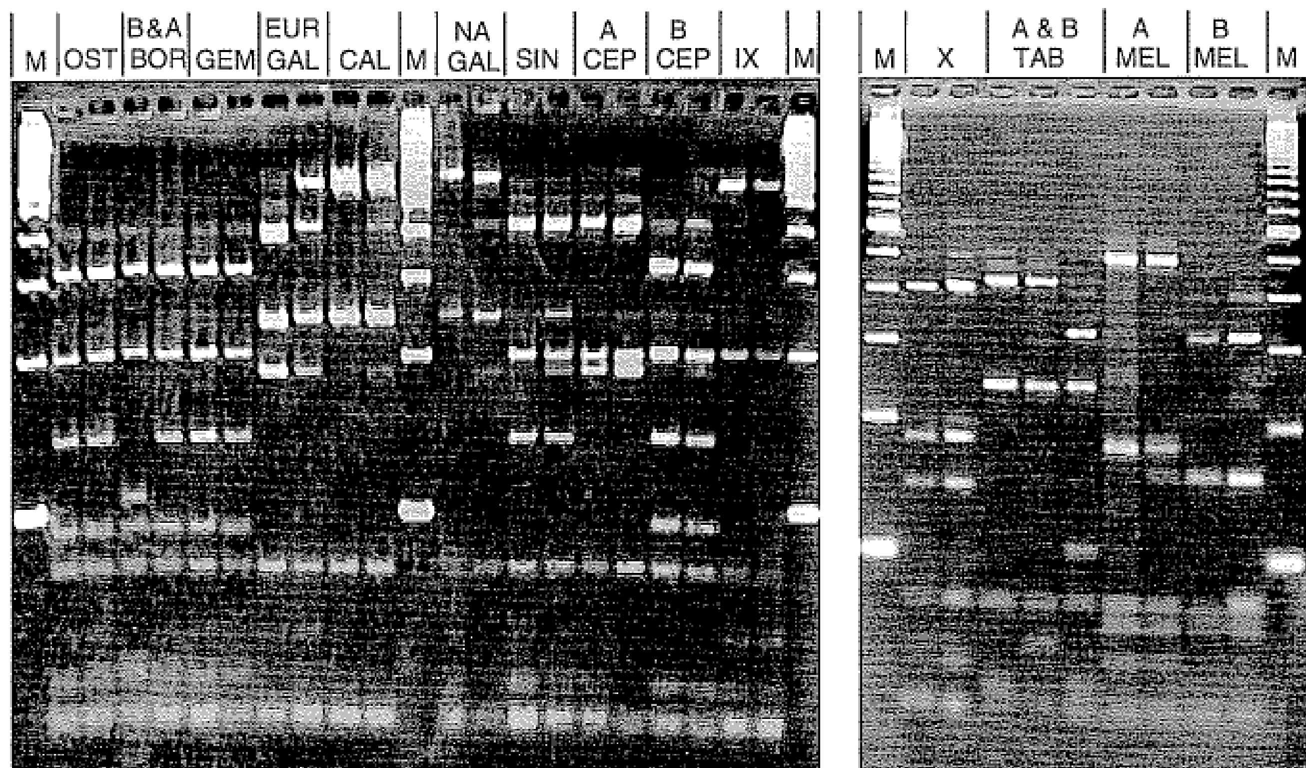


FIG. 1. Ethidium bromide stained agarose gels (3%) of *Alu* I digestion products of the IGS region of representative isolates of *Armillaria*. Markers (M) are 100-bp ladders, the lowest band being 100 bp in size. Lanes are designated with abbreviations for the species listed in TABLE I. Two patterns within a species are denoted as "A" and "B," or "NA" (North American) and "EUR" (European) above the appropriate lanes.

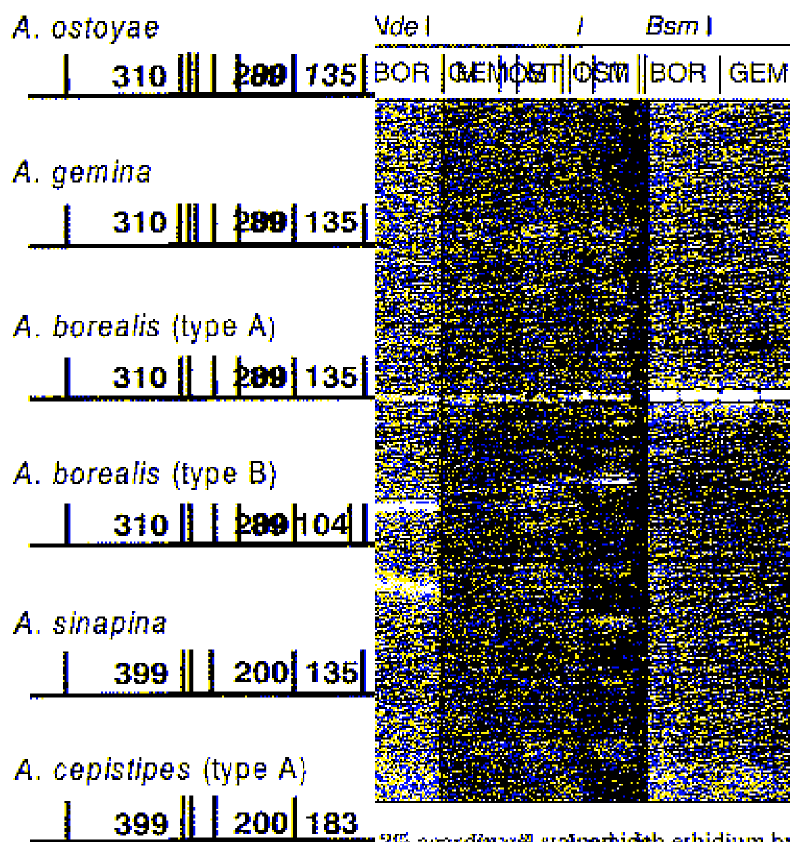
vescens (Anderson and Stasovski, 1992) indicated that the two taxa differ only at a single nucleotide/base pair, and this difference should result in differential restriction by the enzyme *Tha* I. However, we found that the amplified DNA from only some isolates of each taxon were cleaved at this site with *Tha* I, and inconsistencies were also found between two amplified products from the same isolate. Therefore, we were not able to distinguish the amplified DNA from the North American isolates of *A. gallica* and *A. calvescens* using this or any other enzyme.

We attempted amplifications from dried samples and decay without extraction of DNA by scraping the tissues with a pipette tip as described for the amplification from fresh mycelia. We successfully amplified the IGS region from small amounts of 6- to 8-yr-old spore prints of *A. gallica*, gill tissue of a 7-yr-old dried basidiome of *A. mellea*, two 8-yr-old dried basidiomes of *A. ostoyae*, and wood from two oak trees that were decayed by *A. gallica*. Generally, small (barely visible) amounts of material worked better than larger amounts for amplifications. Small pieces of wood were likely present in the scrapings from decay. We also attempted amplifications from the core of dried rhizomorph tissue, but without success; fresh rhizomorph tissue might prove more amenable to the technique. The amplified products from the other tissues were the correct length for the respective species. The *Alu* I restriction patterns were as found in other North American isolates of *A. gallica*, *A. mellea* (type A), and *A. ostoyae* (TABLE I).

DISCUSSION

Digestions of DNA from the IGS region with the restriction enzyme *Alu* I proved reliable for unambiguous identification of pure cultures of most of the *Armillaria* species known to Europe and North America. Because DNA extraction from pure cultures, dried samples or decay was unnecessary, diagnostic restriction patterns could be obtained in a single day. The IGS region was amplified from 8-yr-old spore prints and basidiome tissue, which should allow for nondestructive study of herbarium samples critical to the taxonomy and nomenclature of this difficult genus. Further, the IGS region was amplified directly from decayed wood, which will greatly facilitate rapid and unambiguous identification of fresh field materials for ecological work.

Some care must be taken in interpreting the restric-



A. cepistipes (type A)

399 | 200 | 183

A. cepistipes (type B)

310 | 289 | 135

NABS IX

534 | 200

NABS X

399 | 142 | 183

A. gallica (European)

399 | 240 | 183

A. gallica (N. American)

582 | 240

A. calvescens

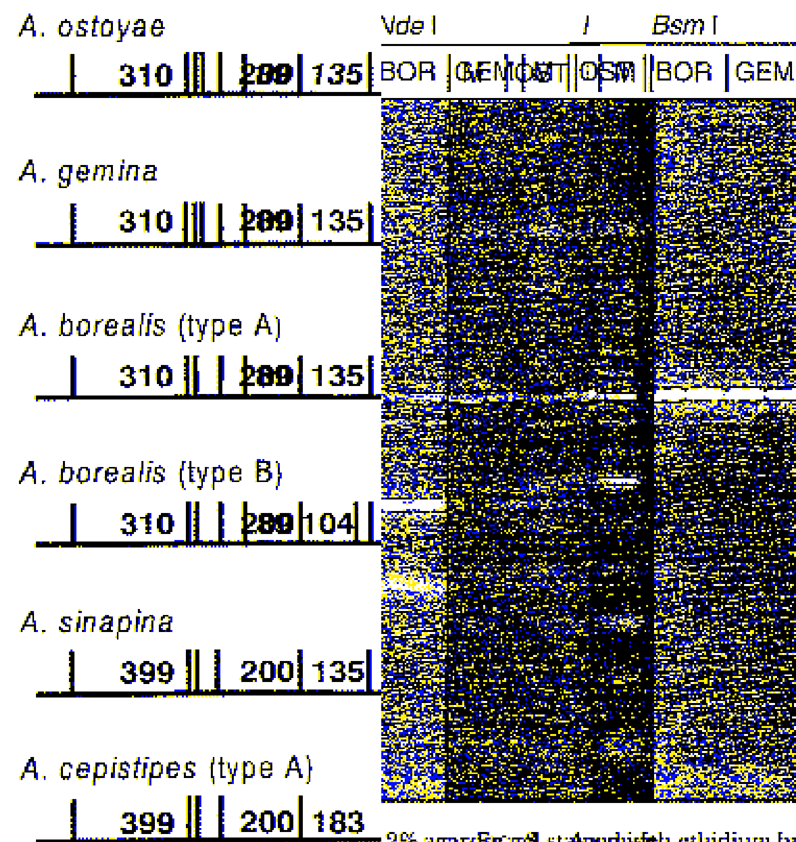
582 | 240

2% agarose gel stained with ethidium br... Nde I and ... digestion of IGS products of ... representative isolates of Armillaria ostoyae ... EM) and A. borealis (BOR). The occurre... undigested DNA fragments in the same ... s incomplete digestion. Markers (M) ar... ith the lowest band being 100 bp in siz...

ns for species identification. Seco... maller than the IGS product were ... in amplifications, and these bands n... ed as restriction fragments. Also, the ... digestion of the PCR product wer... completely digested DNA fragments ... seen, especially with the restrictio... and Nde I. It should be reiterated ... ns were digested in the PCR rea... in the buffer, recommended by the ... the manufacturer of enzyme. Purificatio... n of the PCR product before dige...

←

GS restriction map of the enzyme Alu I ... ces published by Anderson and Stasovsk... n patterns seen in the isolates listed in ... ted in Fig. 1. Numbers designate the a... (bp) of diagnostic fragments. Restrictio... ns and for A. tabescens were not developed.



A. cepistipes (type A)

399 | 200 | 183

A. cepistipes (type B)

310 | 289 | 135

NABS IX

534 | 200

NABS X

399 | 142 | 183

A. gallica (European)

399 | 240 | 183

A. gallica (N. American)

582 | 240

A. calvescens

582 | 240

use of the recommended restriction buffer, use of higher concentrations of the restriction enzyme, and longer digestion periods would give more complete digestions, but these steps would add some expense and considerable time to the technique.

Among the restriction enzymes that we screened, *Alu* I proved the most informative for separating the taxa, but *A. ostoyae, A. gemina* and some of the *A. borealis* isolates had the same restriction pattern. The IGS sequences for the three taxa are similar (Anderson and Stasovski, 1992). The first two species are morphologically similar (Bérubé and Dessureault, 1989) but much different in biology (Rizzo and Harrington, 1993). In northeastern North America, the amplified products from *A. ostoyae,* but not *A. gemina,* should be cleaved by *Bsm* I or *Nde* I. In Europe, where both *A. ostoyae* and *A. borealis* are known, digestions with *Bsm* I could distinguish the two species in most cases. Although the IGS sequence for *A. cepistipes* differs substantially from the above three species (Anderson and Stasovski, 1992), the *A. cepistipes* type B restriction pattern with *Alu* I was the same as the *A. ostoyae* pattern. However, the IGS product of *A. cepistipes* can be distinguished from the other species tested by the presence of a *Hind* II restriction site.

Both *A. borealis* isolates sequenced by Anderson and Stasovski (1992) had the predicted *A. borealis* type B *Alu* I digestion pattern and not the type A pattern. The type A *A. borealis* pattern produced by *Alu* I digestion is identical to that of *A. ostoyae,* but, unlike *A. ostoyae,* all isolates of *A. borealis* lack a *Bsm* I restriction site in this region. However, the amplified DNA from our isolate B177 (Germany) of *A. ostoyae* failed to digest with *Bsm* I, and *A. ostoyae* isolate 337 (Germany) of Anderson and Stasovski (1992) had an IGS sequence lacking the *Bsm* I site. Thus, our *A. ostoyae* isolate B177 and their *A. ostoyae* isolate 337 would be identified as *A. borealis* type A using our technique. Comparisons of the IGS sequences (Anderson and Stasovski, 1992) show that the sequence of isolate 337 is similar to both *A. borealis* and *A. ostoyae.* Thus, it could be speculated that isolates B177 and 337, if accurately identified by pairings, represent evolutionary intermediates between the closely related *A. borealis* and *A. ostoyae.*

The IGS region for *A. cepistipes* type A and type B differed at two *Alu* I restriction sites. Our type A isolates had a restriction pattern consistent with published sequences of isolates 311 (Finland) and 316 (France) of Anderson and Stasovski (1992). Their isolate 304 (Finland), however, had an IGS sequence much different from that of the other two isolates of *A. cepistipes* they studied and had a predicted *Alu* I restriction pattern different from any that we tested. None of the isolates studied by Anderson and Stasovski (1992) had an IGS sequence that would result in the type B *A. cepistipes* pattern with *Alu* I digestion. There are, therefore, at least two, and possibly three, different *A. cepistipes* IGS types.

The distinction between *A. sinapina, A. cepistipes* and NABS XI remains clouded (Anderson et al., 1987; Bérubé and Dessureault, 1988; Guillaumin et al., 1989). *Armillaria sinapina* is known from northern North America (Bérubé and Dessureault, 1988; Shaw and Loopstra, 1988; Mallett, 1990; Blodgett and Worrall, 1992a; Harrington and Rizzo, 1993). *Armillaria cepistipes* is reported from Europe (Guillaumin et al., 1993), but NABS XI (group F *sensu* Morrison) from northwestern North America is at least partially interfertile in pairing studies with *A. cepistipes* and may be the same species (Morrison et al., 1985). We found only one *Alu* I restriction pattern among our *A. sinapina* isolates, and this pattern was predicted by the sequence for *A. sinapina* isolate 48 (New York) of Anderson and Stasovski (1992). The sequence of their *A. sinapina* isolate 205 (British Columbia), however, would give an *Alu* I restriction pattern identical to *A. cepistipes* type A. Unfortunately, we did not have isolates of NABS XI available for testing, nor did Anderson and Stasovski (1992) sequence the IGS region of NABS XI isolates.

The other two undescribed North American taxa of *Armillaria* (NABS IX and X) have unique IGS sequences (Anderson and Stasovski, 1992) and *Alu* I restriction patterns. It should be pointed out, however, that only a limited number of isolates of NABS IX and X were used in these respective studies.

In most cases where two *Alu* I restriction patterns were seen within an *Armillaria* species, a difference at a single restriction site would explain the discrepancy. The geographic origin of the isolates did not appear to correspond with the within-species variation, except in *A. gallica.* The North American *A. gallica* pattern is consistent with the sequence published for the North American isolates 434 (Michigan), 137 (Michigan) and 90 (Vermont) studied by Anderson and Stasovski (1992). Their sequence for the European isolate 332 (France), however, differs substantially from the North American *A. gallica* isolates, and the predicted *Alu* I restriction pattern for this isolate is consistent with the pattern obtained with our European *A. gallica* isolates.

The sequence of the IGS region of the North American *A. gallica* isolates is more similar to the North American species *A. calvescens* than to the sequence in the European *A. gallica* isolate (Anderson and Stasovski, 1992). In fact, the only difference found in the sequence of these two North American species is at a single *Tha* I site, but we found digestion of the IGS region with this restriction enzyme to be unreliable. Basidiomes of the two species are morphologically distinct (Bérubé and Dessureault, 1989), but these species are similar in other respects, including their weak pathogenicity and large, monopodially branched rhi-

zomorphs (Bérubé and Dessureault, 1989; Blodgett and Worrall, 1992a, b; Harrington, 1993; Rizzo and Harrington, 1993). North America, where *A. ostoyae* ... *calvescens* may have ... *gallica* and is better adapted ... forests dominated by ... mains more prevalent ... at locations typically ... of *A. calvescens* (Bérubé and Dessureault, 1989; Blodgett and Worrall, 1992a; Rizzo and Harrington, 1993).

Only the *A. tabescens* amplified product ... of 845 bp. One isolate ... restriction site, but ... the same restriction pattern ... *cens* from Korea also ... The *A. tabescens* isolates ... ica also had the same restriction pattern (data not shown). This ... patterns suggests that ... North America is ... (1992), in spite of ... tween isolates from ... al., 1993).

Each of the *A. mellea* isolates amplified ... product of 875 bp ... restriction patterns ... tions from northeastern ... tern shown by the isolates ... An isolate from Japan ... latter group (data not shown) ... tested had the same ... 1 (data not shown) ... British isolates had ... other *A. mellea* isolates. Thus, there was some variability in IGS restriction patterns that may correspond ... igin of the isolates, but the data are ...

More sampling would likely ... patterns among the ... North America, but ... were likely revealed by ... data, it appears that ... and digestions with other ... guish all the recognized ... continents except the ... *gallica* and *A. calvescens* ... point to questions ... ography and taxonomy ... pathogens.

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LITERATURE CITED

... S. ... and ... Pukkila. 1989. ... ribosomal DNA among biological species ... a genus of ... fungi. *Evolution* ... 1652–166 ...

... Petsche, and M. Smith. 1987. Restriction ... polymorphism ... biological species of ... *mellea*. *Mycologia* 79: 69–76.

... Stasovski. 1992. Molecular phylogeny ... Hemisphere species of *Armillaria*. *Mycologia* 84: 505–5...

... and ... 1988. Morphological ... zation of *Armillaria ostoyae* and *Armillaria* ... nov. *Can. J. Bot.* 66: 2027–2034.

———. 1989. Morphological studies of ... *mellea* complex ... species, *A. gallica* ... *vescens*. *Mycologia* 81: 216–225.

... and ... 1992a. Distribution ... *Armillaria* species in New York. *Pl. Dis.* ... 166–170.

———. 1992b. ... relationships of *Armillaria* ... New York ... 76: 170–174.

... W., ... and T. J. Volk. ... ity among isolates of *Armillaria tabescens* ... erica. *Sydowia* ... 105–116.

... C., and ... Anderson. 1990. Location ... of transcription of the 5S rRNA gene ... *Mycol. Res.* ... 266–269.

... J., J. ... and K. Korhonen. ... interfertility and biological species ... *Armillaria* ... C. G. Shaw, ... USDA Forest Serv. Agric. Handbook No. ... D.C.

... and ... 1989. *Armillaria* ... the northern ... hemisphere. Pp. ... *ceedings 7th IUFRO conference on root and* ... D. J. Morrison, Ed. Forestry Canada, Victoria, British Columbia, Canada.

... N. Anselmi, R. Gregory, ... uisse, S. C. Gr... ... nrieder, M. ... , H. Marxm... on, J. Rishbeth, ... A. Termorshuizen, A. ... n Dam. 1993. Geographic distribution ... of the *Armillaria* species in western Europe. ... *rest Pathol.* 23: 325–341.

... C., ... and F. A. Baker. ... Pp. 81–85. In: *Methods for research on soilborne phytopathogenic fungi* ... L. L. Singleton, J. D. ...

and C. Rush. American Phytopathological Society Press, St. Paul, Minnesota. — intersterility groups occurring in Briti... Canad. J. Bot. 31: 651–653.

———, and D. M. Rizzo. 1992. Armillaria ... Harrington ... Nucl... species from New Hampshire. ... 365–368 ... diploid-haploid pairings of Armillaria ...

Hintikka, V. 1973. A note on the polarity of Armillariella ... 84: 863–869. Mycologi...

mellea. Karstenia 13: 32–39. ——— . 1993. Delineation and biol...

Jahnke, K.-D., G. Bahnweg, and J. Worrall. 1987. Species ... Armillaria ... A. gemina and A. cal... delimitation in Armillaria ... by analysis ... 85: 164–172. Mycologi... of nuclear and mitochondrial DNA ... Trans. Brit. Mycol. Soc. 88: 572–575. ... and C. G. Shaw III. 1988. Identif... pathogenicity of ... Alaskan isolates of A...

Korhonen, K. 1978. Interfertility and clonal size in the Armillariella mellea complex. Karstenia 18: ... Phytopathology 78: 971–974.

Larsen, M. J., M. T. Banik, and H. H. Burdsall. 1992. Clamp connections in Armillaria ... Eur. J. Forest Pathol. 17: 308–311. ... Zur Beurteilung de... Testen mit diploiden Armillari... cies: occurrence and potential ... and J. Smith, and ... Anderson. 1989. Restrictio... species. Mycologia 84: 214–218. ... length polymorphisms in mitochondrial D...

Lee, S. B., and J. W. Taylor. 1990. Isolation of DNA from fungal mycelia and single spores. In: PCR protocols, a guide to methods and applications. ... identification of North American bio... Eds., M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White. Academic Press, San Diego, Calif. ... Mycol. Res. 93: 247–256. ... M., J. Klootwijk, C. A. F. M. de Regt, ... 1981. The primary and secondary str... 26S rRNA. Nucl. Acids Res. 9: 6935–69...

Mallet, K. I. 1990. Host range and geographic distribution of Armillaria root rot pathogens in the Canadian prairie provinces. Canad. J. Forest Res. 20: ... G. A. Wiebe, and H. H. Burdsall. 1991 ... nomenclature, taxonomy, and identification. Pp. 1... In: Armillaria root disease. Eds., C. G. Shaw, and G. A...

Morrison, D. J., A. J. Thomson, D. Chu, ... Sahota, and ... 1985 ... U.S.D.A. Forest Serv. Agric. Handbook No. 691, ... Washington, D.C.

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