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Strain typing of ectomycorrhizal basidiomycetes from subalpine Tyrolean forest areas by random amplified polymorphic DNA analysis

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Abstract The application of random amplified polymorphic DNA (RAPD) analysis for the identifcation of ectomycorrhizal symbionts of spruce (*Picea abies*) belonging to the genera *Boletus*, *Amanita* and *Lactarius* at and below the species level was investigated. Using both fingerprinting [M13, $(GTG)_5$, $(GACA)_4$] as well as random oligonucleotide primers (V1 and V5), a high degree of variability of amplified DNA fragments (band-sharing index 65–80%) was detected between different strains of the same species, hence enabling the identification of individual strains within the same species. The band-sharing index between different species of the same genus (*Boletus, Russula* and *Amanita*) was in the range of 20–30%, and similar values were obtained when strains from different taxa were compared. Thus RAPD is too sensitive at this level of relatonship and cannot be used to align an unknown symbiont to a given taxon. We therefore conclude that RAPD is a promising tool for the identification of individual strains, and could thus be used to distinguish indigenous and introduced mycorrhizal strains from the same species in natural ecosystems.

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Introduction

Spruce (*Picea abies*) is the predominant tree in higher mountainous regions of central Europe. In Austria it accounts for more than 60% of forest trees. Environmental stress has considerably reduced the viability of this population, thereby raising the need for afforestation. The inoculation of forest tree seedlings with ecologically adapted ectomycorrhizal fungi is already established practice in several countries (Kropp and Langlois 1990; Le Tacon et al. 1992), but the results on reforestation sites are still only partially satisfactory. It has been speculated that this may be due to the replacement of the introduced strains by, or recombination with, more aggressive but less beneficial indigenous fungi (Gardes et al. 1991). The identification of the fungal partners involved in these symbioses can thus yield valuable information for breeding and cultivation of the plant. Unfortunately, in the early stages of mycorrhizal interactions and in soil, these fungi are often difficult or even impossible to identify by morphological characteristics alone (Agerer 1993). Molecular biological tools have thus been developed for their determination (Gardes et al. 1991; Bruns and Gardes 1993; Gardes and Bruns 1993; Mehmann et al. 1994).

Most of the methods applied in these studies used gene hybridisation and gene restriction fragment polymorphism as tools that are able to distinguish at the species level (Bruns et al. 1991). However, they are in most cases not able to distinguish between different strains at the infraspecific level. A method suitable for this purpose is random amplified polymorphic DNA (RAPD) analysis (Welsh and McCleland 1990), the ability of which to distinguish between different strains of the same species is now well established (St. Leger et al. 1992; Megnegneau et al. 1993). Its application in the

identification of several endo- (Wyss and Bonfante 1993) and one ectomycorrhizal fungus (*Tuber magnatum* Pico; Lanfranco et al. 1993) has recently been presented.

In the northern-alpine forest areas in Tyrol, Austria, reforestation of spruce (*Picea abies*) is particularly sensitive to the presence of *Boletus* spp., *Amanita* spp. and *Lactarius* spp., and some of these fungi (e.g. *B. edulis* or *L. deterrimus*) are used in afforestation. We have studied whether RAPD is a useful tool to characterise individual strains isolated from this area.

Materials and methods

Fungal strains

The fungal strains investigated in this study and their place and year of harvest are given in Table 1. Filamentous mycelia were obtained by putting pieces of surface-sterilised fruiting bodies into Petri dishes containing Moser-b nutrient agar (Moser 1963).

DNA extraction

To isolate DNA for fingerprinting, fungal mycelia were grown in 9-cm Petri dishes on cellophane sheets placed over the agar. To isolate chromosomal DNA, the fungal mycelium was removed from the cellophane, ground in liquid nitrogen and transferred into an Eppendorf tube containing 500 µl of lysis buffer [50 mM Tris-HCl pH 7.2, 50 mM EDTA, 3% (v/v) SDS, 1% (v/v) 2-mercapto-ethanol]. After vigorous shaking, the suspension was then incubated at 65 °C for 1 h. Thereafter, 250 µl of phenol and 250 µl of chloroform were added, and the mixture centrifuged in an Eppendorf benchtop centrifuge for 15 min at room temperature. The upper aqueous layer was transferred to a fresh Eppendorf tube, 500 µl of chloroform was added and the tube again centrifuged. To the upper aqueous layer, 250 µl of isopropanol and 25 µl of sodium acetate (3 M pH 8.0) were added and the mixture was incubated at –20 °C for 20 min. After centrifugation (see above) for 15 min, the DNA pellet was washed with 70% (v/v) ethanol, and dried in a Speed Vac centrifuge. The dried pellet was resuspended in 100 µl of TE buffer (10 mM Tris-HCl pH 8.0, containing 0.1 mM EDTA), after which 10 µg RNase A was added and the samples stored at –20 °C until use.

RAPD-PCR analysis

Oligonucleotide primers used for RAPD analysis were M13 (5′-GAGGGTGGCGGTTCT-3′), $(GTG)_5$ and $(GACA)_4$, which were originally designed for RFLP fingerprinting (Meyer et al. 1991). Since these are repeated rather than arbitrary nucleotide sequences, we prefer to use the term RAPD fingerprinting for our procedure. We also included two very short arbitrary primers (Caetano-Anolles et al. 1992) named V1 (5′-ACGGTCTTGG-3′) and V5 (5′-TGCCGAGCTG-3′). The optimal amplification conditions were found to be 10 mM Tris-HCl pH 8.8, 50 mM KCl, 4 mM $MgCl_2$, 0.1% Triton X-100, 0.2 mM each of dNTP (dATP, dCTP, dGTP and dTTP), 0.8 mM oligonucleotide primer, 5–25 ng genomic DNA (to be optimised for each strain) and 1 unit of BI-Taq DNA polymerase (Biomedica) in a final reaction volume of 50 µl. With primers M13 and $(GACA)_4$, the amplification protocol was 40 cycles consisting of 20 s denaturation (95 °C), 60 s primer annealing (50 °C) and 30 s extension (72 °C). For the primer $(GTG)_5$, the denaturation during the first five cycles was for 60 s under otherwise identical conditions. With the primers V1 and V5, the amplification protocol was: 20 s denaturation (95 °C), 90 s primer annealing (30 °C), 100 s extension (72 °C) through 35 cycles. The RAPD-PCRs were performed on a Hybaid Thermal Reactor thermocycler.

RAPD product (20 µl) was loaded with 6×loading buffer (0.25% bromophenol blue and 15% ficoll in water) onto a 1.2% agarose gel (containing 0.5 mg/l ethidium bromide) and run in 0.5×TBE at 60 mA constant current. The amplification products were visualised under UV light. The percentage of shared amplified DNA between various isolates was calculated using the following formula: $F = 2*N_{xy}/(N_x + N_y)*100$ [%], where N_{xy} is the number of shared DNA bands between two strains, and N_x and N_y are the total number of bands in each of the samples.

Phylogenetic computation was carried out with the programmes DNADIST (set to Kimura "2" parameter and FITCH in the PHYLIP package (Felsenstein 1989).

Table 1 Mycorrhizal isolates investigated in this study. All species listed form ectomycorrhizae with *Picea abies* (L. Karst.), with the exception of *Boletus aereus*, which is a typical symbiont of *Fagus* sp. and *Quercus* sp., *Lactarius deliciosus*, which is a symbiont of *Pinus* sp. and *L. porninsis*, which is a symbiont of *Larix* sp. All cultures were isolated by F.G. and are maintained at the Forstliche Bundesversuchsanstalt in Innsbruck, Tyrol

Strain	Code	Origin	Year
Amanita muscaria (L. ex Fr.) Hooker	AMUa	Haggen, Tyrol[b]	1980
Amanita muscaria (L. ex Fr.) Hooker	AMUd	Schulterberg, Tyrol[a]	1990
Amanita porphyria (A.&S. ex Fr.) Secr.	APO	Matzenköpfl, Tyrol[b]	1985
Amanita rubescens (Pers. ex Fr.) Gray	ARUa	Klausboden, Tyrol[b]	1974
Amanita rubescens (Pers. ex Fr.) Gray	ARUg	Matzenköpfl, Tyrol[b]	1985
Boletus edulis Bull. ex Fr.	BEDg	Grinzens, Tyrol[b]	1985
Boletus edulis Bull. ex Fr.	BEDk	Stieglreith, Tyrol[b]	1985
Boletus edulis Bull. ex Fr.	BEDp	Paida, Tyrol[b]	1974
Boletus edulis Bull. ex Fr.	BEDt	Schulterberg, Tyrol[a]	1990
Boletus aereus Bull. ex Fr.	BAR	Borgotora, Italy[c]	1985
Boletus erythropus (Fr. ex Fr.) Pers.	BER	Paida, Tyrol[b]	1978
Boletus calopus Fr.	BOC	Ehrwald, Tyrol[b]	1987
Lactarius deterrimus Gröger	LADe	Paida, Tyrol[b]	1985
Lactarius deterrimus Gröger	LADg	Schulterberg, Tyrol[a]	1990
Lactarius deliciosus Fr.	LAL	Mutters, Tyrol[c]	1983
Lactarius porninsis Roll.	LAP	Haggen, Tyrol[c]	1980
Lactarius scrobiculatus (Scop. ex Fr.) Fr.	LAS	Wattener Lizum, Tyrol[b]	1992

[a] Isolates from habitats within the Achenkirch area
[b] Isolates from other parts of Tyrol or Italy
[c] Species that can form ectomycorrhizae with trees other than *Picea abies*

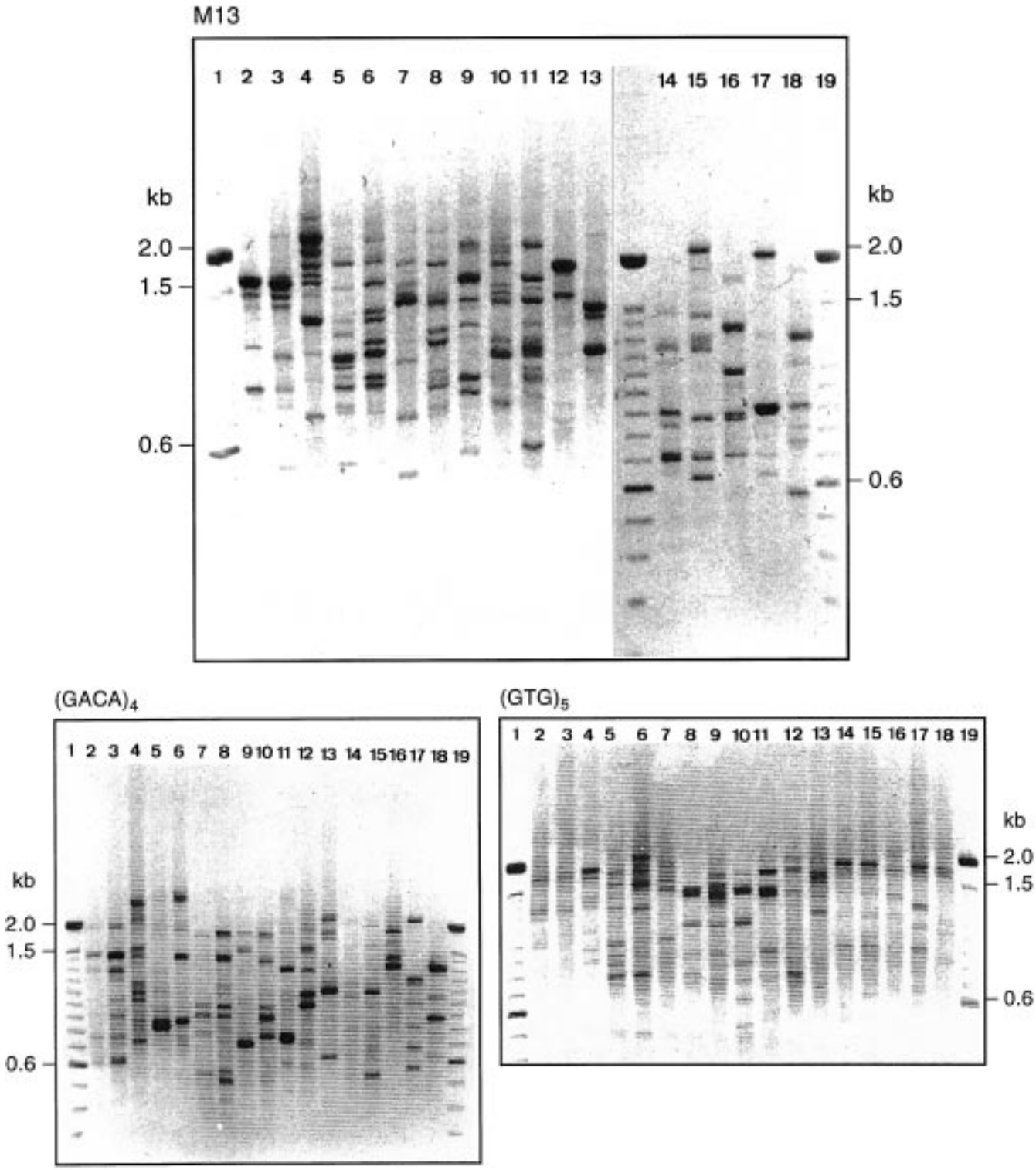

Fig. 1 RAPD amplification products of DNA isolated from different mycorrhizal fungi as indicated by the lane numbers: *2* AMUa, *3* AMUd, *4* APO, *5* ARUa, *6* ARUg, *7* BEDg, *8* BEDk, *9* BEDp, *10* BEDt, *11* BAR, *12* BER, *13* BOC, *14* LADe, *15* LADg, *16* LAL, *17* LAP, *18* LAS. *Lanes 1 and 19* show a 1-kb ladder (the size of major nucleotide bands is given in kb). The primers used for amplification is indicated above each illustration. Colour reversed prints of original polaroid photographs are shown

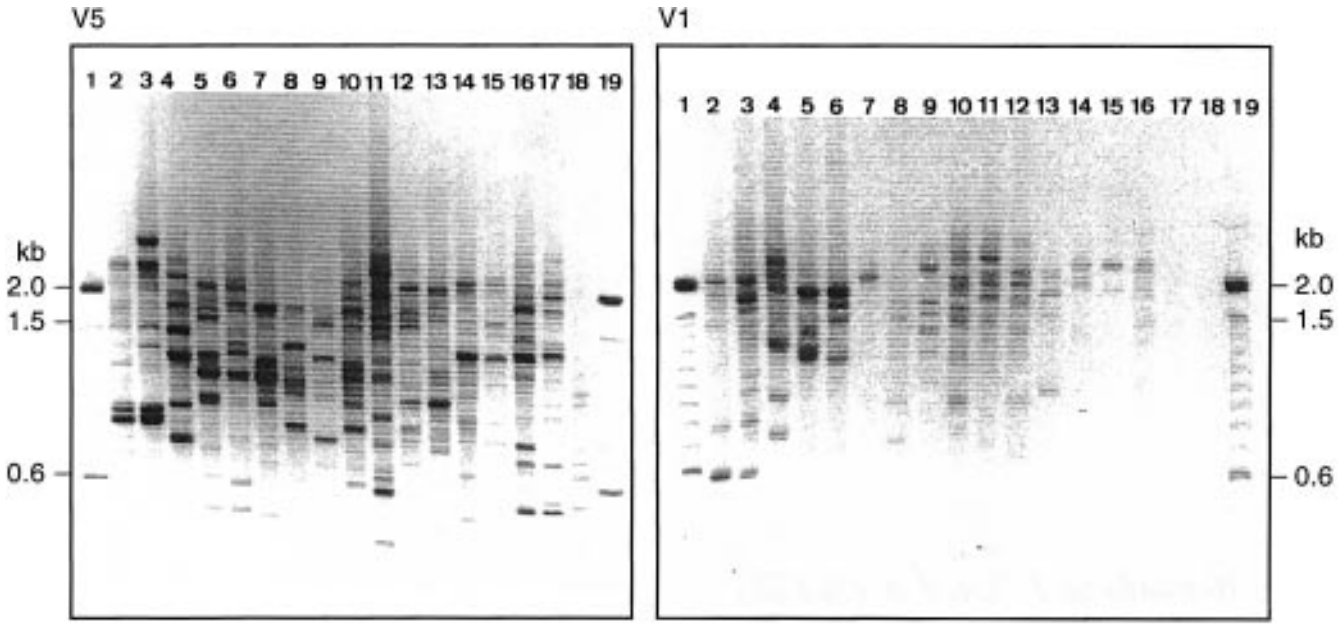

Fig. 1 (Continued) Text see on page 37

Results and discussion

Seventeen isolates belonging to the three different basidiomycetous genera *Amanita (A. muscaria, A. porphyria* and *A. rubescens)*, *Lactarius* (*L. deterrimus, L. deliciosus, L. porninsis* and *L. scrobiculatus*) and *Boletus* (*B. edulis, B. aereus, B. erythropus* and *B. calopus*) were selected for this study. These fungi have in the past been frequently observed as typical specific and nonspecific mycorrhizal symbionts of *Picea abies* in the investigated area (Table 1). Three species, which form mycorrhizae with other trees (i.e. *L. porninsis* with *Larix decidua* Mill., *L. deliciosus* with *Pinus sylvestris* L., and *B. aereus* with *Castanea sativa* and other deciduous trees) were also included. Chromosomal DNA extracted from each of the strains was subjected to RAPD-PCR, using five fingerprinting primers [M13, $(GACA)_4$ and $(GTG)_5$]and two random primers. The fingerprinting primers were chosen because they had already proven useful with other fungi (Lieckfeldt et al. 1993; Arisan-Atac et al. 1995; D. Turner and C.P. Kubicek, unpublished data). Primer M13 was used for most of the preliminary optimisations because it yielded an appropriate number of bands within a well-separable range of molecular sizes. DNA of sufficient quality for RAPD analysis was readily isolated from most species with the exception of *B. erythropus* and *B. calopus.* Among a number of methods recommended for the purification of DNA, separation in low-melting-point agarose and subsequent elution was the most successful with these latter two species.

In the final analyses (Fig. 1), M13 as well as $(GACA)_4$ yielded 8–15 well-separated DNA fragments, in the range of 600–2500 bp for each strain. $(GTG)_5$, on the other hand, yielded only a few clean bands, mostly within a smear, which could not be removed even by several changes in the PCR program or by using different concentrations of template DNA. The number of bands observed with primers V1 and V5 was considerably higher than with the fingerprinting primers (15–20 bands, in the range of 500–3000 bp for each strain). V5 yielded very well-resolved bands, whereas results with V1 were also accompanied by a smear (Fig. 1). Some strains gave a particularly poor fragment pattern with V1, whereas others gave satisfactory results.

On the basis of these findings, the results obtained with primers M13, $(GACA)_4$ and V5 were used for the calculation of band-sharing indices (Table 2). For this purpose, we considered all bands as 1 when present at a given mobility, irrespective of whether they were strong or only faint. In a separate calculation, we arbitrarily attributed a 0.5 value to faint bands. This resulted only in small changes in the similarity coefficients and did not significantly influence the results from this study. In general, variability between strains of the same species was low, as reflected by the high band-sharing index (65–80%). The degree of similarity within *B. edulis* was the lowest, and the comparison of some strains yielded figures even below 50% (e.g. BEDk with BEDp). We have carried out several separate experiments (data not shown) to ensure that this variability was not caused by the DNA extraction. RAPD analysis of several separate DNA preparations from the same strain yielded more than 95% identity, which is clearly above the level of identity observed between different isolates of the same species. We have also separately analysed DNA extracted from mycelia and fruiting bodies of the same isolate, and found that the RAPD bands were highly consistent (band-sharing index $>95\%$). The values of 65–80% between different isolates of the same species,

Table 2 Matrix of percent of RAPD bands shared between individual fungal species. The upper line indicates results with primer M13, the middle line with $(GACA)_4$ and the lowest line with primer V5. Species codes as in Table 1 and Fig. 2

	AMUa	AMUd	APO	ARUa	ARUg	BEDg	BEDk	BEDp	BEDt	BAR	BER	BOC	LADe	LADg	LAL	LAP	LAS
AMUa	100																
AMUd	78	100															
	100	100															
	82	100															
APO	32	48	100														
	17	17	100														
	40	36	100														
ARUa	27	33	40	100													
	32	32	32	100													
	19	14	22	100													
ARUg	36	42	40	86	100												
	32	32	40	80	100												
	32	14	15	79	100												
BEDg	33	20	29	25	25	100											
	22	22	25	0	11	100											
	32	28	30	50	50	100											
BEDk	38	26	33	37	37	78	100										
	27	33	29	17	26	73	100										
	36	31	42	40	40	80	100										
BEDp	40	27	37	39	39	36	48	100									
	22	22	17	11	11	55	64	100									
	36	23	33	40	40	72	73	100									
BEDt	42	27	27	32	24	67	75	52	100								
	30	30	31	10	19	80	83	50	100								
	40	39	32	44	50	81	69	69	100								
BAR	30	36	26	23	31	18	24	42	26	100							
	27	27	38	13	13	27	32	40	24	100							
	26	22	35	34	34	40	38	38	46	100							
BER	17	14	40	22	7	14	12	13	27	0	100						
	38	38	18	24	12	25	30	50	22	62	100						
	34	25	20	39	52	39	36	43	46	32	100						
BOC	0	14	27	33	33	14	12	13	13	13	0	100					
	13	13	27	12	0	25	20	13	22	15	0	100					
	38	29	24	41	41	29	26	26	37	29	49	100					
LADe	22	20	38	25	25	20	26	18	19	9	29	14	100				
	0	0	19	13	13	13	32	27	24	17	31	0	100				
	35	38	27	32	39	32	43	36	34	36	47	49	100				
LADg	12	11	40	17	17	21	27	19	20	10	31	15	74	100			
	32	32	16	30	20	21	44	32	29	13	35	24	63	100			
	39	41	22	21	29	36	40	32	38	34	39	53	77	100			
LAL	29	38	24	10	10	13	32	11	24	11	0	20	25	27	100		
	31	15	21	14	29	15	12	0	13	0	18	0	0	14	100		
	33	29	38	12	24	24	20	13	32	30	33	26	28	24	100		
LAP	0	0	0	11	11	0	11	35	25	12	0	22	40	43	36	100	
	24	24	35	22	22	0	19	12	11	0	0	40	14	33	0	100	
	24	32	28	13	20	40	30	37	35	32	30	33	30	33	46	100	
LAS	22	20	29	42	42	10	35	36	19	18	14	14	40	21	13	27	100
	12	12	44	0	0	35	19	23	21	27	27	13	14	0	17	0	100
	7	0	16	23	31	23	17	17	27	24	21	19	21	15	26	29	100

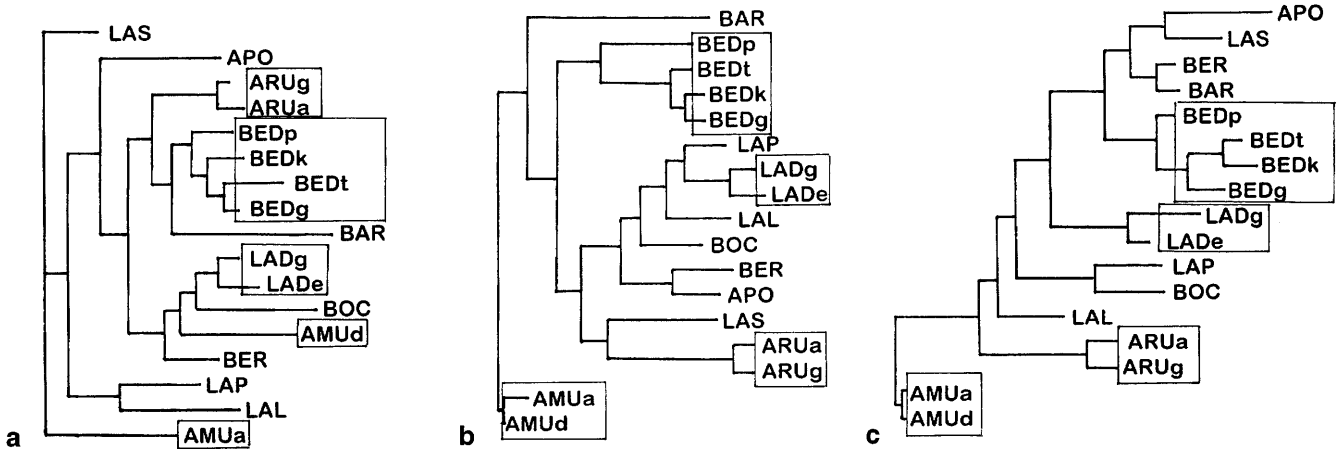

Fig. 2a–c Cladogram of relative genetic distances between the isolates analysed by RAPD in the course of this study with primers M13 (**a**), (GTG)5 (**b**) and V1 (**c**). Distances were calculated by the algorith of Fitch and Margoliash (1967), using PHYLIP (Felsenstein 1967). Data are presented as unrooted trees, optimized from 1591 trees (**a**), 2329 trees (**b**) and 3239 trees (**c**), with an average percent standard deviaton of 14.2 (**a**), 14.6 (**b**) and 12.2% (**c**). The genetic distances between branches are not drawn proportionally. The *boxes* enclose those species for which more than a single isolate was investigated

therefore, indicate a rather high genetic variability, as has been also found with some other fungal genera (Stenlid et al. 1994; St. Leger et al. 1992; Megnegneau et al. 1993), including arbuscular mycorrhizal fungi (Wyss and Bonfante 1993). In most cases, genetic variability has been demonstrated between geographically different isolates, and it is, therefore, interesting that our results only include strains isolated from a geographically very close area.

The band-sharing indices between different species within the same taxon or from another taxon were generally scattered around 25 ± 10%. When we analysed the data by the Fitch-Margoliash algorithm, it was clear that they could not be used to align individual isolates into one of the three fungal genera, as every primer resulted in another placement of isolates within the cladogram. In contrast, the different isolates from one and the same species (with one exception) always clustered together (Fig. 2). We interpret this as an indication that the genetic differences between the species used in this study are too high to be estimated by a method as sensitive as RAPD, and the index value of 25% may, therefore, be considered as a limit of confidence for this method. Others have interpreted figures somewhat below ours in the same way (i.e. 20% in Chapco et al. 1992). This result is less surprising given the fact that RAPD is able to distinguish between physiologically different races of the same species.

In summary, we have demonstrated here the potential of RAPD to distinguish within the same species of ectomycorrhizal fungi, and it may, therefore, complement already established molecular methods used for the identification of mycorrhizal fungi based on amplification and restriction analysis or hybridisation of gene sequences (Gardes et al. 1991; Gardes and Bruns 1993; Bruns and Gardes 1993; Henrion et al. 1994; Mehmann et al. 1994). This high sensitivity of RAPD may prove particularly valuable in the identification of possible recombination events between introduced and indigenous strains, which can greatly influence the fate of introduced strains under natural conditions. A drawback of RAPD is, however, that it cannot be directly applied to DNA extracted from mycorrhizal roots because of interference by the excess of plant DNA present (S.B. Haudek, unpublished data), and hence requires subculture of the fungus prior to its application. The method may nevertheless be useful when the fate of an introduced mycorrhizal symbiont is to be followed under field conditions, or when the identity of a fruiting body with the symbiotic mycelium has to be assessed.

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References

Agerer R (1993) Mycorrhizae: ectomycorrhiza and ectendomycorrhiza. Prog Bot 54:505–529

Arisan-Atac I, Heidenreich E, Kubicek CP (1995) Randomly amplified polymorphic DNA fingerprints identifies subgroups of *Trichoderma viride* and other *Trichoderma* sp. capable of chestnut blight biocontrol. FEMS Microbiol Letts 126:249–256

Bruns TD, Gardes M (1993) Molecular tools for identification of ectomycorrhizal fungi: taxon-specific oligonucleotide probes for sulloid fungi. Mol Ecol 2:233–242

Bruns TD, White TJ, Taylor JW (1991) Fungal molecular systematics. Annu Rev Ecol Syst 22:525–564

Ceatano-Anolles G, Bassam BJ, Gresshoff PM (1992) DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. Bio/Technology 9:553–557

Chapco W, Ashton NW, Martel RKB, Antonoshyn A, Crosby WL (1992) A feasibility study of the use of random amplified polymorphic DNA in the population genetics and systematics of grasshoppers. Genome 35:569–574

Felsenstein J (1989) PHYLIP – Phylogeny interference package (version 3.2). Cladistics 5:164–166

Fitch WM, Margoliash E (1967) Construction of phylogenetic trees. Science 155:279–284

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Gardes M, White TH, Fortin JA, Bruns TD, Taylor JW (1991) Identification of indigenous and introduced symbiotic fungi in ectomycorrhizae by amplification of nuclear and mitochondrial ribosomal DNA. Can J Bot 69:180–190

Henrion B, Di Battista C, Bouchard D, Vairelles D, Thompson BD, Le Tacon F, Martin F (1994) Monitoring the persistence of *Laccaria bicolor* as an ectomycorrhizal symbiont of nursery-grown Douglas fir by PCR of the rDNA intergenic spacer. Mol Ecol 3:571–580

Kropp BR, Langlois C (1990) Ectomycorrhizae in reforestation. Can J For Res 20:438–451

Lanfranco L, Wyss P, Marzachi C, Bonfante P (1993) DNA probes for identification of the ectomycorrhizal fungus *Tuber magnatum* Pico. FEMS Microbiol Letts 114:245–252

Le Tacon F, Alvarez IF, Bouchard D, Henrion B, Jackson RM, Luff S, Parlade JI, Pera J, Stenstrom E, Villeneuve N, Walker C (1992) Variations in field response of forest trees to nursery ectomycorrhizal inoculation in Europe. In: Read DJ, Lewis DH, Fitter A, Alexander IJ (eds) Mycorrhizas in ecosystems. CAB, Wallingford, UK, pp 119–134

Lieckfeldt E, Meyer W, Börner T (1993) Rapid identification and differentiation of yeasts by DNA and PCR fingerprinting. J Basic Microbiol 33:413–426

Megnegneau B, Debets F, Hoekstra RF (1993) Genetic variability and relatedness in the complex group of black *Aspergilli* based on random amplification of polymorphic DNA. Curr Genet 23:232–329

Mehmann B, Brunner I, Braus GH (1994) Nucleotide sequence variation of chitin synthase genes among mycorrhizal fungi and its potential use in taxonomy. Appl Environ Microbiol 60:3105–3111

Meyer W, Koch A, Niemann C, Beyermann B, Epplen JT, Börner T (1991) Differentiation of species and strains among filamentous fungi by DNA fingerprinting. Curr Genet 19:239–242

Moser M (1963) Förderung der Mykorrhizenbildung in der forstlichen Praxis. Mitt Forstl Bundesversuchsanstalt Wien 60:691–720

Stenlid J, Karlsson J-O, Högberg N (1994) Intraspecific genetic variation in *Heterobasidion annosum* revealed by amplification of minisatellite DNA. Mycol Res 98:57–63

St Leger RJ, Allee LL, May B, Staples RC, Roberts DW (1992) World-wide distribution of genetic variation among isolates of *Beauveria* spp. Mycol Res 96:1007–1015

Welsh J, McCleland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 19:861–866

Wyss P, Bonfante P (1993) Amplification of genomic DNA of arbuscular-mycorrhizal (AM) fungi by PCR using short arbitrary primers. Mycol Res 97:1351–1357