The nuclear rDNA intergenic spacer of the ectomycorrhizal basidiomycete *Laccaria bicolor*: structural analysis and allelic polymorphism

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The nuclear rDNA intergenic spacer (IGS) of the ectomycorrhizal basidiomycete *Laccaria bicolor* was amplified and sequenced to identify the source of its intraspecific polymorphism. The IGS was amplified by PCR in several *L. bicolor* strains and shown to exhibit multiple bands and length polymorphism. The IGS loci were shown to segregate in a 1:1 ratio within haploid progenies in three dikaryotic strains, suggesting that divergent IGS haplotypes were present in the two nuclei of these strains. The two haplotypes of *L. bicolor* S238N were sequenced: the β -haplotype was 4160 bp in length, whereas the size of the α -haplotype was estimated to be about 4700 bp. These represent the largest published fungal IGS sequences to date. These sequences can be subdivided into three main regions, IGS₁, 5S rDNA and IGS₂. The IGS sequences are AT-rich and contain numerous occurrences of three types of subrepeats (e.g. T₂AC₃). The length polymorphism, observed between the IGS sequence of the α - and β -haplotypes, results from the insertion of various numbers of a 71 bp subrepeat, called B, occurring in IGS₂. This variation in subrepeat number suggests that the two haplotypes resulted from unequal cross-overs. The *L. bicolor* IGS was aligned with IGS sequences of two other Tricholomataceae (i.e. *Tricholoma matsutake* and *Collybia fusipes*). No sequence similarity was observed between these IGSs, but homologous subrepeats were found in *L. bicolor* and *T. matsutake*. Analysis of IGS length polymorphism is therefore an efficient tool for investigating genetic relationships between genets and within progenies in natural fungal populations.

Keywords: *Laccaria bicolor*, basidiomycete, intergenic spacers, rDNA, repeated DNA

INTRODUCTION

Nuclear rDNA provides useful inter- and intraspecific polymorphisms to type higher fungi. There are multiple copies of 18S, 5·8S, 25S and 5S rRNA genes, which are arranged as head-to-tail repeats separated by non-coding spacers. They are located at one or several loci and have identical sequences due to concerted evolution (Garber *et al.*, 1988). The coding sequences of rRNA are highly conserved between species of ectomycorrhizal fungi, but the internal transcribed spacers (ITS) between

the 18S, 5·8S and 25S RNA sequences, which are removed during processing, and also the intergenic spacers (IGS), residing between each transcription unit, exhibit a high inter- and intraspecific variability (Henrion *et al.*, 1992; Albee *et al.*, 1996; Selosse *et al.*, 1996; Gryta *et al.*, 1997; Martin *et al.*, 1998). The IGS region, which includes the 5S rRNA gene in most fungal species, is worthy of particular attention as it contains a variety of transcriptional regulatory elements and tandem arrays of subrepeating sequence motifs (Baldridge *et al.*, 1992). IGS sequences often contain sufficient variation to allow examination of genetic relationships between fungal populations and individuals (Anderson *et al.*, 1990; Albee *et al.*, 1996; Gryta *et al.*, 1997; Selosse *et al.*, 1998; Guidot *et al.*, 1999). The IGS varies greatly in length among different fungal species, ranging from

Abbreviation: IGS, intergenic spacer.

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about 1 to over 4 kb (Garber *et al.*, 1988). Such length heterogeneity is likely to be a result of the presence of many tandem or dispersed subrepeat domains. However, the long length of fungal IGS sequences may explain the paucity of sequence data available in databases to date. The entire sequence of this region is known only for a few fungal rDNAs, e.g. *Neurospora crassa* (Dutta & Verma, 1990), *Saccharomyces cerevisiae* (Molina *et al.*, 1993), *Filobasidiella* (*Cryptococcus*) *neoformans* (Fan *et al.*, 1995) and *Neotyphodium lolii* (Ganley & Scott, 1998). For the basidiomycetes, the complete IGS sequence has only been characterized for *Collybia fusipes* (B. Marçais, INRA-Nancy, personal communication) and partial sequences are also available for *Tricholoma matsutake* (Hwang & Kim, 1995, 1998) and *Hebeloma cylindrosporum* (Guidot *et al.*, 1999).

Laccaria bicolor ([Maire] Orton) is an ectomycorrhizal basidiomycete (Tricholomataceae) having a haplo-dikaryotic life cycle. The dikaryotic mycelium forms an ectomycorrhizal mutualistic association on tree roots. This symbiosis plays a fundamental role in the biology and ecology of forest trees, affecting growth, water and nutrient absorption, and providing protection from root diseases (Smith & Read, 1997). Questions about the temporal and spatial distributions of populations of this ectomycorrhizal fungus, together with the origin and maintenance of its genetic variation, are therefore critical to understand how populations evolve and disappear at different stages of development of forest ecosystems (Martin *et al.*, 1998; Selosse *et al.*, 1998). Each genotype arises in a unique mating event and then vegetatively expands as a host root-connected mycelium throughout the humus layer of forest soils. Molecular markers have been designed to identify *L. bicolor* and allow the study of its populations in nurseries and plantations (Selosse *et al.*, 1998). Since several different strains are currently used for nursery inoculation of Douglas fir (Villeneuve *et al.*, 1991; Le Tacon *et al.*, 1992), this species provides an ideal model for strain tracking and analysis of population effects of out-planting of inoculated trees.

PCR/RFLP analysis of IGS in *L. bicolor* revealed variation indicative of sequence polymorphisms among isolates in this species (Henrion *et al.*, 1992; Selosse *et al.*, 1996). IGS polymorphisms allowed us to discriminate most genets present in natural populations (Selosse *et al.*, 1998) and haplotypes within progenies (Selosse *et al.*, 1996). In this paper, we report the complete IGS sequence of *L. bicolor* S238N and the analysis of the differences between allelic sequences of this strain to identify the source of its polymorphism.

METHODS

Strains, growth and breeding. A detailed description of the origin of the American strain S238N of *Laccaria bicolor* ([Maire] Orton) (Hymenomycetes, Agaricales, Tricholomataceae) used in this analysis is provided by Di Battista *et al.* (1996). The two other *L. bicolor* strains used are from the Collection of Ectomycorrhizal Fungi (INRA, Nancy, France):

strain 81306 was isolated in 1981 from a sporophore under Douglas fir (*Pseudotsuga menziesii* [Mir.] Franco) at Barbaroux (Haute-Vienne, France) and strain N203 was isolated in 1995 from a sporophore at Saint-Brisson (Nièvre, France) (Selosse *et al.*, 1998). Vegetative dikaryotic mycelia were grown on modified Pachlewski medium according to Henrion *et al.* (1992). Spores of strain N203 were obtained from a sporophore collected under Douglas fir inoculated using the vegetative mycelium of this strain; spores of strain S238N were collected from a sporophore under an inoculated Douglas fir in a glasshouse (Di Battista *et al.*, 1996) and spores of strain 81306 were collected from a sporophore under an inoculated Douglas fir in an experimental plantation at Saint-Brisson (Nièvre, France). Identification of the various sporophores was ascertained by the presence of specific randomly amplified polymorphic DNA (RAPD) markers (Selosse *et al.*, 1998). Haploid progenies (monokaryons) were germinated according to Fries (1983) and mycelium grown on modified Pachlewski medium according to Henrion *et al.* (1992).

DNA extraction, PCR amplification and enzymic digestion. Total DNA was extracted by the hexadecyltrimethylammonium bromide (CTAB)/proteinase K method as described by Henrion *et al.* (1994). The IGS₁ sequence, containing the 3' end of the 25S rDNA and the 25S/5S spacer, was amplified as described by Selosse *et al.* (1996) using primers CNL12/5SA. The IGS₂ sequence, containing the 5S rDNA plus the 5S/18S spacer, was amplified with the primers rev5SA (5'-ATCCACGGCCATAGGACTCTG-3') and revNS1 (5'-GAGACAAGCATATGACTAC-3') with priming sites at the 5' end of the 5S rDNA and at the 5' end of the 18S rDNA, respectively. Primers I1 (5'-TCCAGGTGTACACCAATATGCC-3') and I2 (5'-GGGTTTGTCCGGGAGATATGG-3') were used to amplify the central repeated region VII of IGS₂. Primers were supplied by Bioprobe Systems and Taq DNA polymerase by Appligène-Oncor. Reactions were performed in a Perkin-Elmer GeneAmp 9600 thermocycler under the following conditions: initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 30 s, and extension at 72 °C for 5 min, with a final extension at 72 °C for 10 min. Digestion of PCR products was carried out as described by Henrion *et al.* (1994).

Sequencing. Sequencing of IGS₂ was carried out on two monokaryons from *L. bicolor* S238N, namely HC3 and HC4, carrying different rDNA haplotypes (respectively the α - and β -haplotype; Selosse *et al.*, 1996; Fig. 1a). PCR products were purified using a Qiaquick Spin PCR Purification Kit (Qiagen). The sequencing reactions were performed using the PRISM Ready Reaction Dye Terminator Cycle Sequencing Kit (Perkin-Elmer Applied Biosystems). Sequencing reactions were initially primed using the rev5SA or revNS1 primers and then 12 internal primers were designed for primer walking along the β -type IGS₂. Sequencing redundancy was between 1·7 and 3. The sequencing reaction products were analysed using an ABI model 373S DNA sequencer (Perkin-Elmer Applied Biosystems). Raw sequence data were edited and contigs were assembled using the AutoAssembler (Perkin-Elmer Applied Biosystems) and Sequencher (Gene Codes C.) programs. Regions V, VI and VII of IGS₂ were also sequenced from the α -type IGS₂ of *L. bicolor* S238N. The previously published IGS₁ sequence of *L. bicolor* S238N (GenBank accession no. L25898) was assembled to the IGS₂ sequence using Sequencher. Searches for sequence homologues in the non-redundant DNA database (March 1999) of the National Center for Biotechnology Information (NCBI) was carried out using BLAST 2.0 (Altschul *et al.*, 1997). Multiple sequence alignments were performed with the MultAlin

program (Corpet, 1988) on the IBCP server (Institut de Biologie et Chimie des Protéines, Lyon, France; <http://pbil.ibcp.fr/NPSA/npsa-multalin.html>).

RESULTS

Heterozygosity of *L. bicolor* rDNA revealed by amplification of IGS₂

The 5S rDNA and the intergenic spacer between the 5S and 18S rDNAs (so-called IGS₂) were amplified by PCR in three dikaryotic strains of *L. bicolor*, S238N, 81306 and N203. Two (strains S238N and N203) or three (strain 81306) PCR products were observed after electrophoretic separation (e.g. strain S238N in Fig. 1a, lane 5), suggesting that IGS₂ length heterogeneity existed within individuals. To study further the origin of this length heterogeneity and the inheritance of the amplified fragments, monokaryons germinated from spores of

these three *L. bicolor* strains were analysed (Table 1). For S238N (Fig. 1a, lane 5), 47 monokaryons showed a pattern, referred to as the α -haplotype, with only the 3.9 kb fragment (Fig. 1a, lane 3) and 39 monokaryons exhibited a single 3.4 kb fragment, the β -haplotype (Fig. 1a, lane 4). Five monokaryons produced the α + β parental pattern of strain S238N (Table 1). This latter pattern probably resulted from meiotic recombination within the rDNA repeats (Selosse *et al.*, 1996). For strain N203, which exhibited two fragments after PCR amplification, two haplotypes were also recovered in the haploid progeny, each allowing the amplification of a single fragment (Table 1). In strain 81306, a small 0.5 kb fragment was amplified (data not shown) in addition to two 3.5 and 3.3 kb fragments, as already observed in some *Laccaria proxima* strains (Albee *et al.*, 1996). Monokaryons from this strain showed either the 3.5 kb fragment or the 3.3 and the 0.5 kb fragments (Table 1). These data confirmed and extended our previous results (Selosse *et al.*, 1996), suggesting a meiotic segregation of two IGS₂ haplotypes, α and β , with the fragments amplified in dikaryons corresponding to two allelic IGS₂ haplotypes.

Features of the *L. bicolor* IGS

The sequencing data obtained on the β -type IGS₂ of *L. bicolor* S238N correlated very well with (i) the overall length of IGS₂ spacer and its RFLP pattern (Fig. 1a, lane 2), as determined by agarose gel electrophoresis. Previous PCR amplification of this IGS₂ (Selosse *et al.*, 1996) suggested a longer length, but this was probably due to size overestimation on acrylamide gels of amplified DNA fragments following separation in acrylamide gels (Stellwagen, 1983). The IGS₂ sequence was assembled to the previously sequenced IGS₁ (GenBank accession no. L25898). The total IGS was 4160 bp in length. The junctions between the spacers and coding regions were inferred from sequences reported for other fungi. The sequence includes a small portion of the flanking 25S and 18S coding regions, IGS₁ (Selosse *et al.*, 1996), 5S rDNA and IGS₂ (present study), and its G+C content is 41.8 mol %. The general organization of the *L. bicolor* IGS (Fig. 2) is typical of most fungal, plant and animal ribosomal intergenic spacers (Cordesse *et al.*, 1993). It consists of several repeated sequences and unique regions. Nine different regions with respect to sequence repetition and rRNA gene identity (I–IX; Fig. 2) can be distinguished in the sequence. Region I contains 288 bp of the 3' end of 25S rDNA (46.2% G+C). Region II corresponds to 505 bp of IGS₁ (37% G+C) and contains a series of subrepeats, T₂AC₃, called A. Region III (117 bp in length, 60.7% G+C) corresponds to the 5S rRNA coding sequence. IGS₂ covers regions IV–VIII (3215 bp, 41% G+C) and consists of two central repeated regions (V and VII) and unique regions flanking these repeated sequences. The central repetitive regions extend from nt 1450 to 1592 (subrepeats A, region V) and from nt 1718 to 2602 (subrepeats B, region VII) (Fig. 2). Region IX contains 35 bp of 18S rRNA sequence. The length of region VII

Fig. 1. Agarose gels showing the uncut and digested rDNA IGS₂ (a) or amplified region VII of IGS₂ (b) from *L. bicolor* strain S238N. (a) Lanes: 1, amplified IGS₂ of monokaryon HC3 (α -type) digested by *Rsa*I; 2, amplified IGS₂ of monokaryon HC4 (β -type) digested by *Rsa*I; 3, uncut amplified IGS₂ of monokaryon HC3 (α -type); 4, uncut amplified IGS₂ of monokaryon HC4 (β -type); 5, uncut amplified IGS₂ of the parental strain S238N. (b) Region VII, containing the B subrepeats, was amplified using primers I1 and I2 flanking this central region (see Fig. 2). Amplification and restriction products were separated on 1.5% (w/v) agarose gels. Size markers are shown on the left (ϕ X-174 DNA digested by *Hae*III, and the 3500 and 4250 bp markers are from λ DNA cut by *Eco*RI and *Hind*III).

Table 1. Mendelian segregation of rDNA IGS₂ types (α and β) among monokaryons derived from the *Laccaria bicolor* dikaryotic strains S238N, N203 and 81306

Strain	Size of IGS ₂ haplotypes (kb)		Segregation in the progeny*			
	α	β	α	β	$\alpha + \beta$ †	
S238N	3.9	3.4	47	39	5	(NS)
N203	3.8	3.5	12	17	0	(NS)
81306	3.5	3.3‡	19	37	0	

* (NS), χ^2 not significantly different from 1:1 segregation at the 0.05 level.

† Monokaryons recombinant for the rDNA.

‡ An additional 0.5 kb fragment was also amplified from this haplotype (see text).

Fig. 2. Diagrammatic representation of the organization of IGS regions of *L. bicolor* S238N (β -haplotype) with indication of subrepeats A, B and C. Regions I-IX have been defined based on the occurrence of rDNA coding sequences and subrepeats A, B and C. In region VII, B subrepeats identical in the α - and β -haplotypes are shown as black arrowheads, whereas the grey box containing black arrowheads indicates the B subrepeats of divergent sequence (see Fig. 3a). The diagonally shaded areas indicate regions containing A subrepeats.

for the β -haplotype correlates well with the approximately 960 bp length of the PCR fragment amplified with primers I1 and I2 flanking this region (Fig. 1b).

Fourteen copies of subrepeat A, TA₂C₃, occurred in region V of IGS₂, whereas three were detected in IGS₁ (region II) (Selosse *et al.*, 1996). It may be worth mentioning that this subrepeat was also found in IGS₂ of the ectomycorrhizal *Hebeloma cylindrosporum* (accession no. AJ006148; Guidot *et al.*, 1999) and the telomere hexamer array repeat region of the human chromosome (accession no. U32384). The second subrepeat family, named B, is present 100 bp downstream of the subrepeat A region. This subrepeat block consists of 13 copies of the element B which occur in tandem (Fig. 3a). All units of element B are 70–71 bp in length and consist of three degenerated subrepeats of 20, 19 and 32 bp, except B13 which is truncated. The consensus sequences of the B subrepeats are (identities underlined): Ba, CGGAATAGACTACAAAGTCA; Bb, ATAG-ATAGACTACAAACGC; and Bc, TATAAA-AGACTACAAAAGTCACTACAAAACAT. The Bc element may be a result of incomplete duplication of a Ba- or Bb-like element, as it contains two ACTACA₃ subrepeats. Alignment of IGS₁ and IGS₂ sequences revealed that the B subrepeat can also be interpreted as a repetition of an inverted sequence occurring in IGS₁ (Fig. 3b), from nt 657 to 725.

A third subrepeat, named C, occurred three times in IGS₂ (Fig. 3c). This 88 bp element (restricted to a 36 bp

element in repetition C1) is highly homologous to a repeat sequence of *Tricholoma matsutake* IGS₂ (the DR1 repeat; Hwang & Kim, 1998): 32 of the 34 bp of the central part of motif C are identical to one of the DR1 repeats (*TmDR1a*, Fig. 3c). This motif may correspond to a conserved regulatory sequence for the transcription of 25S rRNA.

With the addition of *L. bicolor*, full-length IGS sequences currently exist for two other members of the Tricholomataceae, *C. fusipes* (B. Marçais, INRA-Nancy, personal communication) and *T. matsutake* (Hwang & Kim, 1998). These sequences ranged in size from 2.2 to over 4.7 kb. The sequence variation prevented unambiguous alignment of these IGSs. As described above, sequence conservation exists only in the region *TmDR1a*.

Comparison of the two IGS₂ haplotypes of *L. bicolor* S238N

RFLP analyses using various enzymes (*AluI*, *HinfI*, *MboI*, *MpsI*, *SauIII*A) showed that the length differences between the amplified α - and β -type IGS₂ of *L. bicolor* S238N always mapped to a single fragment (e.g. the 0.9 or 1.4 kb fragment produced after digestion by *RsaI*; Fig. 1a, lanes 1 and 2). This suggests an almost identical sequence for both haplotypes, with a single insertion responsible for the observed length difference. The variable region, which was not digested by the enzymes tested, corresponds to the central repeat-rich region VII

Fig. 3. Subrepeats found in rDNA IGS₂ of *L. bicolor* S238N. (a) Alignment of the 13 copies of subrepeat B with consensus sequences and indication of its three degenerated subrepeats, Ba, Bb and Bc. (b) Alignment of the consensus sequence of subrepeat B with an inverted part of the IGS₁ sequence (RevIGS1, inverted sequence from nt 657 to 725 of the total IGS). (c) Alignment of the three copies of subrepeat C of *L. bicolor* with two IGS₂ subrepeats from *T. matsutake* (TmDR1a and TmDR1b, accession no. U62538; Hwang & Kim, 1998) and the corresponding consensus sequence. Nucleotide positions of subrepeats are given according to the GenBank *L. bicolor* IGS sequence (see also Fig. 2 for localization of subrepeats).

as shown by PCR amplification using the internal primers designed for sequencing the β -type. The PCR fragment, amplified using primers I1 and I2 flanking repeated region VII, exhibited the expected 0.5 kb length difference between the two haplotypes (Fig. 1b). This inner fragment of the α -type was sequenced and was found to have non-repetitive regions identical to regions VI and VIII of the β -type, flanking a series of 71 bp B subrepeats (region VII) (Fig. 3a). The subrepeats occurring at the 5' and 3' ends of region VII were identical in the two haplotypes, with subrepeats B1, B2 and B3 at the 5' end, and B8 and B9 at the 3' end. The sequence of the inner repeats in the α -haplotype (grey box in Fig. 2) differed from the β -haplotype by point mutations. The sequence of the most central repeats for the β -haplotype could not be exactly established since a sequencing primer cannot be designed due to the high number of repeated motifs. It was therefore not possible to determine the exact number of B subrepeats in α -type IGS₂ by sequence overlapping. However, taking the β -type IGS₂ as the reference, the addition of seven 71 bp B subrepeats could explain the observed 0.5 kb length difference between haplotypes (Figs 1b and 2).

DISCUSSION

The utility of the IGS region in population genetics of ectomycorrhizal fungi will depend on a thorough understanding of its inheritance and mechanisms by

which this region evolved. Length variation in IGS has been reported in many fungi, including the basidiomycetes *Coprinus cinereus* (Wu *et al.*, 1983), *Laccaria proxima* (Albee *et al.*, 1996), *Pleurotus cornucopiae* (Iraçabal & Labarère, 1994) and *H. cylindrosporium* (Gryta *et al.*, 1997), as well as other fungi such as *Cochliobolus heterostrophus* (Garber *et al.*, 1988) and *Fusarium oxysporum* (Appel & Gordon, 1996). The differences are attributed to insertions or deletions in the arrays of subrepeats within the IGS region. We found inter- and intraspecific length variations in IGS₁ (Henrion *et al.*, 1992; Selosse *et al.*, 1996) and IGS₂ (Table 1) of *L. bicolor* and this study revealed that length variations in IGS₂ are mainly due to different numbers of subrepeat B in region VII of this sequence.

This study has shown that the overall structural organization of the rDNA IGS of *L. bicolor* (Fig. 2) is similar to other fungi and plants. As in most basidiomycetes, the 5S rDNA is present in the IGS and is transcribed in the same orientation as the other rRNA genes. IGS₂ is longer than IGS₁ (Albee *et al.*, 1996; Hwang & Kim, 1998; Guidot *et al.*, 1999). IGS₁ and the central part of IGS₂ were composed of tandemly repeated sequence motifs (Figs 2 and 3). Subrepeat A found in IGS₁ (Selosse *et al.*, 1996) and in region V of IGS₂ (this study) was reported in IGS₂ of the ectomycorrhizal *H. cylindrosporium* (Guidot *et al.*, 1999). The presence of very similar, short (20, 19 and 32 bp, respectively) internally repeated sequences within the

71 bp B subrepeat suggests that this subrepeat evolved by amplification of segments of a shorter ancestral subrepeat, then subsequent amplification of the resulting larger units. The amplification of the segments corresponding to the subrepeats may have resulted from recombination, since crossing-over occurs during meiosis (Selosse *et al.*, 1996), or between sister chromatids during the vegetative life cycle (Ganley & Scott, 1998) of *L. bicolor*. Polymerase slippage during replication (Tautz *et al.*, 1986) may have contributed to their formation. The length differences in region VII of the two haplotypes of *L. bicolor* S238N are evidence of unequal crossing-over events, probably due to misalignment of the repeats. The concerted evolution of the repeats probably contributed to the homogenization of the rDNA repeats within each nucleus, leading to fixation of the newly formed IGS [see Ganley & Scott (1998) for a discussion].

The repeated regions found in IGS are probably involved in the regulation of the transcription of rRNA, as described for other eukaryotic IGS (Baldrige *et al.*, 1992). Subrepeat C, which strikingly resembles a sequence from IGS₂ of the agaric species *T. matsutake* (Fig. 3c), may be involved in the transcription of 25S rRNA, although it is lacking in IGS₂ of another Tricholomataceae, *C. fusipes* (B. Marçais, INRA-Nancy, personal communication). This suggests that functional domains within IGSs may be variable even in closely related species. The lack of homology with other sequences from DNA databases is due to the high IGS divergence among higher fungi, but also to the paucity of IGS₂ sequence data for homobasidiomycetes.

This study was undertaken to explain the length variations between IGSs found in genets of *L. bicolor* (Selosse *et al.*, 1998). The insertions and deletions of subrepeats A and B in IGS (Figs 1b and 3a) explain the high level of polymorphisms of this sequence in natural populations (Selosse *et al.*, 1998). Variation of the subrepeat number, as well as the frequent formation of heteroduplexes during amplification of IGS₁ (Selosse *et al.*, 1996), allowed us to use IGS as a mendelian marker for population genetics (Selosse *et al.*, 1998). Together with SCARs (Bonello *et al.*, 1998), IGS amplification will allow us to study fungal populations using DNA extracted from mycorrhizas. Variation in this sequence is mainly located in the central repeated region and specific primers, such as I1 and I2, could therefore be designed for amplifying the different alleles found in local populations (Guidot *et al.*, 1999). IGS therefore appears to be an excellent target sequence for studying intraspecific genetic relationships in ectomycorrhizal basidiomycetes (Selosse *et al.*, 1998; Gryta *et al.*, 1997).

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