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B. Iraçabal · J. Labarère

Restriction site and length polymorphism of the rDNA unit in the cultivated basidiomycete *Pleurotus cornucopiae*

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Abstract In the ribosomal DNA unit of *Pleurotus cornucopiae*, the rDNA coding regions are in the order 5′, 5S-18S-5.8S-25S, 3′, with the 5′ location of the 5S gene differing from its 3′ location found in other basidiomycetes. The most discriminating probe used to study the rDNA polymorphism consisted of a fragment that included the 5S, 18S and part of the 5.8S and 25S genes flanking three intergenic sequences. A high degree of rDNA polymorphism was observed in the seven *P. cornucopiae* dikaryons studied. For the first time within a basidiomycete species, the restrictions maps distinguished two types of rDNA units (I and II). In each rDNA type, length variations in the external intergenic sequence IGS 1 located between the 25S and 5S genes allowed characterization of two different rDNA units in type I and four rDNA units in type II. This suggested that the *P. cornucopiae* rDNA units were derived from two kinds of ancestors (type I and II) by insertion or deletion events (100 – 700 bp) in the IGS 1. In four dikaryotic strains, two rDNA units of the same type (I or II) differing only by the IGS 1 length, were found in a similar number of copies, and presented a meiotic segregation in homokaryotic progeny. In one progeny, some homokaryotic strains possessed two different rDNA units: one with a high copy number and another with a lower one, showing that two different rDNA units could coexist in a single nucleus.

Key words *Pleurotus cornucopiae* · rDNA unit
RFLP · Ribosomal polymorphism

Introduction

Polymorphism of ribosomal RNA (rDNA) has been widely investigated in eukaryotic organisms in order to determine molecular taxonomy in plants, such as wild-type and cultivated beets (Santoni and Bervillé 1992), or to determine phylogenic relationships in the animal kingdom (Field et al. 1988). In most eukaryotes, rDNA is generally organized in clusters of tandemly repeated units (for review, Long and Dawid 1980) composed of conserved rRNA coding regions in the order 5′, 18S-5.8S-25S, 3′ separated by variable intergenic sequences (IGS), and cytologically identified as the nucleolus organizer.

In higher fungi, the principle variations observed in the rDNA unit relate to (1) the size, which varies from 7.7 to 24 kb (Van Heerikhuizen et al. 1985), (2) the copy number per haploid genome, which ranges from 60 to 220 (Cassidy et al. 1984) and (3) the location of the 5S RNA gene, which is located outside the rDNA unit in *Yarrowia lipolytica* (Van Heerikhuizen et al. 1985) and *Cochliobus heterostrophus* (Garber et al. 1988), or in the rDNA unit, generally in the external IGS, such as in the ascomycete *Saccharomyces cerevisiae* (Bell et al. 1977) and in most of the basidiomycetes studied to date: *Coprinus* sp., *Agaricus bisporus* (Pukkila and Cassidy 1987), *Schizophyllum commune* (Buckner et al. 1988), and *Thanatephorus praticola* (Vilgalys and Gonzalez 1990).

In cultivated basidiomycetes, restriction fragment length polymorphism (RFLP) analysis of rDNA has been performed only in the *Agaricus* genus (Hintz et al. 1989): no variability was detected in *A. bisporus* while in two neighbouring species (*A. bitorquis* and *A. campestris*), interstrain length polymorphism was shown in the external IGS. This IGS is known to have evolved more rapidly than the ribosomal genes and to be highly variable among closely related fungal taxa (Rogers et al. 1989) and at the intervarietal level, in both *S. commune* (Specht et al. 1984) and *C. cinereus* (Wu et al. 1983). Since among basidiomycetes, the cultivated mushroom *Pleurotus cornucopiae* is characterized by a high genetic variability (Iraçabal et al. 1991; Iraçabal and Labarère 1993) we decided it would be interesting to determine how this genetic variability could affect the rDNA unit and whether the ribosomal polymorphism could be considered to be a molecular marker suitable for varietal characterization.

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B. Iraçabal · J. Labarère (✉)
Laboratoire de Génétique Moléculaire et Amélioration
des Champignons Cultivés,
Université de Bordeaux II – INRA, CRA. de Bordeaux, B.P. 81,
33883 Villenave d'Ornon Cedex, France

Table 1 Origin, RFLP patterns and types of rDNA units in the *P. cornucopiae* strains studied (*nd*, not determined)

Strains[a]	Origin	Mating types	RFLP patterns	rDNA unit[b]
Dikaryotic				
SM 920501	Commercial strain	*A1 A2 B1 B2*	A	rDNA Ia and Ib
SM 920504	Commercial strain	*A1 A2 B1 B2*	A	rDNA Ia and Ib
SM 67	Wild strain (Créon, Gironde)	*A5 A6 B5 B6*	C	rDNA IIb
SM 69	Wild strain (Montereau, Yonne)	*A6 A8 B2 B7*	D	rDNA IIc and IId
SM 801002	Wild strain (Vergt, Dordogne)	*A3 A4 B3 B4*	A	rDNA Ia and Ib
SM 66	Wild strain (Madirac, Gironde)	*A3 A9 B8 B9*	B	rDNA IIa
SM 800601	Wild strain (Macreu, Gironde)	nd	C	rDNA IIb
Homokaryotic				
HA1	SM 920501 progeny	*A1 B1*	Ia	rDNA Ia
HA2	SM 920501 progeny	*A1 B2*	Ib	rDNA Ib
HA8	SM 920501 progeny	*A2 B2*	Ib	rDNA Ib
HA11	SM 920501 progeny	*A2 B1*	Ib	rDNA Ib
HA13	SM 920501 progeny	*A1 B2*	Ia	rDNA Ia
HA14	SM 920501 progeny	*A2 B1*	Ia	rDNA Ia
HA15	SM 920501 progeny	*A2 B2*	Ia	rDNA Ia
HA18	SM 920501 progeny	*A1 B1*	Ib	rDNA Ib
HE2	SM 69 progeny	*A8 B2*	IId	rDNA IId*
HE3	SM 69 progeny	*A8 B2*	IIc	rDNA IIc
HE4	SM 69 progeny	*A6 B7*	IId	rDNA IId*
HE5	SM 69 progeny	*A8 B7*	IId	rDNA IId*
HE8	SM 69 progeny	*A8 B2*	IId	rDNA IId*
HE11	SM 69 progeny	*A6 B2*	IId	rDNA IId*
HE13	SM 69 progeny	*A6 B7*	IIc	rDNA IIc
HE15	SM 69 progeny	*A6 B7*	IIc	rDNA IIc

[a] The code number corresponds to the nomenclature of the collection of the Laboratoire de Génétique Moléculaire et Amélioration des Champignons Cultivés (GMACC)
[b] In the SM 69 progeny, homokaryons having the rDNA IId unit (*) also possess a lower number of copies of a unit of a similar size to the rDNA IIc unit

In order to study ribosomal polymorphism, the rDNA unit of *P. cornucopiae* carried on two different DNA fragments was used as a RFLP probe with total DNA digested by eight endonucleases (*Bgl*I, *Eco*RI, *Cla*I, *Sma*I, *Nco*I, *Pvu*I, *Sal*I and *Sst*I). Our interstrain polymorphism study of the rDNA unit, performed in seven commercial and wild dikaryotic strains, allowed the discrimination and characterization of each strain according to its type of rDNA units. For the study of the meiotic segregation and inheritance of rDNA polymorphism in homokaryotic progenies, the probe used was the most discriminating fragment of the rDNA unit containing the 5S, 18S and part of the 5.8S and 25S genes flanking three intergenic sequences (IGS 1, IGS 2 and IGS 3).

Materials and methods

Strains and culture conditions

The tetrapolar basidiomycete *P. cornucopiae* belongs to class Agaricales. The seven dikaryotic strains used (Table 1) consisted of two commercial strains provided by a spawn producer and five wild strains from different geographical origins (Iraçabal and Labarère 1993). Homokaryotic strains were obtained from the germination of single basidiospores collected from mature basidiocarps (Labarère et al. 1989). For nucleic acid extraction, vegetative mycelia were cultivated on liquid CYM medium in Roux flasks (Raper and Hoffman 1974).

Nucleic Acids extraction

Total RNA was isolated from *P. cornucopiae* vegetative mycelium using the hot phenol procedure (De Vries et al. 1980). To separate the 18S and 25S RNA, total RNA was electrophoresed in a 1.4% agarose-formaldehyde gel according to Maniatis et al. (1982). For the separation of the 5.8S and 5S RNA, total RNAs were fractionated in a 10% polyacrylamide gel containing 7 *M* urea and run in a 89 m*M* Tris pH 8.3, 89 m*M* boric acid and 2 mM EDTA buffer.

Total DNA was extracted according the *N*-cethyl-*NNN*-trimethyl ammonium method adapted to basidiomycetes (Noël and Labarère 1989).

Plasmid DNA isolation from transformants was obtained by the alkaline lysis method (Maniatis et al. 1982). Endonuclease digestion and the ligation and electrophoresis of DNA in the agarose gel were performed according to Maniatis et al. (1982), or following the manufacturer's recommendations.

Southern and Northern blots

After electrophoresis DNA or RNA was transferred from the agarose gel to a Hybond N+ nylon membrane (Amersham Life Science) as specified by the manufacturer. RNA separated by polyacrylamide gel electrophoresis was electrotransferred to Hybond N+ membranes at an electrical intensity of 2.5 mA/cm^2 for 1 h. The DNA and RNA blots were treated for prehybridization and hybridization according to the manufacturer's recommendations (Amersham Life Science).

DNA and RNA labelling and hybrizidations

Plasmid DNA and restriction fragments excised from Sea Plaque agarose (FMC) used as probes were radiolabelled with 925 kBq α-[^{32}P]-dCTP (111 TBq/mmol Amersham) using the ramdom primer DNA labelling kit (GIBCO-BRL Life Technologies). In order to

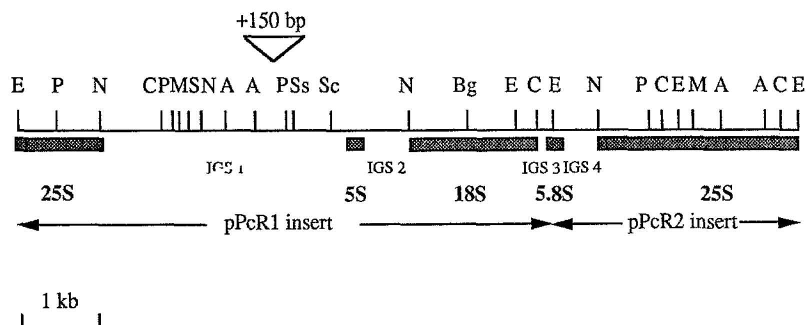

Fig. 1 Restriction and gene map of the rDNA unit of *P. cornucopiae* strain SM 920501. The limits of the pPcR1 and pPcR2 inserts used as probes are indicated by *arrows*. Restriction sites: *A AvaI*, *Bg BglI*, *C ClaI*, *E Eco*RI, *M SmaI*, *N NcoI*, *P PvuI*, *S SalI*, *Sc ScaI Ss SstI*

make a probe with purified 18S and 25S RNA, the ethidium-bromide stained 18S and 25S RNA bands were recovered by electroelution after a 1.4% agarose-formaldehyde gel analysis and then purified by phenol extraction and ethanol precipitation. Ribosomal RNA was specifically labelled by the incorparation of 925 kBq α–[^{32}P]-dCTP (111 TBq/mmol Amersham) into the first strand synthesis of cDNA with the reverse transcriptase (GIBCO-BRL Life Technologies). All probes had a specific radioactivity that was higher than 10^9 cpm/μg DNA. Hybridizations for DNA and RNA were carried out in standard buffer at 65 °C, and the nylon membranes were successively washed as recommanded by the manufacturer (Amersham Life Science). The sizes of the hybridizing fragments detected for each endonuclease digestion were determined by comparing their migration of a molecular weight marker (DNA fragments of the Appligene Raoul marker).

Molecular cloning of the *P. cornucopiae* rDNA unit

The cloning of the *P. cornucopiae* rDNA unit was performed as following. The *Agrocybe aegerita* 18S cDNA (Noël et al. 1991) used as probe on the Southern blot of *Eco*RI total DNA of the dikaryotic strain SM 920501 of *P. cornucopiae* hybridized with a *Eco*RI DNA fragment of 6.5 ± 0.5-kb. The 6.5-kb *Eco*RI DNA fragments were excised from a 0.8% (w/v) low melting point agarose gel (Sea Plaque, FMC) and gel-ligated to the dephosphorylated *Eco*RI pBR322 vector. The ligation mixture was used to transform *Escherichia coli* JM 83 cells according to Hanahan (1985). The screening of recombinant plasmids by Southern hybridizations with *A. aegerita* 18S cDNA allowed selection of a 6800-bp insert composed of two *P. cornucopiae Eco*RI fragments of 6.5-kb and 0.3-kb (pPcR1 insert). A Northern analysis of *P. cornucopiae* total RNA using the pPcR1 insert as the probe revealed a strong hybridization signal with 18S RNA and a faint one with 25S RNA, indicating that the pPcR1 insert overlapped the 18S gene and a small part of the 25S gene. For the cloning of the remaining *Eco*RI DNA fragments constituting the whole rDNA unit of *P. cornucopiae*, denatured *Hpa*II-generated DNA fragments from the pPcR1 insert were used as primers to synthesize radiolabelled cDNAs specific to 18S and 25S RNA. A Southern analysis of *Eco*RI-digested *P. cornucopiae* DNA using the 18S and 25S cDNAs as probes revealed four hybridizing *Eco*RI fragments: the 6.5-kb and the 0.3-kb fragments already cloned in pPcR1 and two additional *Eco*RI fragments of 1.7- and 1.5-kb. The 1.7- and 1.5-kb fragments, together with a 3.2-kb fragment composed of these two adjacent *Eco*RI fragments (pPcR2 insert), were isolated by the screening of an *Eco*RI *P. cornucopiae* gene library constructed in the pBR322 vector with radiolabelled 18S and 25S cDNAs.

A Southern hybridization of the pPcR1 and pPcR2 inserts with either 18S or the 25S cDNA enabled us to locate the 18S gene within the 1.7-kb *Nco*I-ClaI fragment and the 25S gene within the 3.7-kb *Nco*I-*Nco*I fragment. To locate the 5S and 5.8S genes, the 1-kb *Nco*I-*Sca*I fragment between the 25S and 18S genes and the 0.6-kb *Cla*I-*Nco*I fragment between the 18S and 25S genes were individually used to probe a Northern blot of total RNA (separated in a polyacrylamide gel). This allowed the location of the 5S gene between intergenic sequences called IGS 1 and IGS 2 and that of the 5.8S gene between IGS 3 and IGS 4 (Fig. 1).

Results

The nuclear ribosomal DNA of *P. cornucopiae* showed a tandem head to tail organization of 10-kb rDNA units in which the nuclear ribosomal genes were arranged in the order 5′, 5S-18S-5.8S-25S, 3′, and whose the restriction map was determined with the endonucleases *Ava*I, *Bgl*I, *Cla*I, *Eco*RI, *Sma*I, *Nco*I, *Pvu*I, *Sal*I, *Sca*I and *Sst*I (Fig. 1). The complete rDNA unit was carried by the pPcR1 and pPcR2 inserts. The pPcR1 insert consisted of the 5S, 18S and part of the 5.8S and 25S genes flanking three intergenic sequences (IGS 1, IGS2 and IGS 3), while the pPcR2 insert contained the remaining parts of the 5.8S and 25S genes separated by a short intergenic sequence (IGS 4). These two inserts were used as probes for RFLP analysis of dikaryotic strains. To study rDNA unit polymorphism in homokaryotic progenies, we used the most discriminating fragment (pPcR1 insert) carrying the largest intergenic sequence (IGS 1) as probe.

Polymorphism of the rDNA units in dikaryotic strains

To estimate rDNA polymorphism, total DNA of seven *P. cornucopiae* dikaryotic strains (listed on Table 1) was digested by the eight endonucleases that had been used for rDNA unit mapping: *Bgl*I, *Eco*RI, *Cla*I, *Sma*I, *Nco*I, *Pvu*I, *Sal*I and *Sst*I. Southern blots were probed with the two radiolabelled plasmids pPcR1 and pPcR2. In *Bgl*I, *Sal*I and *Sst*I RFLP analysis, a single hybridizing 10-kb fragment was detected, except for strain SM 69, which had an additional 10.7-kb fragment (Fig. 2). RFLP analysis of the five other digestions revealed four specific patterns (Fig. 2, Table 1). Pattern A characterized the two commercial strains (SM 920201 and SM 920504) and the wild strain SM 801002, pattern B, strain SM 66; pattern C, strains SM 800601 and SM 67; and pattern D, strain SM 69. When total DNA from strains with pattern A was digested by *Pvu*I or *Nco*I and probed with the pPcR1 and pPcR2 plasmids, the sum of the sizes of hybridizing fragments of a similar intensity was superior to the unit length (10-kb), suggesting that two different rDNA units were present within this dikaryotic pattern that had the same number of copies. In the same way, the eight RFLP analyses clearly showed the

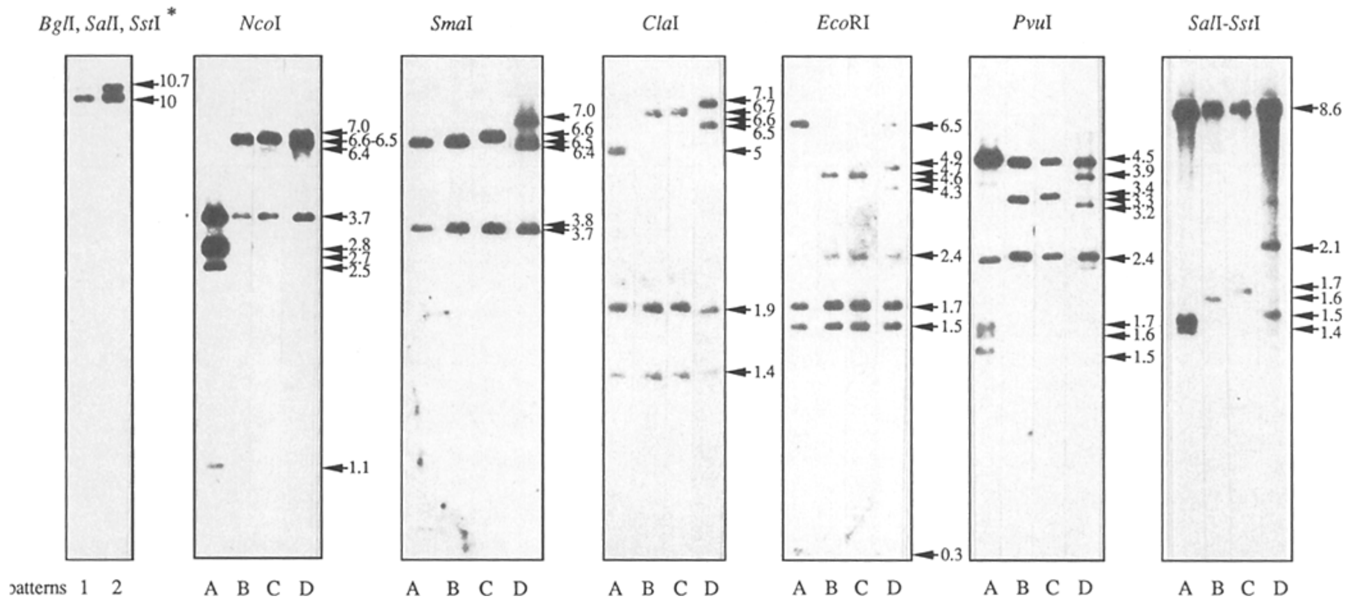

Fig. 2 Autoradiograms of the RFLP patterns (*A*, *B*, *C* and *D*) revealed in seven *P. cornucopiae* dikaryotic strains. Total DNA digested by *BglI*, *SalI*, *SstI*, *EcoRI*, *NcoI*, *SmaI*, *ClaI*, *PvuI* and *SalI-SstI* was hybridized with the radiolabelled plasmids pPcR1a and pPcR2. * In the case of the *BglI*, *SalI*, *SstI* digestions, only two patterns (*1*) and (*2*) were evidenced. The sizes of the restriction fragments are indicated in kb

presence of two different rDNA units in the SM 69 strain (pattern D).

The four RFLP patterns differed both in restriction site and length polymorphism. The results discriminated two different types of rDNA unit restriction maps: (1) the type I detected in pattern A and (2) the type II detected in patterns B, C and D. Type I was defined by the presence of a *EcoRI* site in the 5.8S gene, a *NcoI* site flanking the 18S gene, and four sites (one *ClaI*, two *PvuI* and one *NcoI*) in the IGS 1. Type II was defined by a *EcoRI* site in the IGS 2 (Fig. 3). Length differences in the external IGS 1 were observed for each of both types of rDNA units. Two different rDNA units of type I were visible in pattern A (called rDNA Ia and rDNA Ib); these differed by a 150-bp sequence in the IGS 1 between the *SstI* site and the proximate *AvaI* (Fig. 1). In type II (patterns B, C and D), four different IGS 1 were evidenced (6.5 kb, 6.6 kb, 6.4 kb and 7 kb), which discriminated four rDNA units (rDNA IIa to IId). Considering both the restriction map and the length variations overall, six different rDNA units were discriminated in dikaryotic strains (Table 2). In order to verify whether the length variations were always located in the 5′ region of all of the rDNA units (IGS 1, Fig. 1), RFLP analysis of a double digestion (*SalI-SstI*) was conducted in the seven strains. Each strain had a 8.6-kb fragment that corresponded to the conserved regions and polymorphic fragments of 1.4 kb for rDNA Ia, 1.5 kb for rDNA IIc, 1.55 kb for rDNA Ib, 1.6 kb for rDNA IIa, 1.7 kb for rDNA IIb and 2.1 kb for rDNA IId (Fig. 2; Table 2). The results from

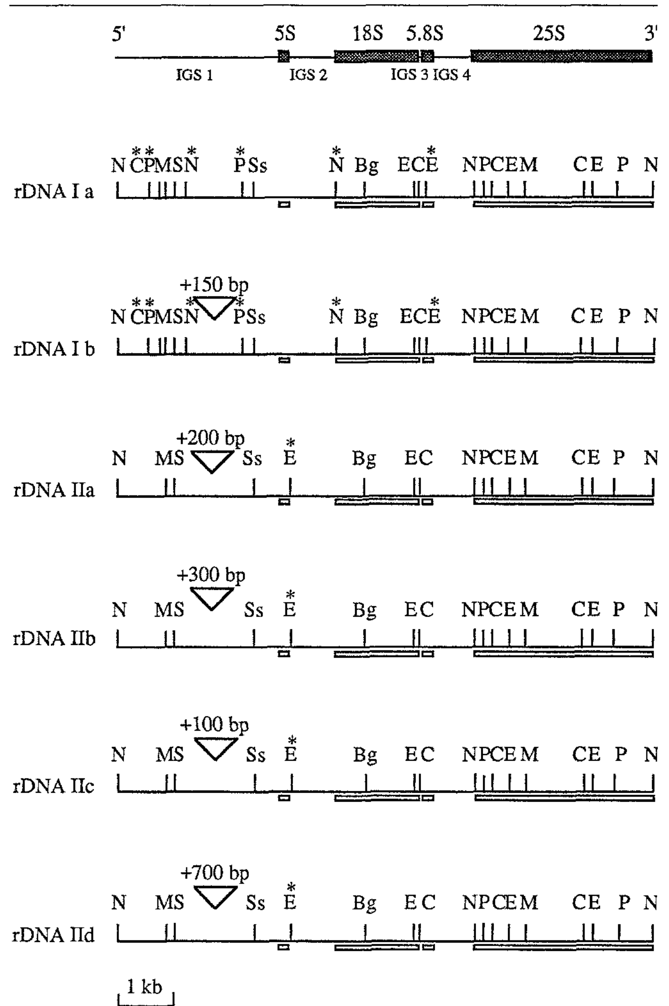

Fig. 3 Restriction site maps of the six rDNA units evidenced in *P. cornucopiae* strains. All maps were established after RFLP analysis of total DNA, digestion by single restriction (*Bg BglI*, *C ClaI*, *E EcoRI*, *M SmaI*, *N NcoI*, *P PvuI*, *S SalI*, *Ss SstI*) and probing by pPcR1 and pPcR2. The sites noted by an asterisk (*) correspond to the sites that differ in the six rDNA units

Table 2 Sizes (in kb) of restriction fragments corresponding to the six *P. cornucopiae* rDNA units, revealed by hybridizations of total digested DNA with the pPcR1 and pPcR2 inserts

rDNA units	rDNA length[a]	Restriction fragments revealed in total DNA digested with:					
		*Nco*I	*Sma*I	*Cla*I	*Eco*RI	*Pvu*I	*Sal*I-*Sst*I
rDNA Ia	10	3.7+2.7+2.5+1.1	3.8+6.2	1.9+4.9+1.4	6.5+1.7+1.5+0.3	4.5+1.6+1.5+2.4	8.6+1.4
rDNA Ib	10.1	3.7+2.8+2.5+1.1	3.8+6.3	1.9+5.0+1.4	6.6+1.7+1.5+0.3	4.5+1.7+1.5+2.4	8.6+1.55
rDNA IIa	10.2	3.7+6.5	3.7+6.5	6.6+1.9+1.4	2.4+4.6+1.7+1.5	4.5+3.3+2.4	8.6+1.6
rDNA IIb	10.3	3.7+6.6	3.7+6.6	6.7+1.9+1.4	2.4+4.7+1.7+1.5	4.5+3.4+2.4	8.6+1.7
rDNA IIc	10.1	3.7+6.4	3.7+6.4	6.5+1.9+1.4	2.4+4.3+1.7+1.5	4.5+3.2+2.4	8.6+1.5
rDNA IId	10.7	3.7+7.0	3.7+7.0	7.1+1.9+1.4	2.4+4.9+1.7+1.5	4.5+3.9+2.4	8.6+2.1

[a] The unit length was determined by summing the restriction fragments hybridizing with the probe composed of the pPcR1 and pPcR2 inserts, whereas hybridizations of total DNA digested by *Bgl*I, *Sst*I or *Sal*I (single restriction site within the cloned rDNA units) revealed a 10-kb (approximate) rDNA unit (Ia, Ib, IIa, IIb and IIc) and a 10.7-kb (approximate) rDNA unit (IId). The sizes are indicated in kb

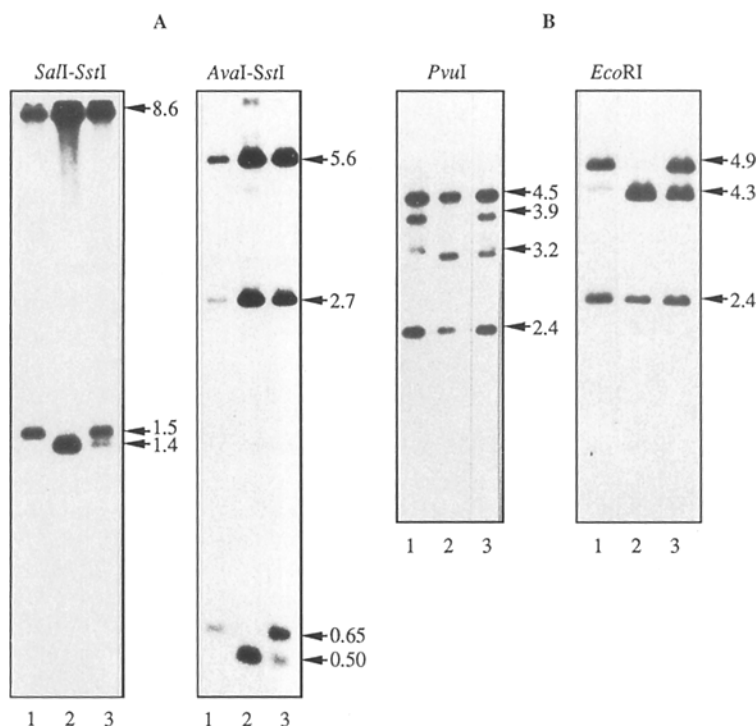

Fig. 4 RFLP autoradiograms of total DNA of homokaryotic progeny and parental *P. cornucopiae* strains, probed with the plasmid pPcR1. **A** Total DNA of homokaryons HA11 (*1*) and HA15 (*2*) and of the parental dikaryotic strain SM 920501 (*3*) digested by *Sal*I-*Sst*I and *Ava*I-*Sst*I. **B** Total DNA of homokaryons HE2 (*1*) and HE3 (*2*) and of the parental dikaryotic strain SM 69 (*3*) digested by *Pvu*I and *Eco*RI. The sizes of the restriction fragments are indicated in kb

this double digestion verified the location of the variable sequence within a *Sal*I-*Sst*I fragment in all rDNA units and determined the size of the additional DNA sequences relative to that of rDNA Ia (Fig. 3): 100 bp in rDNA IIc, 150 bp in rDNA Ib, 200 bp in rDNA IIa, 300 bp in rDNA IIb and 700 bp in rDNA IId.

Polymorphism of the rDNA units in two homokaryotic progenies

Polymorphism analysis of the rDNA units indicated the presence of two different rDNA units in each dikaryotic genome of four strains: rDNA Ia and rDNA Ib for SM 920501, SM 920504 and SM 801002, and rDNA IIc and rDNA IId units for SM 69. In order to determine the inheritance of such polymorphism, the rDNA patterns of eight homokaryotic strains in the progenies of the commercial strain SM 920501 and of the wild strain SM 69 were compared with the parental patterns. The DNA of homokaryons from the SM 920501 progeny digested with *Ava*I-*Sst*I and *Sal*I-*Sst*I were probed with the pPcR1 plasmid. For each digestion, two types of homokaryotic patterns were obtained: one composed of the parental rDNA Ia (for HA1, HA13, HA14 and HA15) and one of the parental rDNA Ib (for HA2, HA8, HA11 and HA18) (Table 1, Fig. 4). In the case of the SM 69 progeny, the DNA of homokaryons were digested with *Pvu*I or *Eco*RI and probed by the pPcR1 plasmid. For each digestion, homokaryotic patterns of HE3, HE13 and HE15 (Table 1, Fig. 4) were composed of the rDNA IIc unit. The HE2, HE4, HE5, HE8 and HE11 patterns were characterized by the large parental rDNA IId unit and by an additional low intensity hybridizing unit whose length was similar to that of rDNA IIc.

Discussion

The high degree of polymorphism of the *P. cornucopiae* rDNA unit is a result of variations in both restriction site and length that affect the intergenic sequences IGS 1 and IGS 2 and the 5.8S gene region. Two types of rDNA restriction maps were evident in the seven strains studied: type I in three strains and type II in four strains. Length variations within the *Sal*I-*Sst*I fragment of IGS 1 allowed the identification of six different rDNA units (two in type I and four in type II) that would be derived from type I or type II ancestors by insertion or deletion events in the external IGS 1. These results, corroborating the extensive genetic variability described in the rDNA units of heterothallic basidiomycetes (Rogers et al. 1989), suggest that the *P. cornucopiae* type I and type II rDNA units belong to two different subpopulations and that they are merely temporarily isolated parts of a continuum, having a common-type ancestror.

The repartition of types I and II in *P. cornucopiae* seems to be geographically dependant: type II is found in all strains originating from Gironde and Yonne, whereas type I is characteristic of the wild strain originating from Dordogne and the two commercial strains. These commercial strains, which have an identical rDNA pattern, also have the same mating-type alleles at both the *A* and *B* incompatibility loci; this supports the idea that they correspond to two isolates of the same strain (Iraçabal and Labarère 1993).

The *P. cornucopiae* rDNA units show numerous variations of restriction sites and a higher variability in the length of insertion sequences. With respect to other basidiomycetes, no polymorphism was observed in *A. bisporus* (Hintz et al. 1989), and the sole variations detected until now in basidiomycetes have been length variations (0.2 – 0.4 kb), which could induce the loss or the addition of sites, such as in *A. campestris* strains (Hintz et al. 1989), in *S. commune* homokaryons (Specht et al. 1984) and in *C. cinereus* homokaryons (Wu et al. 1983). The length variations of the *P. cornucopiae* rDNA unit resulted from the deletions or insertions of sequences located in IGS 1 between the 25S and 18S genes. Such external IGS length polymorphism has already been found in various unrelated organisms as humans (Ranzani et al. 1984) or the plant *Vicia faba* (Rogers et al. 1986) and results generally from a variable number of subrepetitive sequences. It will be interesting to determine by further sequence analysis if such a subrepetitive structure exists in *P. cornucopiae.* It is of interest to note that the *P. cornucopiae* external IGS 1 is adjacent to the 5S RNA gene located in the 5′ region; in all other basidiomycetes studied this gene occupies the 3′ position (Wu et al. 1983; Buchner et al. 1988; Vilgalys and Gonzalez 1990). It is possible that the evolutionary outcome of the 5S genes in filamentous fungi is dependent on flanking regions being potentially subjected to insertion or deletion events such as those identified in *P. cornucopiae* rDNA units.

Among the seven *P. cornucopiae* dikaryons studied, four possess two different rDNA units with a similar copy number in their genome: the two commercial strains and the Dordogne wild strain (rDNA Ia and Ib), and the wild strain SM 69 (rDNA IIc and IId). The coexistence of two different rDNA units in a dikaryotic genome is shown for the first time in a basidiomycete species. It must be noted that in each dikaryon the two rDNA units are always from the same rDNA unit ancestor (type I or II) and differ only by a deletion or insertion sequence in the IGS 1. This supports the idea that such strains arise from crosses between homokaryons belonging to the same progeny, particularly interesting in the case of wild strains, because this would show that there are few interstrain crosses in nature and that most wild dikaryons are derived from anastomosis between homokaryons originating from the same progeny.

Analysis of the meiotic segregation of the rDNA units showed that each homokaryon issuing from SM 920501 possesses only one of the two rDNA parental units, which confirms that each kind of rDNA unit is specific to each nuclear type. However, in SM 69 progeny all of the homokaryons having the large rDNA IId unit also possessed a low copy number of a second rDNA unit that is similar in size to the short parental rDNA IIc unit. As only two kinds of rDNA units were found in parental strain SM 69, it was supposed that this shorter unit is of the IIc type and that a single nucleus of the parental dikaryotic strain can possess two different rDNA units. Moreover, the rDNA IId unit was characterized as having the larger insertion sequence (700 bp); the emergence of a nuclear type possessing two rDNA units in the SM 69 progeny can be explained by deletions occurring within the rDNA IId IGS 1 yielding the shorter rDNA unit.

In conclusion, the *P. cornucopiae* rDNA unit shows a high heterogeneity that is characterized by a restriction site polymorphism of both coding and non-coding regions and by a length polymorphism. The six rDNA units evidenced were considered to be derived from two different ancestors by the deletions or insertions of DNA sequences always located in the 5′ region of the IGS 1, near the 5S RNA gene. It would be interesting to identify the nature of the deletion/insertion sequences (subrepetitive elements, transposon like structures, etc...) responsible for the length polymorphism and to determine its role, notably in transcription or regulation of the rDNA genes. Moreover, the *P. cornucopiae* rDNA unit enabled varietal characterization of dikaryotic and homokaryotic strains and may be used as a suitable genetic marker in view of cultivar protection and genetic improvement in cultivated basidiomycetes.

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