Characterization and comparison of mitochondrial DNAs and rRNAs from *Penicillium urticae* and *P. chrysogenum*

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Mitochondrial DNA (mt DNA) from a patulin producer, *Penicillium urticae* (synonym *P. griseofulvum*), was 27.8 kb $\pm$ 0.6 kb in size by electron microscopy and 27.2 kb by agarose gel electrophoresis. Restriction endonuclease maps for nine restriction enzymes were constructed, and eleven fragments which covered the total range of the mt DNA were cloned into the *Escherichia coli* plasmid vector pUC19. Southern analysis of the native genomes of *P. urticae* and *P. chrysogenum* with six of the cloned fragments as probes indicated similar genome arrangements as well as similar restriction maps. Both the large and small rRNA genes of *P. urticae* and *P. chrysogenum* were located on these restriction maps using Southern hybridization, and the result also supported the similar arrangement. Agarose/formaldehyde gel electrophoresis indicated that the small rRNA was 1.5 kb in size in both species; but, surprisingly, the large rRNA was 4.2 kb in size for *P. urticae* and 3.5 kb for *P. chrysogenum*. These sizes were, respectively, 1.1 kb and 0.4 kb larger than those from the very closely related *Aspergillus nidulans*.

Introduction

Penicillium chrysogenum and *P. urticae* (synonym *P. griseofulvum*) are industrially important filamentous fungi which produce penicillin and griseofulvin, respectively (Mantle, 1987). Moreover, *P. urticae* produces a mycotoxin, patulin, whose biosynthetic pathway is a model for 'polyketide' secondary metabolites (Sekiguchi & Gaucher, 1978; Sekiguchi *et al.*, 1983; Zamir, 1980). In spite of the importance of the genus *Penicillium*, there has been little research on its mitochondrial DNA (mt DNA): restriction maps and autonomously replicating segments (ARSs) of mt DNAs have been reported only for *P. chrysogenum* NRRL 1951 and BC2 (Smith *et al.*, 1984; Saunders *et al.*, 1984; Picknett *et al.*, 1987; Stahl *et al.*, 1987; Saunders & Holt, 1987). These results suggested that *P. chrysogenum* mt DNA varies widely in size and genome order.

In the filamentous fungi, mt ribosomal RNAs (mt rRNAs) of *Neurospora crassa* (Green *et al.*, 1981; Mahler, 1983) and *Aspergillus nidulans* (Köchel & Kuntzel, 1981, 1982; Dyson *et al.*, 1989) have been extensively investigated, and recently the autocatalytic

splicing mechanism of the large rRNA has been focused on (Garriga & Lambowitz, 1984; Lambowitz, 1989). However, there are no reports about even the basic properties of rRNA from the genus *Penicillium*.

In this study, we characterized mt DNAs and rRNAs from *P. urticae* and *P. chrysogenum* and compared them by restriction mapping and Southern hybridization, and by localization of ARSs and rRNA genes.

Methods

Organisms and culture conditions. A patulin producer, *Penicillium urticae* NRRL 2159A, and two penicillin producers, *Penicillium chrysogenum* NRRL 1951 and IAM7119, were used. For slant cultures, glucose/yeast extract agar medium (Sekiguchi & Gaucher, 1977) was used. For submerged cultures, MGG medium [50 g glycerol, 5 g yeast extract (Difco), 0.3 g glucose, 2 g KH₂PO₄, 1 g MgSO₄·7H₂O and 1 g (NH₄)₂SO₄ per litre (pH 6.5)] was used. A suspension of *P. urticae* conidia was inoculated into a 3 litre Sakaguchi flask containing 1 litre of MGG medium, followed by incubation at 28 °C with shaking at 125 r.p.m. (incubator type M100^D; Taiyo). *Escherichia coli* JM109 *recA1* Δ (*lac-proAB*) *endA1* *gyrA96* *thi-1* *hsdR17* *supE44* *relA1* λ^- [*F'*:*traD36 proAB lacZ* ZAM15] (Yanisch-Perron *et al.*, 1985) obtained from Takara was used as the recipient in cloning experiments. *Saccharomyces cerevisiae* SHY3 *a ste-VC9 ura3-52 trp1-289 leu2-3 leu2-112 his3- Δ 1 ade1-101 can1-100* (Botstein *et al.*, 1979) was used as the recipient in yeast transformation. LB medium [5 g yeast extract, 10 g polypeptone and 10 g NaCl per litre (pH 7.5)] for *E. coli* culture, and

Abbreviations: ARS, autonomously replicating segment; mt, mitochondrial.

YPD [10 g yeast extract, 20 g polypeptone and 20 g glucose per litre (pH 6.5)] and SD media [6.7 g yeast nitrogen base w/o amino acids (Difco) and 20 g glucose per litre (pH 5.3)] for yeast culture were used. Plasmids pUC19 (Ap^r Lac⁺) (Yanisch-Perron *et al.*, 1985) and YIp5 (Ap^r Tet^r URA3) (Struhl *et al.*, 1979) were used as vectors.

Isolation of mt DNA. This was done basically as described by Stepień *et al.* (1978), except that the mycelia were disrupted in liquid nitrogen with an Ace homogenizer (Nissei). mt DNA was purified by DAPI (4',6-diamidino-2-phenylindole, Sigma)/CsCl or Hoechst 33258 (Sigma)/CsCl density-gradient ultracentrifugation as described by Cummings *et al.* (1979).

Isolation of total rRNAs and mt rRNAs. mt rRNAs were prepared as described by Wright *et al.* (1982). Total rRNA was isolated from homogenized mycelia. One-tenth volume of 6 M-urea solution containing 3 M-LiCl was added to the homogenized solution and incubated at 0 °C overnight. After centrifugation at 16000 g for 20 min at 0 °C, the pellet was resuspended in 10 vols 0.5% (w/v) SDS/10 mM-Tris/HCl (pH 7.6) and extracted with chloroform/isoamyl alcohol (24:1, v/v). The RNA was precipitated with ethanol and kept at -80 °C in 70% (v/v) ethanol.

Electron microscopy. Samples were prepared by a slight modification of the spontaneous-adsorption method (Lang & Mitani, 1970). Plasmid pBR325 was used as a size reference.

Restriction enzyme analysis. Restriction endonucleases were purchased from Nippon Gene Co. and used according to the recommendations of the supplier.

Transformation of *E. coli* and *S. cerevisiae*. Transformation of *E. coli* JM109 and *S. cerevisiae* SHY3 was done as described by Silhavy *et al.* (1984) and Ito *et al.* (1983), respectively.

Cloning of mt DNA. Standard cloning procedures were done as described by Maniatis *et al.* (1982). Eleven clones which covered the total range of mt DNA of *P. urticae* were constructed in *E. coli* JM109 as follows. Total mt DNA from *P. urticae* was digested with *Eco*RI or *Hind*III, and cloned into the corresponding site of pUC19. The resultant pME4 and pME5 contained the 3.0 kb and 2.0 kb *Eco*RI fragments of *P. urticae* mt DNA, respectively, and pMH2 and pMH7 contained the 4.7 kb and 1.4 kb *Hind*III fragments, respectively. For construction of pMH4, pMH5 and pMH6, the 2.45 kb and 1.9 kb *Hind*III fragments were separated by agarose gel electrophoresis and cloned into the *Hind*III site of pUC19. A *Nco*I site was found in the 1.9 kb *Hind*III fragment of pMH6, but not in that of pMH5. For pME22, mt DNA digested with *Eco*RI and *Pst*I was cloned into the *Eco*RI and *Pst*I sites of pUC19. pME22 contained the 1.9 kb *Eco*RI-*Pst*I insert. For pMB1, the 5.5 kb *Bam*HI-*Sal*I fragment separated by gel electrophoresis was cloned into the *Bam*HI and *Sal*I sites of pUC19. For pMP3, the 2.7 kb *Pst*I fragment purified from *Pst*I-digested mt DNA fragments was further digested with *Sac*I, and cloned into the *Pst*I and *Sac*I sites of pUC19. For pME12, the 8.4 kb *Eco*RI fragment purified from *Eco*RI-digested mt DNA fragments was further digested with *Bam*HI, and cloned into the *Eco*RI and *Bam*HI sites of pUC19. pMP3 and pME12 contained the 1.9 kb *Pst*I-*Sac*I and 3.4 kb *Eco*RI-*Bam*HI fragments, respectively.

Gel electrophoresis of restriction fragments. Gel electrophoresis of restriction DNA fragments was done on submarine 1% (w/v) or 0.3% agarose (type LO-3 and H, respectively; Nippon Gene) gels in a running buffer [0.04 M-Tris/HCl (pH 8.1)/2 mM-EDTA] as described by Sharp *et al.* (1973).

Southern blot hybridization. The DNA probe was labelled with a nick-translation kit (Nippon Gene) containing [α -³²P]dATP (HAS). DNA fragments which had been separated by electrophoresis on 1% agarose

Fig. 1. Electron micrograph of *P. urticae* mt DNA and pBR325. One open circular *P. urticae* mt DNA, and one open and two closed circular pBR325 DNAs (5.996 kb), are shown. Bar, 1 μ m.

gel were transferred to a nylon membrane (magnagraph nylon; Micron Separations Inc.) by the method of Southern (1975). Hybridization was done basically as described by Maniatis *et al.* (1982).

RNA manipulations. Molecular size measurements of RNA by gel electrophoresis were done as described by Lehrach *et al.* (1977) with minor modifications. RNA samples were dissolved in buffered formaldehyde solution [50% (v/v) formamide, 17.5% (v/v) formaldehyde, MOPS buffer (0.02 M-MOPS (pH 7.0)/5 mM-sodium acetate/1 mM-EDTA)], heated at 90 °C for 2 min, and rapidly cooled to room temperature. Two microlitres of loading buffer [50% (v/v) glycerol/1 mM-EDTA/0.4% (w/v) bromophenol blue/0.4% (w/v) xylene cyanol FF] was added to the RNA solution (20 μ l). The RNA samples were put onto agarose/formaldehyde gels (1% agarose/2.2 M-formaldehyde/MOPS buffer) and electrophoresis was done in the MOPS buffer at 100 V for 6 h. RNA Molecular Weight Marker II (Boehringer) was used as a size reference. rRNA probe was extracted from the gels by electroelution (Maniatis *et al.*, 1982) concentrated through NEN-SORB20 (Dupont), and labelled at the 5' terminal with [γ -³²P]ATP (HAS) and T4 polynucleotide kinase (Takara) after dephosphorylation (Maxam & Gilbert, 1980).

Results

Electron microscopy of mt DNA of *P. urticae* 2159A

mt DNA from *P. urticae* 2159A was purified from the upper band after DAPI/CsCl or Hoechst 33258/CsCl ultracentrifugation. An electron micrograph of the mt DNA and pBR325 (5.996 kb; as an internal standard) is shown in Fig. 1. A mt DNA solution prepared by mild disruption of mycelia (30 s homogenization) contained an increased number of circular molecules and moderately degraded size-heterogeneous linear fragments. A histogram of the contour lengths of the circular molecules showed two peaks, corresponding to pBR325 ($2.0 \pm 0.1 \mu$ m, $n = 41$) and mt DNA from *P. urticae* ($9.24 \pm 0.20 \mu$ m, $n = 15$), respectively. From these results, the molecular size of mt DNA was 27.7 ± 0.6 kb.

Table 1. Sizes (kb) of restriction fragments of mt DNAs

Fragment no.	<i>P. urticae</i> 2159A*				<i>P. chrysogenum</i> 1951†		
	<i>NotI</i>	<i>PstI</i>	<i>EcoRI</i>	<i>HindIII</i>	<i>PstI</i>	<i>EcoRI</i>	<i>HindIII</i>
1	18.0	14.0	8.4	10.5	23.2	7.9	6.1
2	9.4	10.5	7.0	4.7	2.7	6.7	4.6
3		2.7	6.8	3.4		6.4	4.3
4			3.0	2.45		2.5	2.8
5			2.0	1.9‡		2.3	2.4
6				1.9‡			1.7§
7				1.36			1.7§
8				0.90			1.25
9							0.87
Total:	27.4	27.2	27.2	27.1	25.9	25.8	25.7

* Digestion with *AvaI*, *BamHI*, *SalI*, *SacI* or *XhoI* produced a 27.2 kb fragment.

† Digestion with *NcoI*, *AvaI*, *BamHI* or *SalI* produced a 25.8 kb fragment. The digestion pattern of mt DNA from *P. chrysogenum* 7119 with *PstI*, *EcoRI*, *HindIII* or *EcoRI* + *HindIII* was identical to that of *P. chrysogenum* 1951.

‡ Fragment nos 5 and 6 were overlapped.

§ Fragment nos 6 and 7 were overlapped.

Restriction enzyme analysis of mt DNAs from *P. urticae* 2159A, and *P. chrysogenum* 1951 and 7119

Table 1 shows the DNA fragment sizes of *P. urticae* and *P. chrysogenum* generated by the restriction endonucleases used in this study. For accurate measurement of the large fragment sizes, 0.3% agarose gels were used. The mean total genome size of *P. urticae* mt DNA calculated from the fragment sizes listed for all enzymes was 27.2 kb, which agrees excellently with the size calculated from electron microscopy. The total genome sizes of *P. chrysogenum* 1951 and 7119 mt DNAs were 25.8 kb, a value similar to that of 27.1 kb obtained by Stahl *et al.* (1987) for *P. chrysogenum* BC2. However, the size of *P. chrysogenum* 1951 mt DNA was quite different from the earlier report of 49 kb by Smith *et al.* (1984). By single, double and triple digestion and agarose gel electrophoresis of native mt DNA (some of the data are shown in Figs 3 and 6), restriction maps of *P. urticae* 2159A and *P. chrysogenum* 1951 were constructed (Fig. 2). The pattern of *P. chrysogenum* 1951 was identical to that of *P. chrysogenum* 7119 and very similar to that of *P. chrysogenum* BC2 except for one extra *HindIII* site (Stahl *et al.*, 1987; Fig. 2). Comparison of the restriction maps of *P. urticae* and *P. chrysogenum* indicates that the mt DNA molecules of these species resemble each other (Fig. 2). The sizes of mt DNAs from the genus *Penicillium* are similar to those of *A. nidulans* (31.5 kb) and *Cephalosporium acremonium* (27 kb), but much smaller than those of other ascomycete genera (Grossman & Hudspeth, 1985).

Cloning of mt DNA fragments from *P. urticae*

P. urticae mt DNA fragments were cloned as described in Methods. Fragment identities were confirmed by agarose gel electrophoresis and Southern hybridization (data not shown). Fig. 2 shows clones containing mt DNA fragments. The eleven clones covered the total range of mt DNA of *P. urticae*.

Southern hybridization of mt DNA from *P. chrysogenum* 1951 with the cloned *P. urticae* mt DNA fragments as probes

To compare the genome order of mt DNA between *P. urticae* and *P. chrysogenum*, *P. chrysogenum* 1951 mt DNA digested with *EcoRI*, *HindIII*, or *EcoRI* + *HindIII* was separated by agarose gel electrophoresis, and Southern transfer and hybridization were done with pME4, pME5, pME12, pMH2, pMH4 and pMB1 as probes. The results are shown in Figs 3 and 4, and summarized in Fig. 2. The probe pME4 hybridized to the EH6 fragment and weakly to the EH3 fragment (fifth and third bands from the top in lane 3, respectively) (Fig. 3, lane 6). The probes pME5, pMH2, and pME12 hybridized to EH3, EH2 and EH5 (third, second, and fourth bands from the top in lane 3, respectively) (Fig. 3, lanes 9, 12 and 15). The probes pMB1 and pMH4 hybridized to EH1 and EH4 (first and third bands, respectively, from the top in Fig. 4, lane 4) (Fig. 4, lanes 7 and 10). Thus the mt DNA fragments of *P. urticae* hybridized at similar regions of mt DNA of *P.*

Fig. 2. Restriction maps and/or cloned fragments of mt DNAs from *P. urticae* and *P. chrysogenum*. I, *P. urticae* 2159A; II, *P. chrysogenum* 1951 and 7119; III, *P. chrysogenum* BC2 (linearized map at *Bam*HI site; Stahl *et al.*, 1987). Clone designation and the inserted fragments of *P. urticae* 2159A mt DNA are shown above the map of I. Numbers of *Eco*RI (E) and *Hind*III (H) fragments shown in blocks correspond to the numbers in Table 1. Fragment sizes from the larger fragment of *P. urticae* mt DNA digested with *Eco*RI + *Hind*III are 5.5, 4.7, 3.0, 2.9, 2.45, 2.0, 1.9, 1.9, 1.12, 0.93, 0.54 and 0.24 kb, and the fragments are designated as EH1 to EH12, respectively (I). The size of the smallest fragment (EH13) of *P. urticae* was not measured. Fragment sizes of *P. chrysogenum* 1951 and 7119 mt DNA digested with *Eco*RI + *Hind*III are 5.6, 4.6, 2.5, 2.4, 2.3, 2.0, 1.7, 1.7, 1.15, 0.87, 0.53, 0.29, 0.24 and 0.17 kb, and the fragments are designated as EH1 to EH14, respectively (II). Thick lines represent the defined coding regions of mt large (L) rRNA and small (S) rRNA. For *P. chrysogenum* mt DNA digested with *Eco*RI + *Hind*III, regions homologous to the cloned fragments are shown by the appropriate shading. A, *Ava*I; B, *Bam*HI; E, *Eco*RI; H, *Hind*III; N, *Nco*I; P, *Pst*I; S, *Sac*I; Sl, *Sall*; X, *Xba*I. The *Nco*I site(s) is undetermined for the mt DNA of *P. chrysogenum* BC2.

chrysogenum 1951 (Fig. 2). These results indicate a similar genome order in these two species.

ARSs of mt DNA from *P. urticae*

mt DNA from *P. chrysogenum* BC2 had at least three regions of autonomous replication function (ARSs) in *S. cerevisiae* (E4, E5, and H1 in Fig. 2 II; Stahl *et al.*, 1987). Fragments estimated from the restriction map to contain the corresponding region were tested for ARS activity. The 2.0 kb *Eco*RI fragment (E5) of pME5 and the 3.4 kb *Eco*RI–*Bam*HI fragment (part of E1) of pME12 of mt DNA from *P. urticae* were subcloned to the *Eco*RI site, and the *Eco*RI and *Bam*HI sites, respectively, of YIp5, which lacks a replication origin in *S. cerevisiae*. The new

plasmids were designated as YIp5E5 and YIp5E12, respectively. These plasmids were used to transform *S. cerevisiae* SHY3 by the method with alkali cations (Ito *et al.*, 1983). *Ura*⁺ transformants were obtained with YIp5E5 and YIp5E12 at frequencies of 7.4×10^2 and 2.3×10^2 per μ g DNA, respectively, but no transformants were obtained with control YIp5 ($< 1 \mu$ g⁻¹). To determine whether the plasmids had been modified, four transformants were randomly chosen from each transformation and used for mini-prep plasmid isolation (Hoffman & Winston, 1987). The lysates were used for transformation of *E. coli* JM109: considerable numbers of transformants were obtained for all samples. Plasmid isolation and restriction enzyme analysis showed the presence of the original plasmid (YIp5E5 or YIp5E12) in

Fig. 3. Southern hybridization analysis of mt DNA from *P. chrysogenum* 1951. Lanes 1 to 3 are 1% agarose gel electrophoresis of *P. chrysogenum* 1951 mt DNA digested with *EcoRI*, *HindIII*, and *EcoRI* + *HindIII*, respectively. Lanes 4, 7, 10 and 13 are Southern hybridization of lane 1; lanes 5, 8, 11 and 14 are of lane 2; and lanes 6, 9, 12 and 15 are of lane 3. Probes for lanes 4 to 6, 7 to 9, 10 to 12 and 13 to 15 are pME4, pME5, pMH2 and pME12, respectively. Migration is from top to bottom. The transfer membrane was repeatedly used after washing with renaturation buffer (RB) [$\times 20$ RB: 50 mM-Tris/HCl (pH 8.0), 2 mM-EDTA, 0.5% (w/v) sodium pyrophosphate, 0.02% (w/v) bovine serum albumin, 0.02% (w/v) Ficoll and 0.02% (w/v) polyvinylpyrrolidone] at 65 °C for 3 h. The sixth band from the top in lane 2 contains the overlapped fragments H6 and H7. The third and sixth bands in lane 3 contain the overlapped fragments EH3 and EH4, and EH7 and EH8, respectively. Fragments smaller than 0.3 kb were not analysed by Southern hybridization. Hybridized fragments are band 5 (E5) in lane 4, band 3 (H3) and band 4 (H4, weak) in lane 5, band 3 (EH3, weak) and band 5 (EH6) in lane 6, band 4 (E4) in lane 7, band 4 (H4) in lane 8, band 3 (EH3) in lane 9, band 2 (E2) in lane 10, band 2 (H2) in lane 11, band 2 (EH2) in lane 12, band 1 (E1) in lane 13, band 3 (H3) in lane 14, and band 4 (EH5) in lane 15.

Fig. 4. Southern hybridization analysis of mt DNA from *P. chrysogenum* 1951. Lanes 2 to 4 correspond to lanes 1 to 3 in Fig. 3, respectively. Lanes 5 and 8 are Southern hybridizations of lane 2; lanes 6 and 9 are of lane 3, and lanes 7 and 10 are of lane 4. Lane 1 is λ *HindIII* fragments. Probes for lanes 5 to 7 and 8 to 10 are pMB1 and pMH4, respectively. Hybridized fragments are band 1 (E1) in lane 5, band 1 (H1) in lane 6, band 1 (EH1) in lane 7, band 3 (E3) in lane 8, band 5 (H5) in lane 9, and band 3 (EH4) in lane 10.

the *E. coli* transformants (data not shown). These results indicated that ARS activities in mt DNA of *P. urticae* are located in the 2.0 kb *EcoRI* fragment (E5) which is homologous to E4 of *P. chrysogenum* (Fig. 2, II), and in

Fig. 5. Electrophoresis of mt rRNAs and cytosol rRNAs from *P. urticae* and *P. chrysogenum* on 1% agarose gel containing 2.2 M-formaldehyde. Lane 1, RNA size markers (7.4, 5.3, 2.8, 1.9 and 1.6 kb; Boehringer). Lane 2, cytosol rRNAs from *S. cerevisiae* AH22, as a control. Lanes 3, 5 and 7, cytosol rRNAs of *P. urticae* 2159A, *P. chrysogenum* 7119 and 1951, respectively. Lanes 4, 6 and 8, mt rRNAs of *P. urticae* 2159A, *P. chrysogenum* 7119 and 1951, respectively. Weak bands (3.4 and 1.8 kb) in lane 4 are contaminants of cytosol rRNAs. Migration is from top to bottom.

Fig. 6. Agarose gel electrophoresis of restriction enzyme digested mt DNA fragments from *P. urticae* and *P. chrysogenum* (a) and Southern hybridization analysis with small mt rRNA as a probe (b). Small mt rRNA was separated on agarose/formamide gel, and extracted and purified as described in Methods. Labelling of small mt rRNA was performed with T4 polynucleotide kinase and [γ - 32 P]ATP, after the removal of 5' phosphate by treatment with calf intestine alkaline phosphatase. Southern transfer and hybridization were done as described in Methods and the results are shown in (b). Lane 0 is *Hind*III-digested λ DNA, lanes 1, 4 and 7 are *Eco*RI-digested mt DNAs, lanes 2, 5 and 8 are *Hind*III-digested mt DNAs, and lanes 3, 6 and 9 are *Eco*RI- and *Hind*III-double-digested mt DNAs. Lanes 1 to 3, 4 to 6, and 7 to 9 are mt DNAs from *P. urticae* 2159A, *P. chrysogenum* 7119 and *P. chrysogenum* 1951, respectively. Lanes 1 to 9 in (a) correspond to lanes 1 to 9 in (b). The third and fifth bands in lane 3 contain overlapped fragments (EH3 and EH4, and EH6, EH7 and EH8, respectively). Overlapped fragments for *P. chrysogenum* shown in lanes 5, 6, 8, and 9 are explained in the legend of Fig. 3.

the 3.4 kb *Eco*RI–*Bam*HI fragment (part of E1). These ARSs were defined as putative replicons only for yeast, but they may be functional in homologous and heterologous systems as described by Tudzynski & Esser (1985).

Molecular sizes of mitochondrial rRNA large and small fragments from *P. urticae* and *P. chrysogenum* strains

Fig. 5 shows the agarose/formaldehyde gel electrophoresis of mitochondrial and cytosol rRNAs from *P. urticae* and two strains of *P. chrysogenum*. The molecular sizes of large and small cytosol rRNAs were 3.4 and 1.8 kb, respectively, among the three penicillia and *S. cerevisiae*. For mitochondrial rRNAs, the size of the small subunit was 1.5 kb among the three penicillia, but the larger subunit was 4.2 kb in *P. urticae*, and 3.5 kb in *P. chrysogenum*. This surprising result is not caused by an artifact of isolation or electrophoresis. Three repeated experiments supported the above results. The size of cytosol rRNAs in *S. cerevisiae* was identical to the published results (Georgiev *et al.*, 1981; Rubtsov *et al.*, 1980). Moreover, electrophoresis on glyoxal gels (Maniatis *et al.*, 1982) also supported the above sizes (data not shown). Since the similarity in genome orders and restriction maps of mt DNA between *P. urticae* and *P. chrysogenum* suggests high conservation of mt DNA, it is interesting that the size of the rRNA large subunit was very different between them.

Location of large and small mt rRNA genes

Southern hybridizations of mt DNAs from *P. urticae* 2159A, and *P. chrysogenum* 7119 and 1951, with labelled small mt rRNA from *P. urticae* are shown in Fig. 6. The hybridization pattern of *P. chrysogenum* 1951 was identical to that of 7119. The small rRNA hybridized to the H2 fragment of *P. urticae* (second band from the top in lane a-2) and H2 of *P. chrysogenum* (second band in lanes a-5 and a-8). However, the probe hybridized to neither H7 nor H8 of *P. urticae* (sixth and seventh bands in lane a-2, respectively), nor to H8 or H9 from *P. chrysogenum* (seventh and eighth bands, respectively, in lanes a-5 and a-8). Thus the small mt rRNA genes of *P. urticae* and *P. chrysogenum* were confined to the 4.7 kb fragment (H2 and EH2 in Fig. 2, I) and 4.6 kb fragment (H2 and EH2 in Fig. 2, II), respectively.

The large rRNA hybridized to the H5 and/or H6 fragment(s) (1.9 kb; fifth band from the top in Fig. 7a, lane 2) and weakly to H4 (fourth band in lane a-2) from *P. urticae*. When the *Hind*III fragments were digested with *Nco*I, the probe hybridized to H5, and also to the 1.4 kb and 0.5 kb *Hind*III–*Nco*I fragments derived from H6. Hybridization to two *Pst*I fragments (14.0 and 10.5 kb) was also observed (data not shown). For *P. chrysogenum*, the large rRNA also hybridized to H6 and H7 (sixth band in lanes 5 and 8; unpublished result) and weakly to H5 (fifth band). Thus the large mt rRNA genes

Fig. 7. Agarose gel electrophoresis of restriction enzyme digested mt DNA fragments from *P. urticae* and *P. chrysogenum* (a) and Southern hybridization analysis with large mt rRNA as a probe (b). Purification and labelling of large mt rRNA were similar to those of small mt rRNA (see Fig. 6). Panel (a) is identical to Fig. 6(a). The nylon membrane after hybridization with the labelled small mt rRNA was washed and reused for hybridization with large mt rRNA as a probe (b). The second band in lane a-1 (E2 and/or E3) is weakly hybridized (lane b-1). However, when the experiment was repeated, it was strongly hybridized.

were confined to the 6.3 kb region (H4–H6) in *P. urticae* (Fig. 2, I) and to the 5.8 kb region (H5–H7) in *P. chrysogenum* (Fig. 2, II). These results indicate that the large and small mt rRNA genes of *P. urticae* are located in a similar region and support the above conclusion of the similar genome arrangement of mt DNA in *P. urticae* and *P. chrysogenum*. The minimal distance between the two mt rRNA genes was 1.36 kb for *P. urticae* and 1.25 kb for *P. chrysogenum*, whereas the distance was 3.1 kb for *A. nidulans* (Netzker *et al.*, 1982).

Discussion

Fungal mitochondrial genomes vary in length between 18.9 kb and 121 kb (Grossman & Hudspeth, 1985). In the genus *Aspergillus*, wide variation of the mitochondrial genome size (26 to 42 kb) was estimated from the sum of the restriction fragment sizes (Kozłowski & Stepień, 1982). In *P. chrysogenum*, previous reports indicated that the genome sizes of mt DNA were 32.2 MDa (49 kb) for strain NRRL 1951 and 27.1 kb for strain BC2, and the restriction enzyme pattern was quite different between these strains (Smith *et al.*, 1984; Stahl *et al.*, 1987). However, our results indicated similarities in size and restriction map of mt DNA between *P. chrysogenum* BC2, and *P. chrysogenum* NRRL 1951 and IAM 7119 (Fig. 2), and the latter two genomes were identical in restriction map and genome order (Figs 2, 6 and 7). Since our cleavage pattern of mt DNA of *P. chrysogenum* 1951 was very clear (Figs 6 and 7), the conflicting result by Smith *et al.* (1984) might have been caused by their crude mt DNA preparations and/or incomplete digestion.

Comparison of mitochondrial genomes between *P. urticae* and *P. chrysogenum* indicated similar genome sizes and arrangements, including locations of rRNA genes and ARSs (Figs 3, 4, 6 and 7).

P. urticae and *P. chrysogenum* had common sizes of cytosol rRNAs, which were also similar to those of *S. cerevisiae* (Fig. 5). For mt rRNA, the size of the small subunit from *P. urticae* was also similar to those from *P. chrysogenum* (Fig. 5) and *A. nidulans* (1.55 kb; Dyson *et al.*, 1989), but smaller than those from *S. cerevisiae* (1.7 kb; Li *et al.*, 1982) and *N. crassa* (2.0 kb; Mannella *et al.*, 1979; Green *et al.*, 1981). In spite of the extensive homology of the mt DNAs and the small subunits of rRNAs between *P. urticae* and *P. chrysogenum* (Figs 2 and 5), the large subunit of mt rRNA was markedly different in size (4.2 kb for *P. urticae* and 3.5 kb for *P. chrysogenum*) (Fig. 5). The large subunit in *P. urticae* mitochondria was 1.1 kb larger than that in the closely related fungi *A. nidulans* (Dyson *et al.*, 1989) and *N. crassa* (Green *et al.*, 1981), 0.8–0.9 kb larger than that in *S. cerevisiae* (Dujon, 1980; Sor & Fukuhara, 1983) and at least 0.5 kb larger than those in plants (Leaver & Gray, 1982; Falconet *et al.*, 1985). The 4.2 kb size of the large mt rRNA in *P. urticae* is, to our knowledge, the largest that has ever been described for mt rRNAs. The markedly larger size was not caused by the mutation of the wild-type *P. urticae* NRRL 2159 to the albino NRRL 2159A, since their mt rRNAs migrated similar distances on agarose/formaldehyde gel electrophoresis (data not shown).

The size difference of mt rRNA may be caused by a nuclear mutation. Splicing is well known in the large

subunits of mt rRNAs from yeasts and fungi (Merten *et al.*, 1980; Mahler, 1983) and nuclear mutations have been found to lead to a deficiency in splicing and accumulation of novel larger-sized precursor rRNAs (Mannella *et al.*, 1979; Garriga *et al.*, 1984). However, these temperature-sensitive mutants lacked respiration at non-permissive temperatures, whereas *P. urticae* grew and respired normally.

In this paper we have presented evidence for a complete clone bank of mt DNA from *P. urticae* and for the presence of ARSs functional in *S. cerevisiae*. This clone bank will be utilized for elucidating replication functions in *A. nidulans* and *P. urticae* and sequencing the rRNA gene of *P. urticae*. Now our research is directed toward the splicing mechanism of large subunits of mt rRNAs from *P. urticae* and *P. chrysogenum*.

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