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

JUNICHI SEKIGUCHI,* TAKESHI OHSKI, HIROKI YAMAMOTO, KAZUMICHI KOICHI and TOSHIRO SHIDA

Department of Applied Biology, Faculty of Textile Science and Technology, Shinshu University, 3-15-1 Tokida, Ueda-shi, Nagano 386, Japan

Received 8 August 1989; revised 3 November 1989; accepted 30 November 1989

Mitochondrial DNA (mt DNA) from a patulin producer, *Penicillium urticae* (synonym *P. griseofulvum*), was 27.8 kb ± 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 & Küntzel, 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 K$_2$HPO$_4$, 1 g MgSO$_4$·7H$_2$O and 1 g (NH$_4$)$_2$SO$_4$ 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 M100P; Taiyo). *Escherichia coli* JM109 recA1 Δ(lac-proAB) endA1 gyrA96 thi-1 hsdR17 supE44 relA1 λ- [F′λtrd36 proAB lacP238] (Yanisch-Perron *et al.*, 1985) obtained from Takara was used as the recipient in cloning experiments. *Saccharomyces cerevisiae* SHY3 a ste-V9 ura3-52 trp1-289 leu2-3 ura3-52 leu2-112 his3-A1 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.
Isolation of mt DNA. This was done basically as described by Stepien 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 32358 (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 tRNA 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 EcoRI or HindIII, and cloned into the corresponding site of pUC19. The resultant pME4 and pME5 contained the 34 kb and 20 kb EcoRI fragments of p. urticae mt DNA, respectively, and pMH2 and pMH7 contained the 47 kb and 1-4 kb HindIII fragments, respectively. For construction of pMH4, pMHS and pMH6, the 1.45 kb and 1-9 kb HindIII fragments were separated by agarose gel electrophoresis and cloned into the HindIII site of pUC19. A Neol site was found in the 1-9 kb HindIII fragment of pMH6, but not in that of pMH5. For pME22, mt DNA digested with EcoRI and PstI was cloned into the EcoRI and PstI sites of pUC19. The resultant pME22 contained the 1-9 kb EcoRI-PstI insert. For pMB1, the 5.5 kb BamHI-SalI fragment separated by gel electrophoresis was cloned into the BamHI and SalI sites of pUC19. pMP3, the 2.7 kb PstI fragment purified from PstI-digested mt DNA fragments was farther digested with SacI, and cloned into the PstI and SacI sites of pUC19. For pME12, the 8.4 kb EcoRI fragment purified from EcoRI-digested mt DNA fragments was farther digested with BamHI, and cloned into the EcoRI and BamHI sites of pUC19. pMP3 and pME12 contained the 1-9 kb PstI-SacI and 3-4 kb EcoRI-BamHI 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 [γ-32P]ATP (HAS). DNA fragments which had been separated by electrophoresis on 1% agarose 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/4% (w/v) xylene cyanol FF] was added to the RNA solution (20 μl). The RNA samples were put onto agarose/formaldehyde gels (1% agarose/2% 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. RNA probe was extracted from the gels by electroleution (Maniatis et al., 1982) concentrated through NENSORB20 (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 32358/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 ± 0.1 μm, n = 41) and mt DNA from P. urticae (9-24 ± 0.20 μm, 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**

<table>
<thead>
<tr>
<th>Fragment no.</th>
<th><em>P. urticae 2159A</em></th>
<th><em>P. chrysogenum 1951</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>NcoI</em></td>
<td><em>PstI</em></td>
</tr>
<tr>
<td>1</td>
<td>18.0</td>
<td>14.0</td>
</tr>
<tr>
<td>2</td>
<td>9.4</td>
<td>10.5</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>2.7</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27.4</td>
<td>27.2</td>
</tr>
</tbody>
</table>

* 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 BamHI 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 EcoRI (E) and HindIII (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 EcoRI + HindIII are 5.5, 4.7, 3.0, 2.9, 2.45, 2.0, 1.9, 1.9-12, 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 EcoRI + HindIII 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 EcoRI + HindIII, regions homologous to the cloned fragments are shown by the appropriate shading. A, Asal; B, BamHI; E, EcoRI; H, HindIII; N, NcoI; P, PstI; S, SacI; Sl, SalI; X, XbaI. The NcoI 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.

**AROs of mt DNA from *P. urticae***

mt DNA from *P. chrysogenum* BC2 had at least three regions of autonomous replication function (AROs) 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 EcoRI fragment (E5) of pME5 and the 3.4 kb EcoRI–BamHI fragment (part of E1) of pME12 of mt DNA from *P. urticae* were subcloned to the EcoRI site, and the EcoRI and BamHI 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 × 10² and 2.3 × 10³ per μg DNA, respectively, but no transformants were obtained with control YIp5 (<1 μg⁻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
**mt DNAs and rRNAs from Penicillium**

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) [× 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.

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 2 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.

5. Electrophoresis of mt rRNAs and cytosol rRNAs from *P. urticae* and *P. chrysogenum* on 1% agarose gel containing 2 M-formaldehyde. Lane 1, RNA size markers (7-4, 5-3, 28, 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.

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
Fragments from gous systems as described by Tudzynski. Chrysogenum the 3:4 kb ARSs were defined as putative replicons only for yeast, cytosol rRNAs in tis interesting that the size of the rRNA large subunit was shown). Since the similarity in genome orders and restriction maps of mt DNA between 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.

**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 RNA 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 HindIII fragments were digested with NcoI, the probe hybridized to H5, and also to the 1-4 kb and 0-5 kb HindIII–NcoI fragments derived from H6. Hybridization to two PsI 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
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 (Kozlowski & 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...

---

**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.
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.

The authors wish to thank Dr Y. Horibe, Kotobuki Pharmaceutical Company, for the use of a Hitachi HUS-5 evaporator and Dr M. Nakagaki for the help in the electron microscopy. They also thank Dr S. W. Peterson, Northern Regional Research Center, United States Department of Agriculture, and the curator of the Microbial Stock Laboratory, Institute of Applied Microbiology, Tokyo University, for gifts of P. chrysogenum NRRL 2951 and IAM 7112, respectively. They also thank Dr H. Moriyama, Kanazawa Institute of Technology for sharing his unpublished data.

References


