The Journal of Microbiology, December 1997, p.264-270
Vol. 35, No. 4
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The Genetic Organization of the Linear Mitochondrial Plasmid mlp1 from *Pleurotus ostreatus* NFFA2

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(Received August 21, 1997 / Accepted October 30, 1997)

The structure of plasmid mlp1, a linear 10.2 kb mitochondrial plasmid of *Pleurotus ostreatus* NFFA2 was determined by restriction enzyme mapping and partial sequencing. The plasmid encodes at least two proteins; a putative RNA polymerase showing homology to yeast mitochondrial RNA polymerase and to viral-encoded RNA polymerases, and a putative DNA polymerase showing significant homology to the family B type DNA polymerases. It also contains terminal inverted repeat sequences at both ends which are longer than 274 bp. A 1.6 kb *EcoRI* restriction fragment of mlp1 containing the putative RNA polymerase gene did not hybridize to the nuclear or mitochondrial genomes from *P. ostreatus*, suggesting that it may encode plasmid-specific RNA polymerase. The gene fragment also did not hybridize with the RNA polymerase gene (RPO41) from *Saccaromyces cerevisiae*. The relationship between genes in mlp1 and those in another linear plasmid pClK1 of *Claviceps purpurea* was examined by DNA hybridization. The result indicates that the genes for DNA and RNA polymerases are not closely related with those in *C. purpurea*.

Key words: Linear mitochondrial plasmid, *Pleurotus ostreatus*, mitochondrial RNA polymerase, B type DNA polymerase

Many linear plasmids or plasmid-like DNAs in mitochondria have been reported in higher plants and fungi (8, 33). Like adenoviruses and certain bacteriophages, these genetic traits consist of double-stranded DNA which has terminal inverted repeats and terminal proteins which are covalently bound to the 5' ends. These proteins are thought to act as primers for DNA replication (33).

Although the number of linear plasmids identified in various species is continuously growing and sequence data are available for different plasmids, very little is known about the specific functions of most of these elements. Only in a few biological systems can a specific role be attributed to a particular linear plasmid. In some yeasts, the killer phenotype has been demonstrated to be due to the expression of genes encoded by certain cytoplasmic plasmids (15, 39, 43). In the true slime mold *Physarum polycephalum*, plasmid mF seems to play a role in promoting mitochondrial fusion (17). In matings between mF$^+$ and mF$^-$ strains, which carry and do not carry the mF plasmid, respectively, recombination occurs between the mF plasmid and the mitochondrial DNA of mF-strains (40). In the ascomycete *Neurospora*, the two mitochondrial elements, kalilo and maranhar, have been reported to be in control of senescence in particular strains. The kalilo plasmid can exist in an autonomously replicating form, AR-kal DNA, which appears to have no detrimental effect on the host. However, the inserted form, IS-kal DNA integrated into mitochondrial DNA results in abnormal mitochondrial function, senescence, and death (3, 11). In a long-lived mutant of *Podospora anserina*, a linear mitochondrial plasmid, pAL2-1 was found to be correlated with an increased life span of the corresponding mutant (13).

Knowledge about the coding capacity of many linear plasmids has been derived from sequence data. Most linear plasmids contain two major and several minor open reading frames (ORFs). One of the major ORFs encodes an RNA polymerase structurally related to known mitochondrial and viral enzymes, whereas the other major ORF codes for a protein-primed DNA polymerase of the family B type.

In previous work (21), we isolated several linear mitochondrial plasmids from various *P. ostreatus*

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strains. They were classified into at least 3 groups, of which the group I plasmids sharing homologous sequences were found ubiquitously in all the *P. ostreatus* strains tested. One of the group 1 plasmids, mlp1 (10.2 kb) of *P. ostreatus* NFFA2, shares homology with similar-sized plasmids from other *Pleurotus* spp. (*P. florida, P. pulmonarius, P. sajor-caju,* and *P. spodoleucus*). To learn more about the biological role and self-replicating function of mlp1, we determined partial nucleotide sequences of this plasmid and analyzed its coding potential. We also investigated homology between mlp1 and other related plasmids and gene sequences.

Materials and Methods

Strains, plasmids, and culture media

P. ostreatus NFFA2 was obtained from the National Federation of Forestry Association in Korea. *Escherichia coli* DH5α was used as a host for recombinant DNAs. The *E. coli* vector used for subcloning was pGEM3Zf(+) or 7Zf(+). pRT19 and pDT 19 plasmids were provided by Professor Paul Tudzynski (Düsseldorf University, Germany) and pJH 41 was provided by Professor S.-H. Jang (Taegu University, Korea). *P. ostreatus* was grown in malt media at 25°C for liquid culture (21). *E. coli* was grown in LB medium containing 100 g/ml ampicillin at 37°C.

Nucleotide sequencing and sequence analysis

Double stranded templates were subcloned into pGEM3Zf(+) or 7Zf(+) and nested deletion series were constructed with the Erase-a-base system (Promega Biotech) according to the manufacturer's instructions. Nucleotide sequencing by dideoxy chain termination method was performed using Sequenase version 2.0 (United Stated Biochemicals). DNA sequence data was analyzed and assembled by using softwares such as DNASIS, BLAST, and Clustal V.

DNA manipulation

Linear mitochondrial plasmid was prepared as described previously (21). Total DNA was prepared by SDS-proteinase K extraction method (6). Mitochondrial genomic DNA was purified by CsCl-bisbenzimide gradient sedimentation (21). Restriction enzymes and T4 DNA ligase were purchased from Poscochem. All enzyme reactions were carried out according to the recommendations of manufacturers

Southern hybridization

Genomic or plasmid DNA was digested with the appropriate restriction enzymes. Following electrophoresis on 0.8% agarose gel in TBE buffer, DNA fragments were transferred onto Hybond N+ membrane (Amersham). The gel-purified probe DNA was nonradioactively labeled by ECL DNA labeling system (Amersham). Hybridization and detection were carried out according to manufacturer's instructions.

Results and Discussion

Restriction mapping and partial sequence analysis

The restriction map of a 10.2 kb mlp1 plasmid, one of the linear mitochondrial plasmid from *P. ostreatus*, is shown in Fig. 1. 9.7 kb internal DNAs between the two *Eco*RI sites at the left and right boundaries were subcloned into pGEM7Zf(+) plasmid. The two end fragments (total ~500 bp) flanking the boundary *Eco*RI sites were not cloned. We determined the sequences internal to the *Eco*RI boundary and a 1.6 kb *Eco*RI fragment (region A) and a 1.2 kb *Hin*dIII/*Eco*RV fragment (region B). The boundary sequences turned out to contain 274 bp terminal inverted repeat (TIR) sequence at each end. We estimate the length of the end fragments flanking the boundary *Eco*RI sites to be about 250 bp which is most likely a continuation of the TIR. We therefore hypothesize that mlp1 contains about 520 bp-long TIR at both ends as usually observed for fungal linear plasmids. However, the exact length of TIR should await the cloning of the end fragments.

The sequences of regions A and B reveal that region A contains a truncated open reading frame

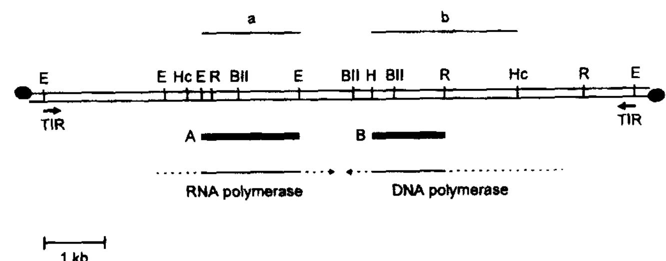

Fig. 1. Restriction map of the 10.2 kb linear plasmid mlp1 from *Pleurotus ostreatus* NFFA2. Sequenced regions (A and B) are indicated by shaded bars. The locations and orientations of putative ORFs are shown by arrows. The regions of undetermined nucleotide sequences are shown by dotted arrows. The terminal inverted repeats (TIR) determined by nucleotide sequencing are shown by arrows. a and b indicate the regions which were used as probes in Southern hybridization (Fig. 4, 5 and 6). E: *Eco*RI, Hc: *Hin*cII, R: *Eco*RV, BII: *Bgl*II, H: *Hin*dIII.

(ORF) for putative RNA polymerase whereas region B contains a different truncated ORF for putative DNA polymerase. The two ORFs are in converging orientation.

Homology search for mlp1 RNA polymerase and DNA polymerase

Comparison of the translated sequence of region A with amino acid sequences in the Genebank/EMBL/DDBJ database revealed sequence similarity with RNA polymerases encoded by the bacteriophages T3, T7, SP6 (25, 32, 35), the nuclear-encoded mitochondrial RNA polymerase (RPO41) of *S. cerevisiae* (30), and RNA polymerases encoded by other linear mitochondrial plasmids of various fungi (12).

In Fig. 2, the translated amino acid sequences of region A are aligned with various RNA polymerase sequences from linear mitochondrial plasmids of *P. anserina* (pAL2-1, 12), *C. purpurea* (pClK1, 36), *N. crassa* (maranhar), *N. intermedia* (kalilo, 7), *Z. mays* (S2, 27), as well as RNA polymerases of *S.*

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                    3                         4
MLP1      PIKLPMICEPKKWSENTTGGY   QISTVNYLNNIPFEINNTMLDY
pAL2-1    PKKMPMMVKPKPYTTRVNGGL   MFDLVNNLSSMGYKINTDVLDF
pClK1     PLKLPMIVAPKPYSKEEHGGY   IYESINKMMAIAFQINKKLLKY
Maranhar  PKALPMVCPPLPWGHGIRGGN   LVDAVNFKSKIPYTINLKLLDF
Kalilo    TIELPMIIPPLEWKIDDNEKI   LYNVYNKLNAFKFKINGDLLSY
S2        EVSDPVITNLTPLSSYR-GGY   VLSAVKKLQRQPVRINKLVYDF
RPO41     PQLLPMLVEPKPWVNWRSGGY   VYDGLNVLGRTPWTVNRKVFDV
T3        PMFQPCVVPPKPWVAITGGGY   VYKAVNLAQNTAWKINKKVLAV
SP6       PAYAPCYIPPRPWRTPFNGGF   VYKAINALQNTQWQINKDVLAV
          .  .*.   ....     ...    .  .. .      .*  ...

                                               5
MLP1      YLPT-FLDFRGRVYPIPVYLSYQGSDLAKIFIIIKNVGLKIDYNNIIQQIVDKNIKNK-LKKLILNDIDYV
pAL2-1    FMPV-NADFRGRVYCTPEYLNYQSTDLAKSLLLFSKPGRMYKKDYIALSYLKMYGGSSFGLD-LKLSANDR
pClK1     YFPL-KLDHRGRLYPITAYFHYQSSELAKALLLFAIPDTIERSDSISIDYLKAYGATCFGNKLDKKSY-DK
Maranhar  YMTV-PIDWRGRFYTSSCALNIQGGELARSLLLFKEGQKLNDI-GLKALKIYTANAFG-LDKRSKEERLDW
Kalilo    YNLNVNIDWRGRIYTQSFYLDYQGSELSLALINLFEGKKLDEKGLFFFYVYGANIYND-GGKFSKKSFQDR
S2        YFPI-FLDFRGRNYRHGP-FHFHERDLVRSLIIFD-----ESDDSAAHTINSDVGDRILHNFLISAAYHKS
RPO41     YFPH-NLDFRGRAYPLSPHFNHLGNDMSRGLLIFWHGKK-LG--PSGLKWLKIHLSNLFGF--DKLPLKDR
T3        WFPY-NNDWRGRVYAVPM-FNPQGNDMTKGLLTLAKGKP-IG--KEGYYWLKIHGANCAGV--DKVPFPER
SP6       YFVY-ANDSRSRVYVQSSTLSPQSNDLGKALLRFTEGRPVNG--VEALKWFCINGANLWGW--DKKIFDVR
          ...   .* *.* *     .  .. .... .. .  .            .    . .  .    .    ..

               6                               7                         8
MLP1     AKEPAQFIS-CLLG   RPILFDATCSGIQHLSALTSDIEIAKLVNLIESTKDEPSD   NLKRSFLKHSIMTVPYN
pAL2-1   AKNKFLFLA-FQIE   YTIQMDATCNGFQHLSLLSLDSNLSKELNLSESTWDDVPK   KIIREIIKKGIMTMPIN
pClK1    AENKFMFLA-YQLE   LPLQLDGTCNGFQHIVLLSNEVKLYSQLNLDGKSKNQDPS   GLSRANIKQAIMTKPYN
Maranhar ADEPLLFLA-CALE   LPILMDATCNGLQHLSAMVNDFVLAEKVNLLKSTENDNPR   NVERCLVKRGLMTITYG
Kalilo   AESPTLLFAAFQLT   MPIFLDATCSGVQHFAAMLLDLELGKYVNLINSGESVNDF   SLNRSLLKKVIMTKSYN
S2       QSKPTFF-EKDIFV   TPWFQDASASAYQYMSYFLLDIDYGIATNLLKKTNTDGRY   IFDRNVVKKNFMPMMYG
RPO41    ADKPWQALAT-CFE   QPVHQDGTCNGLQHYAALGGDVEGATQVNLVPSDKPQDVY   KITRKVVKQTVMTNVYG
T3       QDSPFCFLAF-CFE   LPLAFDGSCSGIQHFSAMLRDEFGGRAVNLLPSETVQDIY   GVTRSVTKRSVMTLAYG
SP6      ADAPYEFLAW-CFE   LPVHQDGSCSGIQHYSAMLRDEVGAKAVNLKPSDAPQDIY   GITRSLTKKPVMTLPYG
         .  .  ...    .    .   *..... *. ..   .      .**  .             *   *   *.  ..
                                                                       .

                9
MLP1     VYWITPSGMKVSVNS
pAL2-1   IMWTLPTGLVVRQSY
pClK1    ITWSLPTGLEVHQQY
Maranhar VNWVTPYGLVIQQKY
Kalilo   LSWSTPDGLELTQRY
S2       VVYSTPVWVTL-QTY
RPO41    AIWTTPLGLPIVQPY
T3       VHWTTPDGFPVWQEY
SP6      LMYTLPTGFILEQKI
           . .*  ..   . .
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Fig. 2. Comparison of amino acid sequences of a 1.6 kb *Eco*RI fragment of mlp1 with several known motifs of RNA polymerase encoded by pAL2-1 of *P. anserina* (12), pClK1 of *C. purpurea* (36), maranhar of *N. crassa* (accession no. X55361), kalilo of *N. intermedia* (7), S2 of *Z. may* (27), the nuclear-encoded mitochondrial RNA polymerase gene (RPO41) of *S. cerevisiae* (30), and bacteriophage T3 (32) and SP6 (25). The domains are numbered according to reference 36. The amino acid residue identical at a given position in all of these organisms is marked by an asterisk, whereas the conserved substitution is marked by a dot.

cerevisiae mitochondria (RPO41, 30) and bacteriophages T3 (32) and SP6 (25). The mlp1 sequence (498 aa) exhibits the highest homology with pAL2-1 of *P. anserina* (26.9% identity) among linear plasmids. It exhibits lower similarity (19.2% identity) with RPO41 of *S. cerevisiae* and the lowest similarity (8.6% identity) with SP6 RNA polymerase. The homologous sequences correspond to domains 3 to 9 of 11 conserved domains of single peptide RNA polymerases. The regions of RNA polymerase that are involved in important biochemical functions have been identified through analysis of T7 RNA polymerase mutants (31). Functional motifs include a region of T7 RNA polymerase that exhibits homology to sigma factor (residues 230~249 in domain I), regions that are hypersensitive to proteases (residues 172~180 and 850~860), a putative helix-turn-helix motif (residues 230~249 between domain II and III), and regions which exhibits homology to other nucleotide polymerase such as pol I (residues 533~556, 625~652 and 804~817 in domain VI, VIII, XI, respectively). At least two regions of RNA polymerase that are involved in promoter binding or recognition have been identified. The asparagine residue at position 748 (N748) in T7 RNA polymerase has been found to interact directly with base pairs at -10 and -11 in the T7 promoter, and this region of the RNA polymerase is located within a putative DNA-binding cleft (37). The other important residue is the codon 159 at the region which exhibits high homology to region 2.4 of the bacterial sigma factor (31). Other key residues include solvent-exposed cysteine residues (C347, C723, and C839) and lysine (K431) for the site of cross-linking to the initiating nucleotide (23, 29).

In Fig. 3, the translated amino acid sequences of region B are aligned with various DNA polymerase sequences from linear mitochondrial plasmids of *P. anserina* (pAL2-1, 12), *C. purpurea* (pClK1, 36), *N. crassa* (maranhar), *N. intermedia* (kalilo, 7), *Z. mays* (S1, 26), *Ascobolus immersus* (pAI2, 20), and adenovirus2 (1) and bacteriophage 29 (2). The mlp1 sequence (352 aa) exhibits the highest homology with pAL2-1 of *P. anserina* (26.1% identity) among linear plasmids. It exhibits the lowest similarity (17.9% identity) with maranhar of *N. crassa*. The sequenced regions of mlp1 corresponds to region Exo III and regions 1 to 3. Direct structural and functional data or indirect evidence based on the presence of homologous regions indicate that in all cases the C-terminal portion (regions 1 to 4) of the DNA polymerases compared (either prokaryotic or eukaryotic, protein-primed or RNA-primed) contains the polymerization activity of the enzyme (2). A central or N-terminal portion which has 3'->5' proofreading exonuclease activity is characterized by the presence of three highly conserved segments (Exo I, II, and III). In some of the DNA polymerases including *E. coli* pol I and HSV DNA polymerase, there is between the N terminus and the

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                EXO III              REGION 1                          REGION 2a
MLP1       KELLKYLNGDIDTLY   SEKPLFKYDFKSHYPNQMRNNPMPGGLPI   EMNKDDISRLIYKLILNSLSGRWGLRD
pAL2-1     FQTLHYLERDLLSLP   YGKNLRYYDVNSTYPFVAKNTMPGHECKY   KANSEGSEKTMTKFLLNSLLGRFGMSM
pClK1      DETLEYLNLDISLHD   STKSYYYYDVNSLYPFAS-IMDIPGLKCT   NPNNVTEKMIA-KLILNSLIGRFGMNI
Maranhar   KETIIYLEKDMKALP   IMNNIFSFDFNSTYPTAM-MMPMPVGMPV   DNTKGSKKQMA-KLILNTLYGRIGMND
Kalilo     KELIKYCEIDTMATP   FGVNIKSYDVNSTYPFAMKYFKMTSGIPK   SPKESPWYYIS-KTTMNSLYGTFGLNS
pAI2       KELKNYCEIDCLALP   FGKNIHSYDINSLYPSAMAKFDMPTGIPL   YTPDDPMYFIA-KLIMNSLYGRFGMDP
S1         EDSLTYLKQDILITP   YGENLYYYDVNSLYPSSM-LDDMPIGKTR   AKGEKALDFIY-KITMNSLYGRFGISP
Adeno      KETLDYCALDVQVTI   IREPLYVYDICGMYASAL-THPMPWGPPL   RDKNQLTRSIA-KLLSNALYGSFATKL
Phi29      PEEYAYIKNDIQIIK   EIGEGMVFDVNSLYPAQMYSRLLPYGEPI   TTSEGAKKQIA-KLMLNSLYGKFASNP
           .. . *.  *    .   .    .*... *. ..      .. . .               .. *. .*.*.*...


               REGION 2b              REGION 3
MLP1       AVQLSAAITAYGRISMAKV   KNISDNLYFGGDTDSFILEKPL
pAL2-1     AVSIAA-VTAYARIFMAQT   MLKNGGNLYYTDTDSIVTDIDT
pClK1      SITTAAAVLSYARIEMAQI   ILENNGTLYYTDTDSIVTDLKL
Maranhar   STSIAAATASWSRILMYKH   MYKHMINSAYTDTDSMRVEKPL
Kalilo     SVPISSATAAYSRMHMSHF   LTKYSNNIYYIDTDGIKVDIDT
pAl2       SVPISAAIAAYSRMIMSEY   LIKYADNLYAVDTDGIKVDIEI
S1         AVQISAAVTGYARIRMHPF   PFISRDDCYYTDTDSVVVEREL
Adeno      PSHLASFVLAWTRAFVSEW   LEDRPLKSVYGDTDSLFVTERG
Phi29      YTPMGVFITAWARFTTITA   AQACYDRIIYCDTDSIHLTGTE
           .  . ..  .. *. .             .. ***.. .
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Fig. 3. Comparison of amino acid sequences of a 1.2 kb *Hin*dIII/*Eco*RV fragment of mlp1 with several known motifs of DNA polymerase encoded by pAL2-1 of *P. anserina* (12), pClK1 of *C. purpurea* (36), maranhar of *N. crassa* (accession no. X55361), kalilo of *N. intermedia* (7), S1 of *Z. may* (26), pAI2 of *Ascobolus immersus* (20), and adenovirus 2 (1), bacteriophage 29 (2). The domains are numbered according to reference 5. The amino acid residue identical at a given position in all of these organisms is marked by an asterisk, whereas the conserved substitution is marked by a dot.

first homologous 3'->5' exonuclease (Exo I), an additional segment containing a 5'->3' exonucleases activity (2).

Plasmid mlp1 appears to be very similar to other linear mitochondrial plasmid (from fungi and higher plants) reported to contains ORFs that may encode the two types of polymerase. It is possible that the linear mitochondrial plasmids have a common ancestor with them because they share similar genome structure and polymerases. Mitochondrial plasmids may resemble viruses either because plasmids and viruses share a common ancestor or because plasmids have evolved under conditions similar to those of viruses.

Search for homologous RNA polymerase gene in the nuclear or mitochondrial genomic DNA

It is possible that linear mitochondrial plasmids represent transposable elements or a replicon derived from the mitochondrial or nuclear genomes. In such cases, homologous genes could be found in the genomic DNAs. This hypothesis was investigated by hybridizing mlp1 DNA probe (probe a in Fig. 1) with total DNAs from *P. ostreatus* containing mitochondrial and nuclear genomic DNA. Restriction enzyme digestion of total DNA extracts is shown in Fig. 4A. In addition to the linear mitochondrial plasmid bands, numerous other bands were produced by the digestion of total DNA, but no hybridization of 1.6 kb *Eco*RI fragment to other bands was observed (Fig. 4B) except to the mlp1 fragment. This indicates that mlp1 has no significant homology with mitochondrial and nuclear DNA sequences.

To confirm this, mitochondrial genomic DNA was isolated from CsCl-bisbenzimide gradient and Southern hybridization was performed with the same probe (Fig. 5A and 5B). No significantly hybridized band was detected except to the contaminated 1.6 kb *Eco*RI (Fig. 5B lane 1) and 5.4 kb *Hin*dIII (Fig. 5B lane 2) fragments of mlp1, respectively. Also, when nuclear or mitochondrial DNA digested with *Bam*HI

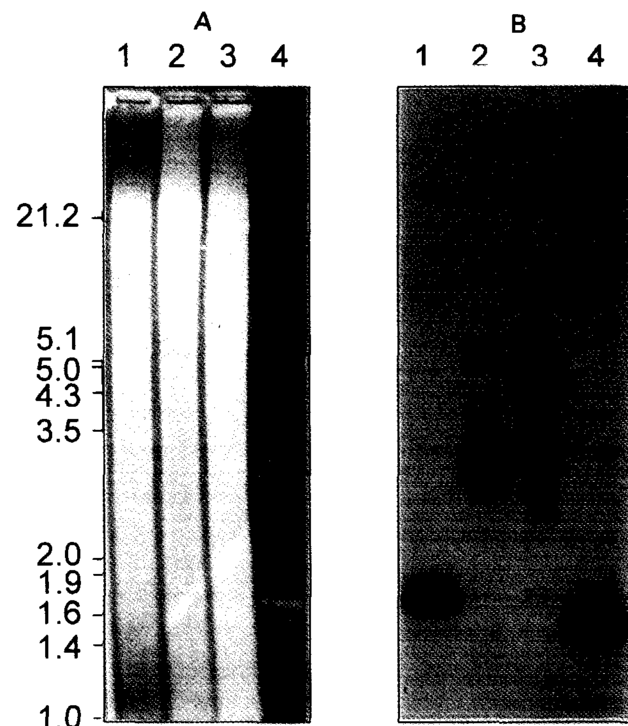

Fig. 4. Restriction digestion of total DNA of *P. ostreatus* and Southern hybridization probed with a 1.6 kb *Eco*RI fragment of mlp1. The size of DNA marker is shown in kb. A. Total DNA from *P. ostreatus* digested with *Eco*RI (lane 1), *Bgl*II (lane 2), and *Eco*RV (lane 3), or purified 1.6 kb *Eco*RI fragment of mlp1 (lane 4) were electrophoresed on 0.8% agarose gel and stained with ethidium bromide. B. Southern blot of the gel hybridized with probe a in Fig. 1.

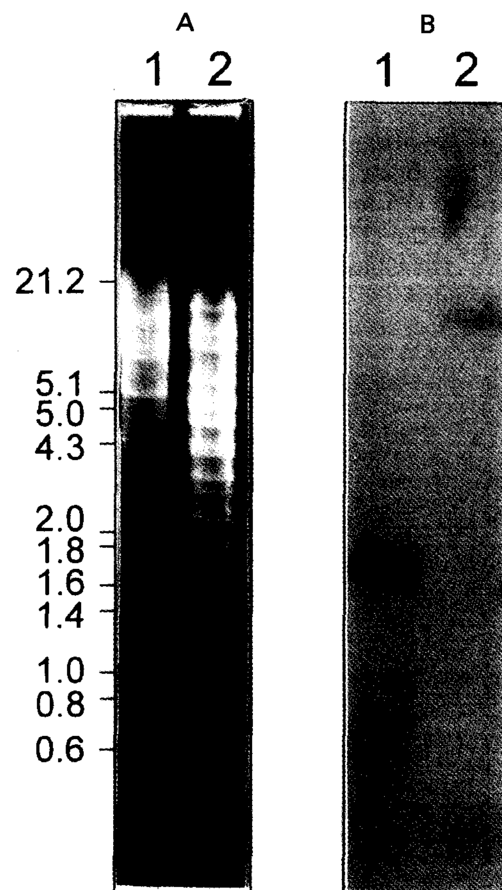

Fig. 5. Restriction digestion of mitochondrial genomic DNA and Southern hybridization probed with a 1.6 kb *Eco*RI fragment of mlp1. The size of DNA marker is shown in kb. A. Mitochondrial genomic DNA digested with *Eco*RI (lane 1) or *Hin*dIII (lane 2) was electrophoresed on 0.8% agarose gel and stained with ethidium bromide. B. Southern blot of the gel hybridized with the same probe as in Fig. 4.

which does not cut mlp1 was hybridized with the same probes as above, there was no hybridized band either (data not shown).

In *Tilletia caries* (22), it has been shown that no sequence homology exists between linear plasmids and either mitochondrial or nuclear DNA. In *Fusarium oxysporum*, linear plasmids were not homologous to its mitochondrial DNA (24). However, homology has been found between plasmids and mitochondrial DNA in higher plants such as *Z. mays* (19), and in fungi such as *Brassica campestris* (42), *N. intermedia* (4), *C. purpurea* (41), *Agaricus bitorquis* (34, 38), *Cochliobolus heterotrophus* (9), and *Aspergillus austeolodamin* (28). In *P. anserina*, sequences homologous to the linear mitochondrial plasmid were found in the nuclear (44) as well as mitochondrial DNA (14).

The kalilo plasmid of *N. intermedia* and the S plasmids of maize appear to be capable interacting actively with their host mitochondrial DNAs by integrating as full-length molecules (4). The S plasmids integrate into the mitochondrial regions homologous to terminal regions of the plasmids. On the other hand, the internal portions of the mitochondrial plasmids of *B. campestris* (42) and of *C. purpurea* (41) hybridize with mitochondrial DNA. This raises interesting questions about the origin of plasmid-homologous fragments and the possible functions that these fragments may have within the mitochondrial DNA.

Homology between mlp1 and other related genes for RNA or DNA polymerases

In Fig. 2, amino acid sequences of a 1.6 kb *Eco*RI fragment exhibit similarity to RNA polymerase of virus, fungi, and plants. To detect any homology in nucleotide sequences between RNA polymerase genes, we performed Southern hybridization. pRT19 contains a portion of RNA polymerase gene from linear plasmid pClK1 of *C. purpurea* (16) and pJH 41 contains a portion of RPO41 gene for mitochondrial RNA polymerase in *S. cerevisiae* (10, 18, 30). In spite of their homology in amino acid sequences, no DNA-DNA hybridization was detected using the 1.6 kb *Eco*RI fragment as probe (data not shown). Also, in the case of DNA polymerase gene, a 2.5 kb *Hin*dIII/*Hin*cII fragment (probe b in Fig. 1) did not hybridize with pDT19 which contains a portion of DNA polymerase gene from pClK1 of *C. purpurea* (data not shown). These negative results may be explained by the relatively low similarity (19.2%, 21.7%, 19.9% identity in RPO41, pClK1 RNA polymerase, and pClK1 DNA polymerase, respectively) in amino acid sequences aligned in Fig. 2 and 3.

Acknowledgments

We thank Prof. P. Tudzynski for providing pRT19 and pDT19 plasmids and Prof. S.-H. Jang for providing pJH41 plasmid. This work was supported by the Korea Science and Engineering Foundation through the Research Center for Molecular Microbiology at Seoul National University.

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