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Organization of Ribosomal RNA Genes from a Loofah Witches' Broom Phytoplasma

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ABSTRACT

Using the technique of integrative mapping with three vectors carrying chromosomal rDNA sequences, one of two rRNA operons of loofah witches' broom (LfWB) phytoplasma was constructed. This is the first complete rRNA operon of a phytoplasma to be reported. The operon has a context of 5′-16S-23S-5S-3′ with a $tRNA^{Ile}$ gene in the ITS and $tRNA^{Val}$ and $tRNA^{Asn}$ genes downstream from the 5S rRNA gene. Although the other operon has not been cloned, the DNA sequence of a PCR-amplified product shows that it has no $tRNA^{Ile}$ gene in the ITS region. The complete nucleotide sequences of 16S, 23S, and 5S rDNA are 1538, 2864, and 113 bp, respectively. Five −10-like sequences, but no −35 sequences, were found within a 494-bp leader region. There was a TG dinucleotide two nucleotides upstream from each −10-like sequence. The existence of a TG dinucleotide at this position has been reported to enhance the efficiency of a promoter without a −35 region. The regions immediately flanking the 5′ and 3′ ends of 16S and 23S rDNA can form long basepaired stems that contain sites for processing by RNase III. No obvious sequence for a rho-dependent or rho-independent termination site was found downstream from the $tRNA^{Asn}$ gene. The transcription may stop within a pyrimidine-rich region, as has been reported for several polypeptide-encoding genes and rRNA operons of archaeobacteria. The presence of the tRNA genes downstream from the 5S rRNA gene in the rRNA operon of LfWB phytoplasma further supports the hypothesis that phytoplasmas are phylogenetically closer to acholeplasmas than to mycoplasmas. The phylogenetic relatedness of LfWB phytoplasma to other phytoplasmas is discussed on the basis of the nucleotide sequence of rRNA genes and ITS.

INTRODUCTION

PHYTOPLASMAS, also known as mycoplasma-like organisms (MLOs), are a group of bacterial pathogens that are wall-less and obligate parasites in the phloem of plants. They have small genomes, ranging from 500 to 1700 kbp, and a relatively low G+C content (Razin, 1985). Inability to culture these organisms *in vitro* makes studies of their biologic and biochemical properties quite difficult, resulting in uncertainty about their taxonomy. Although they are classified as members of Class *Mollicutes* (Sears and Kirkpatrick, 1994), a genus cannot be assigned to them because of the lack of enough information (Tully, 1993).

Recently, molecular genetic analyses have increasingly been employed to identify and differentiate phytoplasmas. Because rRNAs are abundant and their sequences are conserved, the nucleotide sequences of rRNA genes and their PCR DNA products are often used to define these organisms' taxonomy and phylogeny (Lim and Sears, 1989; Kirkpatrick *et al.*, 1992; Kuske and Kirkpatrick, 1992; Gundersen *et al.*, 1994; Razin *et al.*, 1998). The organization of rRNA genes within an operon has been demonstrated to provide additional genetic evidence for the coherence of grouping (Ree *et al.*, 1989; Menke *et al.*, 1991). *Escherichia coli* and *Bacillus subtilis* contain, respectively, 7 and 10 copies of rRNA operons (*rrn*s) in the genome, with a gene order of 5′-16S-23S-5S-3′ (Lindahl and Zengel, 1986; Jinks-Robertson and Nomura, 1987; Srivastava and Schlessinger, 1990; Itaya, 1993). Although this gene order is common, it is not universal. There are three *rrn*s in *Thermus thermophilus* with the order of 5′-16S-3′, 5′-23S-5S-3′, and 5′-23S-5S-3′ (Srivastava and Schlessinger, 1990). Most mollicutes have two copies of *rrn*s with the same gene order as *E. coli* (Bove, 1993). However, *Mycoplasma hyopneumoniae* has only one copy of *rrn*, and the 5S is located far away from the

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16S-23S (Taschke *et al.*, 1986). The rRNA genes in *M. gallisepticum* are separated into three transcription units with the gene order of 5′-16S-23S-5S-3′, 5′-23S-5S-3′, and 5′-16S-3′ (Chen and Finch, 1989). Schneider and Seemuller (1994) analyzed 28 phytoplasmas and found that they all contained two copies of *rrn*s. However, the Western X-disease phytoplasma has only one copy (Kirkpatrick *et al.*, 1987).

Another feature of the *rrn*s is the presence of tRNA genes in the internal transcribed spacer (ITS) between the 16S and 23S rRNA genes. In this region, all of the seven *rrn*s in *E. coli* carry two tRNA genes, whereas only one of the 10 *rrn*s in *B. subtilis* carries two tRNA genes (Ogasawara *et al.*, 1983; Gurtler and Barrie, 1995). No tRNA genes are found in this region of mollicutes with the exception of *Acholeplasma laidlawai* and phytoplasmas (Razin *et al.*, 1998). The ITS in one of two *A. laidlawii rrn*s contains two tRNA genes (Nakagawa *et al.*, 1992). Twenty-six phytoplasmas analyzed by Smart *et al.* (1996) carried one tRNA gene in the ITS.

To further understand the evolution and structure of the phytoplasma genome, we have isolated and characterized several genes from a loofah witches' broom (LfWB) phytoplasma. This paper describes the cloning, mapping, and molecular characterization of an *rrn*. This is the first complete *rrn* to be reported for a phytoplasma. Characterization of the *rrn*s in LfWB phytoplasma should help us to elucidate the regulation of phytoplasma rRNA transcription and provide a close insight into the organisms' phylogeny.

MATERIALS AND METHODS

Bacteria and plant

The LfWB phytoplasmas were maintained by graft inoculation in periwinkles (Chen and Ho, 1997). The original diseased plant was provided by Dr. H.-J. Su, Professor of Department of Plant Pathology, National Taiwan University, Taiwan.

Plant and phytoplasma DNA isolation

DNA from healthy or LfWB phytoplasma-infected periwinkle was isolated using the procedure described by Ko and Lin (1994) and Kollar *et al.* (1990) with minor modifications. The plant branches (15 g) were disinfected with 1% sodium hyperchloric acid, rinsed with distilled water, and then briefly dried with tissue paper. Liquid nitrogen was added to a cooled mortar containing plant tissues, and the tissues were ground by a pestle and then by a coffee grinder. The powder was suspended in 15 ml of extraction buffer (500 mM NaCl, 100 mM Tris HCl, pH 8.0; 10 mM EDTA, 1% N-lauroyl sarcosine) and incubated at 55°C for 2 h. After centrifugation at 10,000 × *g*, at 4°C for 20 min, the supernatant liquid was mixed with 0.6 vol of isopropanol. The mixture was chilled at −20°C for 30 min, and the DNA was precipitated at 10,000 × *g* at 4°C for 20 min. The pellet was resuspended in 5 ml of TE (10 mM Tris HCl, 1 mM EDTA, pH 8.0) containing 0.5% SDS and 500 μg of proteinase K. After incubation at 37°C for at least 1 h, 875 μl of 5 M NaCl and 700 μl of CTAB/NaCl solution (10% CTAB, 0.7 M NaCl) were added. The incubation was continued for another 10 min at 65°C. The mixture was extracted with chloroform-isoamyl alcohol (24:1) three times and then with phenol–chloroform-isoamyl alcohol (25:24:1) twice. The supernatant liquid was mixed with 0.6 vol of isopropanol and chilled at −20°C for 1 h. DNA was precipitated at 12,000 × *g* at 4°C for 20 min. The pellet was rinsed with 75% alcohol, air dried, and then resuspended in 1 ml of TE.

A CsCl-bisbenzimide density gradient was used to separate phytoplasma DNA from host plant DNA, as described by Harrison *et al.* (1991). The gradient was prepared as follows. The DNA solution (in TE), CsCl (g), and bisbenzimide (0.5 mg/ml in TE) were combined at a ratio of 9.5:10.5:1. After centrifugation at 55,000 rpm in a p65VT3 rotor (Hitachi Koki Co., Ltd., Japan) at 20°C for 16 h, the phytoplasma DNA was visualized under UV lights as the uppermost band in the gradient and was collected with a syringe. This DNA fraction was purified once more through the gradient and extracted three times with water-saturated butanol. After addition of three volumes of sterile distilled water and eight volumes of absolute alcohol and chilling at −20°C for 1 h, the DNA was precipitated. The DNA pellet was rinsed with 75% alcohol, dried, and dissolved in 500 μl of TE.

Phytoplasma genomic library construction and screening

The LfWB phytoplasma DNA was partially digested with *Eco*RI or *Hin*dIII and cloned into λZAP (ZAP Express System; Stratagene). Cell package extracts and *in vitro* packaging were prepared as previously described (Ho *et al.*, 1992). The library contained 7.6×10^4 plaque-forming units (pfu) per milliliter with greater than 95% recombinants determined in the presence of isopropyl-β-D-thiogalactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (Xgal). The library was screened for the rRNA gene by plaque hybridization using a ^{32}P-labeled rDNA fragment as the probe. The positive plaque areas were selected and rescreened until a single, isolated plaque could be picked up.

Blot hybridization analysis

Southern blot analysis was performed as previously described (Ho *et al.*, 1992) at 42°C in the presence of 50% formamide and 0.1% SDS. The filter was washed in 0.1× SSC (1× SSC: 15 mM NaCl, 15 mM sodium citrate) containing 0.1% SDS at 50°C. The hybridized bands were detected by exposing the filter to a PhosphoImager screen (PhosphoImager 425; Molecular Dynamics).

Polymerase chain reaction

Two primers—ITS1: 5′AGTCGTAACAAGGTATC3′ (nucleotides 1981~1997) and ITS2: 5′CTTAGTGCCAAGGCATC3′ (nucleotides 2300~2284)—flanking the ITS were made to amplify the DNA fragment from LfWB phytoplasma DNA or cloned DNA template in a PCR. The first cycle of PCR was as follows: denaturation at 94°C for 5 min, annealing at 45°C for 1 min, and extension at 72°C for 1 min. The reaction was continued for 35 cycles: denaturation at 94°C for 1 min, annealing at 45°C for 1 min, and extension at 72°C for 1 min. Finally, the reaction was at 72°C for 5 min. The products were subjected to gel electrophoretic analysis.

DNA sequence determination and analysis

For DNA sequencing, the recombinant phage was converted into a phagemid by *in vivo* excision according to manufacturer's instructions (Stratagene). The sequence of the DNA insert in the recombinant phagemid was determined by a DNA automated sequencer (ABI Prism Model 377, v. 3.0; Applied Biosystems) using a step-by-step procedure in which synthetic primers for forward sequencing were designed from previously released sequences. DNA sequence analysis was performed using DNAStar software (DNASTAR).

Accession Number

The nucleotide sequence of the DNA fragment containing the LfWB phytoplasma *rrn* was deposited in GenBank with Accession Number AF251150.

RESULTS

Cloning of rRNA operon

Clone R4 containing a 1.6-kb insert DNA was obtained using the insert DNA from clone 138 as a probe to screen an *Eco*RI genomic library of LfWB phytoplasma constructed in λ ZAP vector. Clone 138 contained the rRNA genes of *M. genitalium* and was provided by Dr. P.-C. Hu (University of North Carolina at Chapel Hill, NC, USA). The 1.6-kb insert DNA contained a portion of 16S rDNA, ITS, and one-third of the 23S rDNA. The second clone, H1, containing a 4.0-kb insert DNA, was obtained by screening a *Hin*dIII genomic library using the 1.6-kb insert DNA of clone 4 as a probe. This insert DNA carried the complete 16S rDNA and its upstream portion, ITS, and two thirds of 23S rDNA. In order to construct a complete LfWB phytoplasma *rrn*, a 3′ 1.0-kb *Eco*RI-*Hin*dIII fragment from the 4.0-kb insert DNA was used as a probe to screen the *Eco*RI genomic library. Thus, the third clone, R6, containing a 6.0-kb insert DNA, was obtained. The insert DNA contained the other third of 23S rDNA, the entire 5S rDNA, two tRNA genes, and a stretch of sequence whose function was unknown. From a comparison of the nucleotide sequences of the 4.0-kb and 6.0-kb DNA fragments with the *rrn*s of other mollicutes and gram-positive bacteria, the two fragments comprised a complete LfWB phytoplasma *rrn* with a context of 5′-16S-23S-5S-3′, representing a typical rRNA gene organization of prokaryotes (Fig. 1).

Copy number of rRNA operon in LfWB phytoplasma

To find out the copy number of *rrn* in LfWB phytoplasma, the genomic DNA digested with the restriction enzymes *Eco*RI, *Eco*RV, *Hin*dIII, and *Sca*I was probed with the 1.6-kb ^{32}P-labeled *Eco*RI DNA fragment. As shown in Figure 2, there were two bands in the *Eco*RI-digested DNA, one band in the *Eco*RV-digested DNA, and two bands in the *Hin*dIII- and *Sca*I-digested DNAs. The probe DNA contained two adjacent *Sca*I recognition sites and no *Eco*RV or *Hin*dIII recognition sites. The data indicate that there were two copies of *rrn*s: one copy contained a *Hin*dIII recognition site within the fragment corresponding to the probe, and the other (shown in Fig. 1) did not. Figure 3A shows the results of the analysis of the PCR products amplified from LfWB phytoplasma DNA and recombinant plasmid

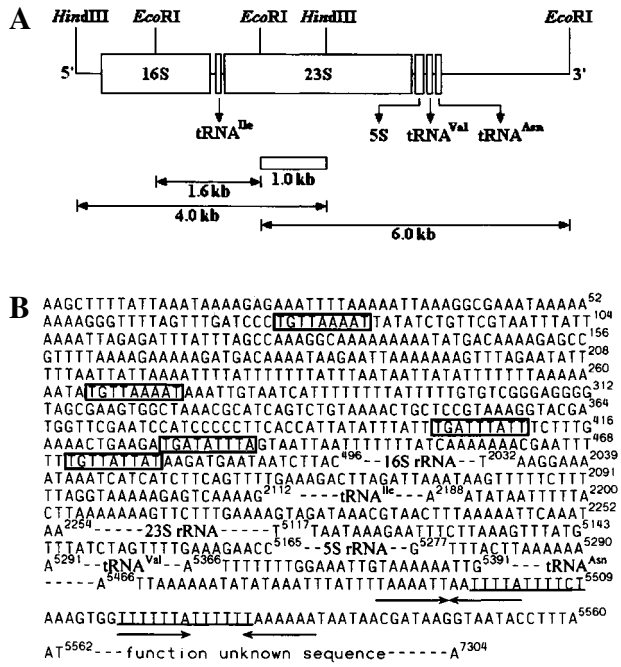

FIG. 1. Structure of *rrn*. (**A**) Restriction map of cloned *rrn*. The 1.6-kb, 4.0-kb, and 6.0-kb DNA fragments were inserted into clones R4, H1, and R6, respectively. The 1.0-kb *Eco*RI-*Hin*dIII fragment used as probe to screen the library for clone R6 is indicated by an open bar. (**B**) Nucleotide sequence of DNA fragment containing *rrn*. Only 7304 bp were determined. The coding regions of the 16S, 23S, and 5S rRNA genes, the tRNA genes, and most regions downstream from the tRNA^{Asn} gene are indicated by broken lines (not to scale). Five −10-like sequences and their immediate TG dinucleotides are boxed. The pyrimidine-rich sequences where the transcription possibly terminates are underlined. The inverted sequences are indicated by arrows.

DNA of clone R4 using primers flanking the ITS. There were two PCR products amplified from the LfWB phytoplasma DNA but only one product from the recombinant plasmid of clone R4. These data further support the presence of two *rrn*s in LfWB phytoplasma and indicate that the one that was not cloned had a short ITS. The data also explain the observation of two bands in *Eco*RI-digested DNA in Southern blotting. The DNA sequence analysis of the short ITS revealed a deletion of 53 nucleotides in the 5′ region of the tRNA^{Ile} gene (Fig. 3B).

Determination of the boundaries of the 16S, 23S, and 5S rRNA genes

The termini of the rRNA genes were determined by alignment of the nucleotide sequence or secondary structure with those of the respective rRNA gene of other microorganisms (Weisburg *et al.*, 1989; Ludwig *et al.*, 1992) (Fig. 4). The complete nucleotide sequence of the LfWB phytoplasma 16S rRNA gene was 1538 bp. Its 5′ and 3′ ends were located at C_{496} and U_{2032}, respectively. The 23S rRNA started at A_{2254} and ended at U_{5117} and had 2864 bp. The nucleotide sequences of 5S rRNA are not conserved, as are those of 16S and 23S rRNA. In this report, the boundaries of 5S rRNA were defined by comparing

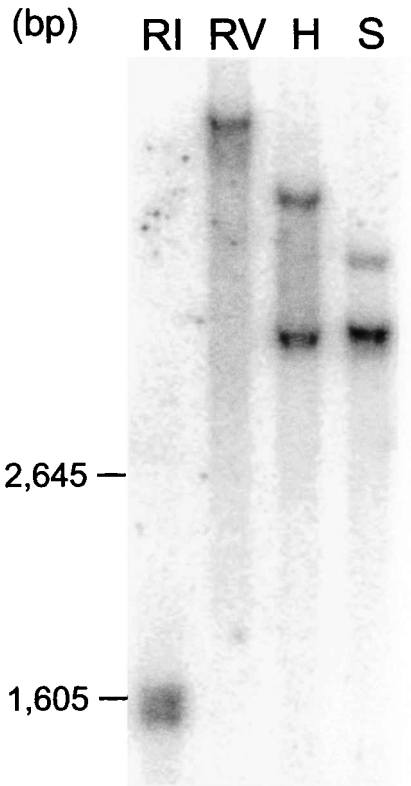

FIG. 2. Southern blot analysis of LfWB phytoplasma genomic DNA. The DNA digested with *Eco*RI (RI), *Eco*RV (RV), *Hin*dIII (H), and *Sca*I (S) was probed by a 1.6-kb ^{32}P-labeled *Eco*RI fragment. The 2,645 and 1,605 are DNA size markers (pGEM DNA marker; Promega).

its sequence with those of *B. subtilis* and *A. laidlawii*, which are phylogenetically close to phytoplasmas (Ogasawara *et al.*, 1983; Rogers *et al.*, 1985). Our 5S rRNA began at C_{5165} and ended at G_{5277} and had 113 bp.

Sequence analysis of the flanking and ITS regions of rRNA operon

To search for the promoter and termination and processing signals, the regions flanking each rRNA gene were analyzed. Five possible −10 sequences, but no −35 sequences, were found within a 494-bp *rrn* leader region (see Fig. 1). The regions immediately flanking the 5′ and 3′ ends of the 16S and 23S rDNA could form long basepaired stems that contained sites for processing by RNase III (Fig. 5). There was a tRNA^{Ile} gene in ITS and a tRNA^{Val} gene as well as a tRNA^{Asn} gene downstream from the 5S rRNA gene. No obvious sequence for a rho-dependent or rho-independent termination site was found downstream from the tRNA^{Asn} gene (see Fig. 1).

DISCUSSION

Currently, it is not possible to grow phytoplasmas in culture medium *in vitro*. In the absence of a proper cultivation method, characterization of cloned DNA fragments provides an important base for understanding the biochemical and physiological properties of these organisms in addition to delineating the structural organization of genes in the genome. The rRNA genes are particularly of interest because their products are essential to all cells, and their sequences are highly conserved. Although the 16S rRNA gene and ITS of phytoplasmas have been extensively investigated for the purpose of phylogenetic studies, no complete 23S rRNA and *rrn* have been reported.

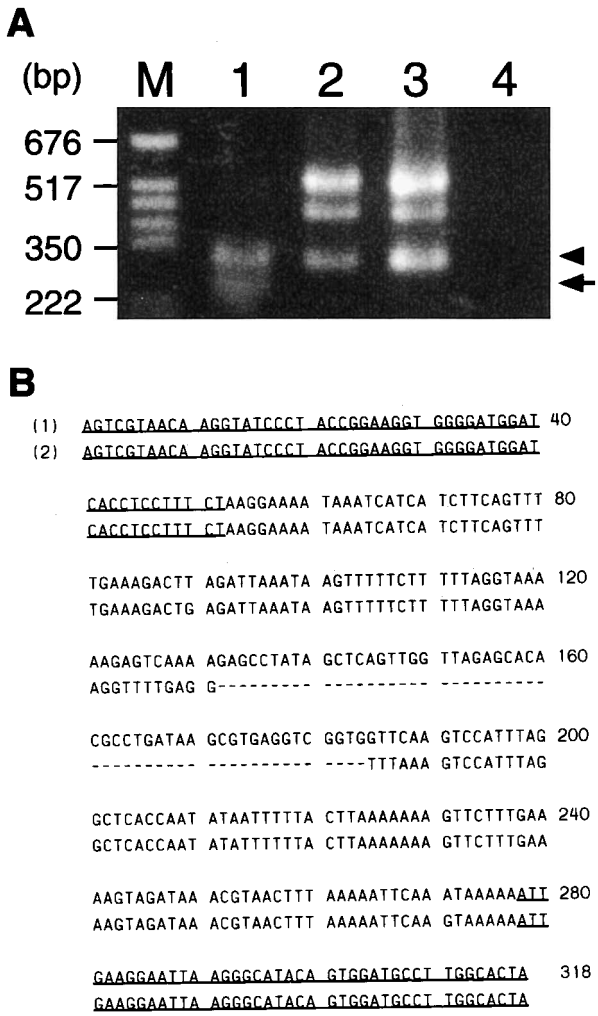

FIG. 3. Analysis of PCR products. (**A**) Gel electrophoretic analysis of products amplified from different DNA templates. Lane 1 = product from LfWB phytoplasma DNA; lane 2 = product from recombinant plasmid of clone R4; lane 3 = product from recombinant plasmid of other clone containing the same insert DNA as clone R4; lane 4 = PCR without a DNA template; lane M = DNA size markers (pGEM DNA marker; Promega). After electrophoresis, the agarose gel was stained with ethidium bromide. Arrowhead points to the DNA product containing ITS with a tRNA^{Ile} gene. Arrow points to the DNA product containing ITS without tRNA^{Ile} gene. (**B**) Nucleotide sequences of DNA products containing ITS. Line 1 = the DNA product with tRNA^{Ile} gene; line 2 = the DNA product without tRNA^{Ile} gene. Dash represents the deleted nucleotides in 5′ region of tRNA^{Ile} gene. Underlined nucleotides are the flanking 16S and 23S rDNA sequences, respectively.

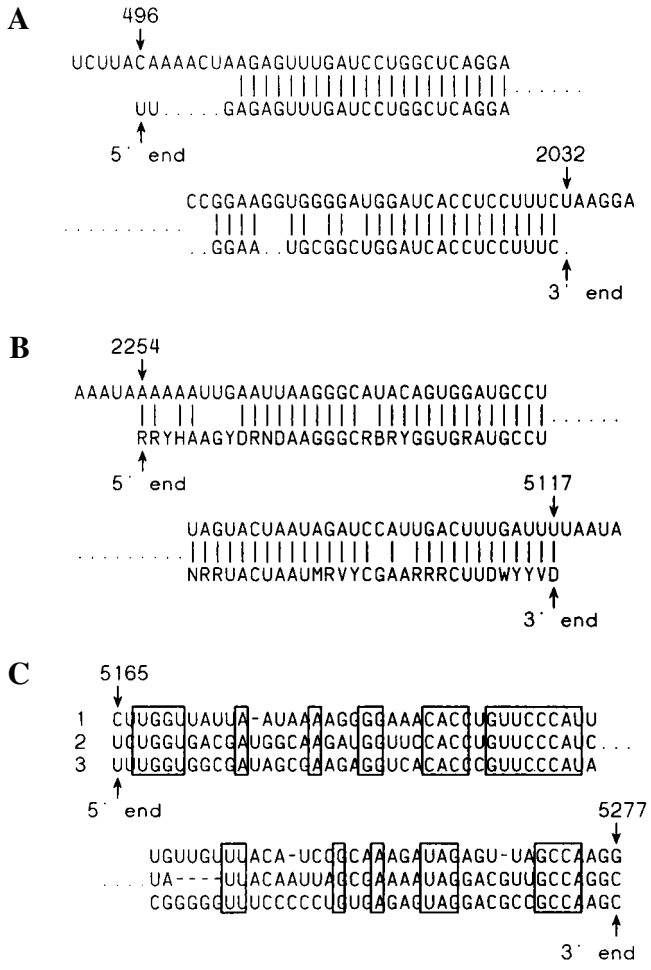

FIG. 4. Terminus determination of rRNA. The 5′ and 3′ portions of LfWB phytoplasma rRNAs were aligned with the corresponding regions of other bacterial rRNAs. (**A**) LfWB phytoplasma 16S rRNA (upper strand) with a 16S rRNA from a representative gram-positive bacterium (bottom strand). (**B**) LfWB phytoplasma 23S rRNA (upper strand) with a 23S rRNA from representative gram-positive bacterium (bottom strand). (**C**) The LfWB phytoplasma 5S rRNA (1) with 5S rRNAs of *A. laidlawii* (2) and *B. Subtilis* (3).

There are two copies of *rrn*s in phytoplasmas (Schneider and Seemuller, 1994) except the Western X-disease MLOs, which carry only one copy of *rrn* (Kirkpatrick *et al.*, 1987). In LfWB phytoplasma, our Southern blot and PCR analyses indicated that there were two copies of *rrn*s. The copy reported in this paper had a $tRNA^{Ile}$ gene in the ITS and no *Hin*dIII recognition site in the 1.6-kb DNA fragment containing the ITS (see Fig. 1). The other copy had a *Hin*dIII recognition site and no $tRNA^{Ile}$ gene in the 1.6-kb DNA fragment. To our knowledge, this is the second report that a phytoplasma ITS contains no $tRNA^{Ile}$ gene. The other one was that of StLL (Schneider *et al.*, 1999).

Because LfWB phytoplasmas are obligate parasites of plants and their rRNA genes have high similarity to those of plant chloroplasts and mitochondria, it is difficult to purify phytoplasma rRNA for terminus analysis using primer extension and nuclease S1 mapping. The 5′ and 3′ termini of the rRNA genes were defined by comparison of the nucleotide sequences or secondary structures with those of other microorganisms. The same approach was used to define the boundaries of the 16S, 23S, and 5S rRNA genes of *Rhodococcus facians* (Pisabarro *et al.*, 1998). The −10 promoter consensus sequence (TATAAT) found in prokaryotic *rrn*s was consistently found in the promoter regions of the mollicutes (Rasmussen *et al.*, 1987). However, it was not found in a 494-bp upstream region of LfWB phytoplasma 16S rDNA. Instead, there were five −10-like sequences in this region (Fig. 1). Although no −35 (TTGACA/T) sequence was found upstream from the −10-like sequence, there was a TG dinucleotide two nucleotides upstream from each −10-like sequence. Previous investigations have shown that the TG dinucleotide at this position is able to enhance the efficiency of a promoter without a −35 region (Keilty and Rosenberg, 1987; Ponnambalam *et al.*, 1988). *Mycoplasma hyopneumoniae* is reported to have the same promoter characteristics (Taschke and Herrmann, 1986). It is possible that each of five −10 sequences in LfBW phytoplasma can promote initiation of rRNA transcription. Alternatively, the one nearest the 16S rDNA is the true −10 sequence, and the other four can be postulated to have a modulating effect on transcription by facilitating the binding of RNA polymerase and the formation of the initiation complex.

Because of the short distance between the 5S rRNA gene and its downstream $tRNA^{Val}$ gene, it was unlikely that a promoter sequence lay within this region. This implied that the $tRNA^{Val}$ and $tRNA^{Asn}$ genes downstream from 5S rRNA were in the same transcription unit as the rRNA genes. No obvious sequence representing a typical rho-dependent or rho-independent termination site was found downstream from the $tRNA^{Asn}$ gene. Transcription termination at indiscrete sites has been re-

FIG. 5. Schematic structure of *rrn* and the putative stem structures surrounding the rRNA genes. The sites for processing by RNAse III are boxed.

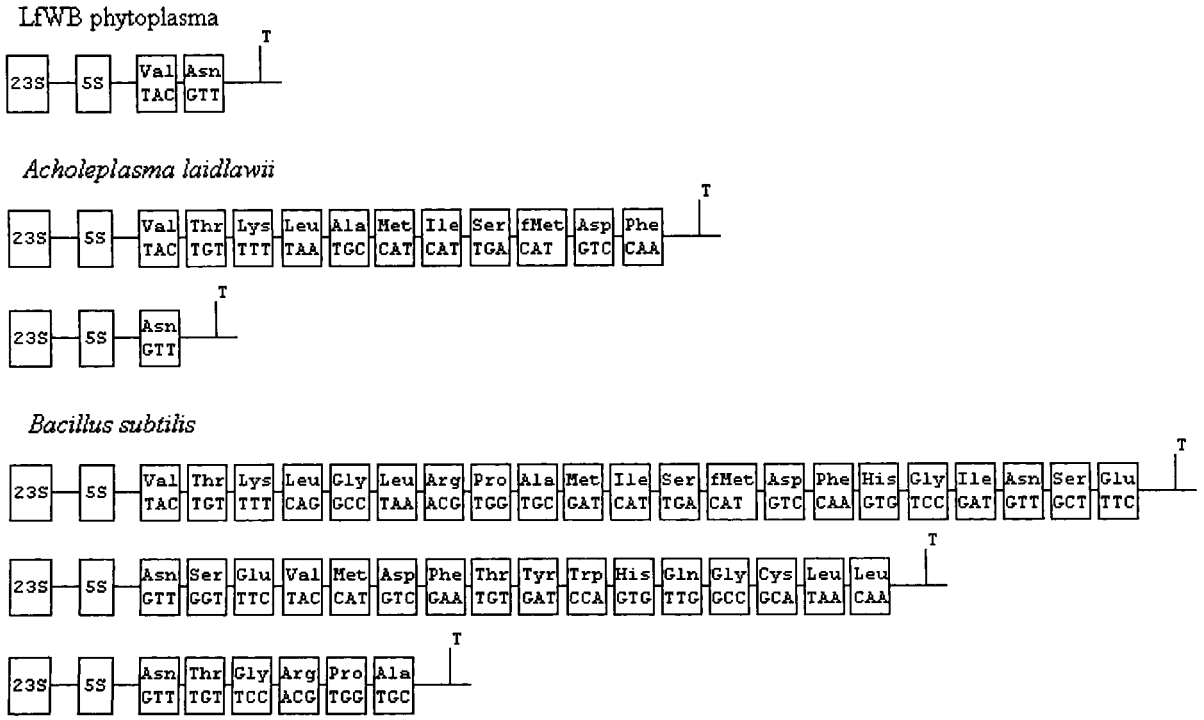

FIG. 6. The arrangement of tRNA genes downstream from the 5S rRNA genes of LfWB phytoplasma, *A. laidlawii*, and *B. subtilis*. The tRNA genes are boxed. T represents the transcription terminator.

ported for several polypeptide-encoding genes and *rrn*s of archaeobacteria, where transcription stops within or near a T-rich sequence (DasSarma *et al.*, 1984; Hui and Dennis, 1985; Chant *et al.*, 1986; Reiter *et al.*, 1988; Brown *et al.*, 1989). It was possible that the transcription of the LfWB phytoplasma *rrn* terminated in one of several pyrimidine-rich regions immediately downstream from the $tRNA^{Asn}$ gene (see Fig. 1). In *M. genitalium*, an *Mgpar* gene is located 100 bp downstream from the 5S rRNA gene (Fraser *et al.*, 1995). However, no ORF was found within a 3.5-kb region downstream from the last $tRNA^{Asn}$ gene in LfWB phytoplasma *rrn*.

Phytoplasmas are classified into 14 groups and 38 subgroups on the basis of restriction fragment length polymorphism analysis of PCR-amplified 16S rDNA sequences, and LfWB phytoplasma was assigned to 16SrVIII-A (Lee *et al.*, 1998). Schneider *et al.* (1999) placed LfWB phytoplasma and StLL in the same cluster within the AshY group on the basis of 16S rRNA sequence homology. Using the ITS DNA sequences of LfWB phytoplasma and the sequences cited in the paper of Schneider *et al.* (1999) and *Acholeplasma palmae* (AF005323) as an outgroup, a phylogenetic tree was constructed by cluster analysis. The LfWB phytoplasma and StLL were in the same cluster; however, the cluster was not in the AshY group (data not shown). This might be attributable to the fact that the ITS sequences were more divergent. In addition to the DNA nucleotide sequence, the arrangement of the tRNA genes downstream from the 5S rRNA gene was used to infer that acholeplasmas evolved from gram-positive bacteria with low G+G content (Tanaka *et al.*, 1991). So far, no tRNA gene has been found downstream from the 5S rRNA gene in mycoplasmas. There were two tRNA genes downstream from the 5S rRNA gene in LfWB phytoplasma. In *A. laidlawii*, there were 11 and 1 tRNA gene(s) in the two *rrn*s, respectively (Tanaka *et al.*, 1991). In *B. subtilis*, 3 of the 10 *rrn*s contained several tRNA genes (Srivastava and Schlessinger, 1990). Data shown in Figure 6 suggest that two tRNA genes in LfWB phytoplasma might have been derived from the 21 tRNA genes of *B. subtilis* by a deletion during evolution, a process that might have similarly occurred in acholeplasmas. These data further support the view that phytoplasmas are phylogenetically closer to acholeplasmas than to mycoplasmas.

Also found in the *rrn* of LfWB phytoplasma were features similar to those of *B. subtilis* (Ogasawara *et al.*, 1983), *M. hyopneumoniae*, and *M.* PG50 (Taschke and Herrmann, 1986; Rasmussen *et al.*, 1987). The regions immediately flanking the 5′ and 3′ ends of the 16S and 23S rDNA could form long base-paired stems that contain sites for processing by RNase III. This suggests that LfWB phytoplasma might have the same rRNA maturation process as in the other three bacteria.

In conclusion, there are two copies of *rrn*s in the LfWB phytoplasma genome, one with and the other without a $tRNA^{Ile}$ gene in the ITS. The copy presented in this paper was the one with a $tRNA^{Ile}$ gene in the ITS and is the first complete phytoplasma *rrn* to be reported. Our data should provide a base to elucidate the regulation of phytoplasma rRNA transcription and a close insight into phytoplasmas' phylogeny.

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