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REVIEW

# Sequence Analysis of the 16S-23S Spacer Region in rRNA Operons of the *Mollicutes*

## *Acholeplasma* has tRNA Genes in the Spacer Region but *Mycoplasma* does not

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*Received July 5, 1993*

We amplified the 16S-23S rRNA spacer region of various mollicutes by the polymerase chain reaction (PCR) and sequenced the PCR products. No tRNA-like sequences were detected in 11 *mycoplasma species and Ureaplasma urealyticum*, but one of the spacer regions of *Acholeplasma laidlawii* PG8 has sequences homologous to the spacer tRNA genes in *Bacillus subtilis*. This is the first evidence of tRNA genes between the 16S-23S rRNA intergenic spacer regions in the class *Mollicutes*.

KEY WORDS: Mycoplasma / Acholeplasma / rRNA / tRNA / PCR

### Introduction

The class *Mollicutes* is a group of the smallest prokaryotes known that are able to grow in cell-free media, lack a cell wall, and have very small genomes with a low G+C content (1, 2). Mollicutes have been analyzed phylogenetically by examination of their 5S rRNA (3, 4) and 16S rRNA (5) sequences.

The 5S and 16S rRNA genes of some *Mycoplasma* species have been sequenced, but the spacer regions between the rRNA genes have not, except for *M. capricolum* (6) and *M. hyopneumoniae* (7). These two species do not contain tRNA genes in their spacer regions, as *Escherichia coli* and *B. subtilis* do (8, 9), but they do have sequences homologous with the postulated recognition sequences of *B. subtilis* for rRNA processing (10). It is of interest to know whether these features are shared by the mollicutes. In a series of study we amplified the region between 16S and 23S DNA of 13 species of the mollicutes by using the polymerse chain reaction (PCR) (11, 12). The nucleotide sequences were identified and analyzed for the tRNA sequence and for other possible secondary structure.

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## Materials and Methods

### 1. *Mollicutes* strains and culture conditions

The *Mollicutes* strains used in this study were *M. arginini* G230, *M. arthritidis* PG6, *M. capricolum* california kid, *M. fermentans* PG18, *M. hominis* PG21, *M. hyopneumoniae* VPP11, *M. hyorhinis* BTS7, *M. neurolyticum* PG28, *M. orale* CH19299, *M. pulmonis* m53, *M. salivarium* PG20, *Ureaplasma urealyticum* T960, and *Acholeplasma laidlawii* PG8 (same progenitor as ATCC 23206). Each strain was grown as described previously (13) at 37℃ in mycoplasmal broth supplemented with 10% unheated horse serum, 5% yeast extract, and 1000 units per ml penicillin G potassium.

### 2. DNA preparation

The cells of mollicutes were harvested by centrifugation at 10,000 x *g* for 30 min and washed once with TE buffer (50 mM Tris-HCl, pH 8.0, and 10 mM EDTA). The washed cells from 1 liter of a culture were resuspended in 100 μl of TE buffer, and the DNA was extracted by hydroxyapatite batch elution (14).

DNA of *M. capricolum* was kindly supplied by Dr. S. Osawa of Nagoya University. Human placental DNA was purchased from Clontech, Inc. (Palo Alto, CA). Mouse DNA was extracted from a BALB/c mouse liver by hydroxyapatite batch elution (14).

### 3. PCR

PCR was performed in a DNA thermal cycler (Perkin Elmer-Cetus, Norwalk, CT) by use of a GeneAmp kit (Perkin Elmer-Cetus), with 1 ng of DNA used as a template. Denaturation was at 94℃ for 30 sec, annealing was at 55℃ for 2 min, and extension was at 72℃ for 1 min. This was repeated for a total of 30 cycles in a volume of 100 μl.

### 4. DNA sequencing

Amplified DNA was used as a template for sequencing in the "snap-cooling" procedure previously described (15). In brief, the amplified DNA and an end-labeled sequencing primer were first denatured and then annealed by a rapid decrease in the temperature. DNA was sequenced by the dideoxy chain-termination reaction (16) with a 7-deaza sequencing kit (Takara Shuzo, Kyoto, Japan). When the sequence was not identified in some positions of the sequence ladder, the amplified DNA was subcloned in pUC118 or pUC119 (17) and sequenced as described above.

### 5. Homology search

Amprogram, DNASIS, developed by Hitachi Software Engineering Co., Ltd. (Yokohama, Japan) was used to find the best alignment of pairs of similar sequences.

### 6. Primer preparation

Primers were synthesized by an automatic DNA synthesizer (Applied Biosystems, Foster City, CA) and purified by anion-exchange chromatography. The sequences of primers F0, R0, R16-2, and R23-1R are as follows:

F0: $^{5'}$GTAATCGCGAATCAGCTATG$^{3'}$
R0: $^{5'}$GACTTATCGCAGGTAGTCAC$^{3'}$
R16-2: $^{5'}$GGCTGGATCACCTCCTTTCT$^{3'}$
R23-1R: $^{5'}$CTCCTAGTGCCAAGGCATCC$^{3'}$

## Results

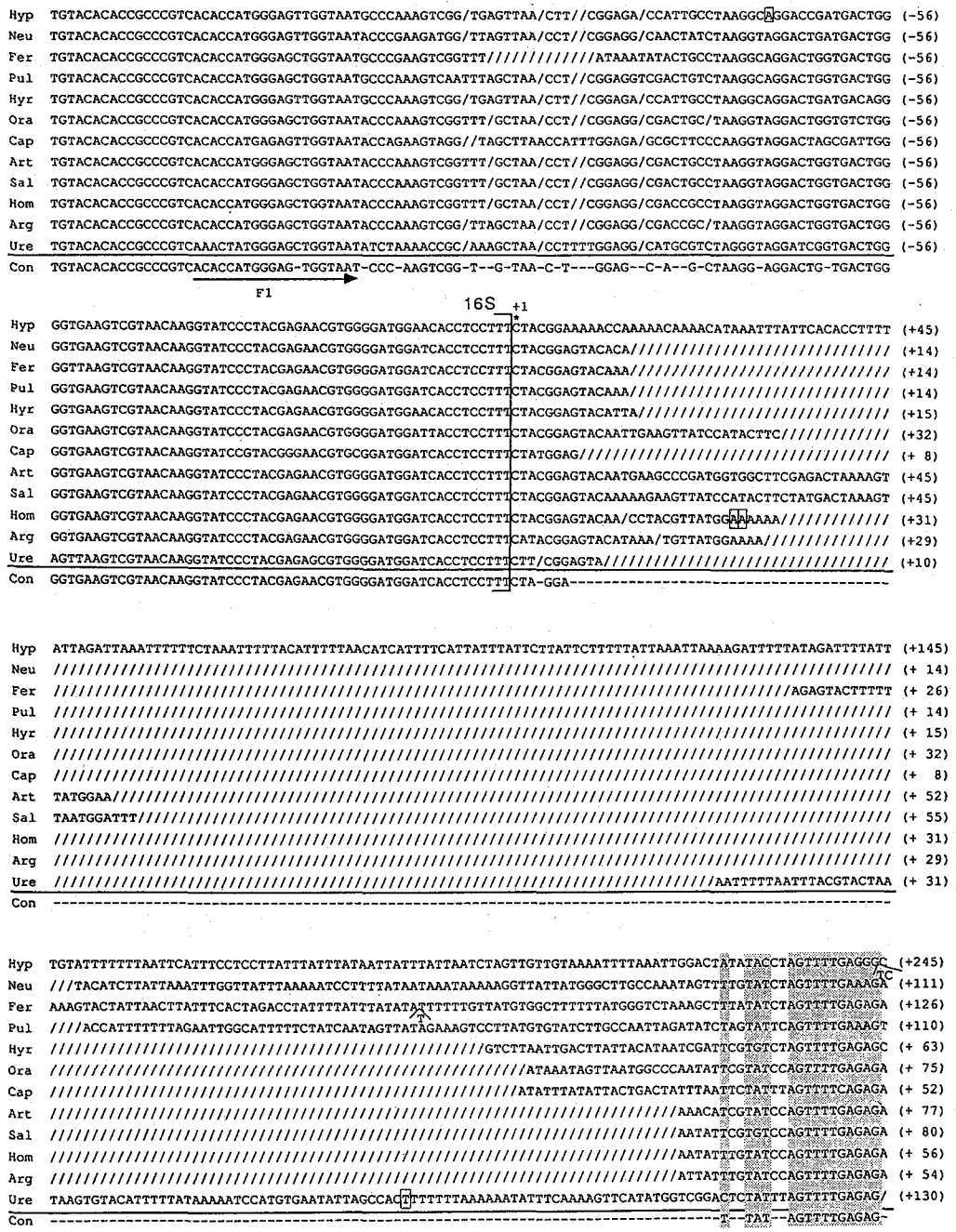
### 1. Amplification of the 16S-23S spacer sequences of 11 mycoplasmas and a ureaplasma

Sequences for the 16S and 23S ribosomal RNA conserved between *E. coli* and *M. capricolum* were identified by comparison of their published sequences (10, 18). PCR primers for the amplification of the spacer region of the mollicutes were selected from these conserved sequences, because sequences conserved in different divisions are likely to be conserved in the same family. The annealing temperature was set at 37℃, lower than the usual 55℃, to allow amplification even if the sequences of the primers were not identical with the corresponding sequences on the chromosome. Selected primers were designated F0 and R0; the positions of these primers in the 16S-23S region are shown in the legend for Fig. 1. Amplified fragments of 450 to 770 bp were obtained after 50 cycles when the genome DNAs from the 11 species of mycoplasmas and a ureaplasma, Ureaplasma *urealyticum* (shown in the legend for Fig. 1), were used as templates.

### 2. Comparison of nucleotide sequences of the 12 species

The nucleotide sequences of the amplified fragments were identified mainly by the direct sequencing of these fragments (Fig. 1), which were compared with each other (19). Sequences in the 16S and 23S regions were highly conserved among the 12 species, as expected. In contrast to the sequences in the 16S and 23S regions, those in the spacer regions diverged somewhat, and the size of the spacer regions was from 183 to 495 bp. By comparison of these spacer sequences, we found three sequences shared by all 12 species, which are shaded in Fig 1.

All of these spacer sequences were investigated for the secondary structure by using a SEQA program (20). Regions of significant sequence similarity were aligned by a combination of visual inspection. Hypothetical secondary structures were edited and plotted from the results deduced from the SEQA program. Several palindromic sequences, which may be responsible for pausing, were identified in the 16S-23S spacer regions of the 11 *Mycoplasma* species examined (Fig. 2). However neither tRNA genes nor their pseudogenes were found, and this is similar to eucaryotic spacers. A box A-like sequence was seen within the rRNA processing sites of all the 11 *Mycoplasma* species and it always located on the loop of the possible secondary stem-loop structures (Fig. 2). A short consensus motif 5'-CTTT (G/A) -3' of the box A is similar to a direct repeat in the controlling region of the *E. coli* lac operon.


Hyp TGTACACACCGCCCGTCACACCATGGGAGTTGGTAATGCCCAAAGTCGG/TGAGTTAA/CTT//CGGAGA/CCATTGCCTAAGGCAGGACCGATGACTGG (-56)
Neu TGTACACACCGCCCGTCACACCATGGGAGTTGGTAATACCCGAAGATGG/TTAGTTAA/CCT//CGGAGG/CAACTATCTAAGGTAGGACTGATGACTGG (-56)
Fer TGTACACACCGCCCGTCACACCATGGGAGCTGGTAATGCCCGAAGTCGGTTT//////////////ATAAATATACTGCCTAAGGCAGGACTGGTGACTGG (-56)
Pul TGTACACACCGCCCGTCACACCATGGGAGCTGGTAATGCCCAAAGTCAATTTAGCTAA/CCT//CGGAGGTCGACTGTCTAAGGCAGGACTGGTGACTGG (-56)
Hyr TGTACACACCGCCCGTCACACCATGGGAGTTGGTAATGCCCAAAGTCGG/TGAGTTAA/CTT//CGGAGA/CCATTGCCTAAGGCAGGACTGATGACAGG (-56)
Ora TGTACACACCGCCCGTCACACCATGGGAGCTGGTAATACCCAAAGTCGGTTT/GCTAA/CCT//CGGAGG/CGACTGC/TAAGGTAGGACTGGTGTCTGG (-56)
Cap TGTACACACCGCCCGTCACACCATGAGAGTTGGTAATACCAGAAGTAGG//TAGCTTAACCATTTGGAGA/GCGCTTCCCAAGGTAGGACTAGCGATTGG (-56)
Art TGTACACACCGCCCGTCACACCATGGGAGCTGGTAATACCCAAAGTCGGTTT/GCTAA/CCT//CGGAGG/CGACTGCCTAAGGTAGGACTGGTGACTGG (-56)
Sal TGTACACACCGCCCGTCACACCATGGGAGCTGGTAATACCCAAAGTCGGTTT/GCTAA/CCT//CGGAGG/CGACTGCCTAAGGTAGGACTGGTGACTGG (-56)
Hom TGTACACACCGCCCGTCACACCATGGGAGCTGGTAATACCCAAAGTCGGTTT/GCTAA/CCT//CGGAGG/CGACCGCCTAAGGTAGGACTGGTGACTGG (-56)
Arg TGTACACACCGCCCGTCACACCATGGGAGCTGGTAATACCCAAAGTCGG/TTAGCTAA/CCT//CGGAGG/CGACCGC/TAAGGTAGGACTGGTGACTGG (-56)
Ure TGTACACACCGCCCGTCAAACTATGGGAGCTGGTAATATCTAAAACCGC/AAAGCTAA/CCTTTTGGAGG/CATGCGTCTAGGGTAGGATCGGTGACTGG (-56)
Con TGTACACACCGCCCGTCACACCATGGGAG-TGGTAAT-CCC-AAGTCGG-T--G-TAA-C-T---GGAG--C-A--G-CTAAGG-AGGACTG-TGACTGG
F1
16S
+1
Hyp GGTGAAGTCGTAACAAGGTATCCCTACGAGAACGTGGGGATGGAACACCTCCTTTCTACGGAAAAACCAAAAACAAAACATAAATTTATTCACACCTTTT (+45)
Neu GGTGAAGTCGTAACAAGGTATCCCTACGAGAACGTGGGGATGGATCACCTCCTTTCTACGGAGTACACA////////////////////////////// (+14)
Fer GGTTAAGTCGTAACAAGGTATCCCTACGAGAACGTGGGGATGGATCACCTCCTTTCTACGGAGTACAAA////////////////////////////// (+14)
Pul GGTGAAGTCGTAACAAGGTATCCCTACGAGAACGTGGGGATGGATCACCTCCTTTCTACGGAGTACAAA////////////////////////////// (+14)
Hyr GGTGAAGTCGTAACAAGGTATCCCTACGAGAACGTGGGGATGGAACACCTCCTTTCTACGGAGTACATTA////////////////////////////// (+15)
Ora GGTGAAGTCGTAACAAGGTATCCCTACGAGAACGTGGGGATGGATTACCTCCTTTCTACGGAGTACAATTGAAGTTATCCATACTTC////////////// (+32)
Cap GGTGAAGTCGTAACAAGGTATCCGTACGGGAACGTGCGGATGGATCACCTCCTTTCTATGGAG////////////////////////////////////// (+ 8)
Art GGTGAAGTCGTAACAAGGTATCCCTACGAGAACGTGGGGATGGATCACCTCCTTTCTACGGAGTACAATGAAGCCCGATGGTGGCTTCGAGACTAAAAGT (+45)
Sal GGTGAAGTCGTAACAAGGTATCCCTACGAGAACGTGGGGATGGATCACCTCCTTTCTACGGAGTACAAAAAGAAGTTATCCATACTTCTATGACTAAAGT (+45)
Hom GGTGAAGTCGTAACAAGGTATCCCTACGAGAACGTGGGGATGGATCACCTCCTTTCTACGGAGTACAA/CCTACGTTATGGAAAAAA////////////// (+31)
Arg GGTGAAGTCGTAACAAGGTATCCCTACGAGAACGTGGGGATGGATCACCTCCTTTCATACGGAGTACATAAA/TGTTATGGAAAA////////////// (+29)
Ure AGTTAAGTCGTAACAAGGTATCCCTACGAGAGCGTGGGGATGGATCACCTCCTTTCTT/CGGAGTA////////////////////////////////// (+10)
Con GGTGAAGTCGTAACAAGGTATCCCTACGAGAACGTGGGGATGGATCACCTCCTTTCTA-GGA----------
Hyp ATTAGATTAAATTTTTTCTAAATTTTTACATTTTTAACATCATTTTCATTATTTATTCTTATTCTTTTTATTAAATTAAAAGATTTTTATAGATTTTATT (+145)
Neu ////////////////////////////////////////////////////////////////////////////////////////////////// (+ 14)
Fer ////////////////////////////////////////////////////////////////////////////////////////////AGAGTACTTTTT (+ 26)
Pul ////////////////////////////////////////////////////////////////////////////////////////////////// (+ 14)
Hyr ////////////////////////////////////////////////////////////////////////////////////////////////// (+ 15)
Ora ////////////////////////////////////////////////////////////////////////////////////////////////// (+ 32)
Cap ////////////////////////////////////////////////////////////////////////////////////////////////// (+ 8)
Art TATGGAA////////////////////////////////////////////////////////////////////////////////////////////// (+ 52)
Sal TAATGGATTT////////////////////////////////////////////////////////////////////////////////////////// (+ 55)
Hom ////////////////////////////////////////////////////////////////////////////////////////////////// (+ 31)
Arg ////////////////////////////////////////////////////////////////////////////////////////////////// (+ 29)
Ure ////////////////////////////////////////////////////////////////////////////AATTTTTAATTTACGTACTAA (+ 31)
Con ----------
Hyp TGTATTTTTTTAATTCATTTCCTCCTTATTTATTTATAATTATTTATTAATCTAGTTGTTGTAAAATTTTAAATTGGACTATATACCTAGTTTTGAGGGC (+245)
TC
Neu ///TACATCTTATTAAATTTGGTTATTTAAAAATCCTTTTATAATAAATAAAAAGGTTATTATGGGCTTGCCAAATAGTTTTGTATCTAGTTTTGAAAGA (+111)
Fer AAAGTACTATTAACTTATTTCACTAGACCTATTTTATTTATATATTTTTTGTTATGTGGCTTTTTTATGGGTCTAAAGCTTTATATCTAGTTTTGAGAGA (+126)
Pul ////ACCATTTTTTTAGAATTGGCATTTTTCTATCAATAGTTATAGAAAGTCCTTATGTGTATCTTGCCAATTAGATATCTAGTATTCAGTTTTGAAAGT (+110)
Hyr ////////////////////////////////////////////////////GTCTTAATTGACTTATTACATAATCGATTCGTGTCTAGTTTTGAGAGC (+ 63)
Ora ////////////////////////////////////////////////////ATAAATAGTTAATGGCCCAATATTCGTATCCAGTTTTGAGAGA (+ 75)
Cap ////////////////////////////////////////////////////ATATTTATATTACTGACTATTTAATTCTATTTAGTTTTCAGAGA (+ 52)
Art ////////////////////////////////////////////////////////////////////////////AAACATCGTATCCAGTTTTGAGAGA (+ 77)
Sal ////////////////////////////////////////////////////////////////////////////AATATTCGTGTCCAGTTTTGAGAGA (+ 80)
Hom ////////////////////////////////////////////////////////////////////////////AATATTTGTATCCAGTTTTGAGAGA (+ 56)
Arg ////////////////////////////////////////////////////////////////////////////ATTATTTGTATCCAGTTTTGAGAGA (+ 54)
Ure TAAGTGTACATTTTTATAAAAATCCATGTGAATATTAGCCAGTTTTTTTTAAAAAATATTTCAAAAGTTCATATGGTCGGACTCTATTTAGTTTTGAGAG/ (+130)
Con ----------T--TAT--AGTTTTGAGAG-

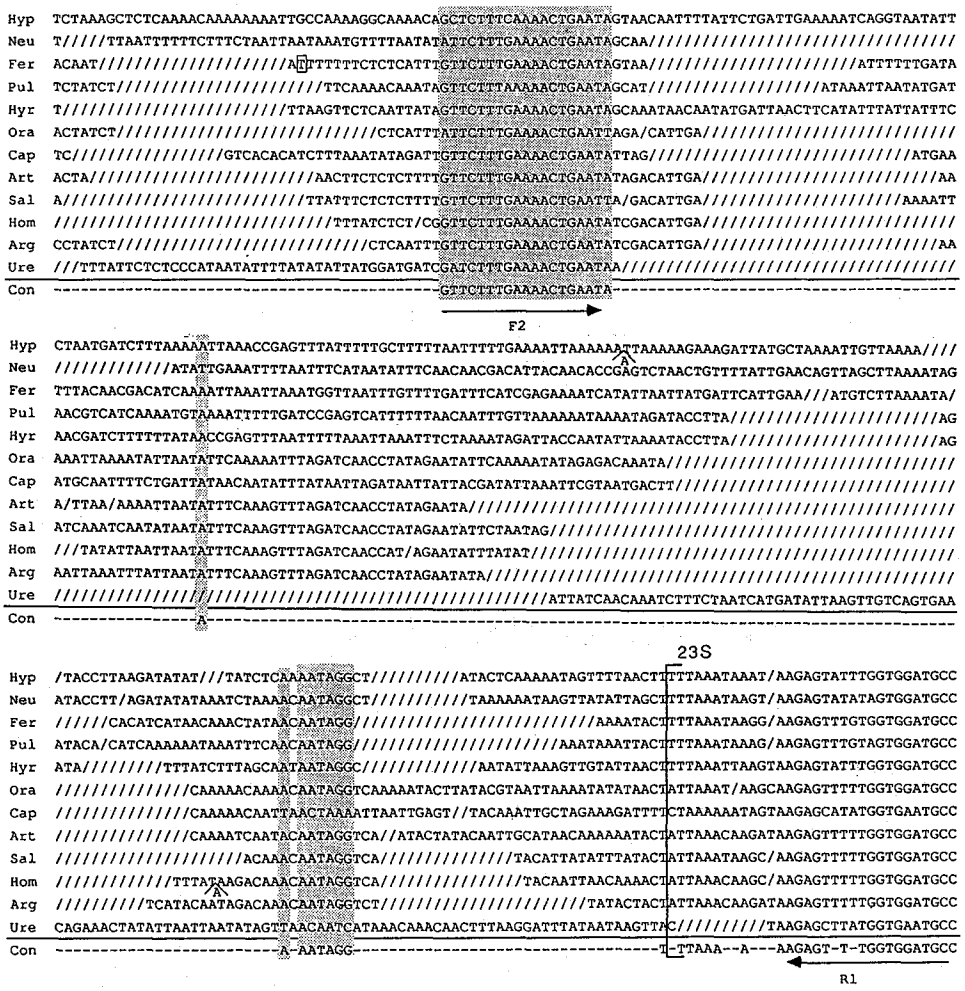



Fig. 1. Nucleotide sequences in the spacer region between the 16S and 23S coding sequences of the 12 mycoplasmal species. Their sequences are aligned from top to bottom in the order of *M. hyopneumoniae* (Hyp), M. neurolyticum (Neu), *M. fermentans* (Fer), *M. pulmonis* (Pul), *M. hyorhinis* (Hyr), *M. orale* (Ora), *M. capricolum* (Cap), *M. arthritidis* (Art), *M. salivarium* (Sal), *M. hominis* (Hom), *M. arginini* (Arg), and *U. urealyticum* (Ure). The sequence of M. capricolum is from Sawada et al (6). In the alignment of the sequences, deletions are introduced so that homologous sequences are lined up in the same column. Boundaries for the 12 species between the 16S or 23S rRNA sequence and the encompassed spacer sequence are drawn in the same position as that defined by Sawada et al (6) for *M. capricolum*. Sequences are numbered from the 5'-end of the spacer sequence. Negative numbers are given for bases on the 16S ribosomal RNA. Positions for the right-end residue are given on the right in each row. Squares are used to mark missing residues in the sequence of *M. hyopneumoniae* reported by Taschke and Herrmann (7) and in another set of sequences of *M. fermentans*, *M. hominis*, and *U. urealyticum*. Additional residues in those sequences are shown below each of the sequences with a mark that shows the insertion position. When 10 or more residues coincide in the same column, that residue was defined as having consensus and is listed in the bottom row. A dash is drawn if there was no consensus. Consensus sequences in the spacer region are shaded. The primer positions are shown by underlining.

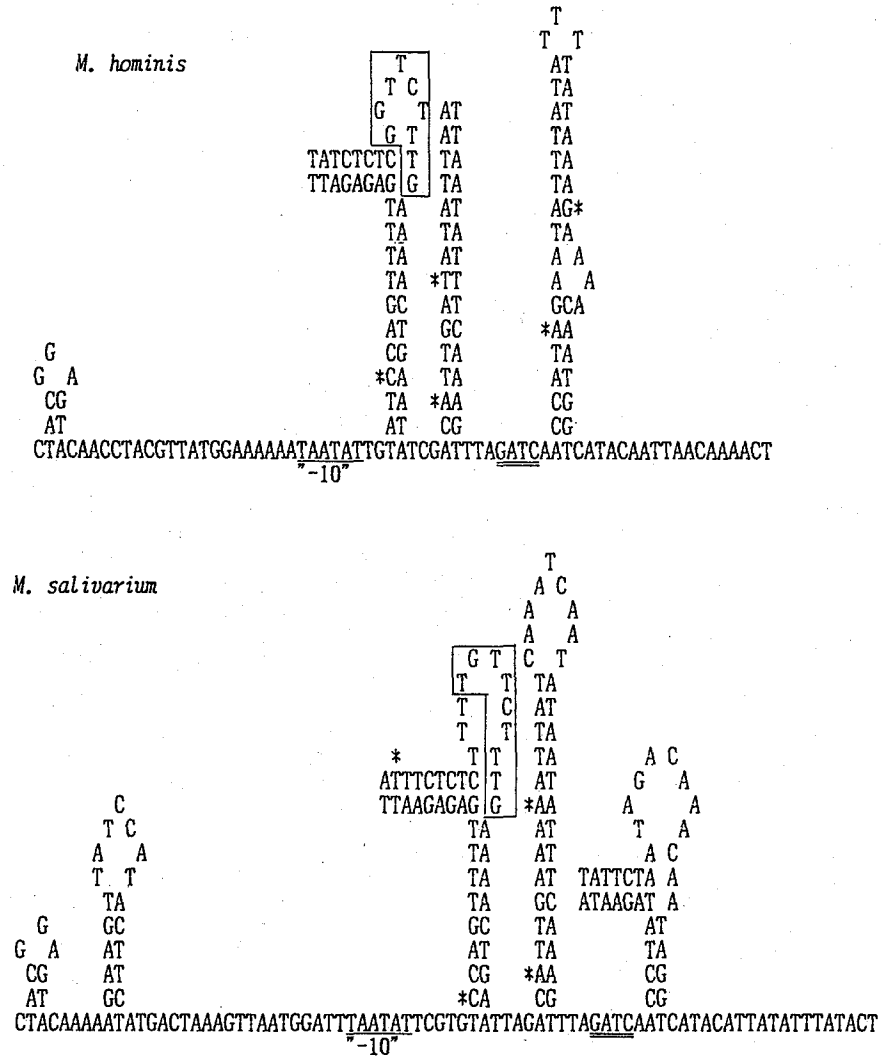



Fig. 2. Hypothetical secondary structure of the spacer region between 16S and 23S rRNA genes of *M. hominis* and *M. salivarium*. The 'box A'-like sequence is boxed. Mismatch pairing is shown by an asterisk.

### 3. Analysis of the 16S-23S spacer sequences of an acholeplasma, *Acholeplasma laidlawii*

PCR primers R16-2 and R23-1R, which can amplify the 16S-23S spacer region of 8 species of *Lactobacillus* including subspecies and non-identified species (21), were used to amplify the spacer region of *A. laidlawii*. Two discrete bands, which corresponds to 440 bp and 230 bp in length, were identified after amplification (Fig. 3). This result shows that

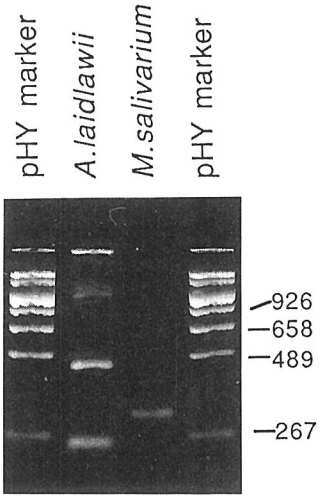



Fig. 3. Identification by polyacrylamide gel electrophoresis of the amplified fragments. *A. laidlawii* gives two discrete bands with a size of 440 bp and 233 bp. Under the same condition for PCR, *M. salivarium* gives a single band of 279 bp. Templates used (1 ng) are shown at the top. Sizes of the pHY markers at both sides are given at right.

*Acholeplasma* has at least two rRNA operons and each of them has different sequences in the 16S-23S spacer region. Larger DNA fragment was designated UL and smaller one was designated US. Each of the amplified products was recovered from an agarose gel and was used as a template for direct sequencing previously described (22).

The two amplified products share the same sequence at the region next to the primers, but DNA fragment of 238 or 31 nucleotide long was inserted between the common sequences in the case of UL or US, respectively (Fig. 4) (23).

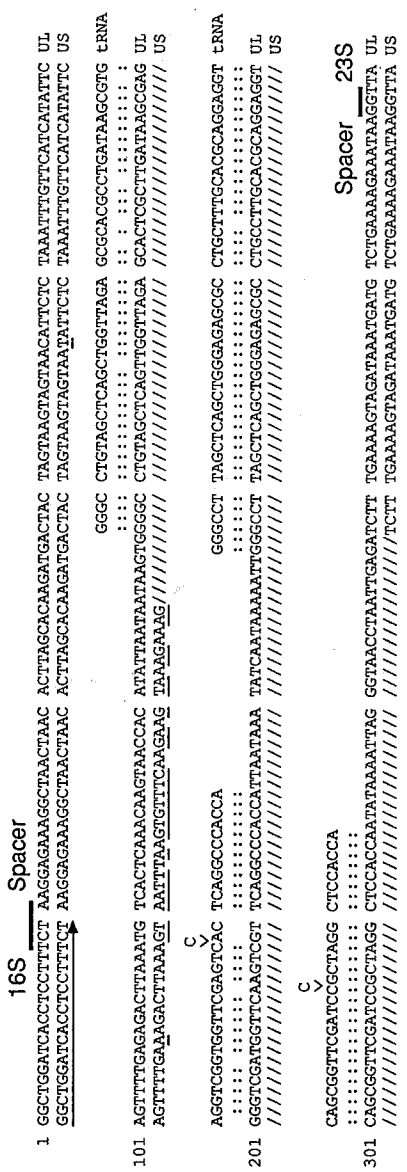



Fig. 4. Nucleotide sequence of the UL and US. Sequences of the UL and US and the tRNA sequence of *B. subtilis* (indicated by tRNA) are aligned to give maximum homology; deletion or insertions are given to the sequence of US or the tRNA sequence in the ribosomal RNA operon of *B. subtilis*, respectively. Deletion is represented by slashes in the line for US sequence and a insertion residue is shown above the tRNA sequence with an insertion signal. Sequences of US that are not coincident with those of UL are underlined. When a residue of the tRNA sequence are coincident with that of the UL insertion sequence, two dots (:) are given between sequence lines. Primer sequences used to amplify the UL and the US are shown by lines with an arrowhead and these sequences do not necessarily represent original sequences of *A. laidlawii*. Boundaries between the 16S or 23S ribosomal RNA sequence and the encompassed spacer sequence are maked by bold lines above the UL sequence at the same position as those defined by Loughney et al. for *B. subtilis* (25).

US has AT rich sequence as *Mycoplasma* (65.2%), but UL does not (57.7%). Because *Acholeplasma* species are considered to be phylogenetically close to *Bacillus* species (24), we compared the nucleotide sequence of the inserted sequence of UL with that of ribosomal RNA operon of *B. subtilis* (25). Two regions in the inserted sequence of UL were homologous to tRNA genes found in *B. subtilis*. 73 residues from the sequences that span from the position 255 to 328 in our coordinates are coincident with those of $tRNA^{Ala}$ (99%) and 66 residues from the sequences that span from the position 157 to 232 are coincident with those of $tRNA^{Ile}$ (87%). If these two regions are transcribed into RNA, they could have a cloverleaf structure of a $tRNA^{Ala}$ and a $tRNA^{Ile}$ (Fig. 5). In contrast, US does not contain tRNA sequence.

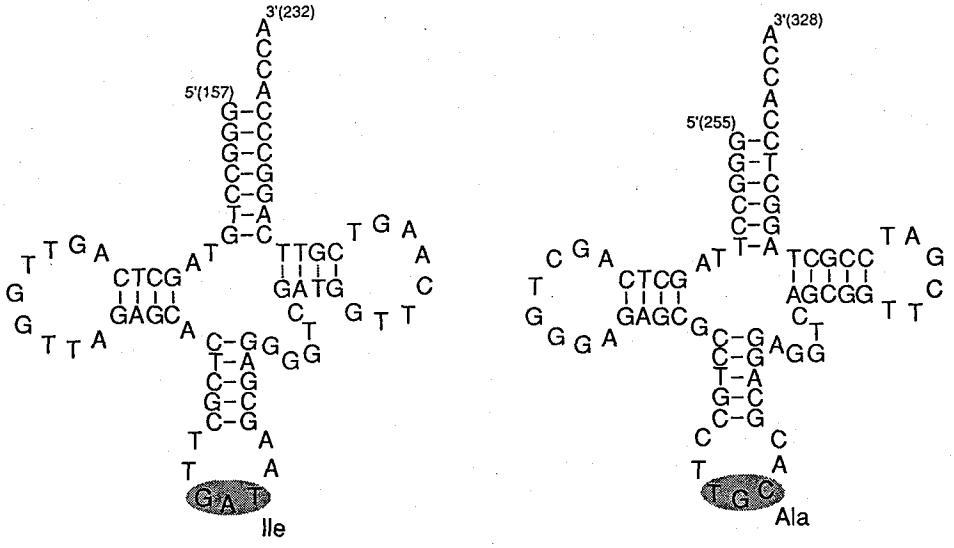



Fig. 5. Possible secondary structure of the insertion sequence in UL. Sequences that correspond to anticodons are shaded. The number given in parenthesis to 5'- and 3'- end residues corresponds to the coordinates in Fig. 1. The AT and GC parings are marked by solid lines and the GT paring is marked by a broken line

These results show that *Acholeplasma* has at least two types of rRNA operons, one with insertion of two tRNA genes ($tRNA^{Ile}$ and $tRNA^{Ala}$) and the other without insertion.

## Discussion

In the sequence analysis of the amplified fragments from *M. fermentans*, *M. hominis*, and *U. urealyticum*, there were two sets of sequence ladders in a restricted area and we found that one set was superimposed on the other. These two sets of ladders were identical, i.e. gave same sequence, but ladder positions in one set were shifted for one base from the other set. The presence of two sets of ladders in an area were observed when the sequences were analyzed from either end of the fragments. This means that these three species have two or more sets of the ribosomal RNA operon. This result concerning the number of operons in U. *urealyticum* is in agreement with the report by Amikam et al (26). *M. capricolum* has two or more sets of this operon (27). There may be multiple sets of this operon in the other species as well, because multiplicity of the operon cannot be detected by this procedure if the sets are identical.

Acholeplasmas are a group of mollicutes, which have been isolated from animals, plants, insects, and saprophytic environments, and are classified as a single genus *Acholeplasma* in the family *Acholeplasmataceae* of the order Acholeplasmatales under the class Mollicutes (28). They differ from most mycoplasmas in the lack of nutritional requirement for sterol and in their ability to grow at temperature as low as 22℃ (29). The genome size for the family *Acholeplasmataceae* species is about $1.9 \times 10^9$ daltons, compared with about $5.0 \times 10^8$ daltons for species of the family *Mycoplasmataceae*. Acholeplasmas as well as most mycoplasmas have been reported to possess low numbers of rRNA genes arranged in the procaryotic fashion, 5'-16S-23S-5S-3' (1). Some eubacteria such as *E. coli* or *B. subtilis* have been reported to include tRNA genes for isoleucine-alanine and/or glutamic acid in some 16S-23S intergenic spacer regions of rRNA operons (25, 30). Therefore, it was of interest to examine whether the genus *Acholeplasma*, which was considered to be phylogenetically closer to the gram-positive eubacteria, has tRNA genes in the 16S-23S intergenic spacer regions. We have examined the nucleotide sequence of the 16S-23S spacers in the two operons of *A. laidlawii*, type species, in the genus *Achopleplasma* and found tRNA genes in the spacer region. The sequences of mycoplasmal tRNA reported so far have more sequence similarity to the corresponding tRNA from *Bacillus* than to that from *Escherichia* (31, 32). The *E. coli* genome has seven rRNA operons which contain either a single $tRNA^{Glu}$ gene or adjacent $tRNA^{Ile}$ and $tRNA^{Ala}$ genes in the 16S-23S spacer region (33, 34). The *B. subtilis* genome has 10 rRNA operons; eight of them do not include any tRNA genes, but two rRNA operons include both the $tRNA^{Ile}$ and $tRNA^{Ala}$ genes (35, 25). Our observation supports that the spacer tRNA genes for isoleucine and alanine demonstrated in *A. laidlawii* have also more similarity to those of *B. subtilis* than to those of *E. coli*. One of the two copies of rRNA operons in plant pathogenic mycoplasma-like organisms (MLO) has been known to contain a single $tRNA^{Ile}$ gene in the 16S-23S spacer region (36). Sequence homology of the spacer $tRNA^{Ile}$ gene between *A. laidlawii* and MLO was 88.2%. It is currently unknown why the spacer tRNA genes are biased to encode isoleucine and alanine.

The 16S-23S spacer sequences of 11 mycoplasmas and a ureaplasma have been deposited to the EMBL Data Library under the accession numbers given in parentheses: *M hyopneumoniae* VPP11 (X58551), *M. neurolyticum* PG28 (X58552), *M. fermentans* PG18 (X58553), *M. pulmonis* m53 (X58554), *M. hyorhinis* BTS7 (X58555), *M. orale* CH19299 (X58556), *M.*

*arthritidis* PG6 (X58557), *M. salivarium* PG20 (X58558), *M. hominis* PG21 (X58559), *M. arginini* G230 (X58560), *U. urealyticum* T960 (X58561). The DDBJ / EMBL / GenBank accession number of UL sequence of *A. laidlawii* PG8 is D13259 and that of US sequence is D13260.

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