Comparison of 16S rRNA Genes within the T960 and Parvo Biovars of *Ureaplasmas* Isolated from Humans

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Two-by-two sequence alignment revealed that the levels of homology between 16S rRNA gene sequences of strains belonging to the two biovars of *Ureaplasma urealyticum* (class *Mollicutes*) ranged from 98.5 to 98.9%. Within the biovars, three serovars of the T960 biovar exhibited levels of homology of $\geq 99.7\%$, and the four serovars of the parvo biovar exhibited levels of homology of $\geq 99.7\%$. A dendrogram of the *Mycoplasma pneumoniae-Ureaplasma* clade of the *Mollicutes* reflected the distinctiveness of the biovars.

Ureaplasmas of the genital tract of humans can act as commensal organisms or pathogens (14). Although these organisms are currently classified as members of a single species, differences in phenotypic and genotypic characteristics clearly divide *Ureaplasma urealyticum* strains into two biovars (9). The parvo biovar comprises serovars 1, 3, 6, and 14 and the T960 biovar comprises serovars 2, 4, 5, and 7 through 13. A recent comparison of the nucleotide sequences of the 16S rRNA genes of serovar 3 and 8 strains, representing the two biovars, revealed a level of homology of 98.8% (13). Exploitation of the single region of multiple-nucleotide variation in these strains allowed us to develop primers for PCRs. Using a PCR, we identified to biovar all laboratory-adapted and wild-type strains of *U. urealyticum* that we examined (6, 13), and our results confirmed the genetic distinctiveness of each biovar. Our next goal was to determine the levels of homology of the highly conserved 16S rRNA gene within and between the two biovars.

The sources and identities of the 14 serovar standard strains of *U. urealyticum* have been described previously (12). The seven strains examined in this study are identified below. The T960 biovar was represented by serovar 2, 5, and 8 strains. Despite serological cross-reactivity (4, 11), serovars 2 and 5 represent the extremes of the genome sizes (5, 7, 10) and restriction map arrangements (10) found in the serovars of the T960 biovar; the serovar 8 strain is the type strain. Because taxonomic changes primarily would affect the designation of the parvo biovar, all four of its serovar standard strains were examined.

Primers which were used previously but now containing *Pst*I or *Xho*I restriction sites were used to amplify target DNA by a PCR. The amplicons were restricted at the *Pst*I and *Xho*I sites and were ligated into similarly restricted pBluescript II vector, which was then transformed into *Escherichia coli* LX1-blue. Transformants of the appropriate size were selected, and each insert was sequenced by the Sanger dideoxy sequencing reaction method. The sequences of the primers and the methods used have been described previously (3). Extraordinary care was taken to verify the sequence at those few sites where divergence occurred. For example, in one area of compression the nucleotide order was ascertained by the sensitivity of the site to restriction endonuclease activity, as confirmed by aga-

rose gel electrophoresis. During the analyses we made changes to the previously published sequence of the gene of the serovar 3 standard (13). These changes decreased the number of differing nucleotides in serovar 3 and 8 genes and thereby increased the calculated level of homology between the biovar representatives from 98.8 to 98.9%. DNA amplification by a PCR and subsequent cloning of the amplicon can introduce errors in the nucleotide sequence. In the final alignment we identified a few sites at which, because of the gain or loss of a single nucleotide, the sequence of a single strain differed from the 16S rRNA sequences of all other strains and all species used for the multiple alignment (Fig. 1). However, these questionable nucleotides did not affect our interpretation of the data, so sequencing additional clones was not necessary.

The *U. urealyticum* serovar 8 standard belongs to the *Ureaplasma* group which is part of the *Mycoplasma pneumoniae-Ureaplasma* clade of the *Mollicutes* (8). We aligned the 16S rRNA sequences of the seven *U. urealyticum* serovar standard strains with the 16S rRNA sequences of four *Mycoplasma* species, two of which belonged to the *M. pneumoniae* group and two of which belonged to the *Ureaplasma* group. A dendrogram (Fig. 1) was prepared, and this dendrogram indicated that the *Ureaplasma* group comprised two subgroups. One of these subgroups contained *Mycoplasma muris* (15) and *Mycoplasma penetrans* (1); the other contained *U. urealyticum*. The latter subgroup contained two clusters, one for the serovars of each biovar. The gene sequences of the parvo biovar cluster were more similar to the gene sequences of the *M. pneumoniae* group (data not shown).

Further examination of the data confirmed that the cytosine residue at position 923 in the *U. urealyticum* serovar 3 sequence, which characterizes the *M. pneumoniae-Ureaplasma*

TABLE 1. 16S rRNA sequence differences for *U. urealyticum* T960 biovar serovar 2, 5, and 8 standard strains^a

Standard strain of serovar:	Nucleotide at position:							% Homology to serovar 8 standard strain
	87	134	199	391	395	820	1448	
8	T	C	G	C	C	C	N ^b	100.0
2	— ^c	T	A	C	A	C	C	99.7
5	T	C	G	A	C	—	T	99.9

^a A total of 1,430 nucleotides were compared. The nucleotide positions were the nucleotide positions in the corrected sequence of the serovar 3 standard strain.

^b N, unidentified nucleotide (14).

^c —, missing nucleotide.

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TABLE 2. 16S rRNA sequence differences for *U. urealyticum* parvo biovar serovar 1, 3, 6, and 14 standard strains

Standard strain of serovar:	Nucleotide(s) at position(s):						% Homology to serovar 3 standard strain
	471	597 and 598	849	1342	1347	1426	
3	G	GT	C	G	A	G	100.0
1	A	GT	T	G	A	— ^b	99.8
6	G	GC	T	G	A	—	99.7
14	A	—T	C	A	G	—	99.7

^a A total of 1,422 nucleotides were compared. The nucleotide positions were the nucleotide positions in the corrected sequence of the serovar 3 standard strain.

^b —, missing nucleotide.

clade, was present in all of the strains tested (15). We also identified sequences unique to *U. urealyticum* that began at position 636 of the serovar 3 gene; 8 of the next 14 nucleotide positions had residues specific for the *U. urealyticum* strains and also for the *Mycoplasma* species that we examined. *U. urealyticum*, but not the *Mycoplasma* species, had two missing nucleotides after position 430 and a characteristic GT at positions 886 and 887. In addition, many single-nucleotide sites were also specific for *U. urealyticum*. The major biovar-specific sequences began at position 217. The gene of the parvo biovar was recognizable by eight residues, TCTTTGTG. The gene of the T960 biovar contained residues at only the first four of these eight positions, but these residues were also biovar specific; these residues were GTTT. This region of the gene was the basis of the differentiating PCRs described previously (12). The two *U. urealyticum* biovars had single-nucleotide differences at positions 128, 241, 848, 1015, 1131, and 1311, and all of the differences were transitions. Except at position 848, these differences were substitutions from G and C residues in the T960 serovars to A and T residues in the parvo serovars, respectively.

The 16S rRNA gene sequences of serovar 2 and 5 strains exhibited 99.7% or greater homology with the serovar 8 sequence (10) (Table 1), and the sequences of serovar 1, 6, and 14 strains exhibited 99.7% or greater homology with the serovar 3 sequence (8) (Table 2). The calculated levels of homology between strains belonging to the two biovars (Table 3) ranged from 98.5 to 98.9%. Thus, the levels of interbiovar homology were markedly lower than the levels of intrabiovar homology. Furthermore, while the interbiovar differences included sites where there were multiple variations, all intrabiovar variations (Tables 1 and 2) occurred at single nucleotide positions.

FIG. 1. Dendrogram based on alignment of the 16S rRNA gene sequences of seven serovars of *U. urealyticum* (Uu) and four species belonging to the *M. pneumoniae-Ureaplasma* clade of the *Mollicutes*. Serovars 2, 5, and 8 represented the T960 biovar, and serovars 1, 3, 6, and 14 represented the parvo biovar of *U. urealyticum*. The *Mycoplasma* species used were *M. penetrans* (pen), *M. muris* (mur), *M. pirum* (pir), and *M. pneumoniae* (pneu). The alignment was based on 1,444 nucleotides of the sequence of the serovar 3 standard strain of *U. urealyticum*. The dendrogram was constructed by using the software program Pileup obtained from the Genetics Computer Group, Inc., Madison, Wis.

The levels of total DNA homology between various serovars of *U. urealyticum* vary from 38 to 96% (2, 5); a break in this range occurs between 60 and 74%. This distribution has contributed genotypic evidence which supports the division of the ureaplasmas into two biovars. The development of the biovar-specific PCR based on the 16S rRNA of a representative of each biovar and subsequent evidence that clinical isolates could invariably be assigned to one of the two biovars (6, 13) further supports the phylogenetic division of the current species. Our data confirm the genetic distinctiveness of multiple serovar strains within each biovar. The taxonomic status of *Ureaplasma* strains isolated from humans is now being reviewed.

The GenBank accession numbers of the sequences of serovar 8 standard strain ATCC 27618 and the serovar 3 standard strain (progenitor of ATCC 27815) are M23935 and L08642, respectively. The amended serovar 3 sequence and five more recently determined nucleotide sequences have also been submitted to the GenBank database. The accession numbers of the latter sequences are as follows: serovar 1 (progenitor of ATCC 27813), U06094; serovar 2 (progenitor of ATCC 27814), U06096; serovar 5 (progenitor of ATCC 27817), U06097; serovar 6 (progenitor of ATCC 27818), U06098; and serovar 14 (ATCC 33697), U06095. The GenBank accession numbers of *M. pneumoniae*, *Mycoplasma pirum*, *M. muris*, and *M. penetrans* sequences are M29061, M23940, M23939, and L10839, respectively.

TABLE 3. Relative levels of homology for 16S rRNA genes of selected serovar standard strains of *U. urealyticum*^a

Standard strain of serovar:	% Homology						
	Parvo biovar				T960 biovar		
	Serovar 1 strain	Serovar 3 strain	Serovar 6 strain	Serovar 14 strain	Serovar 2 strain	Serovar 5 strain	Serovar 8 strain
1	100.0						
3	99.8	100.0					
6	99.8	99.8	100.0				
14	99.6	99.6	99.6	100.0			
2	98.6	98.6	98.6	98.5	100.0		
5	98.6	98.9	98.6	98.6	99.6	100.0	
8	98.8	98.9	98.9	98.7	99.7	99.8	100.0

^a After we eliminated the data for all positions with missing or unspecified residues, 1,394 nucleotides were available for comparison.

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