Phylogenetic Analysis of Genes Coding for 16S rRNA in Mammalian *Ureaplasmas*

RYÔ HARASAWA^{1*} AND GAIL H. CASSELL²

Animal Center for Biomedical Research, Faculty of Medicine, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan,¹ and Department of Microbiology, The University of Alabama at Birmingham, Birmingham, Alabama 35294-2170²

Phylogenetic relationships among species of the genus *Ureaplasma* were elucidated by analyzing 16S rRNA sequence information. The 16S rRNA genes of six strains of the mammalian *Ureaplasma* species were amplified by PCR and were sequenced directly by a primer walking method. The phylogenetic tree based on the nucleotide sequence of the 16S rRNA genes corresponded to the evolutionary history of the host animal species.

The genus *Ureaplasma* in the family *Mycoplasmataceae* is composed of organisms that hydrolyze urea. The following six species have been recognized in the genus *Ureaplasma*: *Ureaplasma urealyticum*, isolated from humans; *Ureaplasma diversum*, isolated from bovines; *Ureaplasma gallorale*, isolated from birds; *Ureaplasma felinum* and *Ureaplasma cati*, isolated from cats; and *Ureaplasma canigenitalium*, isolated from dogs. The accumulated data suggest that the human species *U. urealyticum* consists of two different clusters, the T960 and parvo biovars, which can be distinguished on the basis of the results of polyacrylamide gel electrophoresis of cell proteins (12, 20), DNA-DNA homology data (1), restriction endonuclease cleavage patterns (13), restriction fragment length polymorphism data (4), manganese susceptibility data (14), and DNA modification system data (2). The genome sizes of *U. urealyticum* strains have been estimated to be 760 to 1,170 kbp by pulsed-field gel electrophoresis (9). Two loci for rRNA operons have been determined on the genome of *U. urealyticum* (3). In the present study, we sequenced and compared the 16S ribosomal DNAs (rDNAs) of the mammalian *Ureaplasma* species, including the two biovars of *U. urealyticum*, because phylogenetic analysis of the mycoplasmas has been greatly improved by analyzing 16S rRNA sequences (21).

U. diversum A417^T (T = type strain) was obtained from C. J. Howard and R. N. Gourlay, Institute for Research on Animal Diseases, Compton, England (8). *U. felinum* FT2-B^T, *U. cati* F2^T, and *U. canigenitalium* D6P-C^T were all obtained from our laboratory stock cultures (5, 6). All of the strains were grown aerobically in liquid medium at 37°C as described previously (6).

The almost complete 16S rDNA sequences of *U. diversum*, *U. felinum*, *U. cati*, and *U. canigenitalium* were amplified by PCR by using universal primers F16S (5'-GAGTTTGATCCTGGCTCAGG-3') and R16S (5'-GGGATACCTGTGTTACGACTT-3'). Both strands of the PCR products were sequenced by the dideoxy termination method (18) by primer walking without molecular cloning.

The nucleotide sequences of the 16S rDNAs of the *Ureaplasma* species were aligned by the method of Higgins et al.

(7), using the DNASIS software package (Hitachi Software Engineering Co., Yokohama, Japan). A phylogenetic tree was constructed by the neighbor-joining method (17).

The nucleotide sequences of the 16S rDNAs of strains T960^T and 27 of the two biovars of *U. urealyticum* (19) were obtained from nucleotide sequence databases. The nucleotide sequence of *U. gallorale* was not included in this study since the *U. gallorale* 16S rRNA gene was not amplified by the PCR primers used, which suggests that the avian ureaplasmas may be genetically different from the mammalian ureaplasmas.

The 16S rDNAs of six strains of the mammalian *Ureaplasma* species were aligned for maximum matching (Fig. 1). The alignment of the six *Ureaplasma* strains showed that there were conserved and variable regions in the 16S rDNAs. Nucleotide substitutions were not limited to a particular region of the predicted secondary structures (data not shown). In the present study, we constructed a phylogenetic tree for the mammalian *Ureaplasma* species based on the nucleotide sequences of the 16S rRNA genes (Fig. 2); this tree corresponds to the proposed evolutionary tree of the host animal species (11). However, the phylogenetic tree showed closer relationships (97.3% homology between the strains belonging to the two human *Ureaplasma* biovars, strains T960^T and 27, and 96.8% homology between the two feline *Ureaplasma* species, *U. felinum* and *U. cati*).

Although determinants for host ranges and susceptibilities to mycoplasma infections are currently unknown, *Ureaplasma* species, as well as *Mycoplasma* species, have defined host ranges consisting of specific animal species. Our data suggest that the origin of the mammalian *Ureaplasma* species dates back to about 100×10^6 years ago because the Mammalia, including human, bovine, canine, and feline lines, are thought to have diverged almost simultaneously during the Cretaceous period. Thus, the *Ureaplasma* species seem to have coevolved with their host animal species after this divergence, and rigid host-parasite relationships were established during the evolutionary process.

Nucleotide sequence accession numbers. The nucleotide sequences determined in this study have been deposited in the DDBJ, EMBL, GSDB, and NCBI nucleotide sequence databases under accession numbers D78648 (*U. canigenitalium*), D78649 (*U. cati*), D78650 (*U. diversum*), and D87651 (*U. felinum*).

* Corresponding author. Mailing address: Animal Center for Biomedical Research, Faculty of Medicine, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan. Fax: 81-3-3816-5680. Electronic mail address: rh@tansei.cc.u-tokyo.ac.jp.

	10	20	30	40	50	60	70	80	90	100
<i>U. urealyticum</i> T960	AGTTTGATC	TGGCTCAGG	ATTAACGCTG	CGGCATGCC	TAATACATGC	AAATCGAACG	AAGCCTTTTA	GGCTTAGTGG	TGAACGGGTG	AGTAACACGT
<i>U. urealyticum</i> 27	AGTTTGATC	TGGCTCAGG	ATTAACGCTG	CGGCATGCC	TAATACATGC	AAATCGAACG	AAGCCTTTTA	GGCTTAGTGG	TGAACGGGTG	AGTAACACGT
<i>U. diversum</i> A417	AGTTTGATC	TGGCTCAGG	ATTAACGCTG	CGGCATGCC	TAATACATGC	AAATCGAACG	AAGCCTTTTA	GGCTTAGTGG	TGAACGGGTG	AGTAACACGT
<i>U. felinum</i> FT2-B	AGTTTGATC	TGGCTCAGG	ATTAACGCTG	CGGCATGCC	TAATACATGC	AAATCGAACG	AAGCCTTTTA	GGCTTAGTGG	TGAACGGGTG	AGTAACACGT
<i>U. calli</i> F2	AGTTTGATC	TGGCTCAGG	ATTAACGCTG	CGGCATGCC	TAATACATGC	AAATCGAACG	AAGCCTTTTA	GGCTTAGTGG	TGAACGGGTG	AGTAACACGT
<i>U. canigenitalium</i> D6P-C	AGTTTGATC	TGGCTCAGG	ATTAACGCTG	CGGCATGCC	TAATACATGC	AAATCGAACG	AAGCCTTTTA	GGCTTAGTGG	TGAACGGGTG	AGTAACACGT
	110	120	130	140	150	160	170	180	190	200
<i>U. urealyticum</i> T960	ATCCAACTA	CCCTTAAGTT	GGGATAAAGT	AGTCGAAGA	TAGCTAATA	CGAATAATA	ACATCAATAT	GCATGAGTA	AGATGTAGAA	AGTCGCGTTT
<i>U. urealyticum</i> 27	ATCCAACTA	CCCTTAAGTT	GGGATAAAGT	AGTCGAAGA	TAGCTAATA	CGAATAATA	ACATCAATAT	GCATGAGTA	AGATGTAGAA	AGTCGCGTTT
<i>U. diversum</i> A417	ATCCAACTA	CCCTTAAGTT	GGGATAAAGT	AGTCGAAGA	TAGCTAATA	CGAATAATA	ACATCAATAT	GCATGAGTA	AGATGTAGAA	AGTCGCGTTT
<i>U. felinum</i> FT2-B	ATCCAACTA	CCCTTAAGTT	GGGATAAAGT	AGTCGAAGA	TAGCTAATA	CGAATAATA	ACATCAATAT	GCATGAGTA	AGATGTAGAA	AGTCGCGTTT
<i>U. calli</i> F2	ATCCAACTA	CCCTTAAGTT	GGGATAAAGT	AGTCGAAGA	TAGCTAATA	CGAATAATA	ACATCAATAT	GCATGAGTA	AGATGTAGAA	AGTCGCGTTT
<i>U. canigenitalium</i> D6P-C	ATCCAACTA	CCCTTAAGTT	GGGATAAAGT	AGTCGAAGA	TAGCTAATA	CGAATAATA	ACATCAATAT	GCATGAGTA	AGATGTAGAA	AGTCGCGTTT
	210	220	230	240	250	260	270	280	290	300
<i>U. urealyticum</i> T960	---CGACG	TTTGGATG	AGGGTGGAC	TATCAGATA	TTGGTGAGG	TAATGGCTCA	CAAGTCAAT	ACCGGTAGC	GTACTGAGA	GGTAGAACAG
<i>U. urealyticum</i> 27	TGTGCGACG	TTTGGATG	AGGGTGGAC	TATCAGATA	TTGGTGAGG	TAATGGCTCA	CAAGTCAAT	ACCGGTAGC	GTACTGAGA	GGTAGAACAG
<i>U. diversum</i> A417	---CGACG	TTTGGATG	AGGGTGGAC	TATCAGATA	TTGGTGAGG	TAATGGCTCA	CAAGTCAAT	ACCGGTAGC	GTACTGAGA	GGTAGAACAG
<i>U. felinum</i> FT2-B	---CGACG	TTTGGATG	AGGGTGGAC	TATCAGATA	TTGGTGAGG	TAATGGCTCA	CAAGTCAAT	ACCGGTAGC	GTACTGAGA	GGTAGAACAG
<i>U. calli</i> F2	---CGACG	TTTGGATG	AGGGTGGAC	TATCAGATA	TTGGTGAGG	TAATGGCTCA	CAAGTCAAT	ACCGGTAGC	GTACTGAGA	GGTAGAACAG
<i>U. canigenitalium</i> D6P-C	---CGACG	TTTGGATG	AGGGTGGAC	TATCAGATA	TTGGTGAGG	TAATGGCTCA	CAAGTCAAT	ACCGGTAGC	GTACTGAGA	GGTAGAACAG
	310	320	330	340	350	360	370	380	390	400
<i>U. urealyticum</i> T960	CACAATGGG	ACTGAGACAG	GGCCATACT	CTACGGGAG	CAGCAGTAG	GGAATTTTC	ACAATGGGCG	CAAGCCTTAT	AAGCAATGC	CGGTGAACG
<i>U. urealyticum</i> 27	CACAATGGG	ACTGAGACAG	GGCCATACT	CTACGGGAG	CAGCAGTAG	GGAATTTTC	ACAATGGGCG	CAAGCCTTAT	AAGCAATGC	CGGTGAACG
<i>U. diversum</i> A417	CACAATGGG	ACTGAGACAG	GGCCATACT	CTACGGGAG	CAGCAGTAG	GGAATTTTC	ACAATGGGCG	CAAGCCTTAT	AAGCAATGC	CGGTGAACG
<i>U. felinum</i> FT2-B	CACAATGGG	ACTGAGACAG	GGCCATACT	CTACGGGAG	CAGCAGTAG	GGAATTTTC	ACAATGGGCG	CAAGCCTTAT	AAGCAATGC	CGGTGAACG
<i>U. calli</i> F2	CACAATGGG	ACTGAGACAG	GGCCATACT	CTACGGGAG	CAGCAGTAG	GGAATTTTC	ACAATGGGCG	CAAGCCTTAT	AAGCAATGC	CGGTGAACG
<i>U. canigenitalium</i> D6P-C	CACAATGGG	ACTGAGACAG	GGCCATACT	CTACGGGAG	CAGCAGTAG	GGAATTTTC	ACAATGGGCG	CAAGCCTTAT	AAGCAATGC	CGGTGAACG
	410	420	430	440	450	460	470	480	490	500
<i>U. urealyticum</i> T960	ATGAAGGCTC	TATAGATTGT	AAAGTCTCTT	TATTTGGGAA	AAACGCTTAA	TATAGGAAAT	GATTTTAGAT	TGACTGTACC	ATTGAATAA	TATCGGCTA
<i>U. urealyticum</i> 27	ATGAAGGCTC	TATAGATTGT	AAAGTCTCTT	TATTTGGGAA	AAACGCTTAA	TATAGGAAAT	GATTTTAGAT	TGACTGTACC	ATTGAATAA	TATCGGCTA
<i>U. diversum</i> A417	ATGAAGGCTC	TATAGATTGT	AAAGTCTCTT	TATTTGGGAA	AAACGCTTAA	TATAGGAAAT	GATTTTAGAT	TGACTGTACC	ATTGAATAA	TATCGGCTA
<i>U. felinum</i> FT2-B	ATGAAGGCTC	TATAGATTGT	AAAGTCTCTT	TATTTGGGAA	AAACGCTTAA	TATAGGAAAT	GATTTTAGAT	TGACTGTACC	ATTGAATAA	TATCGGCTA
<i>U. calli</i> F2	ATGAAGGCTC	TATAGATTGT	AAAGTCTCTT	TATTTGGGAA	AAACGCTTAA	TATAGGAAAT	GATTTTAGAT	TGACTGTACC	ATTGAATAA	TATCGGCTA
<i>U. canigenitalium</i> D6P-C	ATGAAGGCTC	TATAGATTGT	AAAGTCTCTT	TATTTGGGAA	AAACGCTTAA	TATAGGAAAT	GATTTTAGAT	TGACTGTACC	ATTGAATAA	TATCGGCTA
	510	520	530	540	550	560	570	580	590	600
<i>U. urealyticum</i> T960	ACTATGTGCC	AGCAGCCGCG	TAATACATA	GGATGCAAGC	TTATCCGGGA	TTACTGGGCG	TAAACAGAG	GCAGGCCGGG	TTTGAAGTT	TGGTATTAAG
<i>U. urealyticum</i> 27	ACTATGTGCC	AGCAGCCGCG	TAATACATA	GGATGCAAGC	TTATCCGGGA	TTACTGGGCG	TAAACAGAG	GCAGGCCGGG	TTTGAAGTT	TGGTATTAAG
<i>U. diversum</i> A417	ACTATGTGCC	AGCAGCCGCG	TAATACATA	GGATGCAAGC	TTATCCGGGA	TTACTGGGCG	TAAACAGAG	GCAGGCCGGG	TTTGAAGTT	TGGTATTAAG
<i>U. felinum</i> FT2-B	ACTATGTGCC	AGCAGCCGCG	TAATACATA	GGATGCAAGC	TTATCCGGGA	TTACTGGGCG	TAAACAGAG	GCAGGCCGGG	TTTGAAGTT	TGGTATTAAG
<i>U. calli</i> F2	ACTATGTGCC	AGCAGCCGCG	TAATACATA	GGATGCAAGC	TTATCCGGGA	TTACTGGGCG	TAAACAGAG	GCAGGCCGGG	TTTGAAGTT	TGGTATTAAG
<i>U. canigenitalium</i> D6P-C	ACTATGTGCC	AGCAGCCGCG	TAATACATA	GGATGCAAGC	TTATCCGGGA	TTACTGGGCG	TAAACAGAG	GCAGGCCGGG	TTTGAAGTT	TGGTATTAAG
	610	620	630	640	650	660	670	680	690	700
<i>U. urealyticum</i> T960	CTAGATGCT	TAAAGTCTAG	TGATATCAAA	AACTGTAAAC	TAGAGTGTA	TAGGGAGTT	GGGAACTCC	ATGTGGAGCG	TAAATAGCG	TAGATATATG
<i>U. urealyticum</i> 27	CTAGATGCT	TAAAGTCTAG	TGATATCAAA	AACTGTAAAC	TAGAGTGTA	TAGGGAGTT	GGGAACTCC	ATGTGGAGCG	TAAATAGCG	TAGATATATG
<i>U. diversum</i> A417	CTAGATGCT	TAAAGTCTAG	TGATATCAAA	AACTGTAAAC	TAGAGTGTA	TAGGGAGTT	GGGAACTCC	ATGTGGAGCG	TAAATAGCG	TAGATATATG
<i>U. felinum</i> FT2-B	CTAGATGCT	TAAAGTCTAG	TGATATCAAA	AACTGTAAAC	TAGAGTGTA	TAGGGAGTT	GGGAACTCC	ATGTGGAGCG	TAAATAGCG	TAGATATATG
<i>U. calli</i> F2	CTAGATGCT	TAAAGTCTAG	TGATATCAAA	AACTGTAAAC	TAGAGTGTA	TAGGGAGTT	GGGAACTCC	ATGTGGAGCG	TAAATAGCG	TAGATATATG
<i>U. canigenitalium</i> D6P-C	CTAGATGCT	TAAAGTCTAG	TGATATCAAA	AACTGTAAAC	TAGAGTGTA	TAGGGAGTT	GGGAACTCC	ATGTGGAGCG	TAAATAGCG	TAGATATATG
	710	720	730	740	750	760	770	780	790	800
<i>U. urealyticum</i> T960	AAGAACACC	GGTGGCGAAG	CGCCCAACTT	GGACTATTA	TGACGCTTA	GGCTCGAAA	TGTGGGAG	AAATAGGAT	TAGATACCTT	AGTAGTCCAC
<i>U. urealyticum</i> 27	AAGAACACC	GGTGGCGAAG	CGCCCAACTT	GGACTATTA	TGACGCTTA	GGCTCGAAA	TGTGGGAG	AAATAGGAT	TAGATACCTT	AGTAGTCCAC
<i>U. diversum</i> A417	AAGAACACC	GGTGGCGAAG	CGCCCAACTT	GGACTATTA	TGACGCTTA	GGCTCGAAA	TGTGGGAG	AAATAGGAT	TAGATACCTT	AGTAGTCCAC
<i>U. felinum</i> FT2-B	AAGAACACC	GGTGGCGAAG	CGCCCAACTT	GGACTATTA	TGACGCTTA	GGCTCGAAA	TGTGGGAG	AAATAGGAT	TAGATACCTT	AGTAGTCCAC
<i>U. calli</i> F2	AAGAACACC	GGTGGCGAAG	CGCCCAACTT	GGACTATTA	TGACGCTTA	GGCTCGAAA	TGTGGGAG	AAATAGGAT	TAGATACCTT	AGTAGTCCAC
<i>U. canigenitalium</i> D6P-C	AAGAACACC	GGTGGCGAAG	CGCCCAACTT	GGACTATTA	TGACGCTTA	GGCTCGAAA	TGTGGGAG	AAATAGGAT	TAGATACCTT	AGTAGTCCAC
	810	820	830	840	850	860	870	880	890	900
<i>U. urealyticum</i> T960	ACCGTAACCG	ATCATCATTA	ATATGTCGGT	GA--ATG	TCGGTGTTGT	AGCTAACGCA	TAAATGATG	TCCTGGGTA	TACATTCGG	AAGAATGAAA
<i>U. urealyticum</i> 27	ACCGTAACCG	ATCATCATTA	ATATGTCGGT	GA--ATG	TCGGTGTTGT	AGCTAACGCA	TAAATGATG	TCCTGGGTA	TACATTCGG	AAGAATGAAA
<i>U. diversum</i> A417	ACCGTAACCG	ATCATCATTA	ATATGTCGGT	GA--ATG	TCGGTGTTGT	AGCTAACGCA	TAAATGATG	TCCTGGGTA	TACATTCGG	AAGAATGAAA
<i>U. felinum</i> FT2-B	ACCGTAACCG	ATCATCATTA	ATATGTCGGT	GA--ATG	TCGGTGTTGT	AGCTAACGCA	TAAATGATG	TCCTGGGTA	TACATTCGG	AAGAATGAAA
<i>U. calli</i> F2	ACCGTAACCG	ATCATCATTA	ATATGTCGGT	GA--ATG	TCGGTGTTGT	AGCTAACGCA	TAAATGATG	TCCTGGGTA	TACATTCGG	AAGAATGAAA
<i>U. canigenitalium</i> D6P-C	ACCGTAACCG	ATCATCATTA	ATATGTCGGT	GA--ATG	TCGGTGTTGT	AGCTAACGCA	TAAATGATG	TCCTGGGTA	TACATTCGG	AAGAATGAAA
	910	920	930	940	950	960	970	980	990	1000
<i>U. urealyticum</i> T960	TCAAAACGG	ATTGACGGGG	ACCCGACAAA	TGGTGGAGG	ATGTTGCTTA	ATTGACAAAT	ACACGTAGAA	CTTACCTAG	TTTGACATC	TATGCAATG
<i>U. urealyticum</i> 27	TCAAAACGG	ATTGACGGGG	ACCCGACAAA	TGGTGGAGG	ATGTTGCTTA	ATTGACAAAT	ACACGTAGAA	CTTACCTAG	TTTGACATC	TATGCAATG
<i>U. diversum</i> A417	TCAAAACGG	ATTGACGGGG	ACCCGACAAA	TGGTGGAGG	ATGTTGCTTA	ATTGACAAAT	ACACGTAGAA	CTTACCTAG	TTTGACATC	TATGCAATG
<i>U. felinum</i> FT2-B	TCAAAACGG	ATTGACGGGG	ACCCGACAAA	TGGTGGAGG	ATGTTGCTTA	ATTGACAAAT	ACACGTAGAA	CTTACCTAG	TTTGACATC	TATGCAATG
<i>U. calli</i> F2	TCAAAACGG	ATTGACGGGG	ACCCGACAAA	TGGTGGAGG	ATGTTGCTTA	ATTGACAAAT	ACACGTAGAA	CTTACCTAG	TTTGACATC	TATGCAATG
<i>U. canigenitalium</i> D6P-C	TCAAAACGG	ATTGACGGGG	ACCCGACAAA	TGGTGGAGG	ATGTTGCTTA	ATTGACAAAT	ACACGTAGAA	CTTACCTAG	TTTGACATC	TATGCAATG

FIG. 1. Nucleotide sequence alignment of the PCR products of the 16S rRNA genes of ureaplasmas. Nucleotides that are identical in two of three sequences are shown as inverted characters. The dashes indicate spaces between adjacent nucleotides introduced for maximum alignment.

FIG. 1—Continued.

FIG. 2. Dendrogram showing degrees of primary nucleotide sequence relatedness among six ureaplasmas, based on the neighbor-joining method.

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