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

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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 ATCC 10974 (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-B1, U. cati F25, and U. canigenitalium D6P-C9 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'-GGTACCTGGTACGACTT-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 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 by 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 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 × 106 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 D78651 (U. felinum).
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.
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REFERENCES


