Phylogenetic Relationships of the Porcine Mycoplasmas *Mycoplasma hyosynoviae* and *Mycoplasma hyopharyngis*

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The phylogenetic positions of the porcine mycoplasmas *Mycoplasma hyosynoviae* and *Mycoplasma hyopharyngis* were determined by using PCR-amplified 16S rRNA gene sequences. *M. hyosynoviae* is a member of the *Mycoplasma hominis* group, while *M. hyopharyngis* belongs to the *Mycoplasma fermentans* group of mollicutes. Neither species is closely related to previously characterized porcine mycoplasmas belonging to the *Mycoplasma hyorhinis* group.

The members of the class *Mollicutes* that have been isolated from porcine hosts include *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis*, both of which are considered respiratory pathogens, and *Mycoplasma flocculare*, a nonpathogenic respiratory organism. *Mycoplasma hyopharyngis* is also a respiratory isolate without obvious pathogenicity (2). In addition to these organisms, *Mycoplasma hyosynoviae*, which is a common inhabitant of the pharynx, tonsils, and nasal cavities, of adult pigs, has been implicated in arthritis in young pigs, which results in economic losses because of reduced growth rates (12). *M. hyopneumoniae*, *M. hyorhinis*, and *M. flocculare* are genetically related and, on the basis of 16S rRNA sequence analysis data, belong to the *M. hyorhinis* phylogenetic group (11) along with nonporcine isolates. In this paper we describe the phylogenetic positions of *M. hyosynoviae* and *M. hyopharyngis*.

Cloning and sequencing of 16S rRNA genes. *M. hyosynoviae* S-16 (= ATCC 25591) was obtained from J. G. Tully (National Institute of Allergy and Infectious Diseases, Frederick, Md.). Cultures were grown in ATCC medium 243 supplemented with heat-inactivated pig serum, Bacto Mucin (Difco Laboratories, Detroit, Mich.), and L-arginine at 37°C until the medium pH increased from 7.0 to 7.8. DNA was isolated by gentle phenol-chloroform extraction of sodium dodecyl sulfate-proteinase K-treated cells. *M. hyopharyngis* H3-6B F^T (T = type strain) was propagated in a similar medium, which contained inactivated horse serum instead of pig serum. Each cell pellet was washed in 70% ethanol, and the resulting dried pellet was subsequently rehydrated and used directly for gene amplification. By using modified universal mollicutes primers (1) which were synthesized so that they contained *Xho*I and *Pst*I restriction sites, the DNA was amplified to produce essentially full-length 16S ribosomal DNA copies. This amplified DNA was appropriately digested, ligated into pBluescript (Stratagene, La Jolla, Calif.), and transformed into *Escherichia coli*, and recombinant colonies were selected. Two *M. hyosynoviae* recombinants and one *M. hyopharyngis* 16S ribosomal DNA recombinant were each grown in 500 ml of Luria-Bertani broth, and plasmid DNA was prepared by using a Wizard Maxiprep column (Promega, Madison, Wis.). Both strands of the insertions of the plasmids were then sequenced, initially with M13 forward and reverse primers and the universal 16S rRNA

primers (6); then the sequences were extended by using primers from conserved sequences of mycoplasmal 16S rRNA or newly synthesized primers as needed.

Phylogenetic analysis. The sequences were analyzed at the Ribosomal Database Project (7) to find closely related bacterial species, and an unrooted tree was constructed by using the maximum-likelihood analysis method (9) at the Ribosomal Database Project and the 16S rRNA sequences of representative mollicutes (Fig. 1). A similar tree was obtained when the sequences were downloaded from GenBank, aligned by using PILEUP (Genetics Computer Group, Madison, Wis.), and subjected to a maximum-parsimony analysis with bootstrapping, using programs from the PHYLIP package (3) (data not shown).

Although *M. hyosynoviae* has been shown previously to exhibit weak cross-hybridization with an *M. hyorhinis* 16S rRNA-specific probe (5), *M. hyosynoviae* is clearly a member of the *Mycoplasma hominis* group (8). This relationship is also reflected by the nutritional requirement of all members of the *M. hominis* group for arginine (4). *Mycoplasma orale*, a commonly isolated member of the human oral microflora (10), is the closest known relative of *M. hyosynoviae*. *M. hyopharyngis* belongs to neither the *M. hyorhinis* group nor the *M. hominis* group; *M. hyopharyngis* belongs to the *Mycoplasma fermentans* group (8), and *Mycoplasma lipophilum* is its closest neighbor.

Our results suggest that while coevolution could explain the relatedness of *M. hyopneumoniae*, *M. hyorhinis*, and *M. flocculare*, *M. hyosynoviae* and *M. hyopharyngis* clearly did not arise from this lineage but presumably independently colonized porcine hosts. Because the species most closely related to *M. hyosynoviae* and *M. hyopharyngis* are human commensal organisms,

FIG. 1. Unrooted phylogenetic tree for *M. hyosynoviae*, *M. hyopharyngis*, and related species, constructed by maximum likelihood. The scale bar indicates branch lengths in units of expected nucleotide substitutions per site.

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the possibility of transmission through agricultural contact cannot be ruled out. It would be interesting to examine nondomesticated relatives of the pig to see if they are colonized by these or related mollicutes.

Nucleotide sequence accession numbers. The nucleotide sequences of *M. hyosynoviae* and *M. hyopharyngis* determined in this study have been deposited in the GenBank database under accession numbers U26730 and U58997, respectively.

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