Phylogenetic Relationships of Salmonella typhi and Salmonella typhimurium Based on 16S rRNA Sequence Analysis

HERNAN R. CHANG,*‡ L. H. LOO,‡ K. JEYASEELAN,‡ L. EARNEST,‡ and E. STACKEBRANDT*‡

Department of Microbiology‡ and Department of Biochemistry,‡ Faculty of Medicine, National University of Singapore, Singapore 0511, Republic of Singapore, and DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, 38124 Braunschweig, Germany

The 16S rRNA gene sequences of Salmonella typhi and Salmonella typhimurium were amplified by PCR, cloned, and sequenced. These sequences were analyzed by comparison with reference organisms from the family Enterobacteriaceae. Both S. typhi and S. typhimurium belong to the gamma subdivision of the class Proteobacteria.

The genus Salmonella is a genus of the family Enterobacteriaceae. The following two different classification systems have been validated for species of the genus Salmonella: (i) the two-species system, with Salmonella enterica and Salmonella bongori; and (ii) the five-species system, with S. bongori, Salmonella choleraesuis, Salmonella enteritidis, Salmonella typhi, and Salmonella typhimurium. In this communication we follow the five-species classification. All species share a high degree of DNA homology (2). By using cross-absorption and antisera reactions to differentiate O (somatic or polysaccharide) and H (flagellar) antigens, workers have defined over 2,000 Salmonella serotypes (18). Some serotypes have a marked host specificity, and some others can infect many animal species besides humans and can produce invasive disease, such as enteric fever. For instance, S. typhi causes enteric (typhoid) fever and is only pathogenic in humans, whereas S. typhimurium causes gastroenteritis and is pathogenic for several mammalian species. Bacteriophage typing has been used to differentiate S. typhi within serotypes (6). Despite their different sensitivities to specific bacteriophages, these bacteria appear to be clonal, as determined by multilocus enzyme electrophoresis and DNA hybridization analysis (11). In order to gain insight into the phylogenetic relationships of both S. typhi and S. typhimurium, we cloned and sequenced their 16S ribosomal RNAs (rRNAs) (5) by using a PCR approach.

Cloning and sequencing of 16S rRNA genes. S. typhi ATCC 19430 was obtained from the American Type Culture Collection (Rockville, Md.), and S. typhimurium NCTC 8391 was obtained from the Culture Collection of the Department of Microbiology, National University of Singapore. Single colonies of these strains were grown in liquid media, and their genomic DNAs were extracted by lysis with lysozyme and sodium dodecyl sulfate followed by isolation with N-cetyl-N,N,N-trimethylammonium bromide (Merck, Darmstadt, Germany) and precipitation with ethanol (16). Each purified DNA was subjected to PCR with universal primers containing flanking EcoRI sites (17). The amplified 16S rDNAs were purified and ligated to the pT7Blue T-vector (Novagen, Inc., Madison, Wis.) according to the manufacturer’s instructions and were transformed by electroporation into competent Escherichia coli. After selection of recombinant colonies, isolated colonies were grown, and their plasmid DNAs were extracted and purified (14). Each purified plasmid DNA was amplified by using a Taq DyeDeoxy terminator cycle sequencing kit in a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.) and was analyzed by using a model 373A automated DNA sequencer (Applied Biosystems, Inc., Foster City, Calif.) and a set of sequencing primers. In addition, following digestion of clones with EcoRI (New England Biolabs, Inc., Beverly, Mass.) and subcloning into pUC19 plasmids, we sequenced both strands of three S. typhi subclones and two S. typhimurium subclones.

Phylogenetic analysis. Pairwise evolutionary distances were computed from the 16S rDNA similarity values (data not shown) by using the correction of Jukes and Cantor (7). The distance matrix method of De Soete (3) and neighbor-joining analysis (4, 13) were used to reconstruct phylogenetic trees from the distance matrix. Bootstrap values, calculated for 300 trees, were generated by using the algorithms Njboot and NJFind. Almost complete 16S rDNA sequences were determined for S. typhi ATCC 19430 and S. typhimurium NCTC 8391; these sequences comprised 99.2% of the Escherichia coli 16S rDNA sequences (1). The two sequences were aligned with the homologous sequences of members of the family Enterobacteriaceae for which almost complete 16S rDNA sequences had been deposited in the EMBL or Ribosomal Database Project (10) (Fig. 1), and similarity values were determined. The sequences of the two Salmonella strains show 99.7% similarity. The five differences are found at positions 572, 609, 1136, 1137, and 1269. The similarity values for the two Salmonella strains and other members of the Enterobacteriaceae range between 97.6 and 91.7%. The phylogenetic trees, generated on the basis of corrected similarity values (7), were identical in that the two Salmonella strains were placed in a phylogenetic cluster that contains Enterobacter cloacae, Escherichia coli and Citrobacter freundii (96.3 to 97.6% similarity). Pantoea agglomerans (about 95% similarity), Serratia marcescens (95.5 to 96% similarity), and Erwinia carotovora (95.2 to 95.7% similarity) were moderately related to the Escherichia coli cluster, while the other strains belonging to the Enterobacteriaceae included in this study formed a separate subline of descent. A phylogenetic dendrogram is shown in Fig. 1.

Analysis of the two Salmonella sequences with unpublished short 16S rDNA stretches of about 300 nucleotides (between positions 300 and 600) from S. typhimurium K-12, S. typhi, S. choleraesuis, S. enteritidis, and “Salmonella dublin” which are deposited in the EMBL revealed 100% sequence similarity (data not shown). The nucleotide sequence of this stretch is so conserved that even the 16S rDNAs of Salmonella species and

* Corresponding author. Present address: Laboratory of Nutrition/Infection. CRI-3, Department of Medicine, Beth Israel Deaconess Medical Center, West Campus/Harvard Medical School, 194 Pilgrim Road, Boston, MA 02115. Phone: (617) 632-0132. Fax: (617) 632-0204. E-mail: rochang@west.bidmc.harvard.edu.

‡ Present address: Laboratorium für Mikrobiologie, Mikrobiologisches Institut, Universität Regensburg, D-93040 Regensburg, Germany.
those of the _Escherichia coli_ strains are either absolutely identical or differ only in one or two nucleotides. Consequently, analysis of this stretch does not allow discrimination of phylogenetic relationships.

Previous DNA-DNA reassociation data (2, 8, 9, 15) and the 16S rDNA sequence similarities correlate very well: (i) both methods place the genus _Salmonella_ in the family _Enterobacteriaceae_; (ii) the close 16S rDNA relationship of _Salmo- nella_ species to members of the genera _Escherichia_ and _Citro- bacter_, shown previously by DNA-DNA reassociation to be about 45% (2), is confirmed; and (iii) the results of both methods indicate that the genera _Erwinia_, _Pantoea_, and _Serratia_ are less closely related to the genera _Salmonella_, _Escherichia_, and _Citrobacter_ and that the genera _Yersinia_, _Hafnia_, _Proteus_, _Xenorhabdus_, and _Photorhabdus_ are even more remotely related. The genus _Salmonella_ contains five validly described species, which are highly related as determined by DNA-DNA reassociation. The high degree of DNA hybridization similarity for _S. typhi_ and _S. typhimurium_ (84%) (2) was confirmed by a high 16S rDNA similarity value (99.7%).

Probes developed for the 23S rDNA of _Salmonella_ species could be used to discriminate between two groups of _Salmo-

---

**FIG. 1.** Phylogenetic dendrogram based on distance matrix analysis of 16S rRNA gene sequences from _S. typhi_ and _S. typhimurium_ strains sequenced in this work and reference strains. Reference sequences of the following organisms were obtained from EMBL: _Escherichia coli_ ATCC 25922 (accession no. X80724), _Photorhabdus luminescens_ DSM 3326 (X82248), _Proteus vulgaris_ IFAM 1731 (X07652), _Xenorhabdus nematophila_ DSM 3370 (X82251), _Yersinia intermedia_ ER-3854 (X75279), and _Yersinia ruckeri_ ATCC 29473 (X75275). Reference sequences of the following organisms were obtained from the Ribosomal Database Project (10), in which no strain designations are indicated for the species: _Citrobacter freundii_ (accession no. M59291), _Erwinia carotovora_ (M59149), _Serratia mace- rescens_ (M59160), _Pantoea agglomerans_ _Hafnia alvei_ (M59155), and _Plesiomonas shigelloides_ (M59159). The sequence of _Enterobacter cloacae_ DSM 30054 was kindly provided by F. A. Rainey.

---

**REFERENCES**