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Nucleotide sequence and comparison of the 5S ribosomal RNA genes of *Rochalimaea henselae*, *R. quintana* and *Brucella abortus*

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The 5S rRNA genes from three human pathogens within the α -2 subgroup of the *Proteobacteria* were characterized. The resulting 5S rRNA sequences were then used to corroborate phylogenetic relationships previously based upon 16S rRNA sequences (1,2). PCR amplification of the 5S rRNA genes from *Rochalimaea henselae* (bacillary angiomatosis), *R. quintana* (trench fever) and *Brucella abortus* (undulant fever) was accomplished by using 15mer primers based on the 5' (GACTTGGTGGTTATG) and 3' (AGACCTGGCAGCGAC) ends of the 5S rRNA gene from the closely-related pathogen, *Bartonella bacilliformis* (Oroya fever) (3). PCR amplification of purified DNA from the three bacteria and control DNA from *B. bacilliformis* produced ~120-bp DNA fragments. Nucleotide sequences of the 5S rRNA genes were subsequently determined by double-stranded sequencing of the PCR products (4) using the 15mer primers employed in PCR amplification. Sequences within the 15-nucleotide primer regions were verified by directly sequencing bacterial chromosomal DNA by Taq dideoxy-terminator cycled sequencing (Applied Biosystems). Results of the sequencing are shown in Figure 1 with an alignment of the three 5S rRNA genes, the 5S rRNA gene of *B. bacilliformis* (3) and the 5S rRNA of *Vibrio cyclospites* (5). The overall sequence identity of the rRNA genes of *R. henselae* and *R. quintana* are 98.3% identical; in good agreement with 16S rRNA sequence identity of 99.1% (2). The *B. bacilliformis* 5S rRNA gene (3) is approximately 92% identical to the two *Rochalimaea* spp., in excellent agreement with the 91.7% sequence identity obtained by sequence comparison of *R. quintana* and *B. bacilliformis* 16S rRNA (1). *Brucella abortus* shows the least homology to other bacteria within the group at 86.6%, but has 95% sequence identity with the 5S rRNA of *V. cyclospites*, a bacterium which is not believed to be a true member of the *Vibrionaceae* (5). A dendrogram showing the predicted phylogenetic relationships between the bacteria is given in Figure 2. These results are very similar to the phylogeny based upon 16S rRNA homology (1,2). Double-stranded DNA sequence can be obtained rapidly from amplified 5S rRNA genes due to their small size relative to 16S rRNA (~120 bp vs. ~1425 nucleotides, respectively). This feature, plus the production of phylogenetic results which are similar to those obtained from 16S rRNA homology, make the 5S rRNA genes an attractive alternative for determining phylogenetic relationships among the α -group of *Proteobacteria*.

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Rh GACTTGGTGGTTATGGCGAGTGAACGACCCGATCCATCCGGAATCG 80
Rq .....C.....
Ba .....AC.CCT.....
Bb .....C.G.CCT.....
Vc .....C.G.CCT.....T.....

Rh GCCTGAAAGCGCTCAGCGCTGATGGTACTTGCTCAAGGACGGCAGA 100
Rq .....T.....
Ba .....T.....T.....G.....
Bb .....C.....C.....AGGA.....
Vc .....C.T.....A.G.....G.....

Rh GTAAGTCGTCGAGGCTC 119
Rq .....
Ba .....C.....
Bb .....G.....A.....
Vc .....G.....C.....

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Figure 1. Nucleotide sequence and alignment of the 5S rRNA genes of *Rochalimaea henselae* (Rh), *R. quintana* (Rq), *Brucella abortus* (Ba), *Bartonella bacilliformis* (Bb) (3) and the 5S rRNA of *Vibrio cyclospites* (Vc) (5). Only bases which differ from the *R. henselae* (Rh) 5S rRNA gene sequence are indicated.

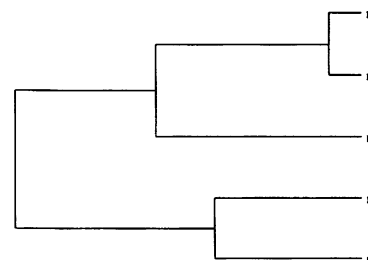


Figure 2. Dendrogram of the bacterial species based on 5S rRNA genes or 5S rRNA sequence. Abbreviations correspond to those of Figure 1.