

Nucleotide sequences of the 23S rRNA genes from *Bordetella pertussis*, *B. parapertussis*, *B. bronchiseptica* and *B. avium*, and their implications for phylogenetic analysis

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Virtually all members of the gram-negative bacillus genus *Bordetella* are pathogenic, being responsible for a variety of diseases in both man and animals. *B. pertussis* causes whooping cough, while *B. parapertussis* infections result in milder, but still serious, respiratory diseases in human (1). *B. bronchiseptica* is pathogenic for both dogs and pigs, and *B. avium* infects birds (2, 3).

While *B. pertussis*, *B. parapertussis* and *B. bronchiseptica* are very closely related and are more correctly considered subspecies (4), *B. avium* is considerably more distant to the group and, as such, constitutes a distinct species classification (5).

We have determined the complete nucleotide sequence of the 23S rRNA genes from *B. pertussis* Tohama, *B. parapertussis* ATCC 15311, *B. bronchiseptica* ATCC 19395 and *B. avium* ATCC 35086. The 23S rRNA genes, including the 5' and 3' flanking regions, were amplified via the polymerase chain reaction (PCR). The resultant amplification products were directly sequenced by the dideoxy termination method. In addition, we performed Southern blot analysis on the genomic DNA of each bacteria, and detected 3 distinct loci for this rRNA in all 4 species (data not shown). We have found the three rRNA gene sequences in closely linked gene sets in the order 16S-23S-5S in the genome of all 4 *Bordetella* species. Furthermore, we have discovered two putative tRNA genes located in the intergenic spacer region between the 16S and 23S sequences. All of the *Bordetella* species examined contain the identical nucleotide sequences for the tRNA^{Ile} and tRNA^{Ala} genes. The 23S rRNA gene of *B. pertussis* is 2876 bp long with a G + C content of 53%. These genes from *B. parapertussis* and *B. bronchiseptica* are 2877 bp in length, also with a G + C composition of 53%. On the other hand, the gene from *B. avium* consists of 2867 bp, with a G + C content of 52.7%.

In Table 1 sequence homologies between the *Bordetella* species and three other purple bacteria are shown. *Rhodobacter capsulatus* (6) belongs to the alpha subclass, whereas *Pseudomonas cepacia* (7) is from the beta subclass and *Pseudomonas aeruginosa* (8) falls in the gamma subclass. A phylogenetic tree based on the DIST values (9) is outlined in Figure 1. The high sequence similarity (>99%) between *B. pertussis*, *B. parapertussis* and *B. bronchiseptica* confirms the close relationship of these organisms. In contrast, the lower homology between *B. avium* and the other three *Bordetella* members (>97%) is in agreement with the results reported by De Ley *et al.* (6).

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Table 1. Sequence homology (upper right-hand triangle) and DIST values (lower left-hand triangle) of 23S rRNA genes from *Bordetella* species and three other purple bacteria.

	<i>B. pertussis</i>	<i>B. parapert.</i>	<i>B. bronch.</i>	<i>B. avium</i>	<i>R. capsula.</i>	<i>P. cepacia</i>	<i>P. aerugin.</i>
<i>B. pertussis</i>	-	99.87	99.60	97.32	68.25	89.63	80.88
<i>B. parapert.</i>	0.0002	-	99.65	97.29	68.25	89.50	80.88
<i>B. bronch.</i>	0.0017	0.0014	-	97.25	67.75	89.38	80.75
<i>B. avium</i>	0.0241	0.0241	0.0241	-	66.63	88.63	80.25
<i>R. capsula.</i>	0.2579	0.2581	0.2583	0.2617	-	68.88	69.75
<i>P. cepacia</i>	0.0912	0.0908	0.0909	0.0975	0.2645	-	82.13
<i>P. aerugin.</i>	0.1687	0.1683	0.1683	0.1737	0.2736	0.1704	-

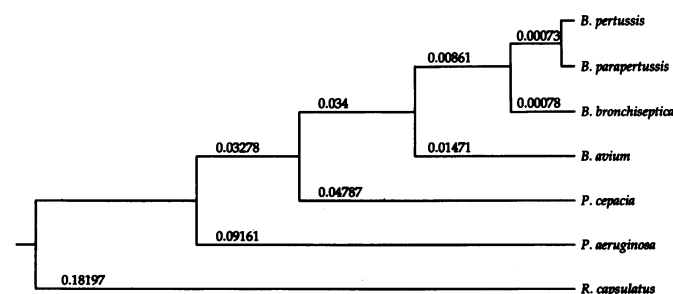


Figure 1. Phylogenetic tree showing the relationship between the *Bordetella* species and three other purple bacteria based on the DIST values calculated.

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[†]X68323, X68368, X70371 and X70370