Draft Genome Sequences of 53 Genetically Distinct Isolates of
Bordetella bronchiseptica Representing 11 Terrestrial and Aquatic Hosts

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Bordetella bronchiseptica infects a variety of mammalian and avian hosts. Here, we report the genome sequences of 53 genetically distinct isolates acquired from a broad range of terrestrial and aquatic animals. These data will greatly facilitate ongoing efforts to better understand the evolution, host adaptation, and virulence mechanisms of B. bronchiseptica.

Bordetella bronchiseptica infects a variety of terrestrial and aquatic animals, having a host range that is remarkably broad compared with that of other Bordetella species. Most frequently, it causes respiratory disease in pigs and dogs, but is also an occasional zoonotic pathogen (1, 2). For some hosts, including birds and several species of wild mammals, its colonization has not been associated with disease (3, 4). The characterization of isolates using multilocus enzyme electrophoresis (5), PvuII ribotyping (6-8), and multilocus sequence typing (8,9) indicates that some genotypes preferentially infect one or a few hosts. Here, we report the genome sequences of 53 B. bronchiseptica isolates selected to maximally represent geographic, host range, and molecular diversity. The isolates originated from terrestrial and aquatic hosts including 10 mammalian and one avian species, and they collectively represent Australia, Asia, Europe, and North America. They include the 32 PvuII ribotypes so far defined (6-8) (K. B. Register, unpublished data), 29 of the 60 multilocus sequence types (STs) currently identified among the B. bronchiseptica species (9) (http://pubmlst.org/bordetella/), and 5 STs not previously associated with the bacterium.

Genomic DNA was prepared (10) and sequenced using a combination of 3- or 5-kb mate-pair Illumina MiSeq 2 × 250-bp and HiSeq 2000 1 × 100-bp paired-end reads. After quality trimming, the reads for each strain (between 2,221,299 and 6,288,700) were assembled with the Celera Assembler 6.1 (11) or the Velvet assembler (12). The underlying consensus sequences and gaps were improved using custom scripts to recruit unmapped reads. All the genomes have between 41 and 296 contigs (median, 117 contigs) (Table 1), with N50 values ranging from 41,868 bp to 281,818 bp (median, 100,254 bp). The overall G+C content is ~68.1%, with genome sizes ranging from 5.04 Mb to 5.83 Mb. The genomes were annotated using the J. Craig Venter Institute (JCVI) prokaryotic annotation pipeline and contain between 4,388 and 5,660 predicted protein-coding genes. Due to their high copy number, the rRNA loci were broken in the assembly, so the exact number of operons could not be confidently enumerated in each strain. All strains have between 50 and 68 tRNAs, consistent with previously published genomes (13). The pangenome of the species was estimated at 10,375 genes, with just over 3,300 genes present in all strains, and an additional 1,084 genes present in >90% of the strains. In contrast, just over 3,300 singleton genes were identified, with a single strain containing as few as 1 and as many as 310 strain-specific genes. The majority of the unique genes encode hypothetical proteins or proteins with functions associated with phage and other mobile elements. These results are typical of species that frequently acquire genes by horizontal transfer.

Whole-genome single-nucleotide polymorphism (SNP) analysis clusters isolates into two main groups corresponding to complexes I and IV (9). All avian isolates cluster in complex IV, while most other nonhuman isolates cluster in complex I. In contrast, the human isolates from North America and Europe are evenly dispersed between complexes I and IV. The results of this study provide a wealth of information useful for understanding the evolution, host adaptation, and virulence mechanisms of B. bronchiseptica.

Nucleotide sequence accession numbers. The sequences of the B. bronchiseptica isolates have been deposited in GenBank under the accession numbers listed in Table 1; the source of each strain is also listed.

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| TABLE 1 Strain descriptions and genome assembly characteristics |
|---------------------------------------------------------------|
| **B. bronchiseptica strain** | **Host** | **No. of contigs** | **Length (bp)** | **GenBank accession no.** | **Repository** |
| 00-P-2730 | Human | 296 | 5,831,418 | JGWG00000000 | NRRL |
| 00-P-2796 | Human | 179 | 5,551,792 | JGJF00000000 | NRRL |
| 345 | Human | 79 | 5,286,504 | JGJF00000000 | NRRL |
| 3E44 | Rabbit | 104 | 5,235,953 | JGJF00000000 | NRRL |
| 7E71 | Horse | 109 | 5,163,549 | JGJF00000000 | NRRL |
| 980 | Unknown | 70 | 5,353,898 | JGJF00000000 | NRRL |
| A1-7 | Rabbit | 184 | 5,265,200 | JGJF00000000 | NRRL |
| B18-5 | Rabbit | 99 | 5,219,442 | JGJF00000000 | NRRL |
| B20-10725633 | Rabbit | 124 | 5,316,022 | JGJF00000000 | NRRL |
| CA90 BB02 | Turkey | 170 | 5,126,467 | JHBU00000000 | Harvill Lab |
| CA90 BB1334 | Turkey | 120 | 5,269,162 | JGJR00000000 | NRRL |
| CARE970018BB | Pig | 121 | 5,240,190 | JGJR00000000 | NRRL |
| D756 | Human | 102 | 5,233,656 | JGJF00000000 | NRRL |
| D989 | Human | 75 | 5,326,241 | JGJF00000000 | NRRL |
| D993 | Human | 197 | 5,278,699 | JGJF00000000 | NRRL |
| E010 | Human | 187 | 5,179,093 | JGJF00000000 | NRRL |
| E012 | Human | 156 | 5,175,924 | JGJF00000000 | NRRL |
| E013 | Human | 119 | 5,099,096 | JGJF00000000 | NRRL |
| E014 | Human | 111 | 5,210,402 | JGJF00000000 | NRRL |
| F-1 | Turkey | 96 | 5,377,336 | JGJF00000000 | NRRL |
| F2 | Turkey | 164 | 5,380,523 | JGJF00000000 | NRRL |
| F4563 | Human | 162 | 5,263,073 | JGJF00000000 | NRRL |
| GA96-01 | Human | 158 | 5,292,152 | JGJF00000000 | NRRL |
| M435/02/3 | Seal | 182 | 5,157,766 | JGJF00000000 | NRRL |
| M85/00/2 | Seal | 159 | 5,157,897 | JGJF00000000 | NRRL |
| MBORD591 | Dog | 271 | 5,151,134 | JGJF00000000 | NRRL |
| MBORD595 | Dog | 107 | 5,214,983 | JGJF00000000 | NRRL |
| MBORD624 | Horse | 213 | 5,306,540 | JGJF00000000 | NRRL |
| MBORD632 | Horse | 164 | 5,148,641 | JGJF00000000 | NRRL |
| MBORD635 | Cat | 55 | 5,092,496 | JGJF00000000 | NRRL |
| MBORD665 | Guinea pig | 51 | 5,147,090 | JGJF00000000 | NRRL |
| MBORD668 | Guinea pig | 59 | 5,149,790 | JGJF00000000 | NRRL |
| MBORD670 | Guinea pig | 80 | 5,169,029 | JGJF00000000 | NRRL |
| MBORD675 | Human | 41 | 5,173,023 | JGJF00000000 | NRRL |
| MBORD678 | Guinea pig | 50 | 5,184,788 | JGJF00000000 | NRRL |
| MBORD681 | Koala | 70 | 5,158,100 | JGJF00000000 | NRRL |

(Continued on following page)
| B. bronchiseptica strain | Host         | No. of contigs | Length (bp) | GenBank accession no. | Repository |
|--------------------------|--------------|----------------|-------------|-----------------------|------------|
| MBORD698                 | Koala        | 52             | 5,151,735   | JGXQ0000000000       | NRRL       |
| MBORD707                 | Turkey       | 56             | 5,138,088   | JGXR0000000000       | NRRL       |
| MBORD731                 | Horse        | 52             | 5,132,633   | JGXS0000000000       | NRRL       |
| MBORD762                 | Guinea pig   | 63             | 5,280,420   | JHBR0000000000       | NRRL       |
| MBORD782                 | Cat          | 90             | 5,134,438   | JGXT0000000000       | NRRL       |
| MBORD785                 | Dog          | 102            | 5,152,034   | JGXU0000000000       | NRRL       |
| MBORD839                 | Dog          | 103            | 5,190,832   | JGXM0000000000       | NRRL       |
| MBORD849                 | Pig          | 68             | 5,216,172   | JGXX0000000000       | NRRL       |
| MBORD901                 | Turkey       | 109            | 5,096,955   | JGX0000000000       | NRRL       |
| MO211                    | Human        | 140            | 5,258,097   | JHOJ0000000000       | Harvill Lab|
| MO275                    | Human        | 131            | 5,032,460   | JHBS0000000000       | NRRL       |
| OSU054                   | Turkey       | 131            | 5,400,773   | JHBO0000000000       | Harvill Lab|
| OSU095                   | Turkey       | 54             | 5,460,040   | JGY0000000000       | NRRL       |
| OSU553                   | Turkey       | 250            | 5,685,971   | JGXZ0000000000       | NRRL       |
| RB630                    | Rabbit       | 48             | 5,312,681   | JGAY0000000000       | NRRL       |
| SBL-F6116                | Human        | 121            | 5,060,248   | JHT0000000000       | NRRL       |
| SO10328                  | Sea otter    | 115            | 5,116,835   | JGYB0000000000       | NRRL       |