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GENOME SEQUENCES

Complete Genome Sequence of *Bordetella bronchiseptica* Strain KM22

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ABSTRACT *Bordetella bronchiseptica* isolate KM22 has been used in experimental infections of swine as a model of clinical *B. bronchiseptica* infection and to study host-to-host transmission. The draft genome sequence of KM22 was reported in 2014. Here, we report the complete genome sequence of KM22.

Bordetella bronchiseptica is a small, coccoid-shaped Gram-negative motile bacterium with a size of about 0.2 to 0.5 μm by 0.5 to 2 μm and is one of 15 species within the genus (http://www.bacterio.net/). *Bordetella pertussis*, *Bordetella parapertussis*, and *Bordetella bronchiseptica* are referred to as the classical *Bordetella* species due to their close genetic relatedness and their ability to colonize the respiratory tract of mammals, contributing to mild to severe respiratory disease (1). Previous studies focusing on the evolution of *Bordetella* species demonstrated that *B. pertussis* and *B. parapertussis* evolved independently from different lineages of a *B. bronchiseptica*-like ancestor (1–4); as a result, *B. pertussis* and *B. parapertussis* represent two of the many examples of human pathogens that evolved from zoonotic sources (5, 6). In addition to their close genetic relatedness, classical *Bordetella* species harbor many of the same virulence factors, which are similarly regulated (2, 4, 7). Despite these similarities, the classical *Bordetella* species differ in traits such as host specificity, disease severity, and duration of infection. *B. pertussis* infects only humans and lacks an animal reservoir and the ability to survive in the environment (8, 9). *B. bronchiseptica* infects a variety of animals, often establishing chronic infections that range from lethal pneumonia to asymptomatic carriage, and is capable of surviving in the environment (10, 11).

B. bronchiseptica strain KM22 was originally isolated in Hungary in 1993 from a swine herd with atrophic rhinitis. Based on multilocus sequence type (MLST) analysis, KM22 is sequence type 7 (ST7), in clonal complex 1 of an MLST-based *Bordetella* phylogeny (3) and harbors a ribotype (12) and pertactin repeat region variant (13) shared with the majority of isolates obtained from swine. KM22 has been successfully used by our laboratory to develop a reproducible swine respiratory disease model reflective of clinical *B. bronchiseptica* infections within swine herds and host-to-host transmission (14–25). The draft genome sequence of KM22 was previously reported (26). We began to employ transcriptomic and proteomic techniques to fully investigate the mechanisms used by KM22 in response to environmental changes and stress. The need to obtain a complete and closed genome sequence for furthering these studies became clear.

Whole-genome sequencing was performed using both the Pacific Biosciences (PacBio) and Illumina Genome Analyzer IIx (GAIIx) platforms. For DNA extraction, a single colony was inoculated into Stainer-Scholte (SS) broth (27) and cultivated at 37°C with shaking at 250 rpm for 36 h. The High Pure template preparation kit (Roche Applied Science, Indianapolis, IN) was used to extract total genomic DNA from the sample, which was subsequently used to prepare both the PacBio and the Illumina libraries. Library preparation for PacBio sequencing was performed following the PacBio

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20-kb insert library preparation protocol (https://www.pacb.com/wp-content/uploads/Procedure-Checklist-20-kb-Template-Preparation-Using-BluePippin-Size-Selection-System.pdf). The 20-kb library was sequenced with a PacBio RS II platform using two single-molecule real-time (SMRT) cells, resulting in 283,436 total reads and an average read length of 7,600 bp. Reads were subsequently assessed for quality using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/).

Whole-genome assemblies were generated using PacBio SMRT Analysis v. 2.3.0 and Canu v. 1.5 (28) software. The average PacBio coverage for the assembled genome was 475×. Assembling the PacBio data resulted in a fully sequenced closed circular chromosome, which was subsequently oriented to start at the *dnaA* gene and trimmed by removing any overlapping sequence. The genome was then polished and error corrected using the Broad Institute's Pilon v. 1.18 software (29) along with the Illumina GAIIx 3,474,442 paired-end sequencing reads, which were previously used for the draft assembly (26). Default parameters were used for all software. The closed KM22 genome was then annotated using NCBI's Prokaryotic Genome Annotation Pipeline (PGAP) (30). The complete genome of KM22 consists of 5,205,646 bp with a G+C content of 68.2%, a total of 4,827 predicted protein coding sequences (CDSs), 9 rRNA operons, 1 transfer-messenger RNA (tmRNA), 3 noncoding RNAs (ncRNAs), and 56 tRNAs.

Data availability. The whole-genome sequence for *Bordetella bronchiseptica* isolate KM22 was deposited in DDBJ/ENA/GenBank under the accession number CP022962. The PacBio read data were deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA398562 and SRA study accession number SRP222122 (run numbers SRR10134673 and SRR10134672). Illumina HiSeq short read sequences have been deposited at the European Nucleotide Archive under accession number ERS027415.

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