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Complete Genome Sequences of *Bordetella pertussis* Vaccine Reference Strains 134 and 10536

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Vaccine formulations and vaccination programs against whooping cough (pertussis) vary worldwide. Here, we report the complete genome sequences of two divergent *Bordetella pertussis* reference strains used in the production of pertussis vaccines.

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Bordetella pertussis is the primary causative agent of whooping cough (pertussis), a respiratory disease most severe in unvaccinated infants. The introduction of vaccines against pertussis dramatically reduced disease incidence worldwide. However, many countries have recently experienced disease resurgence, in part due to genetic divergence of circulating strains. The resulting antigenic mismatch with vaccine references has led many to conclude that *B. pertussis* is evolving under vaccine-driven selection (1–5). Adaptation of *B. pertussis* is complicated by the varied administration of whole-cell and acellular vaccines between countries and the diversity of reference strains used for vaccine production (6–8). Here, we report the complete genome sequences of two such strains used in manufacturing pertussis vaccines: B202 (Lederle Laboratories, strain 134) and B203 (Sanofi-Pasteur MSD, strain 10536) (9).

Whole-genome shotgun sequencing was performed using a combination of the PacBio RSII (Pacific Biosciences, Menlo Park, CA), Illumina HiSeq/MiSeq (Illumina, San Diego, CA), and Argus (OpGen, Gaithersburg, MD) platforms, as described previously (10). Briefly, genomic DNA libraries were prepared for PacBio sequencing using the SMRTbell template prep kit 1.0 and polymerase binding kit P4, while Illumina libraries were prepared using the NEBNext Ultra library prep kit (New England BioLabs, Ipswich, MA). *De novo* genome assembly of filtered reads was performed using the Hierarchical Genome Assembly Process (HGAP version 3; Pacific Biosciences) at 130× and 144× coverage for B202 and B203, respectively. The resulting consensus sequences were determined with Quiver (version 1), manually checked for circularity, and then reordered to match the start of reference strain Tohama I (accession no. CP010964) (10). To ensure accuracy, assemblies were confirmed by comparison to BamHI and KpnI restriction digestion optical maps using the Argus system (OpGen) with MapSolver (version 2.1.1; OpGen) and further polished by mapping either Illumina HiSeq PE-100 or MiSeq PE-300 reads using CLC Genomics Workbench (version 8.5; CLC bio, Boston, MA). Final assemblies were annotated using the NCBI automated Prokaryotic Genome Annotation Pipeline (PGAP).

The average G+C content of both B202 and B203 was 67.1%, with genome sizes of 4,128,979 and 4,134,643 bp, respectively. Genome annotation identified 3,645 protein-coding genes in B202 and 3,636 protein-coding genes in B203. Both genomes encoded three rRNA operons and 51 tRNAs.

The assemblies were distinct from genomes of vaccine reference strains Tohama I (GlaxoSmithKline, accession no. CP010964), CS (China, accession no. CP010963), and 137 (Brazil, accession no. CP010323), which have been sequenced previously (10, 11). B202 and B203 were not related, and their genomes differed from that of Tohama I by multiple rearrangements, as well as 186 and 410 single-nucleotide polymorphisms (SNPs), respectively. The genome of B202 was phylogenetically and structurally similar, but not identical, to other strains with the profile *prn1-ptxP1-ptxA2-ptxB2-fimH1*, such as clinical isolate H375 (accession no. CP010961) (10). B203 appeared to be closely related to Brazilian vaccine strain 137, sharing allele profile *prn7-ptxP2-ptxA4-ptxB2-fimH1*, but differed by 13 SNPs and a single ~74-kb inversion flanked by rRNA operon copies.

The availability of these genome sequences provides added resolution to known diversity among references used in vaccine production and will hopefully aid in the research of immune response to clinical infection in vaccinated patients.

Accession number(s). The complete genome sequences have been deposited at DDBJ/EMBL/GenBank under the accession numbers CP016338 and CP012128 for *B. pertussis* B202 and B203, respectively. The versions described in this paper are the first versions.

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