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PROKARYOTES

Complete Genome Sequences of *Bordetella pertussis* Isolates with Novel Pertactin-Deficient Deletions

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ABSTRACT Clinical isolates of the respiratory pathogen *Bordetella pertussis* in the United States have become predominantly deficient for the acellular vaccine immunogen pertactin through various independent mutations. Here, we report the complete genome sequences for four *B. pertussis* isolates that harbor novel deletions responsible for pertactin deficiency.

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Bordetella pertussis is the causative agent of whooping cough (pertussis), a vaccine-preventable respiratory disease with recent increased case reports in the United States and other developed countries (1). The majority of circulating isolates recovered in the United States do not produce the acellular vaccine immunogen pertactin (Prn) due to one of at least 16 independent mutations to the structural gene *prn*, including point mutations, promoter disruption, and mobile element disruption (2, 3). Here, we report the complete genome sequences of four such Prn-deficient clinical isolates which carry novel *prn* deletion mutations.

Whole-genome shotgun sequencing was performed using a combination of the PacBio RSII (Pacific Biosciences, Menlo Park, CA), MiSeq (Illumina, San Diego, CA), and Argus (OpGen, Gaithersburg, MA) platforms, as described previously (4). Briefly, genomic DNA libraries were prepared for PacBio sequencing using the SMRTbell template prep kit 1.0 and polymerase binding kit P6, 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) (5), with at least 100× coverage. The resulting consensus sequences were determined with Quiver version 1, manually checked for circularity, and then reordered to start at the coding region for glucose-inhibited cell division protein A (*gidA*), consistent with available genome sequences of *B. pertussis*. 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 Illumina MiSeq PE-300 reads using CLC Genomics Workbench version 10.0.1 (CLC bio, Boston, MA). Final assemblies were annotated using the NCBI automated Prokaryotic Genome Annotation Pipeline (PGAP). Partial deletion of the *prn* coding region or upstream promoter sequence was confirmed by Sanger sequencing, as described previously (2).

Isolate characteristics and *prn* mutations are summarized in Table 1. In J625, the deletion spanned the promoter and 5′ end of *prn* and was replaced with a 355-bp sequence fragment that was identical to a region adjacent to an IS*1663* element

TABLE 1 Characteristics of *B. pertussis* isolates and *prn* mutations

Isolate	U.S. state	Yr	*prn* mutation[a]	*prn* location (bp)[b]	Accession no.
H696	CA	2011	*prn2*::del(−1513, 145)	1008517–1011120	CP021402
J078[c]	MN	2013	*prn2*::del(−283, −40)	1079512–1082303	CP021401
J473[c]	NY	2016	*prn2*::del(666, 667)	2979800–2977055	CP021403
J625	VT	2016	*prn2*::del(−292, 1340)[d]	1085097–1086504	CP022362

[a]Numbers in parentheses indicate the position of each mutation (start, stop) relative to the *prn* start codon.
[b]Numbers in the *prn* location column indicate genomic coordinates of the *prn* gene.
[c]Isolates collected through Enhanced Pertussis Surveillance (6).
[d]Replaced with 355-bp fragment identical to 1074011 to 1073657.

upstream. These results add to the diverse catalogue of observed mutations which confer Prn deficiency through independent parallel disruption of *prn*.

Accession number(s). The complete genome sequences have been deposited at DDBJ/EMBL/GenBank under the accession numbers listed in Table 1. The versions described in this paper are the first versions.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. The use of trade names and commercial sources is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention, the Public Health Service, or the U.S. Department of Health and Human Services.

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