



Complete Genome Sequence of *Pasteurella multocida* Sequence Type 394, Isolated from a Case of Bovine Respiratory Disease in Australia

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ABSTRACT Here, we present the completely closed genome sequence of *Pasteurella multocida* 17BRD-035, a bovine respiratory disease (BRD) pathogen from Queensland, Australia, with genes that confer resistance to β -lactams, tilmicosin, and tetracycline. It consists of a single 2,624,884-bp chromosome and an average GC content of 40.23% and belongs to the newly described Rural Industries Research and Development Corporation (RIRDC) sequence type 394.

We present a completely closed genome sequence of a recently catalogued multilocus sequence type (MLST) variant, sequence type 394 (ST394), of *Pasteurella multocida* (strain 17BRD-035) for the RefSeq database. The ST394 designation is based on housekeeping genes used for the *P. multocida* Rural Industries Research and Development Corporation (RIRDC) MLST scheme (1) available via pubMLST. Using the multihost sequence typing scheme developed for *P. multocida* (2), 17BRD-035 represents ST159. *P. multocida* is one of the most frequently isolated bacterial pathogens associated with bovine respiratory disease (BRD) and is increasingly associated with antimicrobial resistance in the feedlot cattle industry, both globally and within Australia (3).

Strain 17BRD-035 was isolated from a diagnostic submission (lung swab from a BRD-affected animal collected postmortem) in Queensland, Australia, in 2017 (3). The strain was maintained on sheep blood agar for routine microbiological assays and grown in brain heart

TABLE 1 Reads generated by sequencing platforms and assembly statistics of the closed genome

| Parameter | Data |
|---|-----------|
| Raw sequence reads | |
| Illumina paired-end read length (nt) | 150 |
| No. of Illumina left reads (R1) | 1,872,270 |
| No. of Illumina right reads (R2) | 1,872,270 |
| Properly paired Illumina reads (%) | 94.91 |
| No. of Nanopore reads (total) | 145,043 |
| No. of Nanopore reads (filtered) | 143,212 |
| Assembly statistics ^a for the closed genome (CP082272) | |
| No. of contigs | 1 |
| Total length of the genome (bp) | 2,624,884 |
| Total no. of reads used | 3,301,309 |
| GC content (%) | 40.23 |
| Mapped reads (%) | 99.65 |
| Avg. coverage depth (\times) | 344 |
| N_{50} for the hybrid assembly (bp) | 2,624,884 |

^a Assembled using Unicycler hybrid assembler.

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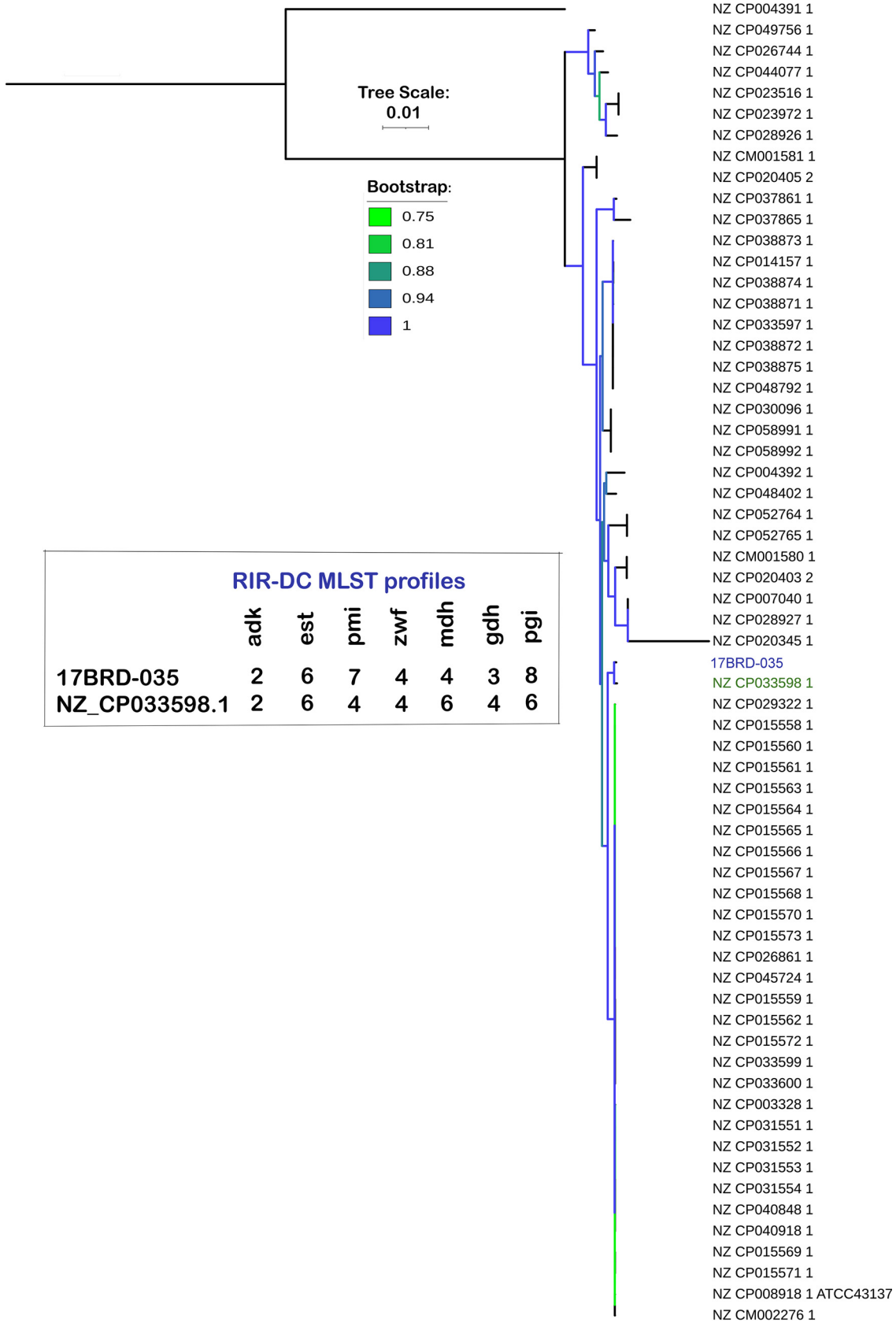


FIG 1 A midpoint-rooted phylogenetic tree of 17BRD-035 and 81 other genomes downloaded from the GenBank RefSeq database constructed using PhyloSift (<https://github.com/gjospin/PhyloSift>). The inset shows the RIRDC-MLST profile of 17BRD-035 (ST395) and the most closely related genome in the database *NZ_CP033598.1* (ST7).

infusion broth for DNA extraction. Details of DNA preparation steps, sequencing kits and software used for bioinformatic analyses, presented in Table 1, are as follows: DNA extraction for Illumina HiSeq (Bioline isolate II genomic DNA extraction kit), HiSeq library preparation (Illumina Nextera XT), Nanopore DNA preparation (XS buffer lysis followed by phenol-chloroform purification [4]), Nanopore sequencing (rapid barcoding sequencing kit [SQK-RBK004], R9.4.1 flow cell [FLO-MIN106]), Nanopore base calling (Guppy [5]), Quality control (QC; FastQC v0.11.9 [6], fastp v0.22.0 [7], and pycoQC 2.5.0.23 [8]), and QC data collection (MultiQC v1.9 [9]). Unless otherwise specified, default parameters were used for all software tools. The genome was assembled *de novo* with the Unicycler v0.4.8 hybrid assembly (10) protocol utilizing both Illumina short read and Nanopore sequence data generated in-house at the University of Technology Sydney sequencing facility. The genome was assembled into a single circular chromosome 2,624,884 bp in length and with an average GC content of 40.23% (GenBank accession [CP082272](#)) (Table 1). Preliminary annotations using Prokka v1.7 (11) revealed the presence of 2,547 coding DNA sequences (CDS) and 23 rRNA and 66 tRNA gene sequences in the genome. It contained β -lactamase-encoding *bla_{ROB-1}* and tetracycline resistance-conferring *tetR/tetH* genes, as well as a number of genes conferring resistance to heavy metals (*corC*, magnesium and cobalt efflux; *mco*, copper oxidase family of genes which confer copper resistance). In addition, the genome contains genes which encode MacA-MacB proteins which constitute a multidrug efflux pump and has been associated with variable macrolide resistance in other Gram-negative bacteria (12–14). Overexpression of these antibiotic efflux pumps may contribute to the nonsusceptibility to tilmicosin recorded by the strain.

Due to the lack of completely closed ST394 genomes in the GenBank RefSeq database, we conducted a phylogenetic analysis of the 17BRD-035 genome with 81 finished genomes in the RefSeq database (accessed on 2 August 2021) using PhyloSift v20141105 (15). 17BRD-035 is most closely related to *P. multocida* strain CQ7 (Fig. 1), GenBank accession number [NZ_CP033598.1](#). This strain was obtained from bovine pneumonic lung tissue originating from Chongqing, China, in 2013. A pairwise BLAST alignment of 17BRD-035 with CQ7 indicated 99.62% nucleotide identity over 83% of the query sequence. Although both 17BRD-035 and CQ7 belong to capsular serotype A (identified in-house on ABRicate v1.0.1 [<https://github.com/tseemann/abricate>] using a manually curated database following a strategy published by Peng et al. 2019 [16]), the CQ7 genome aligns with ST7 of the RIRDC-MLST scheme. ST7 differs from ST394 in the sequences of *pmi*, *mdh*, *gdh*, and *pgi* genes.

Data availability. The complete genome sequence of *P. multocida* 17BRD-035 has been deposited in GenBank under accession number [CP082272](#) and BioSample number [SAMN21014890](#) under BioProject [PRJNA758188](#). Reads for the Hi-Seq run ([SRR17163532](#)) can be accessed in GenBank using accession number [SRX13347427](#). Reads from the Oxford Nanopore run ([SRR17853486](#)) can be accessed in GenBank using accession number [SRX14014314](#).

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