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GENOME SEQUENCES

# Complete Genome Sequences of Three *Mycoplasma gallisepticum* 6/85-like Isolates

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**ABSTRACT** Control of *Mycoplasma gallisepticum* infection can be accomplished through vaccination. However, virulent field strains with genetic markers identical to vaccine strains have been identified. Here, we report the sequencing of three field isolates with genetic markers identical to the *M. gallisepticum* 6/85 vaccine strain.

Infection with *Mycoplasma gallisepticum* often leads to significant economic losses for poultry producers, but vaccination with live attenuated vaccines provides significant protection from disease and losses (1, 2). However, some live attenuated vaccine strains have virulent strains with identical genetic markers. Three virulent *M. gallisepticum* isolates (K4043, K4421A, and K5234) were previously shown to be identical to the 6/85 vaccine strain based on the sequences of their genetic markers (3, 4).

The three *M. gallisepticum* 6/85-like strains were obtained from Stanley Kleven (University of Georgia, retired) as frozen broth cultures, grown at 37°C for a single 1/10 passage upon receipt, and stocked at −80°C. The stocked strains were plated onto Frey's agar (5) at 37°C for 72 h. A single colony of each was grown in Frey's broth to mid-log phase (the phenol red indicator turned orange) for three 1/10 passages, and aliquots of the final passage were pelleted by centrifugation (20,000 × *g*); multiple pellets of each strain were stored at −80°C (6). DNA for genome sequencing was isolated from the bacterial pellets using a DNeasy blood and tissue kit (Qiagen, Inc., Germantown, MD, USA) according to the manufacturer's instructions. The DNA quantity and purity (260/280-nm and 260/230-nm ratios, respectively) were assessed using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Illumina sequencing was conducted by the USDA ARS Genomics and Bioinformatics Research Unit (Stoneville, MS, USA). DNA samples were sheared to 500-bp fragments, libraries were prepared using an Illumina NeoPrep instrument (version 1.0 kit), and 2 × 150 paired-end sequencing was performed using an Illumina NextSeq500 sequencer (Illumina Biotechnology Company, San Diego, CA, USA). The sequences were trimmed using the FastX-Toolkit version 0.0.14 Trimmer, and bases 9 to 144 were retained (7). Sickle version 1.33 was used to filter the paired-end reads with quality and length thresholds of 25 and 20, respectively (8). All software packages were used with default parameters unless otherwise specified. The average sequence quality and length were 34 and 136, respectively, for all sequences. The sequence metrics are given in Table 1.

Genomic DNA for MinION sequencing (Oxford Nanopore Technologies, Oxford, UK) was isolated from duplicate frozen bacterial pellets, as used for the Illumina sequencing. Unsheared genomic DNA was barcoded using the EXP-NBD103 kit and prepared for sequencing using the SQK-LSK108 kit, and all three samples were sequenced together for 48 h using the same FLO-MIN106 flow cell. DNA base calling and barcode sorting were performed using Guppy version 4.2.2 (Oxford Nanopore Technologies). Fastp version 0.20.0 was used to remove sequences shorter than 3,000 bases to facilitate assembly (9). The sequence metrics are given in Table 1. Genome assembly using both the Illumina and MinION data was performed using the hybrid assembly mode of Unicycler version 0.4.8 (10). Overlaps

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**TABLE 1** Accession numbers and genome information

| Parameter | Data for strain: K4043 | K4421A | K5234 |
|---|---|---|---|
| Isolation source (3, 4) | *Meleagris gallopavo*, 1995, Nebraska, USA | *Meleagris gallopavo*, 1997, Michigan, USA | *Gallus domesticus*, 2002, USA |
| GenBank accession no. | CP092250.1 | CP092249.1 | CP092251.1 |
| Illumina sequencing | | | |
| Coverage (×) | 947 | 2,145 | 53 |
| No. of paired-end reads | 6,740,304 | 15,431,938 | 381,216 |
| SRA accession no. | SRX14173698 | SRX14172012 | SRX14175583 |
| MinION raw sequence reads | | | |
| No. of reads | 96,132 | 88,990 | 85,961 |
| Avg length (bp) | 7,345 | 8,549 | 10,583 |
| SRA accession no. | SRX15714068 | SRX15714069 | SRX15714067 |
| MinION filtered reads | | | |
| Coverage (×) | 617 | 651 | 891 |
| No. of reads | 47,974 | 46,196 | 50,105 |
| Avg length (bp) | 12,446 | 13,786 | 17,413 |
| SRA accession no. | SRX14173699 | SRX14172013 | SRX14175584 |
| Length (bp) | 968,336 | 978,181 | 979,308 |
| %G+C | 31.6 | 31.4 | 31.6 |
| No. of tRNAs | 32 | 32 | 32 |
| No. of rRNAs | 6 | 6 | 6 |
| No. of CDSs[a] | 754 | 759 | 759 |

[a] CDSs, coding sequences.

were identified and trimmed automatically using the software. Each genome was assembled into a single circular contig, rotated to start with *dnaA*, and polished automatically using the software. Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline version 6.0 during the genome submission (11, 12). The total genome size and other genome statistics are given in Table 1.

**Data availability.** The annotated genome sequences, along with the raw reads, have been deposited at GenBank. The accession numbers are listed in Table 1.

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