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GENOME SEQUENCES

Whole-Genome Sequencing of Three *M. gallisepticum* Isolates Similar to the 6/85 Vaccine Strain

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ABSTRACT Effective control of *Mycoplasma gallisepticum* infection is accomplished through vaccination with live attenuated vaccines. However, virulent *M. gallisepticum* strains with genetic markers consistent with those of vaccine strains were found in infected flocks. We report here the complete genetic sequences of three isolates that are genetically similar to the 6/85 vaccine strain.

Vaccination with live attenuated *Mycoplasma gallisepticum* vaccines has been shown to be an effective method of preventing disease in commercial layer flocks (1, 2). However, unvaccinated flocks have been shown to be infected with strains with genetic markers consistent with the 6/85 vaccine strain (3, 4). Three field isolates were sequenced to determine their similarity to the 6/85 strain.

The three *M. gallisepticum* strains were a gift from Stanley Kleven (University of Georgia, retired). The strains were grown at 37°C in modified Frey's medium to mid-log phase (as determined by phenol red indicator color change) and stored at −80°C (5, 6). Bacteria were grown on Frey's agar, and a single colony was picked and grown to mid-log phase for three 1/10 passages in Frey's broth. The bacteria were pelleted by centrifugation (10 min at 20,000 × *g*), and the pellets were stored at −80°C. Genomic DNA for sequencing was isolated from the frozen pellets using a DNeasy blood and tissue kit (Qiagen, Inc., Germantown, MD, USA) according to the manufacturer's instructions. A NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to quantitate the DNA concentration and purity (optical density [OD] ratios, 260/280 nm and 260/230 nm).

The USDA ARS Genomics and Bioinformatics Research Unit (Stoneville, MS, USA) performed the Illumina sequencing. The DNA samples were sheared into 500-bp fragments, and 2 × 150-bp paired-end libraries were prepared using an Illumina NeoPrep instrument with the version 1.0 kit (Illumina, San Diego, CA, USA). Sequencing was performed using an Illumina NextSeq 500 sequencer (Illumina). To prepare the sequences for assembly, the ends were trimmed using FastX-Trimmer, and bases 9 to 144 were retained (FastX-Toolkit version 0.0.14). Paired-end sequence files with a quality threshold of 25 and length threshold of 20 were generated using Sickle version 1.33 (7). Default settings were used for all software unless otherwise specified. The sequence quality averaged at least 34, and the average length was 136 bases for all sequences. The sequence file data are provided in Table 1.

Unsheared genomic DNA isolated from duplicate frozen bacterial cells pellets as used for the Illumina sequencing was barcoded and prepared for MinION sequencing using the EXP-NBD103 and SQK-LSK108 kits, and sequencing was performed over 48 h using a FLO-MIN106 flow cell (Oxford Nanopore Technologies, Oxford, UK). DNA base calling and barcode sorting were performed using Guppy version 5.0.7 (Oxford Nanopore Technologies). Filtlong was used to remove sequences shorter than 3,000 bases and decrease the overall number of sequences to 100 million total bases using the "–target_bases" command. The sequences were assembled using both the MinION and Illumina data sets with the hybrid assembly mode of Unicycler version 0.4.8 (8). Each genome was assembled into a single circular contig. Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline

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TABLE 1 Genome information and accession numbers

Parameter	Data for strain: K4631b	K4465	K5263e
Isolation source (species, yr, location)[a]	*Meleagris gallopavo*, 1998, Michigan, USA	*Meleagris gallopavo*, 1997, Ohio, USA	*Gallus domesticus*, 2002, Georgia, USA
GenBank accession no.	CP119260	CP119259	CP119258
Illumina data			
Sequencing coverage (×)	514	3,295	256
No. of paired-end reads	1,911,847	11,847,908	920,691
SRA accession no.	SRX19558972	SRX19558974	SRX19558976
MinION raw read data			
No. of reads	105,624	571,906	380,506
Avg length (bp)	8,003	1,373	1,597
N_{50} (bp)	16,223	3,014	5,622
SRA accession no.	SRX19558973	SRX19558975	SRX19558977
MinION filtered read data			
Coverage (×)	99	102	102
No. of reads	4,129	7,945	7,678
Avg length (bp)	24,227	12,587	13,025
N_{50} (bp)	25,851	12,857	13,261
Genome statistics			
Length (bp)	1,011,242	978,019	976,657
%G+C	31	31	31
No. of tRNAs	32	32	32
No. of rRNAs	8	6	6
No. of CDSs[b]	800	763	767

[a] See references 3 and 4.
[b] CDSs, coding DNA sequences.

version 6.4 during genome submission (9, 10). The total genome size and other genome statistics are given in Table 1.

Data accessibility. The annotated genomes and raw reads were deposited at GenBank and in the Sequence Read Archives, respectively. The accession numbers are listed in Table 1.

REFERENCES

1. Ley DH. 2003. *Mycoplasma gallisepticum* infection, p 722–744. *In* Saif YM, Barnes HJ, Glisson Fadly AM, McDougald LR, Swayne DE (ed), Diseases of poultry, 11th ed. Iowa State Press, Ames, IA.
2. Evans JD, Leigh SA, Branton SL, Collier SD, Pharr GT, Bearson SMD. 2005. Mycoplasma gallisepticum: current and developing means to control the avian pathogen. J Appl Poult Res 14:757–763. https://doi.org/10.1093/japr/14.4.757.
3. Ferguson NM, Hepp D, Sun S, Ikuta N, Levisohn S, Kleven SH, Garcia M. 2005. Use of molecular diversity of Mycoplasma gallisepticum by gene-targeted sequencing (GTS) and random amplified polymorphic DNA (RAPD) analysis for epidemiological studies. Microbiology (Reading) 151:1883–1893. https://doi.org/10.1099/mic.0.27642-0.
4. Throne Steinlage SJ, Ferguson N, Sander JE, Garcia M, Subramanian S, Leiting VA, Kleven SH. 2003. Case report: isolation and characterization of a 6/85-like Mycoplasma gallisepticum from commercial laying hens. Avian Dis 47:499–505. https://doi.org/10.1637/0005-2086(2003)047[0499:ICOAL]2.0.CO;2.
5. Frey ML, Hanson RP, Andrson DP. 1968. A medium for the isolation of avian mycoplasmas. 11. Am J Vet Res 29:2163–2171.
6. Low IE, Eaton MD. 1965. Replication of Mycoplasma pneumoniae in broth culture. J Bacteriol 89:725–728. https://doi.org/10.1128/jb.89.3.725-728.1965.
7. Joshi NA, Fass JN. 2011. Sickle: a sliding-window, adaptive, quality-based trimming toll for FastQ files (version 1.33).
8. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595. https://doi.org/10.1371/journal.pcbi.1005595.
9. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569.
10. Haft DH, DiCuccio M, Badretdin A, Brover V, Chetvernin V, O'Neill K, Li W, Chitsaz F, Derbyshire MK, Gonzales NR, Gwadz M, Lu F, Marchler GH, Song JS, Thanki N, Yamashita RA, Zheng C, Thibaud-Nissen F, Geer LY, Marchler-Bauer A, Pruitt KD. 2018. RefSeq: an update on prokaryotic genome annotation and curation. Nucleic Acids Res 46:D851–D860. https://doi.org/10.1093/nar/gkx1068.