AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Complete Genome Sequences of Two Vaccine Strains and One Field Isolate of *Mycoplasma gallisepticum*

Spencer A. Leigh,[a] Jeff D. Evans,[a] Scott L. Branton[a]

[a]USDA-ARS Poultry Research Unit, Mississippi State, Mississippi, USA

ABSTRACT *Mycoplasma gallisepticum* infection of poultry can cause significant losses for poultry producers. Live attenuated *M. gallisepticum* vaccines mitigate the losses caused by infection, although the antigens that lead to immune protection have not been identified. Here, we report the sequencing of two vaccine strains and one field strain.

Mycoplasma gallisepticum infection of poultry can cause significant pathology and severe economic losses for poultry producers, but its effects can be mitigated by vaccination with a live *M. gallisepticum* vaccine (1, 2). While protection from *M. gallisepticum*-caused disease can be obtained through live *M. gallisepticum* vaccination, the genomic changes that result in effective vaccine strains are still being elucidated.

The 6/85 and ts-11 strains were cultured from the commercial vaccines (Mycovac-L [Merck Animal Health, Madison, NJ, USA] and *M. gallisepticum* vaccine [Merial Select, now part of Boehringer Ingelheim, Duluth, GA, USA], respectively). The mx-4 strain is a field isolate that was obtained over 20 years ago from the Mississippi State Veterinary Diagnostic Laboratory. All three strains were grown on Frey's agar (3), with 6/85 and mx-4 grown at 37°C and ts-11 grown at 30°C. A single colony of each was grown in Frey's broth. Bacteria were grown to mid-log phase, as indicated by the orange color of the phenol red indicator, and pelleted by centrifugation (20,000 × *g*), and the pellets were stored at −80°C (4). DNA for genome sequencing was isolated from bacterial pellets using a DNeasy blood and tissue kit (Qiagen, Inc., Germantown, MD, USA) according to the manufacturer's instructions. DNA quantity and purity ($A_{260/280}$ and $A_{260/230}$ ratios) were assessed using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Illumina sequencing was done by the USDA ARS Genomics and Bioinformatics Research Unit (Stoneville, MS, USA). DNA samples were sheered to 500-bp fragments, libraries were prepared with an Illumina NeoPrep instrument, and 2 × 150-bp paired-end sequencing was performed using an Illumina NextSeq 500 sequencer (Illumina Biotechnology Company, San Diego, CA, USA). Sequences were trimmed with FastX trimmer, and bases 9 to 144 were retained (FastX toolkit version 0.0.14). Sickle version 1.33 was used to filter paired sequences with quality and length thresholds of 25 and 20, respectively (5). All software packages were used with default parameters unless otherwise specified. The average sequence quality was at least 34, and the average length was 136 bases for all sequences. A total of 10,798,646 paired-end reads for 6/85, 19,102,164 paired-end reads for ts-11, and 4,341,771 paired-end reads for mx-4 were used for assembly.

Genomic DNA for MinION sequencing (Oxford Nanopore Technologies, Oxford, UK) was isolated as described above using frozen bacterial cell pellets of the same passage number as that used for Illumina sequencing. Genomic DNA was barcoded and prepared for sequencing using kits EXP-NBD103 and SQK-LSK108, and 1D sequencing was performed for 48 hours using a FLO-MIN106 flow cell. DNA base calling and

Citation Leigh SA, Evans JD, Branton SL. 2019. Complete genome sequences of two vaccine strains and one field isolate of *Mycoplasma gallisepticum*. Microbiol Resour Announc 8:e01237-19. https://doi.org/10.1128/MRA.01237-19.

Editor Vincent Bruno, University of Maryland School of Medicine

Address correspondence to Spencer A. Leigh, spencer.leigh@usda.gov.

Received 30 September 2019
Accepted 8 November 2019
Published 5 December 2019

TABLE 1 Accession numbers and genome information

Strain	Accession no.	Illumina sequencing coverage (×)	Illumina SRA no.	MinION sequencing coverage (×)	MinION SRA no.	Length (bp)	G+C content (%)	No. of tRNAs	No. of rRNAs	No. of CDS[a]
6/85	CP044224	2,671	SRR10165019	241	SRR10165018	994,372	31.6	32	6	764
ts-11	CP044225	4,785	SRR10165017	267	SRR10165016	963,058	31.4	32	6	757
mx-4	CP044226	1,066	SRR10165015	323	SRR10165014	993,340	31.6	33	6	769

[a] CDS, coding sequences.

barcode sorting were performed using Albacore version 2.0.1 (Oxford Nanopore Technologies). Porechop version 0.2.4 was used to remove barcode and adapter sequences (6). Fastqutils from the NGSUtils package version 0.5.9 was used to remove sequences shorter than 3,000 bases to facilitate assembly (7). Average sequence length postfiltering for MinION sequences was >12,000 bases for all genome samples, with 18,390/30,402 sequences retained for 6/85, 16,935/28,466 sequences retained for ts-11, and 21,170/37,143 sequences retained for mx-4. This resulted in greater than 200× coverage for each MinION data set. Genome assembly using both the Illumina and MinION data was performed using the hybrid assembly mode of Unicycler version 0.4.1 (8). Each genome was assembled into a single circular contig. Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline during genome submission (9, 10). The total genome sizes and other genome statistics are given in Table 1.

Data availability. The annotated genomes along with the sequencing reads were deposited in GenBank and the Sequence Read Archive. 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 JR, 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. Frey MC, Hanson RP, Anderson DP. 1968. A medium for the isolation of avian mycoplasma. Am J Vet Res 29:2164–2171.
4. Low IE, Eaton MD. 1965. Replication of Mycoplasma pneumoniae in broth culture. J Bacteriol 89:725–728.
5. Joshi NA, Fass JN. 2011. Sickle: a sliding-window, adaptive, quality-based trimming toll for FastQ files (version 1.33). https://github.com/najoshi/sickle.
6. Wick R. 2017. Porechop: adapter trimmer for Oxford Nanopore reads. https://github.com/rrwick/Porechop.
7. Breese MR, Liu Y. 2013. NGSUtils: a software suite for analyzing and manipulating next-generation sequencing datasets. Bioinformatics 29:494–496. https://doi.org/10.1093/bioinformatics/bts731.
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.