AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Complete Genome Sequences of *Mycoplasma anatis*, *M. anseris*, and *M. cloacale* Type Strains

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ABSTRACT *Mycoplasma anatis*, *M. anseris*, and *M. cloacale* are pathogens of waterfowl. Airsacculitis, nervous disease, and reproductive disorders are the main symptoms in the affected flocks. Here, we report the complete genome sequences of the *M. anatis* (NCTC 10156), *M. anseris* (ATCC 49234), and *M. cloacale* (NCTC 10199) type strains.

Mycoplasma anatis, *M. anseris*, and *M. cloacale* are waterfowl-pathogenic bacteria. *M. anatis* may cause serious nervous symptoms under stress conditions in ducks (1), while airsacculitis, peritonitis, and the increase of embryo lethality were described after experimental inoculation of the pathogen (2). The type strain was isolated from a duck with sinusitis (3). *M. anseris* causes airsacculitis, peritonitis, and embryo lethality (4) and probably has a role in cloaca and phallus inflammation of ganders (5). The type strain was isolated from a flock with a history of phallus inflammation (6). The *M. cloacale* type strain was isolated from a turkey (7), but this species could be isolated from ducks and geese as well (8, 9). Egg infertility is the most common symptom caused by this agent in waterfowl (10). All three of these *Mycoplasma* strains can be transmitted vertically (4, 11, 12). The coexistence of waterfowl-pathogenic mycoplasmas has been described (13).

The *M. anatis* (NCTC 10156), *M. anseris* (ATCC 49234), and *M. cloacale* (NCTC 10199) type strains were purchased directly from the repositories. Cells were grown in Oxoid *Mycoplasma* broth medium (pH 7.8) (Thermo Fisher Scientific, Inc., Waltham, MA) supplemented with 0.5% (wt/vol) sodium pyruvate, 0.5% (wt/vol) glucose, and 0.005% (wt/vol) phenol red and were incubated at 37°C. DNA was extracted with the QIAamp DNA minikit (Qiagen, Inc., Hilden, Germany). DNA libraries were prepared with the Nextera mate pair library preparation kit (Illumina, Inc., San Diego, CA). Two genome sequencing runs were performed on an Illumina NextSeq 500 instrument for each strain, generating 2 × 150-bp (300 cycles) and 2 × 75-bp (150 cycles) mate pair reads. NxTrimm software (14) was used to trim the junction adapters from all the raw mate pair reads, generating shorter paired-end reads as well. First, contigs were generated per strain from the paired-end output data by the SPAdes Genome Assembler 3.11 (15) with the assembly-only option. Then, the paired-end contigs and the trimmed mate pair output data were assembled with the same option, generating the draft genomes. Trimmed reads (mate pair and paired-end) were control mapped to the draft *de novo* genome and curated with Geneious 9.1.8 software (16). Circularization of the contigs was performed by primer pairs and PCR assays specific for the contigs' ends (data not shown), and the PCR products were sequenced on the ABI Prism 3100 (Applied Biosystems, Foster City, CA) automated DNA sequencer. The NCBI Prokaryotic Genome Annotation Pipeline (17) online service was used to annotate the genomes. The rRNA

Received 10 July 2018 **Accepted** 4 September 2018 **Published** 27 September 2018

Citation Grózner D, Forró B, Sulyok KM, Marton S, Kreizinger Z, Bányai K, Gyuranecz M. 2018. Complete genome sequences of *Mycoplasma anatis*, *M. anseris*, and *M. cloacale* type strains. Microbiol Resour Announc 7:e00939-18. https://doi.org/10.1128/MRA.00939-18.

Editor Catherine Putonti, Loyola University Chicago

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TABLE 1 Genome information and GenBank accession numbers of *Mycoplasma anatis*, *M. anseris*, and *M. cloacale* type strains[a]

Strain	GenBank accession no.	SRA no.	Size (bp)	Coverage (×)	G+C content (%)	No. of CDS[b]	No. of rRNAs	No. of tRNAs	No. of ncRNAs[c]
M. anatis (NCTC 10156)	CP030141	SRP155810	956,093	292	26.7	791	6	33	1
M. anseris (ATCC 49234)	CP030140	SRP155813	750,010	1,833	26.4	617	6	32	2
M. cloacale (NCTC 10199)	CP030103	SRP155814	659,552	1,439	27.0	541	4	31	2

[a]The number of tmRNAs was 1 and the number of regulatory elements was 1 for each strain listed.
[b]CDS, coding sequences.
[c]ncRNAs, noncoding RNAs.

and the tRNA genes and the transfer-messenger RNA (tmRNA) genes were verified by RNAmmer (18) and ARAGORN (19), respectively.

The total genome sizes and information concerning the strains are detailed in Table 1. We hope that the presented complete and circularized genomes will improve research of the waterfowl-pathogenic *Mycoplasma* species.

Data availability. The annotated genome sequences were deposited in GenBank, and the raw read data are available in the Sequence Read Archive. The accession numbers are listed in Table 1.

ACKNOWLEDGMENTS

This work was supported by the Lendület (Momentum) program (LP2012-22) of the Hungarian Academy of Sciences. M.G., S.M., and Z.K. were supported by the Bolyai János Fellowship of the Hungarian Academy of Sciences. M.G. was supported by the Bolyai+ Fellowship (ÚNKP-18-4) of the New National Excellence Program of the Ministry of Human Capacities. The funders had no role in study design, analysis and interpretation, decision to publish, or preparation of the manuscript.

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