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Complete Genome Sequence of *Mycoplasma flocculare* Strain Ms42^T (ATCC 27399^T)

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***Mycoplasma flocculare* is a commensal or low-virulence pathogen of swine. The complete 778,866-bp genome sequence of *M. flocculare* strain Ms42^T has been determined, enabling further comparison to genomes of the closely related pathogen *Mycoplasma hyopneumoniae*. The absence of the *p97* and *glpD* genes may contribute to the attenuated virulence of *M. flocculare*.**

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Mycoplasma flocculare is generally considered to be a commensal colonizer of the porcine nasopharynx but an opportunist pneumonic pathogen in coinfections with *Mycoplasma hyopneumoniae* (1). *M. hyopneumoniae* is the agent of swine enzootic pneumonia, a disease with high morbidity that significantly impacts swine production (2). Based on 16S rRNA phylogeny, *M. flocculare* and *M. hyopneumoniae* are each other's closest relatives (3), prompting comparative analysis of representative genomes of these taxa (4). A draft genome sequence of *M. flocculare* ATCC 27716 (15 contigs; 763,948 bp) has been determined and extensively analyzed by comparative genomics and transcriptional profiling (4, 5). Presented herein is the completely assembled genome of the *M. flocculare* type strain Ms42^T (ATCC 27399^T), the parental strain from which isolate ATCC 27716 was derived by three additional filter cloning steps.

Genomic DNA was prepared from strain Ms42^T (obtained from the American Type Culture Collection as ATCC 27399^T) and sequenced at the National Center for Genomics Research (Santa Fe, NM, USA) using the Pacific Biosciences platform. Reads from two SMRT cells were assembled into a single contig using HGAP version 2 (6) with 574× coverage. An approximately 10.9-kb duplication was verified by PCR and Sanger sequencing. The 778,866-bp genome was auto-annotated using the PGAP pipeline at NCBI, followed by manual curation. The complete genome comprises 629 genes: 563 open reading frames (ORFs), 31 pseudogenes (22 independently confirmed with alternate sequencing chemistries), 30 tRNAs, and 3 rRNA genes with the 5S rRNA gene separated from the 16S-23S rRNA operon. The G+C content is 28.94%.

A comprehensive comparative analysis of the genomes of several swine mycoplasmas has been documented (4). In that study, it was noted that although multiple adherence-related genes of the *M. hyopneumoniae* P97/P102 families were present in *M. flocculare*, orthologs encoding the primary cilium adhesin P97 (7, 8) and downstream ORF P102 were absent. The same holds true for parental strain Ms42^T, indicating that the absence of the P97-P102 locus was not due to inadvertent selection of a deletion during subcloning.

Another feature that may contribute to the differential virulence of *M. flocculare* and *M. hyopneumoniae* is the absence of *glpD* (encoding glycerol 3-phosphate oxidase) in *M. flocculare*. Orthologs of *glpD* have been found in multiple *Mycoplasma* species, including the six *M. hyopneumoniae* isolates for which genome sequences are available. The product, GlpD, is responsible for the glycerol-dependent production of H_2O_2. This activity has been shown to be cytotoxic to eukaryotic cells when infected by *Mycoplasma mycoides* subspecies *mycoides* SC biotype (9), *Mycoplasma pneumoniae* (10), and *Mycoplasma gallisepticum* (11), although in the latter study, *glpD* was found to be dispensable for virulence in an infection model. The recent development of genetic tools for *M. hyopneumoniae* (12) should enable the potential roles of *glpD* and H_2O_2 production to be ascertained.

The first completely assembled *M. flocculare* genome will enable more detailed analyses of genome structure and plasticity among the *neurolyticum* cluster of *Mycoplasma* species and provides a complete reference for further postgenomic applications.

Nucleotide sequence accession number. This complete genome sequence has been deposited at DDBJ/EMBL/GenBank under the accession number CP007585.

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