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Complete Genome Sequence of *Mycoplasma haemofelis*, a Hemotropic Mycoplasma▽

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Here, we present the genome sequence of *Mycoplasma haemofelis* strain Langford 1, representing the first hemotropic mycoplasma (hemoplasma) species to be completely sequenced and annotated. Originally isolated from a cat with hemolytic anemia, this strain induces severe hemolytic anemia when inoculated into specific-pathogen-free-derived cats. The genome sequence has provided insights into the biology of this uncultivatable hemoplasma and has identified potential molecular mechanisms underlying its pathogenicity.

First described by Clark in 1942 (basonym *Eperythrozoon felis*) (2), *Mycoplasma haemofelis* is a pathogenic hemotropic mycoplasma (trivial name, hemoplasma) that can induce hemolytic anemia in cats (12). In 2001, it was reclassified to the genus *Mycoplasma* within the Mollicutes class following 16S ribosomal gene phylogenetic analysis (9); however, the positioning of the hemoplasmas within this genus remains controversial (15). To date, the feline hemoplasmas have been uncultivatable *in vitro*.

Genomic DNA from *M. haemofelis* strain Langford 1 was purified from blood taken from an experimentally infected specific-pathogen-free (SPF)-derived cat at a time of high parasitemia (10). This low-passage strain, originally isolated from a clinically anemic cat, has been shown to induce hemolytic anemia in SPF-derived cats (13, 14). Whole-genome shotgun pyrosequencing was performed by generating a standard DNA mate pair library with an 8-kb insert size using a 454 preparation kit (Roche Applied Sciences, Indianapolis, IN) and sequenced with a GS-FLX using Titanium chemistry (454 Life Sciences, Roche Applied Sciences). The 454 reads were assembled with Newbler (August 2010 R&D version of GSAssembler; Roche Applied Sciences), using the "stopjoin" finishing parameters. Remaining intrascaffold gaps were closed using specific PCR and Sanger sequencing to span the sequencing gaps. The final assembly was performed with gap4 (http://staden.sourceforge.net/). Overall sequence coverage was greater than 20-fold. Protein-coding genes were identified with GLIMMER (3) and GENEMARK (8) and tRNA genes (tRNAs) by tRNAscan-SE (7). Putative functions were inferred using BLAST against the National Center for Biotechnology Information databases (1) and InterProScan (4), and metabolic pathways were examined by using KEGG databases (5). Artemis version 12 was used to organize data and facilitate annotation (11). Orthologs/paralogs were defined using ORTHOMCL (6). CRISPI was used to examine the genome for clustered regularly interspaced short palindromic repeats (http://crispi.genouest.org/).

Analysis showed a single circular genome of 1,147,259 bp with a GC content of 38.9%. Of the 1,545 putative proteins identified, 328 (21.2%) matched proteins from other bacterial species. Genes involved in carbohydrate metabolism were limited to enzymes of the glycolytic pathway, with glucose appearing to be the sole energy source. The majority of the pentose phosphate pathway genes appear to be incomplete or absent, suggesting an alternative mechanism for sourcing purine and pyrimidine bases, such as scavenging from the host. Of the uncharacterized hypothetical proteins, 1,115 were arranged in series of paralogous repeats or comprised fragments thereof, with these genes encoding putatively surface-expressed proteins of approximately 200 amino acids. Also identified were 31 tRNAs for all amino acids (including a tryptophan tRNA antisense to the opal codon) and three ribosomal operons.

In summary, we report the first fully sequenced and annotated hemoplasma genome. Further analysis of these data will provide valuable information as to why this pathogen remains highly fastidious, as well as identifying potential immunogenic proteins, pathogenicity factors, and possible mechanisms for host immune system evasion.

Nucleotide sequence accession number. The genome sequence of *M. haemofelis* Langford 1 was deposited in the EMBL database under accession number FR773153.

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