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Complete Genome Sequences of Two Hemotropic Mycoplasmas, *Mycoplasma haemofelis* Strain Ohio2 and *Mycoplasma suis* Strain Illinois[▽]

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We report the complete and fully assembled genomes of *Mycoplasma haemofelis* strain Ohio2 and *Mycoplasma suis* strain Illinois, which are the first available genomes of these uncultivatable hemoplasma species. The single circular chromosomes of 1,152,484 bp and 742,431 bp for *M. haemofelis* and *M. suis*, respectively, are typical of mycoplasma species, having reduced size and low G+C content (38.8% for *M. haemofelis* and 31.1% for *M. suis*). Their metabolic pathways are reduced, with evidence of adaption to the blood environment.

Mycoplasma species (class Mollicutes) are wall-less bacteria characterized by small genomes ranging from 580 to 1,350 kb (1). *Mycoplasma haemofelis* (formerly *Haemobartonella felis*) and *M. suis* (formerly *Eperythrozoon suis*) are hemotropic pathogens that cause acute and chronic diseases in cats and pigs, respectively (2). These organisms define a new clade of mycoplasmas that have a unique tropism for red blood cells (RBCs) of their vertebrate host. The trivial name hemoplasma has been given to these bacteria (3, 4). There are two phylogenetic clusters of hemoplasmas, the haemofelis cluster (defined by *M. haemofelis*) and the suis cluster (defined by *M. suis*), which appear to have descended from a common ancestor. Intriguingly, the sequence similarity of the 16S rRNA genes for hemoplasmas compared to that for other mycoplasmas is relatively low (77% to 84%). The complete sequencing and assembly of these two hemoplasmas were pursued to gain new insights into the evolution, metabolism, and pathogenesis of these unique bacteria.

Genomic DNA was extracted from bacteria isolated from blood samples of an experimentally infected cat (*M. haemofelis*) and pig (*M. suis*). The whole genome of each hemoplasma was sequenced using GS-FLX-454 and Titanium chemistry to sequence a 3-kb paired-end library. First-pass annotation was achieved using the blast2GO and Manatee annotation tools. Analysis of genomic features shows that *M. haemofelis* has characteristics of a typical mycoplasma, including a small genome size of 1,152,484 bp and a low G+C content of 38.8%, as well as codon usage of the opal stop codon (UGA) for tryptophan. The 16S, 23S, and 5S rRNA genes were located one after the other as single copies; 31 tRNAs and the noncoding RNase P gene were identified. There were 1,426 predicted protein coding sequences (CDS). Gene homologies and a GC skew graph show that both of these hemoplasmas have typical *Mollicutes oriC* genes. The single circular genome of *M. suis* is smaller than that of *M. haemofelis* at 742,431 bp and has a lower G+C content of 31.1%. Totals of 783 CDS and 32 tRNAs were detected. While the genome of *M. suis* also had only one copy of each rRNA gene, the 16S rRNA gene is separated and 576 kb upstream of the 23-5 rRNA operon. The majority of *M. haemofelis* and *M. suis* CDS were for hypothetical proteins.

The predicted metabolic pathways of these hemoplasmas are reduced and show evidence of adaptation to the blood environment. These organisms are glycolytic species, relying on energy generation through sugar fermentation and ATP-synthase. The pentose-phosphate pathway and metabolism of cofactors, vitamins, and pyruvate dehydrogenase appear to be missing, and no toxin orthologs were identified. We speculate that hemoplasmas may cause disease by a nutrient scavenger and competition mechanism, which may lead to decreased life span of RBCs. These properties are likely to hold important clues for the design of culture media to support the continuous *in vitro* growth of these fastidious organisms. Further, annotation of these genomic sequences may also provide new insights into the evolution of the hemoplasmas and help us to better understand their pathogenesis.

Nucleotide sequence accession numbers. The complete genome sequences of *M. haemofelis* strain Ohio2 (project number 50029) and *M. suis* strain Illinois (project number 49421) are available in GenBank under accession numbers AEVA00000000 and ADWK01000001, respectively.

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