JB Journals.ASM.org

GENOME ANNOUNCEMENT

Genome Sequence for "*Candidatus* Mycoplasma haemominutum," a Low-Pathogenicity Hemoplasma Species

Emily N. Barker,[a] Alistair C. Darby,[b] Chris R. Helps,[a] Iain R. Peters,[a] Margaret A. Hughes,[b] Alan D. Radford,[c] Marilisa Novacco,[d] Felicitas S. Boretti,[d] Regina Hofmann-Lehmann,[d] and Séverine Tasker[a]

School of Veterinary Sciences, University of Bristol, Langford, United Kingdom[a]; Centre for Genomic Research, Institute of Integrative Biology, University of Liverpool, Liverpool, United Kingdom[b]; School of Biological Sciences, University of Liverpool, Liverpool, United Kingdom[c]; and Vetsuisse Fakultät, Universität Zürich, Zürich, Switzerland[d]

We present the genome sequence of "*Candidatus* Mycoplasma haemominutum" strain Birmingham 1, a low-pathogenicity feline hemoplasma strain.

Hemoplasmas are uncultivatable hemotropic bacteria within the genus *Mycoplasma* (8). "*Candidatus* Mycoplasma haemominutum" was identified as being distinct from *Mycoplasma haemofelis* on the basis of 16S rRNA gene sequence data comparison (12). "*Ca.* Mycoplasma haemominutum" is the most prevalent of the feline-infecting hemoplasmas (10), and infection doesn't usually result in clinical disease in immunocompetent cats, but decreases in hematocrit have been reported (11, 17).

Genomic DNA from "*Ca.* Mycoplasma haemominutum" strain Birmingham 1 was purified from blood taken from an experimentally infected specific-pathogen-free cat at high parasitemia, as described previously (2). This low-passage strain, derived from a naturally infected cat (15), has been used in previous infection kinetics studies (9, 14, 16, 17). Whole shotgun pyrosequencing was performed by generating a standard DNA library with a 3-kb insert size using the 454 library preparation kit (Roche Applied Sciences, Indianapolis, IN) and sequenced on a GS-FLX using Titanium chemistry (454 Life Sciences, Roche Applied Sciences). The 454 reads were assembled with Newbler (August 2010 R&D version; Roche Applied Sciences). The final assembly was performed with gap4 (http://staden.sourceforge.net). Open reading frames were identified with GLIMMER (3) and GENEMARK (7), and tRNA genes were identified by tRNAscan-SE (6). Putative functions were inferred using BLAST against the National Center for Biotechnology Information databases (1) and InterProScan (4). Artemis v12 was used to organize data and facilitate annotation (13). Orthologs/paralogs were defined using ORTHOMCL (5).

Final assembly (>50-fold coverage) of the "*Ca.* Mycoplasma haemominutum" genome gave a single linear contig of 513,880 bp with 35.5% GC content. The GC skew and gene organization, compared to those of other hemoplasma genomes, suggested that a region of ~3,000 bp, comprising the origin of replication and genes *dnaA* and *dnaN*, was missing. Despite many attempts, this region could not be amplified by PCR from "*Ca.* Mycoplasma haemominutum" genomic DNA, and, hence, a circular chromosome could not be confirmed.

Of the 547 putative proteins identified, 219 (40%) matched functionally characterized proteins, 149 (27.2%) matched hypothetical proteins of other bacterial species, and 179 (32.7%) had no matches. Of the matched hypothetical proteins, 38 had predicted biochemical functions. In common with other sequenced hemoplasmas, the number of known functional proteins in the genome is small, with energy metabolism appearing to be restricted to the glycolytic pathway and ATP synthesis. Series of paralogs containing a total of 159 hypothetical protein repeats were present; these included 47 hypothetical proteins that had homologs in *Mycoplasma suis* but not in other hemoplasma or bacterial species, suggesting a recent common ancestor in *M. suis*. Unlike other hemoplasmas, "*Ca.* Mycoplasma haemominutum" lacks an intact GMP synthase pathway, which could impair its ability to synthesize purine nucleotides *de novo*, and lacks a type 1 restriction modification system and DNA methylases, found in *M. suis* and *M. haemofelis*, which could explain why it has markedly fewer paralog repeats (159 versus 229 to 240 in *M. suis* and 1,013 to 1,115 in *M. haemofelis*).

We report the nearly complete annotated "*Ca.* Mycoplasma haemominutum" genome. Feline hemoplasma genomes can now be compared to identify pathogenic determinants.

Nucleotide sequence accession number. The genome sequence of "*Ca.* Mycoplasma haemominutum" strain Birmingham 1 was deposited in the EMBL database under accession number HE613254.

ACKNOWLEDGMENTS

The Roche 454 sequencing and analysis were supported by a grant from the University of Bristol Campaigns and Alumni Fund. The feline samples used in this study were generated from a study funded by the Wellcome Trust (grant no. 077718). E.N.B. was supported by a University of Bristol Postgraduate Research Scholarship and Pfizer Animal Health. M.N. was in part supported by a University of Zurich research grant.

We thank Theres Meili Prodan and Enikoe Goenczi for excellent technical assistance.

REFERENCES

1. **Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ.** 1990. Basic local alignment search tool. J. Mol. Biol. **215**:403–410.
2. **Barker EN, et al.** 2010. Detection of humoral response using a recombinant heat shock protein 70 (dnaK) of *Mycoplasma haemofelis* in experimentally and naturally hemoplasma infected cats. Clin. Vaccine Immunol. **17**:1926–1932.

Received 21 November 2011 **Accepted** 30 November 2011

Address correspondence to Emily N. Barker, emi.barker@bristol.ac.uk.

doi:10.1128/JB.06560-11

3. **Delcher AL, Harmon D, Kasif S, White O, Salzberg SL.** 1999. Improved microbial gene identification with GLIMMER. Nucleic Acids Res. **27**: 4636–4641.
4. **Hunter S, et al.** 2009. InterPro: the integrative protein signature database. Nucleic Acids Res. **37**:D211–D215.
5. **Li L, Stoeckert CJ Jr, Roos DS.** 2003. OrthoMCL: identification of ortholog groups for eukaryotic genomes. Genome Res. **13**:2178–2189.
6. **Lowe TM, Eddy SR.** 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. **25**: 955–964.
7. **Lukashin AV, Borodovsky M.** 1998. GeneMark.hmm: new solutions for gene finding. Nucleic Acids Res. **26**:1107–1115.
8. **Neimark H, Johansson KE, Rikihisa Y, Tully JG.** 2001. Proposal to transfer some members of the genera *Haemobartonella* and *Eperythrozoon* to the genus *Mycoplasma* with descriptions of '*Candidatus* Mycoplasma haemofelis,' '*Candidatus* Mycoplasma haemomuris,' '*Candidatus* Mycoplasma haemosuis' and '*Candidatus* Mycoplasma wenyonii'. Int. J. Syst. Evol. Microbiol. **51**:891–899.
9. **Peters IR, et al.** 2011. Detection of feline haemoplasma species in experimental infections by in-situ hybridisation. Microb. Pathog. **50**:94–99.
10. **Peters IR, Helps CR, Willi B, Hofmann-Lehmann R, Tasker S.** 2008. The prevalence of three species of feline haemoplasmas in samples submitted to a diagnostics service as determined by three novel real-time duplex PCR assays. Vet. Microbiol. **126**:142–150.
11. **Reynolds CA, Lappin MR.** 2007. '*Candidatus* Mycoplasma haemominutum' infections in 21 client owned cats. J. Am. Anim. Hosp. Assoc. **43**: 249–257.
12. **Rikihisa Y, et al.** 1997. Western immunoblot analysis of *Haemobartonella muris* and comparison of 16S rRNA gene sequences of *H. muris*, *H. felis*, and *Eperythrozoon suis*. J. Clin. Microbiol. **35**:823–829.
13. **Rutherford K, et al.** 2000. Artemis: sequence visualization and annotation. Bioinformatics **16**:944–945.
14. **Tasker S, et al.** 2006. Effect of chronic feline immunodeficiency infection, and efficacy of marbofloxacin treatment, on '*Candidatus* Mycoplasma haemominutum' infection. Microbes Infect. **8**:653–661.
15. **Tasker S, et al.** 2001. 16S rDNA comparison demonstrates near identity between an United Kingdom *Haemobartonella felis* strain and the American California strain. Vet. Microbiol. **81**:73–78.
16. **Tasker S, et al.** 2009. Distribution of feline haemoplasmas in blood and tissue following experimental infection. Microb. Pathog. **47**:334–340.
17. **Tasker S, et al.** 2009. Description of outcomes of experimental infection with feline haemoplasmas: haematology, Coombs' testing and blood glucose concentrations. Vet. Microbiol. **139**:323–332.