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Draft Genome Sequence of the First Human Isolate of the Ruminant Pathogen *Mycoplasma capricolum* subsp. *capricolum*

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Mycoplasma capricolum subsp. *capricolum* is a well-known pathogen of small ruminants. A recent human case of septicemia involving this agent raised the question of its potential pathogenicity to humans. We present the first draft genome sequence of a human *Mycoplasma capricolum* subsp. *capricolum* isolate.

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*M*ycoplasma capricolum* subsp. *capricolum* is a known etiologic agent of contagious agalactia in small ruminants, a disease associated with chronic inflammation, arthritis, and mastitis (1). Even though *M. capricolum* subsp. *capricolum* is considered one of the least pathogenic members of the *Mycoplasma mycoides* cluster, disease outbreaks caused by this agent can have a significant impact on goat farming industries due to loss of milk production and increased mortality (2, 3). While *M. capricolum* subsp. *capricolum* infections are well known in sheep and goats, reports of other animal hosts are scarce (4). Recently, the *Mycoplasma capricolum* subsp. *capricolum* strain 14DL0024 was isolated from a hospitalized human displaying symptoms of septicemia (M. Heller, R. Schwarz, G. Noe, J. Jores, A. Fischer, E. Schubert, and K. Sachse, submitted for publication). Here, we report the draft genome sequence of the human *Mycoplasma capricolum* subsp. *capricolum* strain 14DL0024.

Genomic DNA of the bacterial isolate was extracted as reported elsewhere (A. Fischer, I. Santana-Cruz, E. Schieck, H. Gourlé, M. Lambert, H. W. Suvarna Nadendla, R. A. Miller, J. Hegerman, J. Meens, S. Vashee, J. Frey, and J. Jores, submitted for publication). DNA libraries were built using the NEBNext library kit E6070 and sequenced on the Illumina HiSeq 2000 platform, yielding a total of 20,483,838 paired-end reads. Reads were processed, trimmed, and assembled using AdapterRemoval (v1.1) (5), Novobarcode Beta-0.8, and Ray (2.3.1) (6). Lastly, contigs were extended and scaffolded by SSPACE basic (v.2.0) (7), yielding a set of scaffolds of which sequences shorter than 2 kilobases (kb) were discarded. The resulting draft genome yielded 7 scaffolds between 11 and 218 kb with a GC content of 23.7%, an N_{50} of 197,640 bp, and an N_{90} of 120,063 bp. With a total size of 964,668 nucleotides, the draft genome covers 95.5% of the length of the reference genome (GI: 83319253) of 1,010,023 bp, indicating a high level of completion for the assembly.

To verify the species of the isolated bacteria, average nucleotide identity (ANI) analyses (8) were carried out, comparing the draft genome with available genomes of the *Mycoplasma mycoides* cluster. As expected, the analysis revealed the highest average nucleotide identity (97.77%) with *Mycoplasma capricolum* subsp. *capricolum* (GI:83319253), while the closely related *Mycoplasma capricolum* subsp. *capripneumoniae* strains M1601 (GI: 326314730), ILRI181 (GI: 677282260), and F38 (GI:675241189) displayed slightly lower ANI values between 96.15% and 96.16%. The remaining members of the *Mycoplasma mycoides* cluster all exhibited significantly smaller ANI values below 93%, thus confirming the species of the draft genome to be *Mycoplasma capricolum* subsp. *capricolum*.

The draft genome was annotated by RAST (9), which identified 773 protein coding genes, 30 tRNAs, and 7 rRNAs. Fourteen genes were unique to the draft genome compared to the *M. capricolum* subsp. *capricolum* reference. Most notable is the presence of three key genes of type I restriction modification (RM) systems. Encoding the specificity (S), modification (M), and restriction (R) subunits, the three genes comprise the entire genetic basis for type I restriction modification. Considering the absence of RM genes in the reference genome and previous reports linking RM systems with bacterial virulence and immune evasion (10), this might indicate a role of the RM system in human *M. capricolum* subsp. *capricolum* infection. However, additional studies will be needed to support this claim.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. LBMF00000000. The version described in this paper is the first version, LBMF01000000.

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REFERENCES

1. **Bergonier D, Berthelot X, Poumarat F**. 1997. Contagious agalactia of small ruminants: current knowledge concerning epidemiology, diagnosis and control. Rev Sci Tech **16**:848–873.

2. **Gómez-Martín A, Amores J, Paterna A, De la Fe C**. 2013. Contagious agalactia due to *Mycoplasma* spp. in small dairy ruminants: epidemiology and prospects for diagnosis and control. Vet J **198**:48–56. http://dx.doi.org/10.1016/j.tvjl.2013.04.015.

3. **Awan MA, Abbas F, Yasinzai M, Nicholas RAJ, Barbar S, Ayling RD, Attique MA, Ahmed Z**. 2009. Prevalence of *Mycoplasma capricolum* subspecies *capricolum* and *Mysoplasma putrefaciens* in goats in Pishin district of Balochistan. Pak Vet J **29**:179–185.

4. **Nicolas MM, Stalis IH, Clippinger TL, Busch M, Nordhausen R, Maalouf G, Schrenzel MD**. 2005. Systemic disease in Vaal rhebok (*Pelea capreolus*) caused by mycoplasmas in the mycoides cluster. J Clin Microbiol **43**:1330–1340. http://dx.doi.org/10.1128/JCM.43.3.1330-1340.2005.

5. **Lindgreen S**. 2012. AdapterRemoval: easy cleaning of next-generation sequencing reads. BMC Res Notes **5**:337. http://dx.doi.org/10.1186/1756-0500-5-337.

6. **Boisvert S, Raymond F, Godzaridis E, Laviolette F, Corbeil J**. 2012. Ray Meta: scalable *de novo* metagenome assembly and profiling. Genome Biol **13**:R122. http://dx.doi.org/10.1186/gb-2012-13-12-r122.

7. **Boetzer M, Pirovano W**. 2014. SSPACE-LongRead: scaffolding bacterial draft genomes using long read sequence information. BMC Bioinformatics **15**:211. http://dx.doi.org/10.1186/1471-2105-15-211.

8. **Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM**. 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol **57**:81–91. http://dx.doi.org/10.1099/ijs.0.64483-0.

9. **Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD**. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics. **9**:75. http://dx.doi.org/10.1186/1471-2164-9-75.

10. **Gumulak-Smith J, Teachman A, Tu AH, Simecka JW, Lindsey JR, Dybvig K**. 2001. Variations in the surface proteins and restriction enzyme systems of *Mycoplasma pulmonis* in the respiratory tract of infected rats. Mol Microbiol **40**:1037–1044. http://dx.doi.org/10.1046/j.1365-2958.2001.02464.x.