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GENOME SEQUENCES

Genome Sequences of *Mycobacterium tuberculosis* Biovar bovis Strains Ravenel and 10-7428

Syeda A. Hadi,[a] Evan P. Brenner,[a] Rinosh Mani,[b] Mitchell V. Palmer,[c] Tyler Thacker,[d] Srinand Sreevatsan[a]

[a]Pathobiology and Diagnostic Investigation Department, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan, USA
[b]Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Michigan State University, Lansing, Michigan, USA
[c]National Animal Disease Center, U.S. Department of Agriculture, Ames, Iowa, USA
[d]National Veterinary Services Laboratory, U.S. Department of Agriculture, Ames, Iowa, USA

ABSTRACT We report the draft genomes of two *Mycobacterium tuberculosis* biovar bovis strains. Strain Ravenel was isolated in the 1900s and has been shown to be attenuated in cattle. Strain 10-7428 is considered highly pathogenic in cattle and was isolated from a bovine tuberculosis outbreak.

Mycobacterium tuberculosis biovar bovis is the primary cause of bovine tuberculosis, a major disease of cattle. We report the genomes of one virulent strain (10-7428) and one attenuated strain (Ravenel).

Strain Ravenel was isolated in the 1900s from a tuberculous cow and has been maintained in rabbits. Ravenel is virulent in rabbits (1, 2), guinea pigs (2), and mice (2–4) but causes subclinical infections in cattle without tuberculous lesions (5). Strain 10-7428 was isolated from a 2010 outbreak in a Colorado dairy herd (6) and has been maintained by the U.S. Department of Agriculture (USDA) (7).

Ravenel and 10-7428 strains obtained from the USDA National Animal Disease Center were cultured at Michigan State University on Middlebrook 7H10 slants (Hardy Diagnostics, Santa Maria, CA, USA) for 14 days at 37°C in 5% CO_2 before DNA extraction with the soil/fecal DNA miniprep kit (Zymo, Irving, CA, USA) and submission to Novogene for sequencing. Genomic DNA was randomly sheared and library preparation was performed with the NEBNext DNA library preparation kit (New England BioLabs) for paired-end (2 × 150-bp) libraries. Quality checking was performed with a Qubit 2.0 fluorometer, Agilent 2100 Bioanalyzer, and quantitative PCR before Illumina NovaSeq sequencing. Novogene used in-house software (v1.0) for quality control and trimming, removing read pairs with any adapter contamination, pairs for which uncertain nucleotide calls were >10% of the read length, and pairs with Phred Q scores of ≤5 for at least one-half of either read. Further analyses were performed with default parameters unless noted. Kraken 2 (8) mapped 99.56% of Ravenel reads and 99.06% of 10-7428 reads to the *Mycobacterium tuberculosis* complex, using the Kraken 2 suggested default standard database for the widest scope of contamination detection. All reads, regardless of Kraken 2 assignment, were assembled *de novo* using ABySS v2.1.5 (k value of 96) (9). *M. tuberculosis* variant *bovis* AF2122/97 (GenBank accession number LT708304) was used as the reference for assembly correction and scaffolding by RagTag v1.1.0 (10). Assemblies were analyzed by QUAST v5.0.2 (11), and contigs of <200 bp were removed before submission to the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v5.1 (12).

Strain Ravenel yielded 9,074,522 reads and after PGAP annotation included 18 contigs (N_{50} of 4,371,545 bp and coverage of 69×) with a total length of 4,377,551 bp (GC content of the largest contig of 65.6%). PGAP identified 4,058 coding sequences (CDSs), 3 rRNAs, 45 tRNAs, 3 noncoding RNAs, and 192 pseudogenes.

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Address correspondence to Srinand Sreevatsan, sreevats@msu.edu.

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Strain 10-7428 yielded 8,877,919 reads and after PGAP annotation included 20 contigs (N_{50} of 4,360,600 bp and coverage of 113×) with a total length of 4,367,626 bp (GC content of the largest contig of 65.6%). PGAP identified 4,054 CDSs, 3 rRNAs, 45 tRNAs, 3 noncoding RNAs, and 208 pseudogenes.

The 3′ and 5′ ends of the largest contig for both assemblies were subjected to BLAST (12) searches against strain AF2122/97 (GenBank accession number LT708304) and demonstrated perfect overlaps at the start and end of the reference sequence, supporting closed, circular genomes. However, while these large, single contigs span typical genome lengths for both assemblies, additional and distinct smaller contigs may represent true variation and lead us to still consider these draft genomes.

Data availability. Data are available through NCBI BioProject PRJNA713797. GenBank accession numbers are as follows: for raw reads, SRX10318108 for Ravenel and SRX10318109 for 10-7428; for post-PGAP sequences, JAGEUB000000000.1 for Ravenel and JAGEUC000000000.1 for 10-7428.

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REFERENCES

1. Converse PJ, Dannenberg AM, Shigenaga T, McMurray DN, Phalen SW, Stanford JL, Rook GAW, Koru-Sengul T, Abbey H, Estep JE, Pitt MLM. 1998. Pulmonary bovine-type tuberculosis in rabbits: bacillary virulence, inhaled dose effects, tuberculin sensitivity, and *Mycobacterium vaccae* immunotherapy. Clin Diagn Lab Immunol 5:871–881. https://doi.org/10.1128/CDLI.5.6.871-881.1998.
2. Via LE, Lin PL, Ray SM, Carrillo J, Allen SS, Eum SY, Taylor K, Klein E, Manjunatha U, Gonzales J, Lee EG, Park SK, Raleigh JA, Cho SN, McMurray DN, Flynn JL, Barry CE. 2008. Tuberculous granulomas are hypoxic in guinea pigs, rabbits, and nonhuman primates. Infect Immun 76:2333–2340. https://doi.org/10.1128/IAI.01515-07.
3. North RJ, Ryan L, LaCource R, Mogues T, Goodrich ME. 1999. Growth rate of mycobacteria in mice as an unreliable indicator of mycobacterial virulence. Infect Immun 67:5483–5485. https://doi.org/10.1128/IAI.67.10.5483-5485.1999.
4. Waters WR, Palmer MV, Nonnecke BJ, Thacker TC, Scherer CFC, Estes DM, Hewinson RG, Vordermeier HM, Barnes SW, Federe GC, Walker JR, Glynne RJ, Hsu T, Weinrick B, Biermann K, Larsen MH, Jacobs WR. 2009. Efficacy and immunogenicity of *Mycobacterium bovis* ΔRD1 against aerosol *M. bovis* infection in neonatal calves. Vaccine 27:1201–1209. https://doi.org/10.1016/j.vaccine.2008.12.018.
5. Khare S, Hondalus MK, Nunes J, Bloom BR, Adams LG. 2007. *Mycobacterium bovis* Δ*leuD* auxotroph-induced protective immunity against tissue colonization, burden and distribution in cattle intranasally challenged with *Mycobacterium bovis* Ravenel S. Vaccine 25:1743–1755. https://doi.org/10.1016/j.vaccine.2006.11.036.
6. Francisco TI, Orloski KA, Roberts NJ. 2014. Investigation of a *Mycobacterium bovis* outbreak in cattle at a Colorado dairy in 2010. J Am Vet Med Assoc 244:805–812. https://doi.org/10.2460/javma.244.7.805.
7. Waters WR, Thacker TC, Nelson JT, DiCarlo DM, Maggioli MF, Greenwald R, Esfandiari J, Lyashchenko KP, Palmer MV. 2014. Virulence of two strains of *Mycobacterium bovis* in cattle following aerosol infection. J Comp Pathol 151:410–419. https://doi.org/10.1016/j.jcpa.2014.08.007.
8. Wood DE, Lu J, Langmead B. 2019. Improved metagenomic analysis with Kraken 2. Genome Biol 20:257. https://doi.org/10.1186/s13059-019-1891-0.
9. Jackman SD, Vandervalk BP, Mohamadi H, Chu J, Yeo S, Hammond SA, Jahesh G, Khan H, Coombe L, Warren RL, Birol I. 2017. ABySS 2.0: resource-efficient assembly of large genomes using a Bloom filter. Genome Res 27:768–777. https://doi.org/10.1101/gr.214346.116.
10. Alonge M, Soyk S, Ramakrishnan S, Wang X, Goodwin S, Sedlazeck FJ, Lippman ZB, Schatz MC. 2019. RaGOO: fast and accurate reference-guided scaffolding of draft genomes. Genome Biol 20:224. https://doi.org/10.1186/s13059-019-1829-6.
11. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086.
12. Tatusova T, Dicuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569.