

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Publications from USDA-ARS / UNL Faculty

U.S. Department of Agriculture: Agricultural
Research Service, Lincoln, Nebraska

2014

Complete Genome Sequence of *Mycobacterium avium* subsp. paratuberculosis, Isolated from Human Breast Milk

John Bannantine

USDA ARS National Animal Disease Center, john.bannantine@usda.gov

Lingling Li

Pennsylvania State University, lul17@psu.edu

Michael M. Mwangi

Pennsylvania State University

Rebecca Cote

Pennsylvania State University

Juan Antonio Raygoza Garay

Pennsylvania State University

See next page for additional authors

Follow this and additional works at: <https://digitalcommons.unl.edu/usdaarsfacpub>

 Part of the [Agriculture Commons](#)

Bannantine, John; Li, Lingling; Mwangi, Michael M.; Cote, Rebecca; Garay, Juan Antonio Raygoza; and Kapur, Vivek, "Complete Genome Sequence of *Mycobacterium avium* subsp. paratuberculosis, Isolated from Human Breast Milk" (2014). *Publications from USDA-ARS / UNL Faculty*. 2410.
<https://digitalcommons.unl.edu/usdaarsfacpub/2410>

This Article is brought to you for free and open access by the U.S. Department of Agriculture: Agricultural Research Service, Lincoln, Nebraska at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Publications from USDA-ARS / UNL Faculty by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Authors

John Bannantine, Lingling Li, Michael M. Mwangi, Rebecca Cote, Juan Antonio Raygoza Garay, and Vivek Kapur

Complete Genome Sequence of *Mycobacterium avium* subsp. *paratuberculosis*, Isolated from Human Breast Milk

John P. Bannantine,^a Lingling Li,^b Michael Mwangi,^b Rebecca Cote,^b Juan A. Raygoza Garay,^b Vivek Kapur^b

National Animal Disease Center-USDA-ARS, Ames, Iowa, USA^a; Department of Veterinary and Biomedical Science, Pennsylvania State University, University Park, Pennsylvania, USA^b

***Mycobacterium avium* subsp. *paratuberculosis* is the etiologic agent of Johne's disease in ruminants and has also been associated with human Crohn's disease. We report the complete genome sequence of *M. avium* subsp. *paratuberculosis*, isolated from the breast milk of a Crohn's disease patient. This sequence has high identity with characterized strains recovered from cattle.**

Received 20 December 2013 Accepted 6 January 2014 Published 6 February 2014

Citation Bannantine JP, Li L, Mwangi M, Cote R, Raygoza Garay JA, Kapur V. 2014. Complete genome sequence of *Mycobacterium avium* subsp. *paratuberculosis*, isolated from human breast milk. *Genome Announc.* 2(1):e01252-13. doi:10.1128/genomeA.01252-13.

Copyright © 2014 Bannantine et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](http://creativecommons.org/licenses/by/3.0/).

Address correspondence to John P. Bannantine, john.bannantine@ars.usda.gov, or Vivek Kapur, vkapur@psu.edu.

Mycobacterium avium subsp. *paratuberculosis* causes Johne's disease (JD) in cattle, sheep, goats, and other ruminant animals. JD presents as a chronic granulomatous intestinal infection with a worldwide distribution and imposes a significant economic toll on livestock industries (1). *M. avium* subsp. *paratuberculosis* has a complex cell wall structure containing mycolic acids and several lipids similar to those of other members of this genus, yet it is the most slowly growing member. This bacterium often requires 8 to 16 weeks before colonies are visible in culture, which is a major hurdle in diagnostics and therefore in the implementation of optimal JD control measures. Although a well-established domestic and wild animal pathogen, it has also been implicated as a causative agent in human Crohn's disease (2), and even though this link is controversial (3), *M. avium* subsp. *paratuberculosis* isolates have been obtained from humans. For instance, *M. avium* subsp. *paratuberculosis* 4, the isolate whose sequence we report here, was originally isolated from the breast milk of a Crohn's disease patient in 2000 (4).

M. avium subsp. *paratuberculosis* is a member of the *M. avium* complex (5), yet only the subspecies *paratuberculosis* of this complex has been found to cause Johne's disease. Another distinguishing phenotype of *M. avium* subsp. *paratuberculosis* is its requirement for the siderophore mycobactin in laboratory medium for growth. The genomes of cattle, sheep, and human strains of this pathogen have been sequenced (6–9). However, the human strain sequences are only in draft form. The data suggest that all non-sheep isolates of *M. avium* subsp. *paratuberculosis* are highly conserved and thus cluster as a homogeneous group (10), and isolates from humans are thought to cluster with the bovine strains; however, a complete sequence of an isolate from humans has not been available, and this has precluded comprehensive and definitive analyses.

Purified genomic DNA obtained from strain *M. avium* subsp. *paratuberculosis* 4 was subjected to whole-genome shotgun sequencing using 454 Life Sciences GS20 pyrosequencing technology (Roche, Indianapolis, IN). The 88.5 million bp were assembled into ~400 contigs using Newbler assembly software (Roche).

These contigs were assembled into ~50 scaffolds using the *M. avium* subsp. *paratuberculosis* strain K-10 sequence as a reference. Sanger-based sequencing was used to close all gaps in an iterative manner, and areas with low-quality scores were resequenced in order to obtain a single assembled high-quality genome sequence that totals 4.83 Mb. The gaps in each scaffold were first closed by using the Lasergene SeqMan software 9.0 (DNASTar, Madison, WI). The gaps between scaffolds were closed by PCR amplification of the entire insertion sequence (IS) elements or repetitive sequences and flanking region on each end of the scaffolds. An annotation file (Artemis) was generated by Rapid Annotations using Subsystems Technology (<http://rast.nmpdr.org/rast.cgi>), and each coding sequence (CDS) was manually verified (11).

Nucleotide sequence accession number. The whole-genome sequence of *M. avium* subsp. *paratuberculosis* 4 has been deposited at DDBJ/EMBL/GenBank under the accession no. CP005928.1.

ACKNOWLEDGMENTS

This research was supported in part by a competitive research grant from the U.S. Department of Agriculture (no. 2008-55620-18710) and the Johne's Disease Integrated Program (JDIP).

We thank Zhengjin Tu and Wayne Xu at the University of Minnesota for their help on the primary assembly and analysis of *M. avium* subsp. *paratuberculosis* 4.

REFERENCES

1. Chacon O, Bermudez LE, Barletta RG. 2004. Johne's disease, inflammatory bowel disease, and *Mycobacterium paratuberculosis*. *Annu. Rev. Microbiol.* 58:329–363. <http://dx.doi.org/10.1146/annurev.micro.58.030603.123726>.
2. Behr MA, Kapur V. 2008. The evidence for *Mycobacterium paratuberculosis* in Crohn's disease. *Curr. Opin. Gastroenterol.* 24:17–21.
3. Baksh FK, Finkelstein SD, Ariyanayagam-Baksh SM, Swalsky PA, Klein EC, Dunn JC. 2004. Absence of *Mycobacterium avium* subsp. *paratuberculosis* in the microdissected granulomas of Crohn's disease. *Mod. Pathol.* 17:1289–1294. <http://dx.doi.org/10.1038/modpathol.3800184>.
4. Naser SA, Schwartz D, Shafran I. 2000. Isolation of *Mycobacterium avium* subsp. *paratuberculosis* from breast milk of Crohn's disease patients. *Am. J. Gastroenterol.* 95:1094–1095. <http://dx.doi.org/10.1111/j.1572-0241.2000.01954.x>.

5. Paustian ML, Kapur V, Bannantine JP. 2005. Comparative genomic hybridizations reveal genetic regions within the *Mycobacterium avium* complex that are divergent from *Mycobacterium avium* subsp. *paratuberculosis* isolates. *J. Bacteriol.* 187:2406–2415. <http://dx.doi.org/10.1128/JB.187.7.2406-2415.2005>.
6. Bannantine JP, Wu CW, Hsu C, Zhou S, Schwartz DC, Bayles DO, Paustian ML, Alt DP, Sreevatsan S, Kapur V, Talaat AM. 2012. Genome sequencing of ovine isolates of *Mycobacterium avium* subspecies *paratuberculosis* offers insights into host association. *BMC Genomics* 13:89. <http://dx.doi.org/10.1186/1471-2164-13-89>.
7. Li L, Bannantine JP, Zhang Q, Amonsin A, May BJ, Alt D, Banerji N, Kanjilal S, Kapur V. 2005. The complete genome sequence of *Mycobacterium avium* subspecies *paratuberculosis*. *Proc. Natl. Acad. Sci. U. S. A.* 102:12344–12349.
8. Ghosh P, Hsu C, Alyamani EJ, Shehata MM, Al-Dubaib MA, Al-Naeem A, Hashad M, Mahmoud OM, Alharbi KB, Al-Busadah K, Al-Swailem AM, Talaat AM. 2012. Genome-wide analysis of the emerging infection with *Mycobacterium avium* subspecies *paratuberculosis* in the Arabian camels (*Camelus dromedarius*). *PLoS One* 7:e31947. <http://dx.doi.org/10.1371/journal.pone.0031947>.
9. Wynne JW, Bull TJ, Seemann T, Bulach DM, Wagner J, Kirkwood CD, Michalski WP. 2011. Exploring the zoonotic potential of *Mycobacterium avium* subspecies *paratuberculosis* through comparative genomics. *PLoS One* 6:e22171. <http://dx.doi.org/10.1371/journal.pone.0022171>.
10. Biet F, Sevilla IA, Cochard T, Lefrançois LH, Garrido JM, Heron I, Juste RA, McLuckie J, Thibault VC, Supply P, Collins DM, Behr MA, Stevenson K. 2012. Inter- and intra-subtype genotypic differences that differentiate *Mycobacterium avium* subspecies *paratuberculosis* strains. *BMC Microbiol.* 12:264. <http://dx.doi.org/10.1186/1471-2180-12-264>.
11. 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, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 9:75. <http://dx.doi.org/10.1186/1471-2164-9-75>.