



# Complete Genome Sequence of JII-1961, a Bovine *Mycobacterium avium* subsp. *paratuberculosis* Field Isolate from Germany

Petra Möbius,<sup>a</sup> Gabriele Nordsiek,<sup>b</sup> Martin Hölzer,<sup>c</sup> Michael Jarek,<sup>b</sup> Manja Marz,<sup>c</sup> Heike Köhler<sup>a</sup>

Institute of Molecular Pathogenesis, Friedrich-Loeffler-Institut (Federal Research Institute for Animal Health), Jena, Germany<sup>a</sup>; Department of Genome Analysis, Helmholtz Centre for Infection Research, Braunschweig, Germany<sup>b</sup>; RNA Bioinformatics and High-Throughput Analysis, Faculty of Mathematics and Computer Science, Friedrich-Schiller-Universität, Jena, Germany<sup>c</sup>

**ABSTRACT** *Mycobacterium avium* subsp. *paratuberculosis* causes Johne's disease in ruminants and was also detected in nonruminant species, including human beings, and in milk products. We announce here the 4.829-Mb complete genome sequence of the cattle-type strain JII-1961 from Germany, which is very similar to cattle-type strains recovered from different continents.

**M***ycobacterium avium* subsp. *paratuberculosis* is an obligate pathogen in domestic and wild ruminants. It causes a chronic progressive granulomatous enteritis designated Johne's disease (JD), or paratuberculosis, which is distributed worldwide (1). Furthermore, *M. avium* subsp. *paratuberculosis* can opportunistically induce infections in the intestine and other tissues of monogastric host species with (2) and without (3) clinical signs. *M. avium* subsp. *paratuberculosis* also has been detected in humans; its role in Crohn's disease is under continuing critical discussion (4). Based on different phenotypes, genotypes, and host associations, *M. avium* subsp. *paratuberculosis* was divided into two main groups: the cattle type and the sheep type (5). Until now, five complete cattle-type genome sequences from three different regions of the world have been published, with four bovine derived and one human derived: *M. avium* subsp. *paratuberculosis* and *M. avium* subsp. *paratuberculosis* K10 from United States (6, 7), *M. avium* subsp. *paratuberculosis* E1 and *M. avium* subsp. *paratuberculosis* E93 from Egypt (8), and MAP/TANUVAS/TN/India/2008 from India (NCBI GenBank accession number CP015495). A genome sequence from a cattle-type strain originating from Europe has not yet been formally published.

This paper announces the complete genome sequence of the cattle-type field strain JII-1961 from Germany. This strain was isolated in 2003 from an ileocecal lymph node of a clinically diseased cow and was characterized by multitarget genotyping (9). Furthermore, JII-1961 was used as an inoculation strain for a new defined animal model for JD in goats focusing on the period of latent infection (10).

For sequencing, JII-1961 had been grown for 6 weeks in Herrold's egg yolk medium supplemented with mycobactin J. High-quality bacterial genomic DNA was extracted using the cetyltrimethylammonium bromide method (11). Libraries were prepared with a paired-end DNA sample prep kit (Illumina, San Diego, CA, USA), assessed by a 2100 Bioanalyzer (Agilent Technologies) and sequenced on the Illumina GAIIx sequencing platform at the Helmholtz Centre for Infection Research in Braunschweig by running 36 cycles in both directions. Image analysis and base calling were performed using the Genome Analyzer Pipeline 1.4 and CASAVA software 1.7. Altogether, 5,154,552 paired-end reads with a length of 35 bp were assembled using Velvet 0.7.55-1 (12), using *M.*

Received 12 July 2017 Accepted 18 July 2017 Published 24 August 2017

**Citation** Möbius P, Nordsiek G, Hölzer M, Jarek M, Marz M, Köhler H. 2017. Complete genome sequence of JII-1961, a bovine *Mycobacterium avium* subsp. *paratuberculosis* field isolate from Germany. *Genome Announc* 5:e00870-17. <https://doi.org/10.1128/genomeA.00870-17>.

**Copyright** © 2017 Möbius et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Petra Möbius, [petra.moebius@fli.de](mailto:petra.moebius@fli.de).

*avium* subsp. *paratuberculosis* K10 as a reference (GenBank accession no. AE016958). The remaining sequence gaps were closed by PCR and Sanger sequencing (ABI Prism 3730xl DNA analyzer). Further analysis and assembly of these additional data were done by Gap4 (Staden Package 1.6). For genome annotation, the NCBI Prokaryotic Genome Annotation Pipeline was used. In addition, noncoding RNA (ncRNA), rRNA, and tRNA genes were detected by a homology search of Rfam (version 11.0) families using the GORAP pipeline comprising Infernal (version 1.1), RNAmmer (version 1.2), and tRNAscan-SE (version 1.3.1), as described by Möbius et al. (13).

Finally, the complete chromosomal sequence of J11-1961 comprises 4,829,728 bp. The average G+C content is 69.3 mol%. NCBI genome annotation identified and predicted 4,325 protein coding sequences (CDSs), 46 tRNAs, 3 rRNAs, and 3 other ncRNAs. Using different approaches for ncRNA annotation, 46 tRNAs and 3 rRNAs were also detected, but in addition, one transfer-messenger RNA (tmRNA), one RNase P, one bacterial small signal recognition particle (SRP), 15 riboswitches, and 14 other ncRNAs were detected (altogether, 81 noncoding RNAs [13]).

**Accession number(s).** The whole-genome sequence of *Mycobacterium avium* subsp. *paratuberculosis* strain J11-1961 has been deposited at DDBJ/EMBL/GenBank under the accession no. CP022105. The version described in this paper is the first version, CP022105.1.

## ACKNOWLEDGMENTS

We thank Maren Scharfe (HZI, Braunschweig) and Kerstin Steger (FLI, Jena) for skillful technical assistance.

This research was funded by the Friedrich-Loeffler-Institut, Jena, Germany.

## REFERENCES

- Harris NB, Barletta RG. 2001. *Mycobacterium avium* subsp. *paratuberculosis* in veterinary medicine. *Clin Microbiol Rev* 14:489–512. <https://doi.org/10.1128/CMR.14.3.489-512.2001>.
- Stief B, Möbius P, Turk H, Horugel U, Arnold C, Pohle D. 2012. Paratuberculosis in a miniature donkey (*Equus asinus* f. *asinus*). *Berl Munch Tierarztl Wochenschr* 125:38–44. (In German.)
- Kukanich KS, Vinasco J, Scott HM. 2013. Detection of *Mycobacterium avium* subspecies *paratuberculosis* from intestinal and nodal tissue of dogs and cats. *ISRN Vet Sci* 2013:323671. <https://doi.org/10.1155/2013/323671>.
- Chiodini RJ, Chamberlin WM, Sarosiek J, McCallum RW. 2012. Crohn's disease and the mycobacterioses: a quarter century later. Causation or simple association? *Crit Rev Microbiol* 38:52–93. <https://doi.org/10.3109/1040841X.2011.638273>.
- Stevenson K. 2015. Genetic diversity of *Mycobacterium avium* subspecies *paratuberculosis* and the influence of strain type on infection and pathogenesis: a review. *Vet Res* 46:64. <https://doi.org/10.1186/s13567-015-0203-2>.
- 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. <https://doi.org/10.1128/genomeA.01252-13>.
- 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. <https://doi.org/10.1073/pnas.0505662102>.
- Amin AS, Hsu CY, Darwish SF, Ghosh P, Abdel-Fatah EM, Behour TS, Talaat AM. 2015. Ecology and genomic features of infection with *Mycobacterium avium* subspecies *paratuberculosis* in Egypt. *Microbiology* 161:807–818. <https://doi.org/10.1099/mic.0.000051>.
- Möbius P, Fritsch I, Luyven G, Hotzel H, Köhler H. 2009. Unique genotypes of *Mycobacterium avium* subsp. *paratuberculosis* strains of type III. *Vet Microbiol* 139:398–404. <https://doi.org/10.1016/j.vetmic.2009.06.011>.
- Köhler H, Soschinka A, Meyer M, Kather A, Reinhold P, Liebler-Tenorio E. 2015. Characterization of a caprine model for the subclinical initial phase of *Mycobacterium avium* subsp. *paratuberculosis* infection. *BMC Vet Res* 11:74. <https://doi.org/10.1186/s12917-015-0381-1>.
- van Soolingen D, Hermans PW, De Haas PE, Soll DR, Van Embden JD. 1991. Occurrence and stability of insertion sequences in *Mycobacterium tuberculosis* complex strains: evaluation of an insertion sequence dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. *J Clin Microbiol* 29:2578–2586.
- Zerbino DR, Birney E. 2008. Velvet: algorithmus for *de novo* short read assembly using de Bruijn graphs. *Genome Res* 18:821–829. <https://doi.org/10.1101/gr.074492.107>.
- Möbius P, Hölzer M, Felder M, Nordsiek G, Groth M, Köhler H, Reichwald K, Platzer M, Marz M. 2015. Comprehensive insights in the *Mycobacterium avium* subsp. *paratuberculosis* genome using new WGS data of sheep strain J111-386 from Germany. *Genome Biol Evol* 7:2585–2601. <https://doi.org/10.1093/gbe/evv154>.