



Draft Genome Sequence of *Mycolicibacterium* sp. Strain NCC-Tsukiji, Isolated from Blood Culture of a Patient with Malignant Lymphoma

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ABSTRACT A nonidentifiable mycolicibacterium was isolated from a malignant lymphoma patient treated with intensive chemoimmunotherapy. Multilocus sequence analysis showed that this isolate was close to *"Mycolicibacterium (Mycobacterium) ratisbonense,"* but the details of this species were still unknown. Here, we report the draft genome sequence data of *Mycolicibacterium* sp. strain NCC-Tsukiji.

The Mycolicibacterium mucogenicum group (M. mucogenicum, M. aubagnense, and M. phocaicum) comprises rapidly growing acid-fast bacteria (1, 2). This species is commonly isolated from water-associated equipment, such as the drinking water supply (3, 4). The risk factor for infection with M. mucogenicum is immune suppression, including anticancer chemotherapy (5–7). Significant clinical cases are catheter-related bloodstream infections (6, 8). Long-term placement of a catheter and bathing with contaminated water can be a risk for infection (5–7, 9).

We isolated *M. mucogenicum*-related species from a malignant lymphoma patient treated with a long-time course of intensive chemoimmunotherapy. The agent was detected 6 days after culturing blood using the BD Bactec FX system with BD Bactec Plus aerobic medium (Becton, Dickinson, USA). A single clone was isolated on sheep blood agar (Nissui, Japan). First, the isolated colony was submitted to matrix-assisted laser desorption-ionization time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Germany) for identification. In addition, partial 16S rRNA and rpoB genes were amplified by PCR and sequenced with an ABI Prism 3730 DNA analyzer (Applied Systems, USA). NCBI BLAST analysis was then performed to determine the identity of each strain with the BLASTN algorithm using the default parameters. The results of MALDI-TOF MS indicated that the agent was Mycolicibacterium mucogenicum, but the results of the BLAST search using a partial sequence of multilocus genes (a 16S rRNA gene and an rpoB gene) showed it shared high similarity with "Mycolicibacterium (Mycobacterium) ratisbonense," which was isolated from a human urine sample. The 16S rRNA gene and rpoB gene sequences of NCC-Tsukiji shared 100% (1,442/1,442 bp) and 99% (632/633 bp) identities with the sequences of M. ratisbonense (the GenBank/EMBL/ DDBJ accession numbers for the two partial genes are AF055331 and LT718451) (10).

The strain was inoculated on Middlebrook 7H9 broth supplemented with 10% oleic acid-albumin-dextrose-catalase enrichment and incubated at 30°C for 7 days (11–14). Genomic DNA extractions were performed using a NucleoSpin plant II kit (Macherey-Nagel). A DNA sequencing library was prepared using a Nextera XT DNA sample prep

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Received 26 November 2018 Accepted 27 February 2019 Published 4 April 2019 kit (Illumina, Inc., San Diego, CA) according to the manufacturer's instructions. The genomic sequencing was obtained by using Illumina 2×150 -bp paired-end reads. Low-quality reads (quality score, 15), the quality score of short reads (length, <25), and adaptors were removed, and the paired-end reads were assembled using the Platanus version 1.1 package with default parameters. Genome quality was assessed using the QUAST Web interface (15). Automated annotation was carried out with the DDBJ Fast Annotation and Submission Tool (DFAST) (https://dfast.nig.ac.jp/). Average nucleotide identity (ANI) was calculated by JSpeciesWS version 1.2.1 (16, 17).

The total number of paired-end reads was 429,643. The assembly comprised 159 contigs with a total length of 5,752,239 bp and an N_{50} value of 78,866 bp. The GC content was 67.12%.

A total of 5,555 protein-coding sequences (CDS), 2 rRNAs, and 68 tRNAs were predicted. The ANI value compared to *M. mucogenicum* strain CSUR P2099 (GenBank accession number NZ_CYSI00000000) was 91.26%.

Data availability. The scaffold sequences and annotations of *Mycolicibacterium* sp. strain NCC-Tsukiji were deposited at DDBJ/EMBL/GenBank under the accession number BHVW000000000. The version described in this paper is the first version, BHVW010000000. Raw sequence data for strain NCC-Tsukiji were deposited under SRA accession number PRJDB7019.

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REFERENCES

- Adékambi T, Berger P, Raoult D, Drancourt M. 2006. rpoB gene sequencebased characterization of emerging non-tuberculous mycobacteria with descriptions of Mycobacterium bolletii sp. nov., Mycobacterium phocaicum sp. nov. and Mycobacterium aubagnense sp. nov. Int J Syst Evol Microbiol 56:133–143. https://doi.org/10.1099/ijs.0.63969-0.
- Adékambi T, Drancourt M. 2004. Dissection of phylogenetic relationships among 19 rapidly growing *Mycobacterium* species by 16S rRNA, *hsp65*, *sodA*, *recA* and *rpoB* gene sequencing. Int J Syst Evol Microbiol 54: 2095–2105. https://doi.org/10.1099/ijs.0.63094-0.
- Cooksey RC, Jhung MA, Yakrus MA, Butler WR, Adékambi T, Morlock GP, Williams M, Shams AM, Jensen BJ, Morey RE, Charles N, Toney SR, Jost KC, Jr, Dunbar DF, Bennett V, Kuan M, Srinivasan A. 2008. Multiphasic approach reveals genetic diversity of environmental and patient isolates of *Mycobacterium mucogenicum* and *Mycobacterium phocaicum* associated with an outbreak of bacteremias at a Texas hospital. Appl Environ Microbiol 74:2480–2487. https://doi.org/10.1128/AEM.02476-07.
- Thomson RM, Carter R, Tolson C, Coulter C, Huygens F, Hargreaves M. 2013. Factors associated with the isolation of nontuberculous mycobacteria (NTM) from a large municipal water system in Brisbane, Australia. BMC Microbiol 13:89. https://doi.org/10.1186/1471-2180-13-89.
- Adékambi T. 2009. Mycobacterium mucogenicum group infections: a review. Clin Microbiol Infect 15:911–918. https://doi.org/10.1111/j.1469 -0691.2009.03028.x.
- Livni G, Yaniv I, Samra Z, Kaufman L, Solter E, Ashkenazi S, Levy I. 2008. Outbreak of *Mycobacterium mucogenicum* bacteraemia due to contaminated water supply in a paediatric haematology-oncology department. J Hosp Infect 70:253–258. https://doi.org/10.1016/j.jhin.2008.07.016.
- 7. Shachor-Meyouhas Y, Sprecher H, Eluk O, Ben-Barak A, Kassis I. 2011. An

outbreak of *Mycobacterium mucogenicum* bacteremia in pediatric hematology-oncology patients. Pediatr Infect Dis J 30:30–32. https://doi.org/10.1097/INF.0b013e3181ee31d7.

- Hawkins C, Qi C, Warren J, Stosor V. 2008. Catheter-related bloodstream infections caused by rapidly growing nontuberculous mycobacteria: a case series including rare species. Diagn Microbiol Infect Dis 61:187–191. https://doi.org/10.1016/j.diagmicrobio.2008.01.004.
- Kline S, Cameron S, Streifel A, Yakrus MA, Kairis F, Peacock K, Besser J, Cooksey RC. 2004. An outbreak of bacteremias associated with *Myco-bacterium mucogenicum* in a hospital water supply. Infect Control Hosp Epidemiol 25:1042–1049. https://doi.org/10.1086/502341.
- Stackebrandt E, Ebers J. 2006. Taxonomic parameters revisited: tarnished qold standards. Microbiol Today 33:152–155.
- Fukano H, Wada S, Kurata O, Mizuno K, Nakanaga K, Hoshino Y. 2015. Nontuberculous mycobacteriosis in farmed thread-sail filefish *Stephanolepis cirrhifer*. Fish Pathol 50:68–74. https://doi.org/10.3147/jsfp.50.68.
- Fukano H, Wada S, Kurata O, Katayama K, Fujiwara N, Hoshino Y. 2017. *Mycobacterium stephanolepidis* sp. nov., a rapidly growing species related to Mycobacterium chelonae, isolated from marine teleost fish, *Stephanolepis cirrhifer*. Int J Syst Evol Microbiol 67:2811–2817. https:// doi.org/10.1099/ijsem.0.002028.
- Fukano H, Yoshida M, Katayama Y, Omatsu T, Mizutani T, Kurata O, Wada S, Hoshino Y. 2017. Complete genome sequence of *Mycobacterium* stephanolepidis. Genome Announc 5:e00810-17. https://doi.org/10.1128/ genomeA.00810-17.
- Fukano H, Yoshida M, Shimizu A, Iwao H, Katayama Y, Omatsu T, Mizutani T, Kurata O, Wada S, Hoshino Y. 2018. Draft genome sequence of Mycobacterium montefiorense isolated from Japanese black salamander

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(Hynobius nigrescens). Genome Announc 6:e00448-18. https://doi.org/10 .1128/genomeA.00448-18.

- 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.
- Fukano H, Yoshida M, Kazumi Y, Fujiwara N, Katayama K, Ogura Y, Hayashi T, Miyamoto Y, Fujimoto N, Hongsheng W, Mizumoto C, Koizumi

Y, Maeda H, Hiranuma O, Mitarai S, Ishii N, Hoshino Y. 2018. *Mycobacterium shigaense* sp. nov., a slow-growing, scotochromogenic species, is a member of the *Mycobacterium simiae* complex. Int J Syst Evol Microbiol 68:2437–2442. https://doi.org/10.1099/ijsem.0.002845.

 Richter M, Rosselló-Móra R. 2009. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci U S A 106: 19126–19131. https://doi.org/10.1073/pnas.0906412106.