

Whole-Genome Sequence of *Mycobacterium kyorinense*

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We report here the first draft genome sequence of *Mycobacterium kyorinense*, which was described in 2009 and exhibits significant pathogenicity to humans.

Received 9 September 2014 Accepted 10 September 2014 Published 16 October 2014

Citation Ohtsuka K, Ohnishi H, Nozaki E, Pais Ramos J, Tortoli E, Yonetani S, Matsushima S, Tateishi Y, Matsumoto S, Watanabe T. 2014. Whole-genome sequence of *Mycobacterium kyorinense*. *Genome Announc.* 2(5):e01062-14. doi:10.1128/genomeA.01062-14.

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Mycobacterium kyorinense is a slow-growing mycobacterium that was first described in 2009 (1). *M. kyorinense* is closely related to *M. celatum*, *M. branderi*, and *M. fragae*, and exhibits significant pathogenicity for humans, causing pneumonia, lymphadenitis, and arthritis (2–4). Antimicrobial susceptibility tests demonstrated that *M. kyorinense* is generally resistant to rifampin, isoniazid, and ethambutol (4). Further investigation is needed to clarify the genomics, biology, epidemiology, and pathogenicity of this species.

We sequenced the genomic DNA of the *M. kyorinense* type strain KUM060204^T on an Ion PGM system (Life Technologies) and assembled the reads using CLC Genomics Workbench 7.0. A total of 4,133,490 reads were generated, with an average read length of 203 bp, yielding a total sequence of 837,657,777 bp.

The assembled sequences of KUM060204^T comprised 453 contigs, with a combined length of 5,302,980 bp, with a G+C ratio of 66.9%. The average cover depth was 50×, the N_{50} contig size was 53,523, the average contig was 11,706 bp long, and the longest contig was 137,319 bp.

Genome annotation was performed using the RAST prokaryotic genome annotation server (<http://www.nmpdr.org/FIG/wiki/view.cgi/Main/RAST>). RAST predicted 5,405 putative open reading frames, including 5,351 coding sequences and 54 RNAs (46 tRNAs and 8 rRNAs). RAST functional analysis of the predicted protein-coding genes showed 78 genes involved in cell walls and capsules, 64 in membrane transport, 206 in protein metabolism, 93 in DNA metabolism, 141 in virulence and defense, 135 in respiration, 331 in fatty acids, lipids, and isoprenoids, 395 in cofactors, vitamins, prosthetic groups, and pigments, and 356 in amino acids and derivatives.

To explore the molecular mechanism underlying the resistance of *M. kyorinense* to anti-tuberculosis drugs, we selected several genes known to be responsible for resistance to rifampin (*rpoB*), ethambutol (*embB*), and isoniazid (*inhA*, *katG*, and *ahpC*). The sequences of these genes in *M. kyorinense* were compared with those in *M. tuberculosis* H37Rv to clarify whether they contain specific mutations associated with resistance to anti-tuberculosis drugs in *M. tuberculosis*. Analysis of the *rpoB* gene confirmed our

previous finding that KUM060204^T harbors a Ser531Asp amino acid substitution, the most frequent mutation in rifampin-resistant *M. tuberculosis* (4, 5). In contrast, we did not detect a substitution at Met306 of *embB*, the major mutation in ethambutol-resistant *M. tuberculosis* (6). Nor did we find a Ser315Thr substitution of *katG*, a mutation in the regulatory region (nucleotides [nt] –48, –51, –54, –81, and –88) of *ahpC*, a Ser94Ala substitution in the *inhA* gene or a mutation in the regulatory region (nt –15 and –17) of *inhA*, which are common mutations in isoniazid-resistant *M. tuberculosis* (6). These results suggested that the mechanism underlying drug resistance in *M. kyorinense* is significantly different from that in *M. tuberculosis*.

In conclusion, we report the genome sequence of KUM060204^T which to the best of our knowledge is the first genome sequence of the species *M. kyorinense*.

Nucleotide sequence accession numbers. The whole genome sequence of KUM060204^T has been deposited in DDBJ/EMBL/GenBank under the accession numbers [BBKA01000001](https://www.ncbi.nlm.nih.gov/nuccore/BBKA01000001) to [BBKA01000453](https://www.ncbi.nlm.nih.gov/nuccore/BBKA01000453).

ACKNOWLEDGMENTS

This work was supported by a grant from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (23590688).

REFERENCES

- Okazaki M, Ohkusu K, Hata H, Ohnishi H, Sugahara K, Kawamura C, Fujiwara N, Matsumoto S, Nishiuchi Y, Toyoda K, Saito H, Yonetani S, Fukugawa Y, Yamamoto M, Wada H, Sejimo A, Ebina A, Goto H, Ezaki T, Watanabe T. 2009. *Mycobacterium kyorinense* sp. nov., a novel, slow-growing species, related to *Mycobacterium celatum*, isolated from human clinical specimens. *Int. J. Syst. Evol. Microbiol.* 59:1336–1341. <http://dx.doi.org/10.1099/ijs.0.000760-0>.
- Ramos JP, Campos CE, Caldas PC, Ferreira NV, da Silva MV, Redner P, Campelo CL, Vale SF, Barroso EC, Medeiros RF, Montes FC, Galvão TC, Tortoli E. 2013. *Mycobacterium fragae* sp. nov., a non-chromogenic species isolated from human respiratory specimens. *Int. J. Syst. Evol. Microbiol.* 63:2583–2587. <http://dx.doi.org/10.1099/ijs.0.046862-0>.
- Campos CE, Caldas PC, Ohnishi H, Watanabe T, Ohtsuka K, Matsushima S, Ferreira NV, da Silva MV, Redner P, de Carvalho LD, Medeiros RF, Abud Filho JA, Montes FC, Galvão TC, Ramos JP. 2012. First

- isolation of *Mycobacterium kyorinense* from clinical specimens in Brazil. J. Clin. Microbiol. 50:2477–2478. <http://dx.doi.org/10.1128/JCM.00023-12>.
4. Ohnishi H, Yonetani S, Matsushima S, Wada H, Takeshita K, Kuramochi D, Caldas PC, Campos CE, da Costa BP, Ramos JP, Mikura S, Narisawa E, Fujita A, Funayama Y, Kobashi Y, Sakakibara Y, Ishiyama Y, Takakura S, Goto H, Watanabe T. 2013. *Mycobacterium kyorinense* infection. Emerg. Infect. Dis. 19: 508–510. <http://dx.doi.org/10.3201/eid1903.120591>.
 5. Telenti A, Imboden P, Marchesi F, Lowrie D, Cole S, Colston MJ, Matter L, Schopfer K, Bodmer T. 1993. Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*. Lancet 341:647–650. [http://dx.doi.org/10.1016/0140-6736\(93\)90417-F](http://dx.doi.org/10.1016/0140-6736(93)90417-F).
 6. Zhang Y, Telenti Y. 2000. Genetics of drug resistance in *Mycobacterium tuberculosis*, p 235–254. In Hatfull GF, Jacobs WR (ed), Molecular genetics of *Mycobacteria*. ASM Press, Washington, D.C.