

## Genome Sequence of *Mycobacterium massiliense* M18, Isolated from a Lymph Node Biopsy Specimen

Yun Fong Ngeow,<sup>a</sup> Yan Ling Wong,<sup>a</sup> Joon Liang Tan,<sup>b,c</sup> Ramitha Arumugam,<sup>b</sup> Guat Jah Wong,<sup>b</sup> Chia Sui Ong,<sup>c</sup> Kee Peng Ng,<sup>a</sup> and Siew Woh Choo<sup>b</sup>

Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia<sup>a</sup>; Dental Research and Training Unit, Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia<sup>b</sup>; and Faculty of Information Science and Technology, Multimedia University, Melaka, Malaysia<sup>c</sup>

*Mycobacterium massiliense* is a rapidly growing mycobacterial species. The pathogenicity of this subspecies is not well known. We report here the annotated genome sequence of *M. massiliense* strain M18, which was isolated from a lymph node biopsy specimen from a Malaysian patient suspected of having tuberculous cervical lymphadenitis.

*ycobacterium massiliense* is one of three subspecies of *M. abscessus*, the other two being *M. abscessus sensu stricto* and *M. bolletii* (3, 4, 8). These subspecies are closely related genetically but exhibit different drug susceptibilities (3, 7, 9). Their differentiation depends mainly on DNA polymorphism in the *rpoB*, *hsp65*, *sodA*, *recA*, and *secA* genes or in the 16S-23S rRNA internal transcribed spacer (1, 2, 6, 8).

*M. massiliense* has been associated with pulmonary and soft tissue infections, including outbreaks of infections related to surgical procedures and medical devices (3, 7, 9). It has been reported to be susceptible to doxycycline and clarithromycin (3), in contrast to *M. bolletii*, which has been described as highly resistant to antimicrobial drugs, including clarithromycin (4).

We sequenced the complete genome of *M. massiliense* M18 to further study phylogenetic relationships and the genetic factors responsible for pathogenicity. *M. massiliense* M18 was isolated from a lymph node biopsy specimen from a Malaysian patient who was investigated for cervical lymphadenitis. The biopsy specimen tissue showed granulomatous inflammation and acid-fast bacilli which grew on Lowenstein-Jensen medium within a week of incubation at 36°C. The identification of the isolate as *M. massiliense* was based on its *hsp65* gene sequence, which was identical to that of the *M. massiliense* CIP 108297 reference strain, and its *rpoB* gene sequence, which showed an only 2-bp difference from the reference strain.

To sequence the genome of *M. massiliense* M18, we used a shotgun sequencing method and Illumina Genome Analyzer 2X technology. A total of 19,111,625 Illumina sequencing reads were generated. These short sequences were assembled with Genomics Workbench 4.9, resulting in 34 contigs with the following quality measurements: an N25 contig size of 1,797,984 bp, an N50 contig size of 833,393 bp, and an N75 contig size of 218,930 bp. Automated annotation was done by using the Rapid Annotation and Subsystem Technology (RAST) server (5).

The *M. massiliense* M18 genome sequence is 4,886,939 bp in length with 4,853 predicted coding sequences. There are 45 tRNAs and 3 rRNAs as predicted by the RAST pipeline. The automated annotation of this genome by the RAST server revealed that this genome may contain many genes encoding proteins that are categorized in the subsystem category of amino acids and derivatives (412 genes), followed by cofactors, vitamins, prosthetic groups, and pigments (324 genes). There are 37 genes encoding products that may be involved in virulence, disease, and defense, of which 24 are linked with resistance to antibiotics and toxic compounds and 13 are involved in invasion and intracellular resistance.

**Nucleotide sequence accession numbers.** This whole-genome shotgun project has been deposited in the GenBank database under accession no. AJSC00000000. The version described in this paper is the first version, AJSC01000000.

## ACKNOWLEDGMENTS

This work was supported by research grants UM.C/HIR/MOHE/08 and UM.C/625/1/HIR/004 from the University of Malaya, Kuala Lumpur, Malaysia.

## REFERENCES

- Adékambi T, Colson P, Drancourt M. 2003. *rpoB*-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria. J. Clin. Microbiol. 41:5699–5708.
- 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.
- Adékambi T, et al. 2004. Amoebal coculture of "Mycobacterium massiliense" sp. nov. from the sputum of a patient with hemoptoic pneumonia. J. Clin. Microbiol. 42:5493–5501.
- Adékambi T, Berger P, Raoult D, Drancourt M. 2006. *rpoB* gene sequence-based 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.
- Aziz RK, et al. 2008. The RAST Server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75.
- 6. Devulder G, Perouse de Montclos M, Flandrois JP. 2005. A multigene approach to phylogenetic analysis using the genus Mycobacterium as a model. Int. J. Syst. Evol. Microbiol. 55:293–302.
- Kim HY, et al. 2007. Outbreak of Mycobacterium massiliense infection associated with intramuscular injections. J. Clin. Microbiol. 45:3127–3130.
- Macheras E, et al. 2009. Inaccuracy of single-target sequencing for discriminating species of the *Mycobacterium abscessus* group. J. Clin. Microbiol. 47:2596–2600.
- Simmon KE, et al. 2007. Identification of an emerging pathogen, *Mycobacterium massiliense*, by *rpoB* sequencing of clinical isolates collected in the United States. J. Clin. Microbiol. 45:1978–1980.

Received 26 April 2012 Accepted 16 May 2012 Address correspondence to Siew Woh Choo, Ichoo@um.edu.my. Copyright © 2012, American Society for Microbiology. All Rights Reserved. doi:10.1128/JB.00712-12