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SHORT COMMUNICATION

Whole Genome Sequence Analysis of a *Mycobacterium iranicum*, a Newly Identified Non-Tuberculosis Mycobacteria, Strain SBH312 Isolated in Sabah, Malaysia

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ABSTRACT

In 2021, tuberculosis (TB) incidence partially rebounded but remained 12% lower compared with 2019, in which 10 million new TB cases have been reported worldwide. In Malaysia, 20 – 30% of the total TB cases were reported in Sabah. In this communication, we are reporting the whole genome sequence of *Mycobacterium iranicum* strain SBH312, isolated from a 49-year-old male suffering from a pulmonary infection. This is the first report of *M. iranicum*, non-tuberculous mycobacteria infecting humans, from Sabah, state of Malaysia. Phylogenetic analysis showed that the *M. iranicum* strain SBH312 was closely related to strains from Iran and Peninsular Malaysia.

INTRODUCTION

Mycobacterium iranicum is a newly identified non-tuberculous mycobacteria first reported from Iran in 2013 (Shojaei et al., 2013). Recently it has been proposed to move this species to the new genus *Mycolicibacterium* (Gupta et al., 2018). Till now, this pathogen has been reported in Russia (Lyamin et al., 2020), the USA (Kalra et al., 2022), Spain (Balakrishnan et al., 2013), France (Grandjean et al., 2017), Germany (Becker et al., 2018), Italy, Sweden, Netherlands, Greece (Shojaei et al., 2013), Saudi Arabia (Varghese et al., 2017), China (Zhang et al., 2019), Japan (Inagaki et al., 2016), and Malaysia (Tan et al., 2013) in patients

with pulmonary infections. However, it is not restricted to pulmonary infections but has also been detected to cause septic arthritis and tenosynovitis, bacteremia (Grandjean et al., 2017), skin granuloma (Becker et al., 2018), and peritonitis (Inagaki et al., 2016). Here, we report a strain of *M. iranicum* for the first time isolated in Sabah, its whole genome sequence analysis and relatedness with strains isolated from other places.

MATERIALS AND METHODS

A 49-year-old male from Kota Kinabalu, Sabah, Malaysia had suspected pulmonary tuberculosis in April 2017 detected through a TB mobile programme after the patient showed general symptoms of tuberculosis infection, including feelings of sickness or weakness, weight loss, fever, night sweats, coughing and chest pain. Sputum was collected and the patient was positive for *Mycobacterium tuberculosis* complex by GeneXpert MDR/RIF (Cepheid, Sunnyvale, CA, USA). The sputum was cultured in 7H9 Middlebrook medium using BACTEC MGIT 320 (Becton-Dickinson, Oxford, United Kingdom).

For further understanding and identification of the strain infecting the patient, whole genome sequencing was performed. Genomic DNA was extracted using Masterpure Complete DNA and RNA purification kit (Epicenter, Inc., Madison, Wisconsin, USA) according to the manufacturer's instructions. The concentration and quality of the extracted DNA were determined by Nanodrop 2000c spectrophotometer (ThermoFisher Scientific, USA) and Qubit® 2.0 fluorometer (Invitrogen, ThermoFisher Scientific, USA), respectively. The genome DNA was sequenced using the Illumina HiSeq 4000 paired-end sequencing platform.

Genomic bioinformatics analysis was performed, including genome assembly, annotation, and phylogenetic analysis based on a few genomes to identify closely related strains. The quality of the sequence read was checked by FastQC. All the raw reads were pre-processed using BBMap version 38.43 tools (Bushnell et al., 2017), whereby the adapters were trimmed and the reads <50bp were removed, based on the Phred with a quality below Q30 using BBDuk.sh. *De novo* assembly was performed using SPAdes version 3.11.1 (Bankevich et al., 2012) software. NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova et al., 2016) was utilized to annotate the generated contigs.

A total of 11 genomes of *M. iranicum* strains were extracted from GenBank for SNP-based phylogenetic analysis, and core-SNP was generated by the kSNP3 package (Gardner et al., 2015). The entire SNP matrix was used for phylogenetic analysis, using the maximum likelihood method available in MEGA X (Sudhir et al., 2018). The analysis used a general time-reversible (GTR) model, nucleotide sequences were aligned and the maximum likelihood method was used. The *Mycobacterium tuberculosis* strain H37Rv was used as an outgroup. The significance of branching was assessed by bootstrap analysis of 1,000 replicates. The numbers adjacent to nodes represent the bootstrap values (%); values less than 70% have not shown in this figure The scale bar shows the genetic distance, which is expressed as nucleotide substitutions per site. This study was approved by the ethics committee at the Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah [JKEtika 2/16 (6)].

RESULTS

The genome was sequenced until 99% completion of the genome using 332 times sequencing coverage reads. A total of 4,886,925 paired reads (~1GB) of a 300-bp insert-size library was generated from Illumina HiSeq 4000. The data sequence was deposited in the Sequence Read Archive (accession number SRR25526524) under biosample accession number SAMN32036409 and bio project accession number PRJNA908658. This whole-genome shotgun project was deposited at DDBJ/ENA/GenBank under Accession No. JAPQYE00000000.1.

De *novo* assembly generated 90 contigs of 99% of the draft genome, with a genome size of 6,516,536 bp and an N_{50} value of 404,830 bp. The GC content of the genome was 66.07%. The genome consisted of a total of 6,331 genes, including 6.275 predicted coding sequences (CDSs) and 56 RNAs (46 tRNAs and 10 rRNAs).

Phylogenetic analysis showed that *M. iranicum* strain SBH321 clustered with strains DSM45541 (LQPC00000000.1) from Iran and UM_TJL (AUWT00000000.1) from Malaysia (Figure 1).

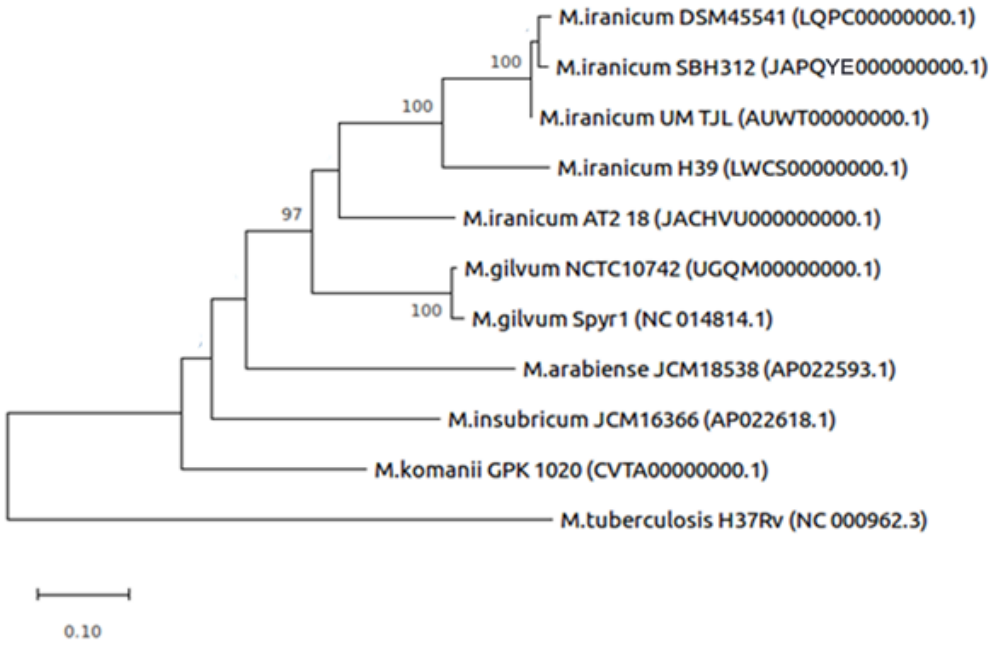

Figure 1 Phylogeny of Mycobacterium species including *M. iranicum* SBH312 strain clustering with strain DSM45541 (LQPC00000000.1) from Iran and UM_TJL (AUWT00000000.1) from Malaysia

DISCUSSION

Genus *Mycobacterium* has been further categorized into strict pathogens (*Mycobacterium tuberculosis* complex) and potential pathogens (non-tuberculous mycobacteria, NTMs) (Chai et al., 2018). NTMs, also known as environmental mycobacteria, are widely distributed in the environment and are passed on to humans by ingestion, inhalation, and inoculation from such sources. *M. iranicum* is a species of the phylum actinobacteria (Gram-positive bacteria with high guanine and cytosine content, one of the dominant phyla of all bacteria), and belongs to the genus *Mycobacterium*.

The comparative genomic analysis of Malaysian clinical isolate against representative mycobacterial species suggests its environmental origin that might have evolved into a consequential human pathogen (Tan et al., 2014). *M. Iranium* not only has been isolated from the environment (Lymperopoulou et al., 2017) but has also recently been isolated from animals (Dibaj et al., 2018), indicating the possible emergence of a new zoonosis. There are a few strains of *M. iranicum* reported as clinical isolates from clinical specimens such as M05 (HQ009482) (Shojaei et al., 2013) and UM_TJL (AUWT00000000.1) from Malaysia (Tan et al., 2013; Tan et al., 2014). This report shows that *M. iranicum* is a new species of NTM, which may infect people by producing similar symptoms as *M. tuberculosis*. More studies are needed to determine whether our strain is from zoonotic or environmental sources.

CONCLUSION

A new strain of *M*. iranicum, SBH312 was isolated from a suspected pulmonary tuberculosis patient from Sabah, Malaysia. This strain was closely related to the strains from Iran and Peninsular Malaysia. This *M. iranicum* needs further clarification of its status as an emerging human pathogen. Also, the clinical significance of this *M. iranicum* is still unclear.

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REFERENCES

Balakrishnan, N., Tortoli, E., Engel, S. L., & Breitschwerdt, E. B. (2013). Isolation of a novel strain of *Mycobacterium iranicum* from a woman in the United States. *Journal of Clinical Microbiology*, *51* (2), 705 – 707. https://doi.org/10.1128/JCM.02560-12

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., Nikolenko, S. I., Pham, S., Prjibelski, A. D., Pyshkin, A. V., Sirotkin, A. V., Vyahhi, N., Tesler, G., Alekseyev, M. A., & Pevzner, P. A. (2012). SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology: A Journal of Computational Molecular Cell Biology*, 19 (5), 455 – 477. https://doi.org/10.1089/cmb.2012.0021

Becker, S. L., Halfmann, A., Schilling, L., Mawlood, D. A., Janssen, E., Reischl, U., Vogt, T., & Müller, C. S. (2018). *Mycobacterium iranicum* infection in a patient with fish tank granuloma: A first case report. *European Journal o Dermatology: EJD*, *28* (2), 238 – 239. https://doi.org/10.1684/ejd.2017.3196

Bushnell, B., Rood, J., & Singer, E. (2017). BBMerge – Accurate paired shotgun read merging via overlap. *PloS one*, *12* (10), e0185056. https://doi.org/10.1371/journal.pone.0185056

Chai, Q., Zhang, Y., & Liu, C. H. (2018). *Mycobacterium tuberculosis*: An adaptable pathogen associated with multiple human diseases. *Front Cell Infect Microbiol*., *8*, 158. https://doi.org/10.3389/fcimb.2018.00158

Dibaj, R., Shojaei, H., & Narimani, T. (2020). Identification and molecular characterization of mycobacteria isolated from animal sources in a developing country. *Acta Tropica*., *204*, 105297. https://doi.org/10.1016/j.actatropica.2019.105297

Gardner, S. N., Slezak, T., & Hall, B. G. (2015). kSNP3.0: SNP detection and phylogenetic analysis of genomes without genome alignment or reference genome. *Bioinformatics*, *31*, 2877 – 2878. https://doi.org/10.1093/bioinformatics/btv271

Gupta, R. S., Lo, B., & Son, J. (2018). Phylogenomics and comparative genomic studies robustly support division of the genus Mycobacterium into an emended genus Mycobacterium and four novel genera. *Frontiers in Microbiology*, *9*, 67. https://doi.org/10.3389/fmicb.2018.00067

Inagaki, K., Mizutani, M., Nagahara, Y., Asano, M., Masamoto, D., Sawada, O., Aono, A., Chikamatsu, K., & Mitarai, S. (2016). Successful Treatment of peritoneal dialysis-related peritonitis due to *Mycobacterium iranicum*. *Internal Medicine* (Tokyo, Japan), *55* (14), 1929 – 1931. https://doi.org/10.2169/internalmedicine.55.5219

Kalra, S., & Rubinstein, I. (2022). Mycobacterium iranicum pulmonary disease in an elderly patient with extensive usual interstitial pneumonia: A case report. *Clinical Case Reports*, *10* (10), e6329. https://doi.org/10.1002/ccr3.6329

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, *35*, 1547 – 1549. https://doi.org/10.1093/molbev/msy096

Lapierre, S. G., Toro, A., & Drancourt, M. (2017). *Mycobacterium iranicum* bacteremia and hemophagocytic lymphohistiocytosis: A case report. BMC research notes, 10(1), 372. https://doi.org/10.1186/s13104-017-2684-8

Lyamin A. V. (2020). Rare species in the structure of acid-resistant members of the order actinomycetales isolated from clinical material. *Russian Clinical Laboratory Diagnostics*, *65* (2), 111–115. https://doi.org/10.18821/0869-2084-2020-65-2-111-115

Lymperopoulou, D. S., Coil, D. A., Schichnes, D., Lindow, S. E., Jospin, G., Eisen, J. A., & Adams, R. I. (2017). Draft genome sequences of eight bacteria isolated from the indoor environment: Staphylococcus capitis strain H36, S. capitis strain H65, S. cohnii strain H62, S. hominis strain H69, Microbacterium sp. strain H83, Mycobacterium iranicum strain H39, Plantibacter sp. strain H53, and Pseudomonas oryzihabitans strain H72. *Standards in Genomic Sciences*, *12*, 17. https://doi.org/10.1186/s40793-017-0223-9

Shojaei, H., Daley, C., Gitti, Z., Hashemi, A., Heidarieh, P., Moore, E. R. B., Naser, A. D., Russo, C., van Ingen, J., & Tortoli, E. (2013). *Mycobacterium iranicum* sp. nov., a rapidly growing scotochromogenic species isolated from clinical specimens on three different continents. *International Journal of Systematic and Evolutionary Microbiology*, *63* (Pt 4), 1383 – 1389. https://doi.org/10.1099/ijs.0.043562-0

Tan, J. L., Ng, H. F., Wee, W. Y., Ang, M. Y., Wong, G. J., Ngeow, Y. F., & Choo, S. W. (2013). First whole-genome sequence of *Mycobacterium iranicum*, a newly reported mycobacterial species. *Genome Announcements*, *1* (5), e00732-13. https://doi.org/10.1128/genomeA.00732-13

Tan, J. L., Ngeow, Y. F., Wee, W. Y., Wong, G. J., Ng, H. F., & Choo, S. W. (2014). Comparative genomic analysis of *Mycobacterium iranicum* UM_TJL against representative mycobacterial species suggests its environmental origin. *Scientific Reports*, *4*, 7169. https://doi.org/10.1038/srep07169

Tatusova, T., DiCuccio, M., Badretdin, A., Chetvernin, V., Nawrocki, E. P., Zaslavsky, L., Lomsadze, A., Pruitt, K. D., Borodovsky, M., & Ostell, J. (2016). NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Research*, *44* (14), 6614 – 6624. https://doi.org/10.1093/nar/gkw569

Varghese, B., Enani, M., Shoukri, M., AlThawadi, S., AlJohani, S., & Al-Hajoj, S. (2017). Emergence of rare species of nontuberculous Mycobacteria as potential pathogens in Saudi Arabian clinical setting. *PLoS Neglected Tropical Diseases*, *11* (1), e0005288. https://doi.org/10.1371/journal.pntd.0005288

Zhang, Q., Shao, D. F., Wang, M. H., Li, W. Q., Wang, H. Y., Rana, M. A., Chen, Y., Liu, C. H., Zhang, S., Jin, Y. X., Zhang, C., Zhu, X. Y., Fu, A. S., & Ge, Y. L. (2019). Hemoptysis and high-density shadow of both lungs combined with elevated serum G-Lipopolysaccharide misdiagnosed as bronchiectasis with gram-negative bacterium infection ultimately confirmed as *Mycobacterium iranicum* infection by CT-guided percutaneous lung biopsy and next generation sequencing (NGS): A case report and literature review. *Clinical Laboratory*, *65* (9), 10.7754/Clin.Lab.2019.190320. https://doi.org/10.7754/Clin.Lab.2019.190320