



Draft Genome Sequence of *Mycolicibacterium* sp. Strain 018/SC-01/001, Isolated from the Marine Sponge *Iotrochota* sp.

Ji Fa Marshall Ong,^a Lik Tong Tan^a

^aNatural Sciences and Science Education, National Institute of Education, Nanyang Technological University, Singapore, Singapore

ABSTRACT Here, we report the draft genome sequence of a marine bacterium, *Mycolicibacterium* sp. strain 018/SC-01/001, isolated from the marine sponge *Iotrochota* sp. collected from the Singapore Strait. The analysis of the bacterial genome using the bioinformatics tool antiSMASH 4.0.2 revealed the presence of a number of unique natural product biosynthetic pathways.

There has been an increasing number of discoveries of structurally novel bioactive compounds with significant therapeutic activities that are produced by microbes isolated from marine samples, such as marine sediments and benthic invertebrates (1–5). Preliminary investigation carried out at our laboratory has revealed that there are potentially novel compounds produced by slow-growing marine bacterial strains associated with marine sponges collected in the Singapore Strait (4). Here, we have sequenced the genome of a representative *Mycolicibacterium* sp. 018/SC-01/001 strain isolated from samples of the marine sponge *Iotrochota* sp., collected using a rectangular dredge from the seabed surface in the Singapore Strait (1.155500°N, 103.735833°E) on 27 April 2017.

Homogenate from the sponge sample was processed in sterile artificial seawater, plated onto starch casein agar, and incubated at 25°C for 39 days before the marine bacterial colony was isolated and purified. Amplification of the 16S rRNA gene with the universal primers 27F (5'-GAGTTTGATCCTGGCTCAG-3') and 1525R (5'-AGAAAGGAGGT GATCCAGCC-3') and subsequent gene sequencing confirmed that isolate 018/SC-01/001 is a member of the *Mycolicibacterium* genus.

Genomic DNA (gDNA) was obtained from a culture of *Mycolicibacterium* sp. strain 018/SC-01/001 incubated at 25°C for 9 days in Difco marine agar. The gDNA was isolated using a Quick-DNA fungal/bacterial microprep kit (Zymo Research), followed by ethanol precipitation in order to obtain good-quality gDNA material prior to library preparation. Sequencing was performed using the 2 × 150-bp paired-end format on the MiSeq platform at Axil Scientific Pte. Ltd. (Singapore). The library was prepared using a Nextera XT library preparation kit (Illumina) following the manufacturer's instructions. The library (22.8 nM) was sequenced using a 300-cycle MiSeq reagent v2 microkit (Illumina), yielding an average sequencing coverage of 40× and total reads of 3 Mb. The quality and quantity of the reads were determined using an Agilent TapeStation 4200 instrument, PicoGreen, and quantitative PCR (qPCR). Default parameters were used for all software unless otherwise specified. An initial annotation was made using the NCBI Prokaryotic Genome Annotation Pipeline using the best-placed reference protein set and GeneMarkS-2+ (6). The draft genome was found to be 5,781,079 bp in length, with a GC content of 68% and an N_{50} value of 198,717. A total of 49 contigs were obtained, with 48 containing protein-encoding genes, and there were 5,559 putative genes in total.

The sequence was examined using antiSMASH 4.0.2 (7, 8). A bacterial database was set as the reference depository, and gene cluster BLAST comparative analysis and the

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Address correspondence to Lik Tong Tan, liktong.tan@nie.edu.sg.

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secondary metabolism gene family (secondary metabolism clusters of orthologous groups [smCOGs]) functions were selected with strict detection parameters that detect only well-defined clusters containing all required parts. A total of 16 biosynthetic gene clusters were identified and predicted, as follows: there are three clusters encoding the biosynthetic pathway for type 1 polyketide synthase (T1PKS), two type 1 polyketide synthase, nonribosomal peptide synthetase (T1PKS, NRPS) gene clusters, two type 3 polyketide synthase (T3PKS) gene clusters, two nonribosomal peptide synthetase (NRPS) gene clusters, three nonribosomal peptide synthetase-like (NRPS-like) gene clusters, two terpenes, one bacteriocin, and one arylpolyene cluster.

The *Mycolicibacterium* sp. 018/SC-01/001 genome sequence and the number of unique secondary metabolite biosynthetic gene clusters suggest that the bacterial strain may be a promising source of potentially novel molecules, as supported by the mass spectrometry-based metabolomics analysis of the organic extracts using the molecular networking platform (9).

Data availability. The raw sequence data have been deposited and made publicly available at DDBJ/ENA/GenBank under accession number [PRJNA551549](https://doi.org/10.1093/nar/gkw569). The SRA data details have been deposited at DDBJ/ENA/GenBank under accession number [SRX6373421](https://doi.org/10.1093/nar/gkr466). The whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under accession number [VKAA00000000](https://doi.org/10.1093/nar/gkx319). The version described in this paper is the first version, VKAA01000000.

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REFERENCES

1. Thomas TR, Kavlekar DP, LokaBharathi PA. 2010. Marine drugs from sponge-microbe association—a review. *Mar Drugs* 8:1417–1468. <https://doi.org/10.3390/md8041417>.
2. Hameş-Kocabaş EE, Uzel A. 2012. Isolation strategies of marine-derived actinomycetes from sponge and sediment samples. *J Microbiol Methods* 88:342–347. <https://doi.org/10.1016/j.mimet.2012.01.010>.
3. Muralidharan A, Josyula VR, Hariharapura RC. 2018. Exploring the potential of marine microbes in clinical management of Alzheimer's disease: a road map for bioprospecting and identifying promising isolates. *Life Sci* 208:149–160. <https://doi.org/10.1016/j.lfs.2018.07.036>.
4. Ong JFM, Goh HC, Lim SC, Pang LM, Chin JSF, Tan KS, Liang ZX, Yang L, Glukhov E, Gerwick WH, Tan LT. 2019. Integrated genomic and metabolomic approach to the discovery of potential anti-quorum sensing natural products from microbes associated with marine samples from Singapore. *Mar Drugs* 17:72. <https://doi.org/10.3390/md17010072>.
5. Malve H. 2016. Exploring the ocean for new drug developments: marine pharmacology. *J Pharm Bioall Sci* 8:83–91. <https://doi.org/10.4103/0975-7406.171700>.
6. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>.
7. Medema MH, Blin K, Cimermancic P, de Jager V, Zakrzewski P, Fischbach MA, Weber T, Takano E, Breitling R. 2011. antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic Acids Res* 39:W339–W346. <https://doi.org/10.1093/nar/gkr466>.
8. Blin K, Wolf T, Chevrette MG, Lu X, Schwalen CJ, Kautsar SA, Suarez Duran HG, de los Santos ELC, Kim HU, Nave M, Dickschat JS, Mitchell DA, Shelest E, Breitling R, Takano E, Lee SY, Weber T, Medema MH. 2017. antiSMASH 4.0—improvements in chemistry prediction and gene cluster boundary identification. *Nucleic Acids Res* 45:36–41. <https://doi.org/10.1093/nar/gkx319>.
9. Yang JY, Sanchez LM, Rath CM, Liu X, Boudreau PD, Bruns N, Glukhov E, Wodtke A, de Felicio R, Fenner A, Wong WR, Linington RG, Zhang L, Debonis HM, Gerwick WH, Dorrestein PC. 2013. Molecular networking as a dereplication strategy. *J Nat Prod* 76:1686–1699. <https://doi.org/10.1021/np400413s>.