



Draft Genome Sequences of Three Sponge-Associated Actinomycetes Exhibiting Antimycobacterial Activity

Daniela Tizabi,^a Ana Sosa,^a Tsvetan Bachvaroff,^a Russell T. Hill^a

^aInstitute of Marine and Environmental Technology, University of Maryland Center for Environmental Science, Baltimore, Maryland, USA

ABSTRACT Marine actinomycetes (order *Actinomycetales*) are of interest as a promising source of pharmaceuticals. The genomes of three novel sponge-associated actinomycetes exhibiting antimycobacterial activity, *Brevibacterium* sp. strain XM4083, *Micrococcus* sp. strain R8502A1, and *Micromonospora* sp. strain XM-20-01, were sequenced in an effort to identify compounds responsible for growth-inhibiting activity.

Actinomycetes produce an extensive arsenal of compounds with pharmaceutical potential (1–4). Approximately 70% of all known antibiotics are derived from actinomycetes (5). These bacteria exist in a diverse range of environments, both terrestrial and aquatic (3–5). Marine actinomycetes are of particular interest to drug discovery efforts, as advances in culturing techniques increasingly facilitate access to previously underexplored rare species (3, 4). In the case of sponge symbionts, the compounds they produce may aid in the defense of their nonmotile hosts. Novel strains of *Brevibacterium*, *Micrococcus*, and *Micromonospora* isolated from the giant barrel sponge *Xestospongia muta* showed promising growth inhibition against several *Mycobacterium* spp. in a screen performed as part of this study. Representatives from these genera produce secondary metabolites with potent bioactivity (1, 6–12). Genomic analysis was performed to elucidate the bioactive capabilities of these bacteria and predict which bioactive compound(s) may be produced by these strains.

Microbial strains were previously isolated from *X. muta* samples collected by SCUBA divers in Conch Reef, Key Largo, FL, in July 2001 and June 2004 (13) and stored at –80°C. Strains were assigned taxonomic classifications after initial isolation, and these were confirmed at the time of this study on the basis of 16S rRNA gene sequence analysis. Strains were cultured in International *Streptomyces* Project 2 (ISP2) medium for 10 to 16 days and incubated at 30°C with shaking at 150 rpm. Organic extracts were prepared by ethyl acetate extraction and produced zones of inhibition in a disk diffusion assay against at least one of three test strains, *Mycobacterium smegmatis* MC² 155, *Mycobacterium marinum* 927, and *Mycobacterium tuberculosis* H37Ra. DNA was extracted from the *Brevibacterium* and *Micromonospora* strains using the UltraClean microbial DNA isolation kit. Phenol-chloroform extraction was required for *Micrococcus* DNA isolation. Genomic DNA was sequenced on the MiSeq sequencer (Illumina) using the MiSeq version 2.4.0.4 reagent kit (300 cycles for *Brevibacterium* sp. and *Micromonospora* sp. and 500 cycles for *Micrococcus* sp.). The Nextera DNA Flex library prep kit (100 ng DNA) was used to prepare the libraries for the *Brevibacterium* and *Micromonospora* strains, while the Nextera XT library prep kit (1 ng DNA) was used to prepare the *Micrococcus* library. Reads were assembled *de novo* using SPAdes version 3.11.1 (14), which includes a built-in BayesHammer read error correction tool. Contigs were filtered primarily based on coverage, followed by match identity after comparison with the Nucleotide BLAST database. Genome annotation was performed using PATRIC version 3.5.41 (15), and biosynthetic gene clusters (BGCs) were identified using antiSMASH version 5.0 (16) (Table 1). Default parameters were used for all software programs.

Citation Tizabi D, Sosa A, Bachvaroff T, Hill RT. 2019. Draft genome sequences of three sponge-associated actinomycetes exhibiting antimycobacterial activity. *Microbiol Resour Announc* 8:e00858-19. <https://doi.org/10.1128/MRA.00858-19>.

Editor Frank J. Stewart, Georgia Institute of Technology

Copyright © 2019 Tizabi et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Russell T. Hill, hill@umces.edu.

Received 18 July 2019

Accepted 5 August 2019

Published 22 August 2019

TABLE 1 Biosynthetic gene clusters identified for novel actinomycetes with antiSMASH

Strain	Pigment	No. of BGCs identified ^a	Order of BGCs (similarity to known cluster [%])
<i>Brevibacterium</i> sp. XM4083	Yellow	2	Carotenoid (57), ectoine (75)
<i>Micrococcus</i> sp. R8502A1	Yellow	1	Carotenoid (66)
<i>Micromonospora</i> sp. XM-20-01	Initially pink, then black	7	Diazepinomicin (75), sioxanthin (100), alkyl-O-dihydrogeranyl-methoxyhydroquinones (71), sioxanthin (100), micromonolactam (100), micromonolactam (100), micromonolactam (100)

^a Only BGCs with at least 40% similarity to known clusters on antiSMASH were considered.

For *Brevibacterium* sp. strain XM4083, MiSeq sequencing generated 4,324,102 read pairs, from which SPAdes assembled 102 contigs. Based on coverage, the final assembly contained 13 contigs ranging in size from 5,527 bp to 1,292,640 bp (N_{50} , 761,026 bp), with average k-mer coverage of 192-fold, yielding a genome of ~4.0 Mb with a G+C content of 68.02%. Annotation with PATRIC identified 3,782 genes, including 3,732 protein-coding sequences (CDS), 47 tRNA genes, and 3 rRNA genes.

For *Micrococcus* sp. strain R8502A1, MiSeq sequencing generated 2,607,861 read pairs, from which SPAdes generated 609 contigs. The final assembly contained 128 contigs ranging in size from 239 bp to 137,025 bp (N_{50} , 38,085 bp), with average k-mer coverage of 158-fold, yielding a genome of ~2.4 Mb with a G+C content of 72.91%. Annotation with PATRIC identified 2,373 genes, including 2,322 CDS, 49 tRNA genes, and 2 rRNA genes.

For *Micromonospora* sp. strain XM-20-01, MiSeq sequencing generated 1,565,813 read pairs, from which SPAdes generated 30,851 contigs. The final assembly contained 149 contigs ranging in size from 250 bp to 343,851 bp (N_{50} , 105,009 bp), with average k-mer coverage of 32-fold, yielding a genome of ~6.7 Mb with a G+C content of 72.86%. Annotation with PATRIC identified 6,401 genes, including 6,347 CDS, 51 tRNA genes, and 3 rRNA genes.

Data availability. This whole-genome shotgun project was deposited at DDBJ/ENA/GenBank under the accession no. [VFYQ0000000](https://doi.org/10.1038/ja.2005.1), [VIGS0000000](https://doi.org/10.1038/ja.2005.1), and [VFYR0000000](https://doi.org/10.1038/ja.2005.1) for *Micromonospora* sp. XM-20-01, *Micrococcus* sp. R8502A1, and *Brevibacterium* sp. XM4083, respectively. Raw reads for *Micromonospora* sp. XM-20-01, *Micrococcus* sp. R8502A1, and *Brevibacterium* sp. XM4083 were deposited in the NCBI SRA under the accession no. [SRR9208918](https://doi.org/10.1038/ja.2005.1), [SRR9208919](https://doi.org/10.1038/ja.2005.1), and [SRR9208920](https://doi.org/10.1038/ja.2005.1), respectively. They pertain to BioProject accession no. [PRJNA543824](https://doi.org/10.1038/ja.2005.1).

ACKNOWLEDGMENTS

We thank the BioAnalytical Services Laboratory at the Institute of Marine and Environmental Technology for performing sequencing.

D.T. was supported by a Ratcliffe Environmental Entrepreneur Fellowship. A.S. was supported by a Maryland Sea Grant Fellowship.

REFERENCES

- Bérdy J. 2005. Bioactive microbial metabolites. *J Antibiot* 58:1–26. <https://doi.org/10.1038/ja.2005.1>.
- Genilloud O. 2018. Mining actinomycetes for novel antibiotics in the omics era: are we ready to exploit this new paradigm? *Antibiotics* 7:85. <https://doi.org/10.3390/antibiotics7040085>.
- Hug J, Bader C, Remškar M, Cirnski K, Müller R. 2018. Concepts and methods to access novel antibiotics from actinomycetes. *Antibiotics* 7:44. <https://doi.org/10.3390/antibiotics7020044>.
- Fenical W, Jensen PR. 2006. Developing a new resource for drug discovery: marine actinomycete bacteria. *Nat Chem Biol* 2:666–673. <https://doi.org/10.1038/nchembio841>.
- Solecka J, Zajko J, Postek M, Rajnisz A. 2012. Biologically active secondary metabolites from actinomycetes. *Cent Eur J Biol* 7:373–390. <https://doi.org/10.2478/s11535-012-0036-1>.
- Talukdar M, Das D, Bora C, Bora TC, Deka Boruah HP, Singh AK. 2016. Complete genome sequencing and comparative analysis of broad-spectrum antimicrobial-producing *Micromonospora* sp. HK10. *Gene* 594: 97–107. <https://doi.org/10.1016/j.gene.2016.09.005>.
- Carlson S, Marler L, Nam SJ, Santarsiero BD, Pezzuto JM, Murphy BT. 2013. Potential chemopreventive activity of a new macrolide antibiotic from a marine-derived *Micromonospora* sp. *Mar Drugs* 11:1152–1161. <https://doi.org/10.3390/md11041152>.
- Carro L, Nouioui I, Sangal V, Meier-Kolthoff JP, Trujillo ME, Montero-Calasanz MC, Sahin N, Smith DL, Kim KE, Peluso P, Deshpande S, Woyke T, Shapiro N, Kyrpidis NC, Klenk H, Göker M, Goodfellow M. 2018. Genome-based classification of micromonosporae with a focus on their biotechnological and ecological potential. *Sci Rep* 8:525. <https://doi.org/10.1038/s41598-017-17392-0>.
- Motta AS, Brandelli A. 2002. Characterization of an antibacterial peptide produced by *Brevibacterium linens*. *J Appl Microbiol* 92:63–70. <https://doi.org/10.1046/j.1365-2672.2002.01490.x>.
- Choi EJ, Kwon HC, Ham J, Yang HO. 2009. 6-Hydroxymethyl-1-

- phenazine-carboxamide and 1,6-phenazinedimethanol from a marine bacterium, *Brevibacterium* sp. KMD 003, associated with marine purple vase sponge. *J Antibiot* 62:621–624. <https://doi.org/10.1038/ja.2009.92>.
11. Ramalingam V, Varunkumar K, Ravikumar V, Rajaram R. 2019. Production and structure elucidation of anticancer potential surfactin from marine actinomycete *Micromonospora marina*. *Process Biochem* 78:169–177. <https://doi.org/10.1016/j.procbio.2019.01.002>.
 12. Braesel J, Crnkovic CM, Kunstman KJ, Green SJ, Maienschein-Cline M, Orjala J, Murphy BT, Eustáquio AS. 2018. Complete genome of *Micromonospora* sp. strain B006 reveals biosynthetic potential of a Lake Michigan actinomycete. *J Nat Prod* 81:2057–2068. <https://doi.org/10.1021/acs.jnatprod.8b00394>.
 13. Montalvo NF, Mohamed NM, Enticknap JJ, Hill RT. 2005. Novel actinobacteria from marine sponges. *Antonie Van Leeuwenhoek* 87:29–36. <https://doi.org/10.1007/s10482-004-6536-x>.
 14. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
 15. Wattam AR, Davis JJ, Assaf R, Boisvert S, Brettin T, Bun C, Conrad N, Dietrich EM, Disz T, Gabbard JL, Gerdes S, Henry CS, Kenyon RW, Machi D, Mao C, Nordberg EK, Olsen GJ, Murphy-Olson DE, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Vonstein V, Warren A, Xia F, Yoo H, Stevens RL. 2017. Improvements to PATRIC, the all-bacterial bioinformatics database and analysis resource center. *Nucleic Acids Res* 45:D535–D542. <https://doi.org/10.1093/nar/gkw1017>.
 16. 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:W36–W41. <https://doi.org/10.1093/nar/gkx319>.