AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Draft Genome Sequences of *Blastococcus* sp. Clones TML/M2B and TML/C7B, with Different Motilities, Isolated in a Laboratory

Hsiao-Mei Liao,[a] Bingjie Li,[a] Shien Tsai,[a] Hebing Liu,[a] Guo-Chiuan Hung,[a] Shyh-Ching Lo[a]

[a]Tissue Microbiology Laboratory, Division of Cellular and Gene Therapies, Office of Tissues and Advanced Therapies, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, Maryland, USA

ABSTRACT Two novel *Blastococcus* sp. clones, TML/M2B and TML/C7B, with 2 different stable growth phenotypes, were isolated from a laboratory tissue culture. The draft genome sequences generated through genomic sequencing of clones TML/M2B and TML/C7B contain 4 and 2 contigs, respectively. The respective genome sizes are 4.10 Mb and 4.11 Mb, with G+C contents of 74.17% and 74.14%, respectively.

The genus *Blastococcus*, a member of the *Geodermatophilaceae* family, contains Gram-positive bacteria isolated mainly from soils, marine sediment, and stone interiors (1–3). In a rare case, an unclassified *Blasctococcus* sp. was isolated from a plant (4). The life cycle of *Blastococcus* spp. contains several cell forms, including motile rod and vibroid, as well as nonmotile cocci and coccoid aggregates (5). Here, we report the draft genome sequences of two clones of *Blastococcus* spp. isolated from a flask of cell culture in a laboratory.

In a prolonged laboratory tissue/cell culture experiment, we found that a flask of cell culture contained unusual microbes. The microorganisms grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (R10) were identified as Gram-positive bacteria (1, 5) and formed orange-pigmented colonies on the R10 agar plates. We performed single-colony picking and isolated a clone growing mostly as motile forms, designated TML/M2B, and a clone growing mostly as aggregated nonmotile coccoid forms, designated TML/C7B (microscopic observations). Genomic DNA of these two bacterial clones was prepared with DNAzol reagent (Thermo Fisher) using the manufacturer's protocol designed for Gram-positive bacteria. Genomic DNA libraries were prepared with a Nextera DNA Flex library prep kit (Illumina) and subjected to genomic sequencing using an Illumina MiSeq instrument with 2 × 150-bp pair-ended sequencing mode. Raw reads were trimmed and assembled into 336 and 302 contigs using the Trim Reads and *De Novo* Assembly tools of CLC Genomics Workbench version 20.0.4. The blastn results of the assembled contigs revealed identical 16S rRNA sequences in these two clones that best matched that of *Blastococcus saxobsidens* strain DD2 (6, 7) in GenBank, with 99.8% identity.

To further improve the completeness of the draft genome sequences, we performed Oxford Nanopore MinION sequencing. Genomic DNA libraries were prepared using a rapid sequencing library preparation kit (SQK-RAD004; Oxford Nanopore Technologies), and sequencing was run using Flowcell version R9.4 (FLO-MIN106; Oxford Nanopore Technologies) for a 48-hour running time. Guppy version 3.2.8 was used to perform base calling to convert MinION FAST5 outputs to FASTQ files. The raw reads in the FASTQ files were assembled into contigs using the *De Novo* Assemble Long Reads tool of CLC Genomics Workbench version 20.0.4 with default settings. The *de novo* assembly generated 4 contigs for clone TML/M2B and 2 contigs for clone TML/C7B. The contigs were then polished using the paired-end reads of the respective

Citation Liao H-M, Li B, Tsai S, Liu H, Hung G-C, Lo S-C. 2021. Draft genome sequences of *Blastococcus* sp. clones TML/M2B and TML/C7B, with different motilities, isolated in a laboratory. Microbiol Resour Announc 10:e00121-21. https://doi.org/10.1128/MRA.00121-21.

Editor Kenneth M. Stedman, Portland State University

Address correspondence to Hsiao-Mei Liao, Hsiao-mei.liao@fda.hhs.gov, or Shyh-Ching Lo, shyhching.lo@gmail.com.

Received 1 February 2021
Accepted 3 March 2021
Published 25 March 2021

TABLE 1 Characterization of draft genome sequences of *Blastococcus* sp. clones TML/M2B and TML/C7B

Characteristic	Data for:	
	***Blastococcus* sp. TML/M2B**	***Blastococcus* sp. TML/C7B**
Motility	Highly motile	Slightly motile
Morphology	Rod and vibroid dominant	Cocci and coccoid dominant
Contig count	4	2
No. of MiSeq reads	8,331,290	6,831,770
No. of minION reads	636,000	312,725
Draft genome length (bp)	4,114,186	4,100,268
N_{50} (bp)	1,784,222	2,703,711
G+C content (%)	74.17	74.14
Avg coverage (×)	230	134
BioProject accession no.	PRJNA685785	PRJNA685785
BioSample accession no.	SAMN17093989	SAMN17093990
SRA accession no.	SRR13283331, SRR13283332	SRR13283329, SRR13283330
GenBank Accession no.	JAENZZ000000000	JAEOAA000000000
PATRIC comprehensive genome analysis annotation		
No. of annotated CDs[a]	4,071	4,036
No. of functional proteins	2,605	2,585
No. of hypothetical proteins	1,466	1,451
No. of annotated tRNAs	48	49
No. of annotated rRNAs	8	6
No. of repeat regions	74	46

[a] CDs, coding sequences.

clones generated from Illumina MiSeq sequencing to improve the correctness (Polish with Reads Tool, CLC Genomics Workbench version 20.0.4).

Whole-genome comparison (whole-genome alignment tools, CLC Genomics Workbench version 20.0.4) revealed that clones TML/M2B and TML/C7B had 86.45% average nucleotide identity (ANI) compared to *Blastococcus saxobsidens* strain DD2, and the ANI between these two clones was 99.98%. The genomes were submitted for comprehensive genome analysis using PATRIC version 3.6.8 (8) for annotation. Our findings are summarized in Table 1.

Data availability. The raw reads and draft genome contigs of the clones *Blastococcus* sp. TML/M2B and TML/C7B have been deposited in the Sequence Read Archive (SRA) and GenBank under BioProject no. PRJNA685785, SRA no. SRR13283329, SRR13283330, SRR13283331, and SRR13283332, and GenBank accession no. JAENZZ000000000 and JAEOAA000000000.

ACKNOWLEDGMENTS

The Illumina MiSeq sequencing was supported by the Protein and Nucleic Acid Laboratory under the Facility for Biotechnology Resources, Center for Biologics Evaluation and Research, Food and Drug Administration. This research was supported in part by an appointment to the Research Fellowship Program at the Office of Tissue and Advanced Therapies/Center for Biologics Evaluation and Research, U.S. Food and Drug Administration (FDA), administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the FDA.

REFERENCES

1. Normand P, Daffonchio D, Gtari M. 2014. The family Geodermatophilaceae, p 361–379. *In* Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (ed), The Prokaryotes: Actinobacteria. Springer, Berlin, Germany. https://doi.org/10.1007/978-3-642-30138-4_180.
2. Hezbri K, Louati M, Nouioui I, Gtari M, Rohde M, Sproer C, Schumann P, Klenk HP, Ghodhbane-Gtari F, Montero-Calasanz MDC. 2016. Blastococcus capsensis sp. nov., isolated from an archaeological Roman pool and emended description of the genus Blastococcus, B. aggregatus, B. saxobsidens, B. jejuensis and B. endophyticus. Int J Syst Evol Microbiol 66:4864–4872. https://doi.org/10.1099/ijsem.0.001443.

3. Hezbri K, Nouioui I, Rohde M, Sproer C, Schumann P, Gtari M, Klenk HP, Montero-Calasanz MDC, Ghodhbane-Gtari F. 2018. Blastococcus xanthinilyticus sp. nov., isolated from monument. Int J Syst Evol Microbiol 68:1177–1183. https://doi.org/10.1099/ijsem.0.002646.
4. Zhu WY, Zhang JL, Qin YL, Xiong ZJ, Zhang DF, Klenk HP, Zhao LX, Xu LH, Li WJ. 2013. Blastococcus endophyticus sp. nov., an actinobacterium isolated from Camptotheca acuminata. Int J Syst Evol Microbiol 63:3269–3273. https://doi.org/10.1099/ijs.0.049239-0.
5. Urzi C, Salamone P, Schumann P, Rohde M, Stackebrandt E. 2004. Blastococcus saxobsidens sp. nov., and emended descriptions of the genus Blastococcus Ahrens and Moll 1970 and Blastococcus aggregatus Ahrens and Moll 1970. Int J Syst Evol Microbiol 54:253–259. https://doi.org/10.1099/ijs.0.02745-0.
6. Chouaia B, Crotti E, Brusetti L, Daffonchio D, Essoussi I, Nouioui I, Sbissi I, Ghodhbane-Gtari F, Gtari M, Vacherie B, Barbe V, Medigue C, Gury J, Pujic P, Normand P. 2012. Genome sequence of Blastococcus saxobsidens DD2, a stone-inhabiting bacterium. J Bacteriol 194:2752–2753. https://doi.org/10.1128/JB.00320-12.
7. Sghaier H, Hezbri K, Ghodhbane-Gtari F, Pujic P, Sen A, Daffonchio D, Boudabous A, Tisa LS, Klenk HP, Armengaud J, Normand P, Gtari M. 2016. Stone-dwelling actinobacteria Blastococcus saxobsidens, Modestobacter marinus and Geodermatophilus obscurus proteogenomes. ISME J 10:21–29. https://doi.org/10.1038/ismej.2015.108.
8. Davis JJ, Wattam AR, Aziz RK, Brettin T, Butler R, Butler RM, Chlenski P, Conrad N, Dickerman A, Dietrich EM, Gabbard JL, Gerdes S, Guard A, Kenyon RW, Machi D, Mao C, Murphy-Olson D, Nguyen M, Nordberg EK, Olsen GJ, Olson RD, Overbeek JC, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomas C, VanOeffelen M, Vonstein V, Warren AS, Xia F, Xie D, Yoo H, Stevens R. 2020. The PATRIC Bioinformatics Resource Center: expanding data and analysis capabilities. Nucleic Acids Res 48:D606–D612. https://doi.org/10.1093/nar/gkz943.