

Draft genome of *Streptomyces globosus* OSY-MM, a broad-spectrum antimicrobial producer isolated from a cave in South Central Kentucky

Gabriella J. Gephart,¹ Ahmed G. Abdelhamid,² Matthew J. Mezydlo,³ Steven J. Carlson,³ Zach L. Hawkins,¹ Ahmed E. Yousef^{1,3}

AUTHOR AFFILIATIONS See affiliation list on p. 2.

ABSTRACT *Streptomyces globosus* OSY-MM was originally isolated from a soil sample collected from a cave in South Central Kentucky and was found to display strong, broad-spectrum antimicrobial activity. *S. globosus* OSY-MM has a 7,277,920 bp genome with 6,476 genes, including biosynthetic gene clusters encoding the production of streptothricin, kirromycin, and venezuelin-like antibiotics.

KEYWORDS *Streptomyces globosus*, cave isolate, streptothricin, genome, antimicrobial agents

Streptomyces spp. are well known for producing a large variety of secondary metabolites, including many antimicrobial agents (1). *Streptomyces globosus* OSY-MM was originally isolated from an undisturbed cave passage in South Central Kentucky and was determined to be a prolific antimicrobial producer, as determined by the spot-on-lawn bioassay against *Escherichia coli* K-12 and *Listeria innocua* ATCC 33090 (2). Liquid chromatography-mass spectrometry analysis suggested that *S. globosus* OSY-MM produces the antibiotic streptothricin D. Importantly, streptothricin research is on the rise due to the potential to combat drug-resistant gram-negative bacteria after largely being overlooked due to toxicity concerns (3).

S. globosus OSY-MM was grown on tryptic soy agar (BD, Sparks, MD, USA) at 30°C for 96 hours. Resulting spores were then transferred to International *Streptomyces* Project-2 (ISP-2) medium containing 0.4% yeast extract (BD), 1% malt extract (BD), and 0.4% dextrose (Fisher Chemical, Fair Lawn, NJ, USA). The inoculated ISP-2 medium was incubated at 30°C for 48 hours with shaking at 200 rpm. Genomic DNA (gDNA) was extracted from 8 mL of culture using a DNA extraction kit (QIAamp DNA Mini Kit; Qiagen, Germantown, MD, USA) and was cleaned up according to the instructions of the manufacturer. Quantity and quality of gDNA were measured using a spectrophotometer (NanoVue Plus; Biochrom, Holliston, MA, USA). The gDNA was sequenced using a long-read technology (Oxford Nanopore Technologies; Plasmidsaurus, <https://www.plasmidsaurus.com>). Read statistics (602,875 reads, 1,042,797,863 bases, and coverage of 143x) were provided by Plasmidsaurus. The genome was assembled using Flye 2.9.6 (4). Additionally, short-read sequencing was performed in our laboratory using the Illumina MiSeq platform, as described previously (5). Briefly, libraries were prepared using the Nextera DNA Flex Kit (Illumina, Madison, WI, USA) and were indexed using the Nextera DNA CD Index Kit (Illumina). The libraries were quantified using a fluorimeter (Qubit 4; Invitrogen, Waltham, MA, USA) before sequencing. Paired-end raw reads (2 × 150 bp) were produced. A total of 291,171,146 bp were sequenced, resulting in a coverage of 40x. The Nanopore assembly was polished twice using Racon 1.5.0 (6), and the Nanopore assembly and Illumina reads were compiled utilizing Pilon 1.20 (7). The polished genome was assessed for quality using QUAST 5.3.0 (8) and was determined to contain two

Editor André O. Hudson, Rochester Institute of Technology, Rochester, New York, USA

Address correspondence to Ahmed E. Yousef, yousef.1@osu.edu.

The authors declare no conflict of interest.

Received 4 February 2026

Accepted 13 April 2026

Published 18 May 2026

Copyright © 2026 Gephart 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/).

contigs (6,695,794 and 582,126 bp), having 74% GC content and an N50 value of 6,695,794 bp. Default parameters were applied for all software used. It was concluded that *S. globosus* OSY-MM has a 7,277,920 bp genome with 6,476 genes and 6,382 coding sequences.

The assembled genome was mined for biosynthetic gene clusters (BGCs) associated with secondary metabolite production utilizing the software antiSMASH 8.0 (9). *S. globosus* OSY-MM contained BGCs encoding the production of streptothricin-, kirromycin-, and venezuelin-like antibiotics. The Genome Taxonomy Database 2.3.2 (10) and Type Strain Genome Server (11) were used for taxonomic classification. In conclusion, *S. globosus* OSY-MM genome encodes novel or rare variants of antimicrobial agents, which may be useful in combating antibiotic-resistant pathogenic bacteria.

AUTHOR AFFILIATIONS

¹Department of Food Science and Technology, The Ohio State University, Columbus, Ohio, USA

²Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan, USA

³Department of Microbiology, The Ohio State University, Columbus, Ohio, USA

AUTHOR ORCIDs

Ahmed E. Yousef  <http://orcid.org/0000-0003-2094-3199>

AUTHOR CONTRIBUTIONS

Gabriella J. Gephart, Conceptualization, Data curation, Formal analysis, Investigation, Validation, Visualization, Writing – original draft, Writing – review and editing | Ahmed G. Abdelhamid, Conceptualization, Formal analysis, Methodology, Validation, Writing – review and editing | Matthew J. Mezydlo, Conceptualization, Data curation, Investigation, Methodology, Writing – review and editing | Steven J. Carlson, Conceptualization, Data curation, Validation, Writing – original draft | Zach L. Hawkins, Conceptualization, Data curation, Investigation, Methodology | Ahmed E. Yousef, Conceptualization, Formal analysis, Funding acquisition, Project administration, Resources, Writing – review and editing

DATA AVAILABILITY

The genome of *Streptomyces globosus* OSY-MM was deposited under GenBank accession number [JBTZS0000000000](#). The linked BioProject, BioSample, SRA (Illumina), and SRA (Nanopore) accession numbers are [PRJNA1414647](#), [SAMN54911780](#), [SRR36994418](#), and [SRR36994417](#), respectively.

REFERENCES

1. Yu Y, Tang B, Dai R, Zhang B, Chen L, Yang H, Zhao G, Ding X. 2018. Identification of the streptothricin and tunicamycin biosynthetic gene clusters by genome mining in *Streptomyces* sp. strain fd1-xmd. *Appl Microbiol Biotechnol* 102:2621–2633. <https://doi.org/10.1007/s00253-018-8748-4>
2. He Z, Kislá D, Zhang L, Yuan C, Green-Church KB, Yousef AE. 2007. Isolation and identification of a *Paenibacillus polymyxa* strain that coproduces a novel lantibiotic and polymyxin. *Appl Environ Microbiol* 73:168–178. <https://doi.org/10.1128/AEM.02023-06>
3. Morgan CE, Kang YS, Green AB, Smith KP, Dowgiallo MG, Miller BC, Chiaraviglio L, Truelson KA, Zulauf KE, Rodriguez S, Kang AD, Manetsch R, Yu EW, Kirby JE. 2023. Streptothricin F is a bactericidal antibiotic effective against highly drug-resistant gram-negative bacteria that interacts with the 30S subunit of the 70S ribosome. *PLoS Biol* 21:e3002091. <https://doi.org/10.1371/journal.pbio.3002091>
4. Lin Y, Yuan J, Kolmogorov M, Shen MW, Chaisson M, Pevzner PA. 2016. Assembly of long error-prone reads using de Bruijn graphs. *Proc Natl Acad Sci USA* 113:E8396–E8405. <https://doi.org/10.1073/pnas.1604560113>
5. Gephart GJ, Abdelhamid AG, Yousef AE. 2024. Genome sequences of six lactic acid bacteria from artisanal cheese reveal biosynthetic gene clusters encoding antimicrobial compounds. *Microbiol Resour Announc* 13:e0070924. <https://doi.org/10.1128/mra.00709-24>
6. Vaser R, Sović I, Nagarajan N, Šikić M. 2017. Fast and accurate *de novo* genome assembly from long uncorrected reads. *Genome Res* 27:737–746. <https://doi.org/10.1101/gr.214270.116>
7. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One* 9:e112963. <https://doi.org/10.1371/journal.pone.0112963>
8. Mikheenko A, Pribelski A, Saveliev V, Antipov D, Gurevich A. 2018. Versatile genome assembly evaluation with QAST-LG. *Bioinformatics* 34:i142–i150. <https://doi.org/10.1093/bioinformatics/bty266>

9. Blin K, Shaw S, Vader L, Szenei J, Reitz ZL, Augustijn HE, Cediél-Becerra JDD, de Crécy-Lagard V, Koetsier RA, Williams SE, et al. 2025. antiSMASH 8.0: extended gene cluster detection capabilities and analyses of chemistry, enzymology, and regulation. *Nucleic Acids Res* 53:W32–W38. <https://doi.org/10.1093/nar/gkaf334>
10. Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH. 2022. GTDB-Tk v2: memory friendly classification with the genome taxonomy database. *Bioinformatics* 38:5315–5316. <https://doi.org/10.1093/bioinformatics/btac672>
11. Meier-Kolthoff JP, Göker M. 2019. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nat Commun* 10:2182. <https://doi.org/10.1038/s41467-019-10210-3>