AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Draft Genome Sequences of Three Antibiotic-Producing Soil Bacteria, *Staphylococcus pasteuri* WAM01, *Peribacillus butanolivorans* WAM04, and *Micrococcus yunnanensis* WAM06, with Growth-Inhibiting Effects against Commensal *Neisseria* Strains

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ABSTRACT We report the isolation, identification, and assemblies of three antibiotic-producing soil bacteria (*Staphylococcus pasteuri*, *Peribacillus butanolivorans*, and *Micrococcus yunnanensis*) that inhibit the growth of *Neisseria* commensals in coculture. With pathogenic *Neisseria* strains becoming increasingly resistant to antibiotics, bioprospecting for novel antimicrobials using commensal relatives may facilitate discovery of clinically useful drugs.

Antibiotic resistance (AR) in *Neisseria gonorrhoeae*, the Gram-negative pathogen responsible for the sexually transmitted infection gonorrhea, is a worldwide threat to public health. Resistance to all therapeutics that have been recommended for empirical treatment has emerged (1, 2), and only two drugs, namely, zoliflodacin (currently in phase 3 trials [3, 4]) and gepotidacin (in phase 2 trials [5, 6]), are in development as alternative options. Bioprospecting for antibiotics produced by microbes in soil communities could uncover novel inhibitory compounds against the gonococcus and other important human pathogens (7, 8). This approach can be implemented in undergraduate classrooms as an inquiry-based exercise, which was previously demonstrated by the Small World Initiative (9, 10), Tiny Earth (11, 12), and academic groups (13–16). Developed protocols screen for soil bacteria that produce antibiotics effective against "safe" bacteria (biosafety level 1 [BSL1]), which may also have inhibitory properties against pathogens within the same genus (e.g., ESKAPE pathogens [17, 18]). Here, we expand this methodology to *Neisseria*, using BSL1 commensals as proxies for pathogens, and identify three soil microbes (WAM01, WAM04, and WAM06) that inhibit commensal *Neisseria* growth as part of an undergraduate-level classroom exercise in the Thomas H. Gosnell School of Life Sciences at the Rochester Institute of Technology (RIT) (BIOL126-Introductory Biology Laboratory).

Soil samples were collected from Geneseo, New York (USA), and included sediment from an agricultural drainage ditch located at Big Tree Farm (42.798, −77.846) and soil from under an oak tree at the end of Main Street (42.792, −77.815). From these samples, serial dilutions were prepared on 50% tryptic soy agar (TSA), and individual colonies were isolated after 1 week of incubation at room temperature. Commensal *Neisseria* strains were obtained from the Centers for Disease Control and Prevention (CDC) and Food and Drug Administration (FDA) AR Isolate Bank *Neisseria* species matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) verification panel, including AR-0944 (*Neisseria cinerea*), AR-0951

Editor Vanja Klepac-Ceraj, Wellesley College

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The authors declare no conflict of interest.

Received 30 June 2022
Accepted 19 August 2022
Published 12 September 2022

TABLE 1 Strain attributes and genome assembly overview of the soil bacteria isolated in this study[a]

Strain	Isolation site	Species	ZOI with:			No. of reads	Total assembly length (bp)	No. of contigs	Coverage (×)	N_{50} (bp)	No. of coding domains	No. of tRNAs	GC content (%)	SRA accession no.	GenBank accession no.
			AR-0944 (*Neisseria cinerea*)	AR-0951 (*Neisseria mucosa*)	AR-0953 (*Neisseria subflava*)										
WAM01	Big Tree Farm ditch, Geneseo, NY	*Staphylococcus pasteuri*	+	−	+	3,795,320	2,563,252	34	444.20	520,728	2,541	63	31.49	SRR19571305	JAMWYJ000000000
WAM04	Main Street oak, Geneseo, NY	*Peribacillus butanolivorans*	−	−	+	3,332,040	5,994,962	213	166.74	84,680	5,885	82	38.03	SRR19571306	JAMWYI000000000
WAM06	Main Street oak, Geneseo, NY	*Micrococcus yunnanensis*	+	+	+	2,289,342	2,127,231	249	322.86	23,464	2,038	41	72.5	SRR19571307	JAMWYH000000000

[a] All assembly statistics are based on contigs of ≥500 bp.

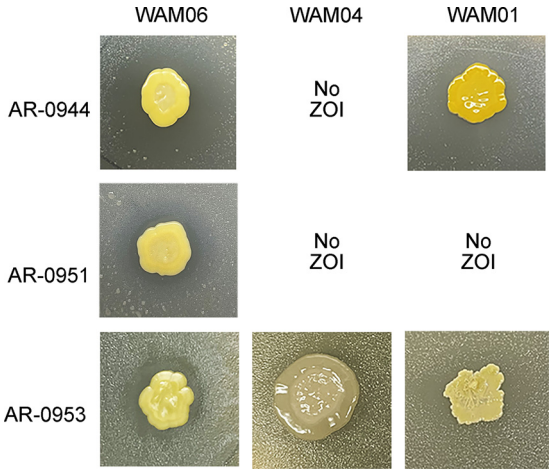

FIG 1 Competitive growth assay on 50% TSA for soil bacteria and commensal *Neisseria* strains. *Micrococcus yunnanensis* (WAM06) shows clear ZOIs against all three of the commensal *Neisseria* species tested. The *Peribacillus butanolivorans* (WAM04) isolate slightly inhibited the growth of one species, and the *Staphylococcus pasteuri* isolate inhibited the growth of two.

(*Neisseria mucosa*), and AR-0953 (*Neisseria subflava*), which were previously characterized and their draft assemblies published (19). Commensal *Neisseria* strains were plated as a lawn on 50% TSA and were subsequently inoculated with a patch (1 cm by 1 cm) of the soil bacterial strains WAM01, WAM04, and WAM06. Resultant cocultures were incubated at 28°C for 1 week, and the presence or absence of zones of inhibition (ZOIs) was recorded (Table 1 and Fig. 1). WAM06 produced a ZOI against all commensal *Neisseria* strains tested.

After incubation for 1 week at room temperature on 50% TSA, DNA was purified from isolates using the Thermo Fisher Scientific PureLink genomic DNA minikit after lysis in Tris-EDTA buffer with 0.5 mg/mL lysozyme and 3 mg/mL proteinase K. The Illumina Nextera XT kit was used to prepare libraries, which were pooled and sequenced using a 600-cycle v3 cartridge (2×300 bp) on the Illumina MiSeq platform at the RIT Genomics Core. Default parameters were used for all analyses except where otherwise noted. Paired-end sequencing resulted in an average of 3.13 ± 0.77 million reads, with an average read length of 185.63 ± 50.25 per library. Library quality was assessed using FastQC v0.11.9 (20), and SPAdes v3.14.1 (21) was used for *de novo* assembly. Assembly statistics were generated with QUAST (http://quast.sourceforge.net/quast), excluding contigs of $<$500 bp, and are reported in Table 1. Open reading frames (ORFs) were annotated using the GenBank Prokaryotic Genome Annotation Pipeline (PGAP) v5.2 (22) (Table 1), which was also used to assign genera and species, as follows: WAM01, *Staphylococcus pasteuri*; WAM04, *Peribacillus butanolivorans*; WAM06, *Micrococcus yunnanensis*. Further characterization of the anti-*Neisseria* compounds produced by the bacteria reported here will be reported in a future publication.

Data availability. The genome assemblies and raw reads are available in GenBank and the SRA, respectively, under the accession numbers listed in Table 1. All code is accessible at https://github.com/wadsworthlab/2022-soil-bacteria.

ACKNOWLEDGMENTS

We acknowledge the generous support for this study provided by the RIT College of Science and the Thomas H. Gosnell School of Life Sciences. The funders had no role in study design, data collection and analysis, the decision to publish, or preparation of the manuscript.

We thank Girish Kumar at the RIT Genomics Core for providing sequencing support.

REFERENCES

1. Mortimer TD, Grad YH. 2019. Applications of genomics to slow the spread of MDR *Neisseria gonorrhoeae*. Ann N Y Acad Sci 1435:93–109. https://doi.org/10.1111/nyas.13871.
2. Unemo M, Nicholas RA. 2012. Emergence of multidrug-resistant, extensively drug-resistant and untreatable gonorrhea. Future Microbiol 7:1401–1422. https://doi.org/10.2217/fmb.12.117.
3. Bradford PA, Miller AA, O'Donnell J, Mueller JP. 2020. Zoliflodacin: an oral spiropyrimidinetrione antibiotic for the treatment of *Neisseria gonorrheae*, including multi-drug-resistant isolates. ACS Infect Dis 6:1332–1345. https://doi.org/10.1021/acsinfecdis.0c00021.
4. Unemo M, Ahlstrand J, Sánchez-Busó L, Day M, Aanensen D, Golparian D, Jacobsson S, Cole MJ, European Collaborative Group. 2021. High susceptibility

to zoliflodacin and conserved target (GyrB) for zoliflodacin among 1209 consecutive clinical *Neisseria gonorrhoeae* isolates from 25 European countries, 2018. J Antimicrob Chemother 76:1221–1228. https://doi.org/10.1093/jac/dkab024.
5. Scangarella-Oman NE, Hossain M, Dixon PB, Ingraham K, Min S, Tiffany CA, Perry CR, Raychaudhuri A, Dumont EF, Huang J, Hook EW, III, Miller LA. 2018. Microbiological analysis from a phase 2 randomized study in adults evaluating single oral doses of gepotidacin in the treatment of uncomplicated urogenital gonorrhea caused by *Neisseria gonorrhoeae*. Antimicrob Agents Chemother 62:e01221-18. https://doi.org/10.1128/AAC.01221-18.
6. Taylor SN, Morris DH, Avery AK, Workowski KA, Batteiger BE, Tiffany CA, Perry CR, Raychaudhuri A, Scangarella-Oman NE, Hossain M, Dumont EF. 2018. Gepotidacin for the treatment of uncomplicated urogenital gonorrhea: a phase 2, randomized, dose-ranging, single-oral dose evaluation. Clin Infect Dis 67:504–512. https://doi.org/10.1093/cid/ciy145.
7. Schneider YK. 2021. Bacterial natural product drug discovery for new antibiotics: strategies for tackling the problem of antibiotic resistance by efficient bioprospecting. Antibiotics 10:842. https://doi.org/10.3390/antibiotics10070842.
8. Strobel G, Daisy B. 2003. Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev 67:491–502. https://doi.org/10.1128/MMBR.67.4.491-502.2003.
9. Barral AM, Makhluf H, Broderick NA, Kurt EL. 2016. The Small World Initiative™: an innovative crowdsourcing platform for antibiotics. FASEB J 30:665.13.
10. Barral AM, Makhluf H, Soneral P, Gasper B. 2014. Small World Initiative: crowdsourcing research of new antibiotics to enhance undergraduate biology teaching (618.41). FASEB J 28:618.41. https://doi.org/10.1096/fasebj.28.1_supplement.618.41.
11. Basalla J, Harris R, Burgess E, Zeedyk N, Wildschutte H. 2020. Expanding Tiny Earth to genomics: a bioinformatics approach for an undergraduate class to characterize antagonistic strains. FEMS Microbiol Lett 367:fnaa018. https://doi.org/10.1093/femsle/fnaa018.
12. Hurley A, Chevrette MG, Acharya DD, Lozano GL, Garavito M, Heinritz J, Balderrama L, Beebe M, DenHartog ML, Corinaldi K, Engels R, Gutierrez A, Jona O, Putnam JHI, Rhodes B, Tsang T, Hernandez S, Bascom-Slack C, Blum JE, Price PA, Davis D, Klein J, Pultorak J, Sullivan NL, Mouncey NJ, Dorrestein PC, Miller S, Broderick NA, Handelsman J. 2021. Tiny Earth: a big idea for STEM education and antibiotic discovery. mBio 12:e03432-20. https://doi.org/10.1128/mBio.03432-20.
13. de Groot PWJ, Fernández-Pereira J, Sabariegos R, Clemente-Casares P, Parra-Martínez J, Cid VJ, Moreno DA. 2019. Optimizing Small World Initiative service learning by focusing on antibiotics-producing actinomycetes from soil. FEMS Microbiol Lett 366:fnaa019. https://doi.org/10.1093/femsle/fnaa019.
14. Cavanaugh NT, Parthasarathy A, Wong NH, Steiner KK, Chu J, Adjei J, Hudson AO. 2021. *Exiguobacterium* sp. is endowed with antibiotic properties against Gram positive and negative bacteria. BMC Res Notes 14:230. https://doi.org/10.1186/s13104-021-05644-2.
15. Schroeter MN, Gazali SJ, Parthasarathy A, Wadsworth CB, Miranda RR, Thomas BN, Hudson AO. 2021. Isolation, whole-genome sequencing, and annotation of three unclassified antibiotic-producing bacteria, *Enterobacter* sp. strain RIT 637, *Pseudomonas* sp. strain RIT 778, and *Deinococcus* sp. strain RIT 780. Microbiol Resour Announc 10:e00863-21. https://doi.org/10.1128/MRA.00863-21.
16. Steiner KK, Parthasarathy A, Wong NH, Cavanaugh NT, Chu J, Hudson AO. 2020. Isolation and whole-genome sequencing of *Pseudomonas* sp. RIT 623, a slow-growing bacterium endowed with antibiotic properties. BMC Res Notes 13:370. https://doi.org/10.1186/s13104-020-05216-w.
17. De Oliveira DM, Forde BM, Kidd TJ, Harris PN, Schembri MA, Beatson SA, Paterson DL, Walker MJ. 2020. Antimicrobial resistance in ESKAPE pathogens. Clin Microbiol Rev 33:e00181-19. https://doi.org/10.1128/CMR.00181-19.
18. Mulani MS, Kamble EE, Kumkar SN, Tawre MS, Pardesi KR. 2019. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. Front Microbiol 10:539. https://doi.org/10.3389/fmicb.2019.00539.
19. Fiore MA, Raisman JC, Wong NH, Hudson AO, Wadsworth CB. 2020. Exploration of the *Neisseria* resistome reveals resistance mechanisms in commensals that may be acquired by *N. gonorrhoeae* through horizontal gene transfer. Antibiotics 9:656. https://doi.org/10.3390/antibiotics9100656.
20. Andrews S. 2021. FastQC: a quality control tool for high throughput sequence data. https://www.bioinformatics.babraham.ac.uk/projects/fastqc.
21. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski 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.
22. 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.