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Permalink
https://escholarship.org/uc/item/4698x3cf

Journal
Microbiology resource announcements, 7(2)

ISSN
2576-098X

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Publication Date
2018-07-01

DOI
10.1128/mra.00871-18

Peer reviewed

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GENOME SEQUENCES

High-Quality Draft Genome Sequences of Eight Bacteria Isolated from Fungus Gardens Grown by *Trachymyrmex septentrionalis* Ants

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ABSTRACT For their food source, *Trachymyrmex septentrionalis* ants raise symbiotic fungus gardens that contain bacteria whose functions are poorly understood. Here, we report the genome sequences of eight bacteria isolated from these fungus gardens to better describe the ecology of these strains and their potential to produce secondary metabolites in this niche.

Fungus-growing ants (tribe *Attini*) form symbioses with a cultivar fungus belonging to the genus *Leucoagaricus*, which they grow in underground fungus gardens as their essential food source (1). Other bacteria also inhabit these fungus gardens and provide nutrients to the cultivar fungus, at least in some cases (2–5). These bacteria have the genetic potential to produce secondary metabolites that may mediate interspecific interactions in fungus gardens, although this remains poorly understood (6).

Trachymyrmex septentrionalis is the northernmost fungus-growing ant and occurs throughout the eastern United States (7). Its colonies are relatively small (~1,000 ants/colony) and subsist largely on caterpillar frass, oak catkins, and some fresh plant material (8). The *T. septentrionalis* fungus garden microbiome remains poorly characterized (9). We therefore isolated several bacteria from *T. septentrionalis* fungus gardens and sequenced their genomes to better understand their potential functions within this symbiotic niche.

T. septentrionalis fungus gardens were collected in Florida, New Jersey, and North Carolina following established protocols (10). Fungus garden fragments were resuspended in phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4), and bacteria were isolated on tryptic soy agar (Difco; adjusted to pH 6) using the spread plate technique. Genomic DNA was extracted from each isolate, and their 16S rRNA genes were PCR amplified as described previously (11). PCR amplicons were Sanger sequenced at the University of Connecticut DNA Biotechnology Center, and the resulting sequences were compared to the NCBI nonredundant database (12) to identify each strain.

Genomes from eight *T. septentrionalis* fungus garden bacteria were sequenced at the Department of Energy Joint Genome Institute (JGI). Pacific Biosciences (PacBio) SMRTbell libraries were constructed following the manufacturer's protocol and sequenced using a PacBio RS instrument. The resulting reads were assembled using the HGAP pipeline version 2.3.0_p5. Genes were predicted using Prodigal (13) and GenePRIMP (14) and annotated using the UniProt (15), TIGRFAMs (16), Pfam (17), KEGG (18), COG (19), InterPro (20), and IMG nonredundant (21) databases. Noncoding RNAs were annotated using tRNAScanSE (22), INFERNAL (23), and the IMG's

Received 18 June 2018 **Accepted** 25 June 2018 **Published** 19 July 2018

Citation Kopac S, Beatty H, Gialopsos P, Huntemann M, Clum A, Spunde A, Pillay M, Palaniappan K, Varghese N, Mikhailova N, Stamatis D, Reddy TBK, Daum C, Ng V, Ivanova N, Kyrpides N, Woyke T, Klassen JL. 2018. High-quality draft genome sequences of eight bacteria isolated from fungus gardens grown by *Trachymyrmex septentrionalis* ants. Microbiol Resour Announc 7:e00871-18. https://doi.org/10.1128/MRA.00871-18.

Editor Julie C. Dunning Hotopp, University of Maryland School of Medicine

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TABLE 1 GenBank accession numbers and metadata for the strains sequenced in this study

Strain name	Collection location	Collection date (day/mo/yr)	Genome size (Mb)	Coverage (×)	No. of contigs	GenBank accession no.
Serratia sp. JKS000199	Wekiwa Springs State Park, FL, USA	20/5/13	5.12	178	1	LT907843
Enterobacteriaceae sp. JKS000233	Wekiwa Springs State Park, FL, USA	20/5/13	5.46	117	6	PEES00000000
Enterobacteriaceae sp. JKS000234	Wekiwa Springs State Park, FL, USA	20/5/13	5.45	70	5	OCMY00000000
Pantoea sp. JKS000250	Wekiwa Springs State Park, FL, USA	20/5/13	4.87	90	3	QICZ00000000
Serratia sp. JKS000296	Wekiwa Springs State Park, FL, USA	20/5/13	5.15	93	4	OCMX00000000
Burkholderia sp. JKS000303	Wharton State Forest, NJ, USA	25/6/14	8.16	99	6	PDBZ00000000
Bacillus sp. JKS001846	Singletary Lake State Park, NC, USA	10/6/15	5.96	217	2	FWYG00000000
Micrococcaceae sp. JKS001869	Paynes Creek Historic State Park, FL, USA	16/11/14	2.59	239	2	PDBY00000000

rRNA gene models (15). Additional gene prediction and annotation were performed using the IMG's ER platform (24).

The sequenced bacteria belong to the genera *Bacillus*, *Burkholderia*, *Pantoea*, and *Serratia* and poorly resolved taxa within the families *Enterobacteriaceae* and *Micrococcaceae* (Table 1). The genome of *Serratia* sp. JKS000199 was assembled into a single contig and is therefore complete. All other genomes were assembled into 2 to 6 contigs and are therefore high-quality drafts. These bacteria likely include both persistent and transient colonists of *T. septentrionalis* fungus gardens. The genome sequences of these strains will inform future studies of their ecology in *T. septentrionalis* symbiosis and how secondary metabolites might mediate interspecific interactions within this niche.

Data availability. The whole-genome shotgun projects for strains JKS000199, JKS000233, JKS000234, JKS000250, JKS000296, JKS000303, JKS001846, and JKS001869 have been deposited in DDBJ/EMBL/GenBank under the accession numbers listed in Table 1.

ACKNOWLEDGMENTS

This research was funded by Department of Energy Joint Genome Institute grant CSP 1652, NSF grant IOS-1656475, and the University of Connecticut. The work conducted by the U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility, is supported under Contract number DE-AC02-05CH11231. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

We thank the Florida, New Jersey, and North Carolina Departments of Environmental Protection for permission and assistance with our collections on state lands. We also thank Lee Deininger, Sarah Goldstein, and Kevin Lee for their assistance collecting ants in New Jersey and North Carolina and Cameron Currie and Heidi Horn for assistance collecting ants in Florida.

REFERENCES

1. Mueller UG, Rehner SA, Schultz TR. 1998. The evolution of agriculture in ants. Science 281:2034–2038. https://doi.org/10.1126/science.281.5385.2034.
2. Pinto-Tomás AA, Anderson MA, Suen G, Stevenson DM, Chu FST, Cleland WW, Weimer PJ, Currie CR. 2009. Symbiotic nitrogen fixation in the fungus gardens of leaf-cutter ants. Science 326:1120–1123. https://doi.org/10.1126/science.1173036.
3. Suen G, Scott JJ, Aylward FO, Adams SM, Tringe SG, Pinto-Tomás AA, Foster CE, Pauly M, Weimer PJ, Barry KW, Goodwin LA, Bouffard P, Li L, Osterberger J, Harkins TT, Slater SC, Donohue TJ, Currie CR. 2010. An insect herbivore microbiome with high plant biomass-degrading capacity. PLoS Genet 6:e1001129. https://doi.org/10.1371/journal.pgen.1001129.
4. Aylward FO, Burnum KE, Scott JJ, Suen G, Tringe SG, Adams SM, Barry KW, Nicora CD, Piehowski PD, Purvine SO, Starrett GJ, Goodwin LA, Smith RD, Lipton MS, Currie CR. 2012. Metagenomic and metaproteomic insights into bacterial communities in leaf-cutter ant fungus gardens. ISME J 6:1688–1701. https://doi.org/10.1038/ismej.2012.10.
5. Aylward FO, Suen G, Biedermann PHW, Adams AS, Scott JJ, Malfatti SA, del Rio TG, Tringe SG, Poulsen M, Raffa KF, Klepzig KD, Currie CR. 2014. Convergent bacterial microbiotas in the fungal agricultural systems of insects. mBio 5:e02077-14. https://doi.org/10.1128/mBio.02077-14.
6. Klassen JL. 2014. Microbial secondary metabolites and their impacts on insect symbioses. Curr Opin Insect Sci 4:15–22. https://doi.org/10.1016/j.cois.2014.08.004.
7. Rabeling C, Cover SP, Johnson RA, Mueller UG. 2015. A review of the North American species of the fungus-gardening ant genus *Trachymyrmex* (Hymenoptera: Formicidae). Zootaxa 4029:1–53.
8. Seal JN, Tschinkel WR. 2007. Complexity in an obligate mutualism: do fungus-gardening ants know what makes their garden grow? Behav Ecol Sociobiol 61:1151–1160. https://doi.org/10.1007/s00265-006-0328-4.
9. Ishak HD, Miller JL, Sen R, Dowd SE, Meyer E, Mueller UG. 2011. Microbiomes of ant castes implicate new microbial roles in the fungus-growing ant *Trachymyrmex septentrionalis*. Sci Rep 1:204. https://doi.org/10.1038/srep00204.

10. Sosa-Calvo J, Jesovnik A, Okonski E, Schultz TR. 2015. Locating, collecting, and maintaining colonies of fungus-farming ants (Hymenoptera: Myrmicinae: Attini). Sociobiology 62:300–320. https://doi.org/10.13102/sociobiology.v62i2.300-320.
11. Cafaro MJ, Poulsen M, Little AEF, Price SL, Gerardo NM, Wong B, Stuart AE, Larget B, Abbot P, Currie CR. 2011. Specificity in the symbiotic association between fungus-growing ants and protective *Pseudonocardia* bacteria. Proc R Soc B Biol Sci 278:1814–1822. https://doi.org/10.1098/rspb.2010.2118.
12. Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. 2017. GenBank. Nucleic Acids Res 45:D37–D42. https://doi.org/10.1093/nar/gkw1070.
13. Hyatt D, Chen G-L, LoCascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 11:119. https://doi.org/10.1186/1471-2105-11-119.
14. Pati A, Ivanova NN, Mikhailova N, Ovchinnikova G, Hooper SD, Lykidis A, Kyrpides NC. 2010. GenePRIMP: a gene prediction improvement pipeline for prokaryotic genomes. Nat Methods 7:455–457. https://doi.org/10.1038/nmeth.1457.
15. UniProt Consortium. 2017. UniProt: the universal protein knowledgebase. Nucleic Acids Res 45:D158–D169. https://doi.org/10.1093/nar/gkw1099.
16. Haft DH, Selengut JD, Richter RA, Harkins D, Basu MK, Beck E. 2013. TIGRFAMs and genome properties in 2013. Nucleic Acids Res 41:D387–D395. https://doi.org/10.1093/nar/gks1234.
17. Finn RD, Coggill P, Eberhardt RY, Eddy SR, Mistry J, Mitchell AL, Potter SC, Punta M, Qureshi M, Sangrador-Vegas A, Salazar GA, Tate J, Bateman A. 2016. The Pfam protein families database: towards a more sustainable future. Nucleic Acids Res 44:D279–D285. https://doi.org/10.1093/nar/gkv1344.
18. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. 2017. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res 45:D353–D361. https://doi.org/10.1093/nar/gkw1092.
19. Tatusov RL, Fedorova ND, Jackson JD, Jacobs AR, Kiryutin B, Koonin EV, Krylov DM, Mazumder R, Mekhedov SL, Nikolskaya AN, Rao BS, Smirnov S, Sverdlov AV, Vasudevan S, Wolf YI, Yin JJ, Natale DA. 2003. The COG database: an updated version includes eukaryotes. BMC Bioinformatics 4:41. https://doi.org/10.1186/1471-2105-4-41.
20. Finn RD, Attwood TK, Babbitt PC, Bateman A, Bork P, Bridge AJ, Chang H-Y, Dosztányi Z, El-Gebali S, Fraser M, Gough J, Haft D, Holliday GL, Huang H, Huang X, Letunic I, Lopez R, Lu S, Marchler-Bauer A, Mi H, Mistry J, Natale DA, Necci M, Nuka G, Orengo CA, Park Y, Pesseat S, Piovesan D, Potter SC, Rawlings ND, Redaschi N, Richardson L, Rivoire C, Sangrador-Vegas A, Sigrist C, Sillitoe I, Smithers B, Squizzato S, Sutton G, Thanki N, Thomas PD, Tosatto SCE, Wu CH, Xenarios I, Yeh L-S, Young S-Y, Mitchell AL. 2017. InterPro in 2017—beyond protein family and domain annotations. Nucleic Acids Res 45:D190–D199. https://doi.org/10.1093/nar/gkw1107.
21. Huntemann M, Ivanova NN, Mavromatis K, Tripp HJ, Paez-Espino D, Palaniappan K, Szeto E, Pillay M, Chen I-MA, Pati A, Nielsen T, Markowitz VM, Kyrpides NC. 2015. The standard operating procedure of the DOE-JGI microbial genome annotation pipeline (MGAP v.4). Stand Genomic Sci 10:86. https://doi.org/10.1186/s40793-015-0077-y.
22. Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25:955–964.
23. Nawrocki EP, Eddy SR. 2013. Infernal 1.1: 100-fold faster RNA homology searches. Bioinformatics 29:2933–2935. https://doi.org/10.1093/bioinformatics/btt509.
24. Markowitz VM, Mavromatis K, Ivanova NN, Chen I-MA, Chu K, Kyrpides NC. 2009. IMG ER: a system for microbial genome annotation expert review and curation. Bioinformatics 25:2271–2278. https://doi.org/10.1093/bioinformatics/btp393.