





## Genome Sequence of Gordonia Phage Yvonnetastic

Welkin H. Pope, Anshika Bandyopadhyay, Meghan L. Carlton, Meghan T. Kane, Niyati J. Panchal, Yvonne C. Pham, Zachary J. Reynolds, Michael S. Sapienza, Brian A. German, Jill E. McDonnell, Claire E. Schafer, Victor J. Yu, Emily C. Furbee, Sarah R. Grubb, Marcie H. Warner, Matthew T. Montgomery, Rebecca A. Garlena, Daniel A. Russell, Deborah Jacobs-Sera, Graham F. Hatfull

Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Gordonia bacteriophage Yvonnetastic was isolated from soil in Pittsburgh, PA, using Gordonia terrae 3612 as a host. Yvonnetastic has siphoviral morphology and a genome of 98,136 bp, with 198 predicted protein-coding genes and five tRNA genes. Yvonnetastic does not share substantial sequence similarity with other sequenced bacteriophage genomes.

Received 9 May 2016 Accepted 20 May 2016 Published 7 July 2016

Citation Pope WH, Bandyopadhyay A, Carlton ML, Kane MT, Panchal NJ, Pham YC, Reynolds ZJ, Sapienza MS, German BA, McDonnell JE, Schafer CE, Yu VJ, Furbee EC, Grubb SR, Warner MH, Montgomery MT, Garlena RA, Russell DA, Jacobs-Sera D, Hatfull GF. 2016. Genome sequence of *Gordonia* phage Yvonnetastic. Genome Announc 4(4):e00594-16. doi:10.1128/genomeA.00594-16.

Copyright © 2016 Pope et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Welkin H. Pope, welkin@pitt.edu.

Gordonia spp. are implicated in foaming of wastewater in treatment plants (1–3) and are associated with opportunistic infections in hospital catheters (4, 5). Seventeen bacteriophages of Gordonia have been isolated, sequenced, and deposited in GenBank (6–9), and although these represent several different genome types, it is unclear whether the overall genomic diversity of the Gordonia phage population is similar to that reported for mycobacteriophages (10–15). The Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) is a course-based research experience in which undergraduates perform authentic research through isolation and characterization of viruses using hosts of the phylum Actinobacteria, and it provides an opportunity for isolation and characterization of Gordonia phages (16).

Yvonnetastic was isolated from soil through direct plating of a filtered soil extract on lawns of Gordonia terrae 3612. Its morphology is siphoviral, with an isometric head and a tail of 400 nm in length. After purification and amplification, DNA was extracted and sequenced using an Illumina MiSeq platform with 140-bp single-end reads. Reads were assembled using Newbler into one major contig of 98,136 bp with discrete ends and 10-base single-stranded 3' extensions with the sequence 5'-CGCGAAGCTC. Protein-coding genes were predicted using Glimmer (17), GeneMark (18), DNA Master, and Phamerator (19), and tRNAs were predicted using Aragorn version 1.3 (20); functional assignments were made using BLAST (21) and HHpred (22) against the publically available databases GenBank, the Protein Database, and pFamA. Yvonnetastic's genome has 59.7% G+C content, somewhat lower than that of its host (67.8%), and it contains 198 predicted protein-coding genes and five tRNA genes. Functional assignments were made to 42 (21%) of the protein-coding genes. The majority of the Yvonnetastic genes are transcribed left to right, with the exception of genes in the leftmost 3 kbp and rightmost 10 kbp of the genome.

Yvonnetastic shows little similarity to currently sequenced phage genomes in its nucleotide sequence, and of the 198 pre-

dicted protein-coding gene products, <50% have amino acid similarity to genes encoded in ~1,500 sequenced phages of actinobacterial hosts. Those that do primarily match gene products encoded in other *Gordonia* phages and in mycobacteriophages. Among the putative predicted gene functions are the virion structure and assembly proteins, two glycosyltransferases, five HNH endonucleases, a RecET recombination system, an endonuclease VII, and an exonuclease VII.

The Yvonnetastic genome is unusual in that a lysis cassette including L-alanyl-D-glutamate peptidase, glycoside hydrolase, and holin genes is located amid the minor tail protein genes, and a putative lysin B gene is located near the right end of the genome, separated by >50 kbp from the other lysis genes. We also note that the putative integrase gene (95) is embedded within a long operon of closely linked rightwards-transcribed genes, and we have not been able to identify a putative *attP* site or predict a chromosomal *attB* site for phage integration. We have also been unable to identify putative immunity repressor gene, and the only predicted regulatory protein is the 67-residue gp109, which contains a predicted helix-turn-helix DNA binding motif.

**Nucleotide sequence accession number.** The Yvonnetastic genome is available from GenBank under the accession no. KU963248.

## **FUNDING INFORMATION**

This work, including the efforts of Graham F. Hatfull, was funded by Howard Hughes Medical Institute (HHMI) (54308198).

## **REFERENCES**

- 1. De los Reyes FL, III, Raskin L. 2002. Role of filamentous microorganisms in activated sludge foaming: relationship of mycolata levels to foaming initiation and stability. Water Res 36:445–459. http://dx.doi.org/10.1016/S0043-1354(01)00227-5.
- De los Reyes FL, III, Rothauszky D, Raskin L. 2002. Microbial community structures in foaming and nonfoaming full-scale wastewater treatment plants. Water Environ Res 74:437–449. http://dx.doi.org/10.2175/106143002X140233.
- 3. Petrovski S, Seviour RJ, Tillett D. 2011. Prevention of Gordonia and

- Nocardia stabilized foam formation by using bacteriophage GTE7. Appl Environ Microbiol 77:7864–7867. http://dx.doi.org/10.1128/AEM.05692
- Blaschke AJ, Bender J, Byington CL, Korgenski K, Daly J, Petti CA, Pavia AT, Ampofo K. 2007. *Gordonia* species: emerging pathogens in pediatric patients that are identified by 16S ribosomal RNA gene sequencing. Clin Infect Dis 45:483–486. http://dx.doi.org/10.1086/520018.
- Grisold AJ, Roll P, Hoenigl M, Feierl G, Vicenzi-Moser R, Marth E. 2007. Isolation of *Gordonia terrae* from a patient with catheter-related bacteraemia. J Med Microbiol 56:1687–1688. http://dx.doi.org/10.1099/ imm.0.47388-0.
- Dyson ZA, Tucci J, Seviour RJ, Petrovski S. 2015. Lysis to kill: evaluation
  of the lytic abilities, and genomics of nine bacteriophages infective for
  Gordonia spp. and their potential use in activated sludge foam biocontrol.
  PLoS One 10:e0134512. http://dx.doi.org/10.1371/journal.pone.0134512.
- Liu M, Gill JJ, Young R, Summer EJ. 2015. Bacteriophages of wastewater foaming-associated filamentous *Gordonia* reduce host levels in raw activated sludge. Sci Rep 5:13754. http://dx.doi.org/10.1038/srep13754.
- Petrovski S, Seviour RJ, Tillett D. 2011. Characterization of the genome of the polyvalent lytic bacteriophage GTE2, which has potential for biocontrol of *Gordonia-*, *Rhodococcus-*, and *Nocardia-*stabilized foams in activated sludge plants. Appl Environ Microbiol 77:3923–3929. http:// dx.doi.org/10.1128/AEM.00025-11.
- 9. Petrovski S, Tillett D, Seviour RJ. 2012. Genome sequences and characterization of the related *Gordonia* phages GTE5 and GRU1 and their use as potential biocontrol agents. Appl Environ Microbiol 78:42–47. http://dx.doi.org/10.1128/AEM.05584-11.
- 10. Jacobs-Sera D, Marinelli LJ, Bowman C, Broussard GW, Guerrero Bustamante C, Boyle MM, Petrova ZO, Dedrick RM, Pope WH, Science Education Alliance-Phage Hunters Advancing Genomics, Evolutionary Science Sea-phages Program, Modlin RL, Hendrix RW, Hatfull GF. 2012. On the nature of mycobacteriophage diversity and host preference. Virology 434:187–201.
- 11. Mageeney C, Pope WH, Harrison M, Moran D, Cross T, Jacobs-Sera D, Hendrix RW, Dunbar D, Hatfull GF. 2012. Mycobacteriophage Marvin: a new singleton phage with an unusual genome organization. J Virol 86: 4762–4775. http://dx.doi.org/10.1128/JVI.00075-12.
- 12. Pope WH, Anders KR, Baird M, Bowman CA, Boyle MM, Broussard GW, Chow T, Clase KL, Cooper S, Cornely KA, Dejong RJ, Delesalle VA, Deng L, Dunbar D, Edgington NP, Ferreira CM, Weston Hafer K, Hartzog GA, Hatherill JR, Hughes LE, Ipapo K, Krukonis GP, Meier CG, Monti DL, Olm MR, Page ST, Peebles CL, Rinehart CA, Rubin MR, Russell DA, Sanders ER, Schoer M, Shaffer CD, Wherley J, Vazquez E, Yuan H, Zhang D, Cresawn SG, Jacobs-Sera D, Hendrix RW, Hatfull GF. 2014. Cluster M mycobacteriophages Bongo, PegLeg, and Rey with unusually large repertoires of tRNA isotypes. J Virol 88:2461–2480. http://dx.doi.org/10.1128/JVI.03363-13.
- 13. Pope WH, Bowman CA, Russell DA, Jacobs-Sera D, Asai DJ, Cresawn SG, Jacobs WR, Hendrix RW, Lawrence JG, Hatfull GF, Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science, Phage Hunters Integrating Research and Education, Mycobacterial Genetics Course. 2015. Whole genome comparison of a large col-

- lection of mycobacteriophages reveals a continuum of phage genetic diversity. Elife 4:e06416.
- 14. Pope WH, Ferreira CM, Jacobs-Sera D, Benjamin RC, Davis AJ, DeJong RJ, Elgin SC, Guilfoile FR, Forsyth MH, Harris AD, Harvey SE, Hughes LE, Hynes PM, Jackson AS, Jalal MD, MacMurray EA, Manley CM, McDonough MJ, Mosier JL, Osterbann LJ, Rabinowitz HS, Rhyan CN, Russell DA, Saha MS, Shaffer CD, Simon SE, Sims EF, Tovar IG, Weisser EG, Wertz JT, Weston-Hafer KA, Williamson KE, Zhang B, Cresawn SG, Jain P, Piuri M, Jacobs WR Jr, Hendrix RW, Hatfull GF, 2011. Cluster K mycobacteriophages: insights into the evolutionary origins of mycobacteriophage TM4. PLoS One 6:e26750. http://dx.doi.org/10.1371/journal.pone.0026750.
- 15. Pope WH, Jacobs-Sera D, Russell DA, Peebles CL, Al-Atrache Z, Alcoser TA, Alexander LM, Alfano MB, Alford ST, Amy NE, Anderson MD, Anderson AG, Ang AA, Ares M Jr, Barber AJ, Barker LP, Barrett JM, Barshop WD, Bauerle CM, Bayles IM, Belfield KL, Best AA, Borjon A Jr, Bowman CA, Boyer CA, Bradley KW, Bradley VA, Broadway LN, Budwal K, Busby KN, Campbell IW, Campbell AM, Carey A, Caruso SM, Chew RD, Cockburn CL, Cohen LB, Corajod JM, Cresawn SG, Davis KR, Deng L, Denver DR, Dixon BR, Ekram S, Elgin SC, Engelsen AE, English BE, Erb ML, Estrada C, Filliger LZ. 2011. Expanding the diversity of mycobacteriophages: insights into genome architecture and evolution. PLoS One 6:e16329. http://dx.doi.org/10.1371/journal.pone.0016329.
- 16. Jordan TC, Burnett SH, Carson S, Caruso SM, Clase K, DeJong RJ, Dennehy JJ, Denver DR, Dunbar D, Elgin SC, Findley AM, Gissendanner CR, Golebiewska UP, Guild N, Hartzog GA, Grillo WH, Hollowell GP, Hughes LE, Johnson A, King RA, Lewis LO, Li W, Rosenzweig F, Rubin MR, Saha MS, Sandoz J, Shaffer CD, Taylor B, Temple L, Vazquez E, Ware VC, Barker LP, Bradley KW, Jacobs-Sera D, Pope WH, Russell DA, Cresawn SG, Lopatto D, Bailey CP, Hatfull GF. 2014. A broadly implementable research course in phage discovery and genomics for first-year undergraduate students. mBio 5:e01051-13. http://dx.doi.org/10.1128/mBio.01051-13.
- 17. Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics 23:673–679. http://dx.doi.org/10.1093/bioinformatics/btm009.
- Besemer J, Borodovsky M. 2005. GeneMark: Web software for gene finding in prokaryotes, eukaryotes and viruses. Nucleic Acids Res 33: W451–W454. http://dx.doi.org/10.1093/nar/gki487.
- Cresawn SG, Bogel M, Day N, Jacobs-Sera D, Hendrix RW, Hatfull GF. 2011. Phamerator: a bioinformatic tool for comparative bacteriophage genomics. BMC Bioinformatics 12:395. http://dx.doi.org/10.1186/1471 -2105-12-395.
- Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res 32:11–16. http://dx.doi.org/10.1093/nar/gkh152.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410. http://dx.doi.org/10.1016/ S0022-2836(05)80360-2.
- Söding J, Biegert A, Lupas AN. 2005. The HHpred interactive server for protein homology detection and structure prediction. Nucleic Acids Res 33:W244–W248. http://dx.doi.org/10.1093/nar/gki408.