AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Complete Genome Sequence of *Burkholderia gladioli* Myophage Mana

Brenda Godoy,[a] Guichun Yao,[a] Tram Le,[a] Maria Guadalupe Vizoso-Pinto,[b] Jason Gill,[a] Carlos Gonzalez,[a] Mei Liu[a]

[a]Center for Phage Technology, Texas A&M University, College Station, Texas, USA

[b]Infection Biology Lab, Instituto Superior de Investigaciones Biológicas, National Council of Scientific and Technological Research, National University of Tucuman, San Miguel de Tucumán, Argentina

ABSTRACT *Burkholderia gladioli* is known to cause respiratory tract infections in cystic fibrosis patients. Here, we describe the annotation of the 38,038-bp genome sequence of Mana, a P2-like phage of *B. gladioli*. Understanding the genomic characteristics of phages infecting pathogens like *B. gladioli* can lead to advancements in phage therapy.

Burkholderia gladioli is a ubiquitous Gram-negative bacterium (1). Though initially recognized as a plant pathogen, *B. gladioli* has been found to infect the human respiratory tract, predominantly attacking cystic fibrosis patients and other immunocompromised individuals (2). Here, we discuss the genome of *B. gladioli* phage Mana, in an effort to investigate the potential clinical applications of phages to bacterial infections (3).

Bacteriophage Mana was isolated from a soil sample collected from Champaign County, IL, using *B. gladioli* strain ATCC 19302 as the host with the soft agar overlay method, and phage purification was carried out by picking and replating isolated plaques for three rounds on soft agar overlay seeded with the host strain as described previously (4). The host strain was grown at 37°C in tryptic nutrient broth or agar. After phage isolation, phage genomic DNA was extracted from the polyethylene glycol (PEG)-precipitated phage particles and purified using a Wizard DNA cleanup kit as previously described (5), and libraries were prepared with 300-bp inserts using a Swift BioSciences 2S Turbo kit followed by Illumina MiSeq sequencing using v2 300-cycle chemistry. FastQC was used for quality control of the total 519,288 raw sequence reads (www.bioinformatics.babraham.ac.uk/projects/fastqc). The genome sequence was then assembled using SPAdes v3.5.0 (6), to 415.1-fold coverage, and closed using PCR and Sanger sequencing of the product amplified by the primers 5′-CCGACTCGTGGCCTAAA-3′ and 5′-TCTTCACGGATGGACACG-3′. Structural annotation was performed using Glimmer v3 and MetaGeneAnnotator v1.0 to identify the gene sequences, while ARAGORN v2.36 was used to detect tRNAs (7–9). The function of genes was predicted using BLAST v2.9.0 against the NCBI nonredundant (nr) and Swiss-Prot databases, with a maximum E value of 0.001 (10, 11). In addition, InterProScan v5.33 and TMHMM v2.0 were used for functional predictions by conserved domains and transmembrane domains, respectively (12, 13). progressiveMauve v2.4 was used to calculate the genome-wide DNA sequence similarity between Mana and other phages (14). These annotation tools were accessed on the CPT Galaxy and Web Apollo interfaces (15–17), and all analyses were conducted with default settings.

Phage Mana has a genome length of 38,038 bp, a coding density of 94%, and a G+C content of 64%. Structural and functional annotation predicted 64 protein-coding sequences, with 42 of these sequences having an assigned putative function. The Mana genome contains no predicted introns. There were no predicted tRNA-coding sequences. Phage Mana has identifiable P2-like baseplate proteins, a tail tube, a tail sheath, and a tape measure protein, strongly indicating that Mana is a myophage. Mana shows similarity, on both

Citation Godoy B, Yao G, Le T, Vizoso-Pinto MG, Gill J, Gonzalez C, Liu M. 2021. Complete genome sequence of *Burkholderia gladioli* myophage Mana. Microbiol Resour Announc 10:e00402-21. https://doi.org/10.1128/MRA.00402-21.

Editor Simon Roux, DOE Joint Genome Institute

Address correspondence to Mei Liu, meiliu@tamu.edu.

Received 20 April 2021
Accepted 30 April 2021
Published 20 May 2021

the DNA and protein levels, to previously characterized P2-like *Burkholderia* phages. Among these phages, Mana shares 39 similar proteins with phage vB_BceM_AP3 (GenBank accession no. KP966108) (18) and 34 and 36 similar proteins (BLASTP at an E value of $<$0.001) with KS5 (GU911303) and KL3 (GU911304), respectively (19). Most of the functions of the Mana genes coincide with the functions of the genes of a P2 phage. Mana was found to have a tape measure protein gene, with a translational frameshift near an upstream chaperone protein gene. Mana was also discovered to contain two predicted holin genes. These genes resemble those of *Salmonella* phage Epsilon15, a podophage (20). The lysis cassette is completed with a downstream endolysin gene and an o-spanin gene embedded within an i-spanin gene.

Data availability. The genome sequence of phage Mana was deposited under GenBank accession no. MT701591.1 and BioSample accession no. SAMN14609638. The BioProject accession number is PRJNA222858, and the SRA accession number is SRR11558334.

ACKNOWLEDGMENTS

This work was supported by funding from the National Science Foundation (award no. EF-0949351 and DBI-1565146). Additional support came from the Center for Phage Technology (CPT), an Initial University Multidisciplinary Research Initiative supported by Texas A&M University and Texas AgriLife, and the Department of Biochemistry and Biophysics (https://cpt.tamu.edu/).

We are grateful for the advice and support of the CPT staff. This announcement was prepared in partial fulfillment of the requirements for BICH464 Phage Genomics, an undergraduate course at Texas A&M University.

REFERENCES

1. Kennedy MP, Coakley RD, Donaldson SH, Aris RM, Hohneker K, Wedd JP, Knowles MR, Gilligan PH, Yankaskas JR. 2007. *Burkholderia gladioli*: five year experience in a cystic fibrosis and lung transplantation center. J Cyst Fibros 6:267–273. https://doi.org/10.1016/j.jcf.2006.10.007.
2. Segonds C, Clavel-Batut P, Thouverez M, Grenet D, Le Coustumier A, Plesiat P, Chabanon G. 2009. Microbiological and epidemiological features of clinical respiratory isolates of *Burkholderia gladioli*. J Clin Microbiol 47:1510–1516. https://doi.org/10.1128/JCM.02489-08.
3. Sulakvelidze A, Alavidze Z, Morris JG, Jr. 2001. Bacteriophage therapy. Antimicrob Agents Chemother 45:649–659. https://doi.org/10.1128/AAC.45.3.649-659.2001.
4. Adams MH. 1959. Bacteriophages. Interscience Publishers, New York, NY.
5. Summer EJ. 2009. Preparation of a phage DNA fragment library for whole genome shotgun sequencing. Methods Mol Biol 502:27–46. https://doi.org/10.1007/978-1-60327-565-1_4.
6. 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.
7. Delcher AL, Harmon D, Kasif S, White O, Salzberg SL. 1999. Improved microbial gene identification with GLIMMER. Nucleic Acids Res 27:4636–4641. https://doi.org/10.1093/nar/27.23.4636.
8. Noguchi H, Taniguchi T, Itoh T. 2008. MetaGeneAnnotator: detecting species-specific patterns of ribosomal binding site for precise gene prediction in anonymous prokaryotic and phage genomes. DNA Res 15:387–396. https://doi.org/10.1093/dnares/dsn027.
9. Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res 32:11–16. https://doi.org/10.1093/nar/gkh152.
10. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST+: architecture and applications. BMC Bioinformatics 10:421. https://doi.org/10.1186/1471-2105-10-421.
11. The UniProt Consortium. 2018. UniProt: the universal protein knowledgebase. Nucleic Acids Res 46:2699. https://doi.org/10.1093/nar/gky092.
12. Jones P, Binns D, Chang H-Y, Fraser M, Li W, McAnulla C, McWilliam H, Maslen J, Mitchell A, Nuka G, Pesseat S, Quinn AF, Sangrador-Vegas A, Scheremetjew M, Yong S-Y, Lopez R, Hunter S. 2014. InterProScan 5: genome-scale protein function classification. Bioinformatics 30:1236–1240. https://doi.org/10.1093/bioinformatics/btu031.
13. Krogh A, Larsson B, von Heijne G, Sonnhammer ELL. 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 305:567–580. https://doi.org/10.1006/jmbi.2000.4315.
14. Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One 5:e11147. https://doi.org/10.1371/journal.pone.0011147.
15. Ramsey J, Rasche H, Maughmer C, Criscione A, Mijalis E, Liu M, Hu JC, Young R, Gill JJ. 2020. Galaxy and Apollo as a biologist-friendly interface for high-quality cooperative phage genome annotation. PLoS Comput Biol 16:e1008214. https://doi.org/10.1371/journal.pcbi.1008214.
16. Dunn NA, Unni DR, Diesh C, Munoz-Torres M, Harris NL, Yao E, Rasche H, Holmes IH, Elsik CG, Lewis SE. 2019. Apollo: democratizing genome annotation. PLoS Comput Biol 15:e1006790. https://doi.org/10.1371/journal.pcbi.1006790.
17. Jalili V, Afgan E, Gu Q, Clements D, Blankenberg D, Goecks J, Taylor J, Nekrutenko A. 2020. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2020 update. Nucleic Acids Res 48:W395–W402. https://doi.org/10.1093/nar/gkaa434.
18. Roszniowski B, Latka A, Maciejewska B, Vandenheuvel D, Olszak T, Briers Y, Holt GS, Valvano MA, Lavigne R, Smith DL, Drulis-Kawa Z. 2017. The temperate Burkholderia phage AP3 of the Peduovirinae shows efficient antimicrobial activity against B. cenocepacia of the IIIA lineage. Appl Microbiol Biotechnol 101:1203–1216. https://doi.org/10.1007/s00253-016-7924-7.
19. Lynch KH, Stothard P, Dennis JJ. 2010. Genomic analysis and relatedness of P2-like phages of the Burkholderia cepacia complex. BMC Genomics 11:599. https://doi.org/10.1186/1471-2164-11-599.
20. Chang JT, Schmid MF, Haase-Pettingell C, Weigele PR, King JA, Chiu W. 2010. Visualizing the structural changes of bacteriophage Epsilon15 and its Salmonella host during infection. J Mol Biol 402:731–740. https://doi.org/10.1016/j.jmb.2010.07.058.