





Draft Genome Sequences of Eight Pseudomonas aeruginosa **Corneal Infection Isolates**

Vishnu Raghuram, a,b Joanna B. Goldbergb,c

a Microbiology and Molecular Genetics Program, Graduate Division of Biological and Biomedical Sciences, Laney Graduate School, Emory University, Atlanta, Georgia, USA Department of Pediatrics, Division of Pulmonary, Allergy and Immunology, Cystic Fibrosis, and Sleep, Emory University School of Medicine, Atlanta, Georgia, USA cEmory Children's Center for Cystic Fibrosis Research, Emory University and Children's Healthcare of Atlanta, Atlanta, Georgia, USA

ABSTRACT Pseudomonas aeruginosa is the major cause of bacterial keratitis, a sightthreatening ocular infection that can occur in contact lens wearers, as well as in others. Here, we report the draft genomes of 8 different P. aeruginosa corneal isolates, adding to the list of publicly available corneal infection-associated P. aeruginosa genomes.

seudomonas aeruginosa is a Gram-negative opportunistic pathogen that causes infections in immunocompromised people. While well known for its ability to cause infections of the respiratory tract, P. aeruginosa is relatively understudied in the context of bacterial keratitis (infection of the cornea). P. aeruginosa is the major cause of bacterial keratitis, occurring in 71% of all infection-positive contact lens wearers (1, 2). Electron microscopic studies have shown that P. aeruginosa adheres to and penetrates corneal epithelial cells (3). A number of exo-enzymes secreted by P. aeruginosa, such as PrpL, PASP, ExoS, ExoT, and ExoU, are associated with increased virulence and can cause corneal damage, potentially leading to permanent vision loss (4-7). However, the exact mechanisms responsible for P. aeruginosa-associated eye infections are not fully understood.

There is a need for more corneal isolates in the pool of sequenced P. aeruginosa strains. The Pseudomonas Genome Database (8) has over 4,800 genomes, out of which only 2 are reported to be corneal isolates. Here, we report the genome sequence of 8 additional P. aeruginosa isolates collected from human eye infections by the Bascom Palmer Eye Institute (Miami, FL) (9, 10), contributing to the diversity of sequenced P. aeruginosa clinical isolates. Our goal is to identify genetic features common to corneal isolates and in the future understand the roles that particular factors play in corneal virulence.

All isolates were incubated at 37°C overnight in 3 ml lysogeny broth (LB) with shaking. Genomic DNA was isolated using a Qiagen DNeasy blood and tissue kit. Samples were then sequenced using an Illumina NextSeq 500 instrument (Microbial Genome Sequencing Center, University of Pittsburgh), producing 151-bp paired-end reads. Sequencing libraries were prepared by the sequencing center as described by Baym et al. (11). Quality trimming was performed using Trim Galore! v0.6.2, and reads with a quality score of >27 were selected for assembly using SKESA v2.3.0 (12–14). These draft assemblies were then fed into SPAdes v3.13.1 with the "-trusted-contigs" parameter, along with reads having a quality score of >20 as the input (15). The "-cov-cutoff" parameter in SPAdes was set to "auto." Quality assessment for the resulting assemblies was performed using QUAST v5.0.2 (16). The assemblies were then annotated using NCBI Prokaryotic Genome Annotation Pipeline v4.9 (17). Default parameters were used for all software unless otherwise noted.

The final assemblies have an average coverage of 80×, with 110 contigs and 66.2%

Citation Raghuram V, Goldberg JB. 2020. Draft genome sequences of eight *Pseudomonas* aeruginosa corneal infection isolates. Microbiol Resour Announc 9:e01253-19. https://doi.org/ 10.1128/MRA.01253-19.

Editor Catherine Putonti, Loyola University

Copyright © 2020 Raghuram and Goldberg. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Joanna B. Goldberg, joanna.goldberg@emory.edu.

Received 8 October 2019 Accepted 17 November 2019 Published 2 January 2020



TABLE 1 Genome assembly statistics of 8 P. aeruginosa isolates recovered from human corneal infections

Strain	GenBank	SRA	No. of	No. of	Total				Assembly
name	accession no.	accession no.	raw reads	contigs	length (bp)	GC (%)	N ₅₀ (bp)	L ₅₀	coverage (×)
6073	VOLA00000000	SRR9904145	2,110,378	154	6,920,359	66.1	97,264	18	75
6206	VOKZ00000000	SRR9904146	2,375,999	121	6,901,724	66.05	138,064	19	85
6354	VOJG00000000	SRR9904143	1,786,529	89	6,334,363	66.51	136,410	14	72
6382	VOJF00000000	SRR9904144	2,777,128	95	6,539,906	66.32	144,880	17	109
6389	VOKY00000000	SRR9904149	2,243,631	110	6,537,335	66.31	112,407	17	84
6436	VOJE00000000	SRR9904150	2,058,162	120	6,700,809	66.16	115,309	17	77
6452	VOJD00000000	SRR9904147	2,475,116	109	6,743,767	66.1	141,768	17	90
6487	VOJC00000000	SRR9904148	2,110,378	86	6,338,660	66.53	114,717	17	74

GC content. All strains were positive for the exo-enzymes PrpL, PASP, and ExoT. All strains except 6487 were positive for ExoU, and only strain 6487 was positive for ExoS.

Data availability. The accession numbers and assembly statistics of these isolates are listed in Table 1. The BioProject accession number is PRJNA558357.

ACKNOWLEDGMENTS

Isolates were kindly provided by Gerald B. Pier (Harvard Medical School, Brigham and Women's Hospital, Boston, MA).

This research was supported in part by a grant from the National Institutes of Health (R21 EY028320 to J.B.G.).

REFERENCES

- Gellatly SL, Hancock RE. 2013. Pseudomonas aeruginosa: new insights into pathogenesis and host defenses. Pathog Dis 67:159–173. https://doi.org/10.1111/2049-632X.12033.
- Green M, Apel A, Stapleton F. 2008. Risk factors and causative organisms in microbial keratitis. Cornea 27:22–27. https://doi.org/10.1097/ICO .0b013e318156caf2.
- Fleiszig SM, Zaidi TS, Fletcher EL, Preston MJ, Pier GB. 1994. Pseudomonas aeruginosa invades corneal epithelial cells during experimental infection. Infect Immun 62:3485–3493.
- 4. Fleiszig SM, Wiener-Kronish JP, Miyazaki H, Vallas V, Mostov KE, Kanada D, Sawa T, Yen TS, Frank DW. 1997. *Pseudomonas aeruginosa*-mediated cytotoxicity and invasion correlate with distinct genotypes at the loci encoding exoenzyme S. Infect Immun 65:579–586.
- O'Callaghan RJ, Engel LS, Hobden JA, Callegan MC, Green LC, Hill JM. 1996. Pseudomonas keratitis. The role of an uncharacterized exoprotein, protease IV, in corneal virulence. Invest Ophthalmol Vis Sci 37:534–543.
- Marquart ME, Caballero AR, Chomnawang M, Thibodeaux BA, Twining SS, O'Callaghan RJ. 2005. Identification of a novel secreted protease from *Pseudomonas aeruginosa* that causes corneal erosions. Invest Ophthalmol Vis Sci 46:3761–3768. https://doi.org/10.1167/iovs.04-1483.
- Lakhundi S, Siddiqui R, Khan NA. 2017. Pathogenesis of microbial keratitis. Microb Pathog 104:97–109. https://doi.org/10.1016/j.micpath.2016.12.013.
- Winsor GL, Griffiths EJ, Lo R, Dhillon BK, Shay JA, Brinkman FS. 2016. Enhanced annotations and features for comparing thousands of *Pseudomonas* genomes in the *Pseudomonas* genome database. Nucleic Acids Res 44:D646–D653. https://doi.org/10.1093/nar/qkv1227.
- Preston MJ, Fleiszig SM, Zaidi TS, Goldberg JB, Shortridge VD, Vasil ML, Pier GB. 1995. Rapid and sensitive method for evaluating *Pseudomonas aeruginosa* virulence factors during corneal infections in mice. Infect Immun 63:3497–3501.

- Fleiszig SM, Zaidi TS, Preston MJ, Grout M, Evans DJ, Pier GB. 1996. Relationship between cytotoxicity and corneal epithelial cell invasion by clinical isolates of *Pseudomonas aeruginosa*. Infect Immun 64:2288–2294.
- Baym M, Kryazhimskiy S, Lieberman TD, Chung H, Desai MM, Kishony R. 2015. Inexpensive multiplexed library preparation for megabase-sized genomes. PLoS One 10:e0128036. https://doi.org/10.1371/journal.pone .0128036.
- Andrews S. 2010. FASTQC: a quality control tool for high throughput sequence data. https://www.bioinformatics.babraham.ac.uk/projects/ fastqc/.
- Souvorov A, Agarwala R, Lipman DJ. 2018. SKESA: strategic k-mer extension for scrupulous assemblies. Genome Biol 19:153. https://doi.org/10.1186/s13059-018-1540-z.
- Martin M, Rahmann S. 2012. From cutadapt to sequencetools (Sqt): a versatile toolset for sequencing projects. EMBnet J 17. https://doi.org/ 10.14806/ej.17.B.272.
- 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.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086.
- 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.

Volume 9 Issue 1 e01253-19 mra.asm.org **2**