





# Draft Genome Sequences of Phenotypically Distinct *Janthinobacterium* sp. Isolates Cultured from the Hudson Valley Watershed

Alexandra M. Bettina,<sup>a</sup> Georgia Doing,<sup>b</sup> Kelsey O'Brien,<sup>c</sup>  Gabriel G. Perron,<sup>a</sup>  Brooke A. Jude<sup>a</sup>

<sup>a</sup>Reem-Kayden Center for Science and Computation, Program of Biology, Bard College, Annandale-on-Hudson, New York, USA

<sup>b</sup>Department of Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, New Hampshire, USA

<sup>c</sup>Pediatric Infectious Disease Division, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA

**ABSTRACT** Investigation of the Hudson Valley watershed reveals many violacein-producing bacteria. These are of interest for their biotherapeutic potential in treating chytrid infections of amphibians. The draft whole-genome sequences for seven *Janthinobacterium* isolates with a variety of phenotypes are provided in this study.

The Hudson Valley watershed is home to a large number of vibrantly colored bacterial isolates (1). Many of these Gram-negative *Bacillus* species produce violacein. Production is mediated via a five-gene operon, *vioA–E* (2). Violacein production was first described in *Chromobacterium violaceum* in 1927 (3), and violacein has been widely investigated for its potential biotherapeutic properties; studies have demonstrated bacterial killing (4), fungal killing (5, 6), antiviral activity (7), tumoral cytotoxicity (8, 9), and antinematodal action (10).

Strains BJB1, BJB301, BJB303, BJB304, BJB312, BJB426, and BJB446 were obtained by plating Hudson Valley freshwater sources on R2A agar and incubating at 22 to 25°C for 48 h. All strains initially presented as violet-pigmented colonies and were cultured on R2A, LB, and 1% tryptone agar media. All strains have been characterized for phenotypic behaviors related to growth, motility, quorum sensing, biofilm production, and violacein expression (our unpublished data). Density-dependent phenotypes, including biofilm production and violacein expression, may provide the microorganism with the ability to persist and thrive in a freshwater environment (11).

Genomic DNA extraction was completed with the Qiagen Genra Puregene Yeast/Bact. kit according to the manufacturer's protocol. Paired-end Illumina libraries (150 bp) were prepared and HiSeq sequencing was completed using the Illumina HiSeq 4000 instrument (Wright Labs, Huntington, PA). Reads were assembled with a modified version of a previously published local pipeline (12). Adapters and contaminants were scanned for and removed when present. Reads were subsequently quality filtered using BBDuk from the BBMap package version 37.50, keeping to a Q score cutoff of 10 (<https://sourceforge.net/projects/bbmap>). A draft whole-genome assembly was built using SPAdes version 3.11.0 (13) using k-mer sizes of 21, 33, 55, 77, 99, and 127. Contigs shorter than 500 bp or those composed of fewer than four reads were subsequently filtered out of the assembly.

The draft whole-genome assemblies ranged from a high of 70 contigs for BJB426 to a low of 22 contigs for BJB446 (Table 1). The average  $N_{50}$  value for all seven optimal assemblies was 940,382 bp, with two genomes, those of BJB301 and BJB446, resulting in high  $N_{50}$  values of 1,349,355 bp and 3,551,037 bp, respectively (Table 1). The average genome size is predicted to be 6.36 Mbp in length, with an average G+C content

**Received** 15 November 2017 **Accepted** 16 November 2017 **Published** 18 January 2018

**Citation** Bettina AM, Doing G, O'Brien K, Perron GG, Jude BA. 2018. Draft genome sequences of phenotypically distinct *Janthinobacterium* sp. isolates cultured from the Hudson Valley watershed. *Genome Announc* 6:e01426-17. <https://doi.org/10.1128/genomeA.01426-17>.

**Copyright** © 2018 Bettina et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Brooke A. Jude, [bjude@bard.edu](mailto:bjude@bard.edu).

**TABLE 1** Characteristics of the isolates in this study

Isolate	Origin	Location	No. of contigs	Genome size (bp)	G+C content (%)	$N_{50}$ (bp)	Median read depth (×)	Avg no. of CDSs	GenBank accession no.
BJB1	Water	Esopus, Hudson Valley, NY	56	6,353,752	63.35	369,198	730	5,718	PDZO00000000
BJB301	Water	Fishkill Creek, Hudson Valley, NY	24	6,386,165	62.78	1,349,355	504	5,665	PDZJ00000000
BJB303	Water	North Tivoli Bay, Red Hook, NY	48	6,275,771	63.36	319,682	577	5,680	PDZI00000000
BJB304	Water	Norrie Pond, Hudson Valley, NY	62	6,466,089	62.72	218,724	67	5,773	PDZN00000000
BJB312	Water	Dutchess County, NY (vernal pool)	38	6,224,897	62.61	488,122	864	5,559	PDZM00000000
BJB426	Water	Staatsburg, NY	70	6,413,115	62.47	286,558	156	5,711	PDZL00000000
BJB446	Water	Echo Valley, Hudson Valley, NY	22	6,403,346	62.67	3,551,037	316	5,701	PDZK00000000

of 62.85%, comparable to those of published *Janthinobacterium* species (Table 1) (14, 15).

The assembled contigs were annotated using three methods, a local pipeline running the Prokka genome annotation software (16), the RASTtk annotation software, via the PATRIC pipeline (17, 18), and the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (19). The 16S rRNA BLAST results for all seven strains aligned most closely with other *Janthinobacterium* species. Annotations across platforms yielded an average of 5,686 coding sequences (CDSs). As expected, a violacein biosynthesis operon (*vioA–E*) was present in all strains. Additionally, all genomes contained genes involved in the bacterial quorum sensing cascade, *jqsA*, *qseC*, and *qseS* (10, 15), as well as twitching motility (*pilT*, *pilJ*, *pilH*, and *pilG*), correlating with the phenotypes observed on media.

Further investigation of this large population of related strains may lead to insights into the therapeutic potential of violacein-producing strains.

**Accession number(s).** The whole-genome assemblies have been deposited at DDBJ/ENA/GenBank under the accession numbers listed in Table 1.

## ACKNOWLEDGMENTS

Many thanks go to Veronica Steckler and Liza Miller for collection water and initial growth of strains.

B.A.J. was provided funding by the New York State Water Resources Institute. Support for training was provided by GCAT-SEEK. GCAT-SEEK has been supported by U.S. National Science Foundation award DBI-1248096: RCN-UBE—GCAT-SEEK: the Genome Consortium for Active Undergraduate Research and Teaching Using Next-Generation Sequencing. K.O. and G.D. were provided summer research support by the Bard Summer Research Institute.

## REFERENCES

- Agate L, Beam D, Bucci C, Dukashin Y, Jo'Beh R, O'Brien K, Jude BA. 2016. The search for violacein-producing microbes to combat *Batrachochytrium dendrobatidis*: a collaborative research project between secondary school and college research students. *J Microbiol Biol Educ* 17:70–73. <https://doi.org/10.1128/jmbe.v17i1.1002>.
- Antônio RV, Creczynski-Pasa TB. 2004. Genetic analysis of violacein biosynthesis by *Chromobacterium violaceum*. *Genet Mol Res* 3:85–91.
- Reilly J, Pyne G. 1927. On the pigment produced by *Chromobacterium violaceum*. *Biochem J* 21:1059–1064. <https://doi.org/10.1042/bj0211059>.
- Huang JP, Mojib N, Goli RR, Watkins S, Waites KB, Ravindra R, Andersen DT, Bej AK. 2012. Antimicrobial activity of PVP from an Antarctic bacterium, *Janthinobacterium* sp. Ant5-2, on multi-drug and methicillin resistant *Staphylococcus aureus*. *Nat Prod Bioprospect* 2:104–110. <https://doi.org/10.1007/s13659-012-0021-4>.
- Becker MH, Brucker RM, Schwantes CR, Harris RN, Minbiole KPC. 2009. The bacterially produced metabolite violacein is associated with survival of amphibians infected with a lethal fungus. *Appl Environ Microbiol* 75:6635–6638. <https://doi.org/10.1128/AEM.01294-09>.
- Haack FS, Poehlein A, Kröger C, Voigt CA, Piepenbring M, Bode HB, Daniel R, Schäfer W, Streit WR. 2016. Molecular keys to the *Janthinobacterium* and *Duganella* spp. interaction with the plant pathogen *Fusarium graminearum*. *Front Microbiol* 7:1668. <https://doi.org/10.3389/fmicb.2016.01668>.
- Andrighetti-Fröhner CR, Antônio RV, Creczynski-Pasa TB, Barardi CR, Simões CM. 2003. Cytotoxicity and potential antiviral evaluation of violacein produced by *Chromobacterium violaceum*. *Mem Inst Oswaldo Cruz* 98:843–848. <https://doi.org/10.1590/S0074-02762003000600023>.
- Kodach LL, Bos CL, Durán N, Peppelenbosch MP, Ferreira CV, Hardwick JCH. 2006. Violacein synergistically increases 5-fluorouracil cytotoxicity, induces apoptosis and inhibits Akt-mediated signal transduction in human colorectal cancer cells. *Carcinogenesis* 27:508–516. <https://doi.org/10.1093/carcin/bgi307>.
- Masuelli L, Pantanella F, La Regina G, Benvenuto M, Fantini M, Mattered R, Di Stefano E, Mattei M, Silvestri R, Schippa S, Manzari V, Modesti A, Bei R. 2016. Violacein, an indole-derived purple-colored natural pigment produced by *Janthinobacterium lividum*, inhibits the growth of head and neck carcinoma cell lines both *in vitro* and *in vivo*. *Tumour Biol* 37:3705–3717. <https://doi.org/10.1007/s13277-015-4207-3>.
- Hornung C, Poehlein A, Haack FS, Schmidt M, Dierking K, Pohlen A, Schulenburg H, Blokesch M, Plener L, Jung K, Bonge A, Krohn-Molt I, Utpatel C, Timmermann G, Spieck E, Pommerening-Röser A, Bode E, Bode HB, Daniel R, Schmeisser C, Streit WR. 2013. The *Janthinobacterium* sp. HH01 genome encodes a homologue of the *V. cholerae* CqsA and *L. pneumophila* LqsA autoinducer synthases. *PLoS One* 8:e55045. <https://doi.org/10.1371/journal.pone.0055045>.
- Besemer K. 2015. Biodiversity, community structure and function of

- biofilms in stream ecosystems. *Res Microbiol* 166:774–781. <https://doi.org/10.1016/j.resmic.2015.05.006>.
12. Shrestha SD, Guttman DS, Perron GG. 2017. Draft genome sequences of 10 environmental *Pseudomonas aeruginosa* strains isolated from soils, sediments, and waters. *Genome Announc* 5:e00804-17. <https://doi.org/10.1128/genomeA.00804-17>.
  13. 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>.
  14. Koo H, Strobe BM, Kim EH, Shabani AM, Kumar R, Crowley MR, Andersen DT, Bej AK. 2016. Draft genome sequence of *Janthinobacterium* sp. Ant5-2-1, isolated from Proglacial Lake Podprudnoye in the Schirmacher Oasis of East Antarctica. *Genome Announc* 4:e01600-15. <https://doi.org/10.1128/genomeA.01600-15>.
  15. Shoemaker WR, Muscarella ME, Lennon JT. 2015. Genome sequence of the soil bacterium *Janthinobacterium* sp. KBS0711. *Genome Announc* 3:e00689-15. <https://doi.org/10.1128/genomeA.00689-15>.
  16. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
  17. Wattam AR, Davis JJ, Assaf R, Boisvert S, Brettin T, Bun C, Conrad N, Dietrich EM, Disz T, Gabbard JL, Gerdes S, Henry CS, Kenyon RW, Machi D, Mao C, Nordberg EK, Olsen GJ, Murphy-Olson DE, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Vonstein V, Warren A, Xia F, Yoo H, Stevens RL. 2017. Improvements to PATRIC, the all-bacterial bioinformatics database and analysis resource center. *Nucleic Acids Res* 45:D535–D542. <https://doi.org/10.1093/nar/gkw1017>.
  18. Wattam AR, Abraham D, Dalay O, Disz TL, Driscoll T, Gabbard JL, Gillespie JJ, Gough R, Hix D, Kenyon R, Machi D, Mao C, Nordberg EK, Olson R, Overbeek R, Pusch GD, Shukla M, Schulman J, Stevens RL, Sullivan DE, Vonstein V, Warren A, Will R, Wilson MJC, Yoo HS, Zhang C, Zhang Y, Sobral BW. 2014. PATRIC, the bacterial bioinformatics database and analysis resource. *Nucleic Acids Res* 42:D581–D591. <https://doi.org/10.1093/nar/gkt1099>.
  19. 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>.