Draft Genome Sequence of a *Chitinimonas* Species from Hudson Valley Waterways That Expresses Violacein Pigment

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**ABSTRACT** *Chitinimonas* spp. are Gram-negative bacilli that are observed in freshwater and soil sources. A number of *Chitinimonas* species have been characterized, including the green-pigmented *Chitinimonas viridis*. The isolate described here, BJB300, was obtained from a freshwater source in the Hudson Valley, NY. BJB300 is the first *Chitinimonas* isolate expressing violacein, a pigment with biotherapeutic potential.

*Chitinimonas* bacteria are Gram-negative, motile bacilli that are found in freshwater and soil and include *Chitinimonas koreensis*, *Chitinimonas taiwanensis*, and *Chitinimonas viridis* (1–3). Isolates are often associated with chitin or organisms with chitinous carapaces (2). *Chitinimonas* spp. are capable of producing chitinases, whose production in other organisms is regulated by quorum sensing (4).

Hudson Valley (NY) freshwater samples were cultured at 22 to 25°C on Reasoner's 2A (R2A) medium, and they displayed vibrantly colored bacterial isolates, including violet colonies (5). The pigment violacein is produced via the expression of a five-gene biosynthetic operon, *vioABCDE* (6). Since its initial characterization, violacein has been studied for its utility in biotherapeutics, most notably for its killing effect on invasive chytrid isolates (7).

*Chitinimonas* sp. strain BJB300 was isolated from a freshwater source on R2A agar and incubated at 22 to 25°C for 48 hours. The isolate grew as diffuse, irregularly shaped, violet-pigmented colonies that could be maintained successfully on R2A agar and 1% tryptone yet is unable to grow on Lennox lysogeny broth (8).

Genomic DNA extraction was completed with a Puregene yeast/bacteria kit (Qiagen). A 150-bp paired-end Illumina library was generated and sequenced on an Illumina HiSeq 4000 sequencer (Wright Labs, Huntington, PA), resulting in 2 Gbp of sequence. DNA was also isolated using the DNeasy blood and tissue kit (Qiagen), and a library was constructed (see SRA accession numbers), without shearing, using the Nanopore rapid sequencing kit (catalog number SQN-RAD004; Oxford). The library was sequenced with the Nanopore MinION device (Oxford), yielding 18.5 million bases. Reads from both sequencing runs were archived through the NCBI and uploaded to the Galaxy Web platform, using the public server at http://usegalaxy.eu, for analysis (Table 1). All programs were run on Galaxy-EU using standard installations except where noted. Illumina sequences were analyzed with FastQC (9) and trimmed using fastp (10), while Nanopore adapters were trimmed using Porechop (11). Unicycler (v. 0.4.6) was used for assembly, removing contigs shorter than 500 bp in length (12, 13). Sequences were mapped back to the assembly using Bowtie 2 and visualized with Tablet, with all contigs having least 230X coverage (14, 15).

The draft genome is 111 contigs. The *N*₅₀ value of the assembly is 246,315 bp. The genome size is predicted to be 5.04 Mbp, with a G+C content of 54.56%. The G+C percentages in the literature for *Chitinimonas* species range from that for *C. viridis* (59.8% G+C) to that for *C. koreensis* (65.0% G+C) (1, 3). Analysis with PlasFlow identified...
33 potential plasmid sequences, many with G+C contents divergent from the reported average (16).

Contigs were annotated using Prokka (v. 1.13.3) (17), RASTrK (https://www.patricbrc.org) (18, 19), and the NCBI Prokaryotic Genome Annotation Pipeline (20). Annotations averaged 4,652 coding sequences (CDS). A BLAST search of the 16S rRNA found that it was 94% identical to that of C. koreensis. As expected, the violacein operon was identified, describing the purple colony pigmentation. Additionally, a chitinase gene was noted, pointing to a functional ability to degrade chitin similar to that of other Chitinimonas isolates.

This report places Chitinimonas into a group of strains capable of producing violacein, enlarging the cohort of bacterial strains available for bioremediation and biotherapeutic purposes. Further analysis of this strain and its biological properties is ongoing.

Data availability. SRA files for Illumina sequencing (SRA accession number SRS2670610) have been deposited, as well as those for Nanopore sequencing (SRA accession number SRA6461400). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number VDCU00000000. The version described in this paper is the second version, VDCU02000000.

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REFERENCES

1. Kim B-Y, Weon H-Y, Yoo S-H, Chen W-M, Kwon S-W, Go S-J, Stackebrandt E. 2006. Chitinimonas koreensis sp. nov., isolated from greenhouse soil in Korea. Int J Syst Evol Microbiol 56:1761–1764. https://doi.org/10.1099/ijs.0.04163-0.
2. Chang S-C, Wang J-T, Vandamme P, Hwang J-H, Chang P-S, Chen W-M. 2004. Chitinimonas taiwanensis gen. nov., sp. nov., a novel chitinolytic bacterium isolated from a freshwater pond for shrimp culture. Syst Appl Microbiol 27:43–49. https://doi.org/10.1078/0723-2020-00252.
3. Joung Y, Lee BI, Kang H, Kim H, Joh K. 2014. Chitinimonas vindis sp. nov., isolated from a mesotrophic artificial lake. Int J Syst Evol Microbiol 64:1123–1126. https://doi.org/10.1099/ijs.0.055442-0.
4. Kim IS, Yang SY, Park SK, Kim YC. 2017. Quorum sensing is a key regulator for the antifungal and biocontrol activity of chitinase-producing Chromobacterium sp. C61. Mol Plant Pathol 18:134–140. https://doi.org/10.1111/mpp.12379.
5. 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.
6. António RV, Creczynski-Pasa TB. 2004. Genetic analysis of violacein biosynthesis by Chromobacterium violaceum. Genet Mol Res 3:85–91.
7. Becker MH, Brucker RM, Schwantes CR, Harris RN, Minbiole K. 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.
8. Lennox ES. 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology 1:190–206. https://doi.org/10.1016/0042-6822(55)90016-7.
9. Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/.
10. Chen S, Zhou Y, Chen Y, Gu J. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:884–890. https://doi.org/10.1093/bioinformatics/bty560.
11. Wick RR. 2018. Porechop: adapter trimmer for Oxford Nanopore reads. https://github.com/rrwick/Porechop.
12. Afgan E, Baker D, Batut B, van den Beek M, Bouvier D, Cech M, Chilton J. 2018. Biocontainers: modular containers for scientific applications. https://github.com/biocontainers/Chitinimonas
J. Clements D, Coraor N, Grüning BA, Guerler A, Hillman-Jackson J, Hiltemann S, Jallil V, Rasche H, Sranzio N, Goecs J, Taylor J, Nekrutenko A, Blankenberg D. 2018. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. Nucleic Acids Res 46:W537–W544. https://doi.org/10.1093/nar/gky379.

13. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595. https://doi.org/10.1371/journal.pcbi.1005595.

14. Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359. https://doi.org/10.1038/nmeth.1923.

15. Milne I, Stephen G, Bayer M, Cock PJA, Pritchard L, Cardle L, Shaw PD, Marshall D. 2013. Using Tablet for visual exploration of second-generation sequencing data. Brief Bioinform 14:193–202. https://doi.org/10.1093/bib/bbs012.

16. Krawczyk PS, Lipinski L, Dziembowski A. 2018. PlasFlow: predicting plasmid sequences in metagenomic data using genome signatures. Nucleic Acids Res 46:e35. https://doi.org/10.1093/nar/gkx1321.

17. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.

18. Wattam AR, Abraham D, Daliz O, Disz T, 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. 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.

20. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zalessky 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.