



Draft Genome Sequence of *Janthinobacterium* sp. Strain PC23-8, Isolated from a Freshwater Stream Impacted by Acid Mine Drainage

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ABSTRACT We present the draft genome sequence of *Janthinobacterium* sp. strain PC23-8, a bacterium isolated from freshwater stream sediment downstream from acid mine drainage. The 6.4-Mb genome sequence of this strain contains several secondary metabolite gene clusters, including one similar to the cyclic peptide jagaricin, synthesized by *Janthinobacterium agaricidamnosum*.

For genome mining studies aimed at drug discovery, extreme environments may yield less typical bacterial species as sources of novel antimicrobials (1–3). In our study, sediment was collected from Davidson Creek (Beaver County, PA, USA) at a site impacted by acid mine drainage. Diluted sediment samples were spread on Trypticase soy agar (TSA) plates to allow formation of single colonies at 25°C. Isolates were colony purified and subjected to 16S rRNA sequencing (Genewiz, South Plainfield, NJ). EzBioCloud was used to find the closest related taxonomic groups (4). Isolate PC23-8 was a nonpigmented, Gram-negative, motile bacillus that showed homology (~99%) to several well-described *Janthinobacterium* species, including the red-brown-pigmented *J. svalbardenesis*, the purple-pigmented *J. lividum* strains, and the nonpigmented *J. agaricidamnosum*.

The genome sequence was generated at ACGT, Inc. (Wheeling, IL). Genomic DNA was isolated from colonies grown on TSA plates using the MasterPure complete DNA/RNA purification kit (Epicentre catalog number MC85200) and purified using the DNA Clean & Concentrator kit (Zymo Research). For paired-end (PE) sequencing, genomic DNA was fragmented using ultrasonication (~500 bp) and used to construct a library with a NEXTflex rapid DNA-Seq library preparation kit (Bioo Scientific). Genomic DNA was also used to prepare a Nextera mate pair (MP; 4- to 10-kb) library. Final fragmentation of the library was performed using ultrasonication to a size of ~500 bp. Both libraries were size selected for 600 to 800 bp using the BluePippin system (Sage Science, Beverly, MA) and verified with a 2100 bioanalyzer (Agilent, Santa Clara, CA). The PE and MP libraries were sequenced using HiSeq and NextSeq, respectively.

For bioinformatic analyses, default settings were used unless otherwise specified. Illumina reads were demultiplexed (bcl2fastq v2.17, Illumina, San Diego, CA) and trimmed (Trim Galore v4.1; Babraham Bioinformatics, Cambridge, UK; q = 30, PE lower limit = 50, MP lower limit = 35). Trimmed reads, assembled using SPAdes v3.7.1 (t = 56, mismatch = careful) (5), totaled 3,489,090,776 bp for the PE library and 1,390,229,081 bp for the MP library. Sequence coverage was estimated at 766fold. Contigs shorter than 1,000 bp were discarded. The assembled genome consisted of 12 contigs. The overall size of the draft sequence was 6,386,497 bp with 11 physical gaps. Using RAST v2.0 (6), the N_{50} value of the genome assembly was found to be 326,467 bp, and the genome contained 5,600 protein-coding and 93 RNA genes. antiSMASH v5.0 (stringent) (7) predicted one gene cluster to generate a cyclic peptide related to the antifungal jagaricin (8). Other predicted clusters coded for novel pep**Citation** Clark CE, Banyas MG, Noell SE, Essig DA. 2019. Draft genome sequence of *Janthinobacterium* sp. strain PC23-8, isolated from a freshwater stream impacted by acid mine drainage. Microbiol Resour Announc 8:e00850-19. https://doi.org/10.1128/MRA .00850-19.

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Received 16 July 2019 Accepted 18 September 2019 Published 10 October 2019 tides, including several bacteriocins and a beta-lactone. Using the Genome-to-Genome Distance Calculator (GGDC; formula 2) (9), PC23-8 was found to be a species distinct from *J. svalbardenesis* (27.5% sequence identity), *J. lividum* (26.4% sequence identity), and *J. agaricidamnosum* (22.9% sequence identity), since values were less than the same-species threshold (70%).

Data availability. This whole-genome shotgun project has been deposited in GenBank under the accession number NZ_NJGY00000000. The version described in this paper is the first version. The SRA accession numbers for the PE and MP libraries used to assemble the genome are SRR10043460 and SRR10043459, respectively.

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REFERENCES

- Pidot SJ, Coyne S, Kloss F, Hertweck C. 2014. Antibiotics from neglected bacterial sources. Int J Med Microbiol 304:14–22. https://doi.org/10.1016/ j.ijmm.2013.08.011.
- Cragg GM, Newman DJ. 2001. Natural product drug discovery in the next millennium. Pharmaceutical Biology 39:8–17. https://doi.org/10.1076/ phbi.39.s1.8.0009.
- Keller M, Zengler K. 2004. Tapping into microbial diversity. Nat Rev Microbiol 2:141–150. https://doi.org/10.1038/nrmicro819.
- Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J. 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA and whole genome assemblies. Int J Syst Evol Microbiol 67:1613–1617. https://doi .org/10.1099/ijsem.0.001755.
- 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.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edward RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL,

Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.

- Weber T, Blin K, Srikanth D, Krug D, Kim HU, Bruccoleri R, Lee SY, Fischbach MA, Müller R, Wohlleben W, Breitling R, Takano E, Medema MH. 2015. antiSMASH 3.0: a comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res 43:W237–W243. https:// doi.org/10.1093/nar/gkv437.
- Graupner K, Scherlach K, Bretschneider T, Lackner G, Roth M, Gross H, Hertweck C. 2012. Imaging mass spectrometry and genome mining reveal highly antifungal virulence factor of mushroom soft rot pathogen. Angew Chem Int Ed Engl 51:13173–13177. https://doi.org/10.1002/ anie.201206658.
- Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. 2013. Genome sequencebased species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14:60. https://doi.org/10.1186/1471 -2105-14-60.