Two Draft Genome Sequences of *Chromobacterium violaceum* Isolates from the Rio Negro

Auricélia Matos da Gama,a,b Luiz Gustavo de Almeida,a Tetsuo Yamane,b Beny Spiraa

aDepartamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil
bEscola Superior de Ciências da Saúde, Curso de Farmácia, Universidade do Estado do Amazonas, Manaus, Amazonas, Brazil

ABSTRACT The draft genome sequences of two *Chromobacterium violaceum* strains isolated from the Rio Negro are reported here. These bacteria carry most genetic systems associated with the production of bioactive compounds, but unlike other *C. violaceum* strains, they lack a dedicated operon for arsenic resistance.

The betaproteobacterium *Chromobacterium violaceum*, found in tropical environments around the globe, forms violet colonies on rich solid media due to the production of violacein (1, 2). *C. violaceum* can cause infections in humans ranging from mild diarrhea to death (3, 4). In addition to violacein, *C. violaceum* produces several potential biotechnology products (5). Though not considered oligotrophic, *C. violaceum* thrives in nutrient-scarce environments, such as the Rio Negro.

Water samples were collected from the Rio Negro at the Amazon Basin (Manaus, Brazil) and spread on L-agar plates. Two violet colonies that are prototrophic, ampicillin resistant, and grow under low-phosphate conditions (6) were isolated. The isolates were designated CV1192 and CV1197.

Genomic DNA was extracted using the Wizard genomic DNA purification kit (Promega) and quantified in a Qubit fluorimeter (Life Technologies). Genome sequencing was performed by the MicrobesNG facility (Birmingham, UK). The genomic DNA library was prepared using the Nextera XT library prep kit (Illumina). Libraries were sequenced on an Illumina HiSeq platform using a 250-bp paired-end protocol. Reads were adapter trimmed using Trimmomatic 0.30, with a sliding window quality cutoff of Q15 (7), and *de novo* genome assembly was carried out with SPAdes (version 3.7) (8). General genome features and annotation were obtained using the Geneious 10.3 and Prokka (9) softwares, with the genome of *C. violaceum* ATCC 12472 (GenBank accession number NC_005085) as a reference.

Each genome comprised 58 contigs, with 50 contigs larger than 1,000 bp. The genome lengths of strains CV1192 and CV1197 are, respectively, 4,386,139 bp (64.9% G+C content) and 4,388,579 bp (65.1% G+C content). Both contain 4,405 coding DNA sequences (CDSs). Of these, 61.08% were assigned a putative function, and the remainder were considered hypothetical. Twenty-five rRNAs and 98 tRNA sequences were annotated. One intact prophage region was detected in both sequences using the PHASTER algorithm (10).

The genomes of CV1192 and CV1197 were, respectively, 92.3% and 92.4% identical to that of *C. violaceum* ATCC 12472. The genomes of both strains lack 61 open reading frames (ORFs) that are present in the ATCC 12472 strain. Of significance, an Rhs-like protein (CV_1238) that is involved in bacteriocin synthesis, a phenazine biosynthesis protein (CV_2663), and the *arsRCB* operon (CV_2438, CV_2439, and CV_2440) are missing in both isolates. Most other missing ORFs code for structural phage proteins or...
secretion systems. All other genetic elements associated with the production of antibiotics and bioactive secondary metabolites are present in both genomes.

Unlike CV1192 and CV1197, the ATCC 12472 and other C. violaceum strains carry a functional arsRCB operon, which is involved in arsenic detoxification (11). The concentration of As in the waters of the Rio Negro is approximately 0.05 mg/m³ (0.36 nM), being the lowest As concentration among all Amazon rivers (12). The loss of the arsRCB operon in CV1192 and CV1197 appears to be a striking example of negative (purifying) selection. Likewise, Chromobacterium amazonense, another bacterium isolated from the Rio Negro, lacks an arsRCB operon.

Accession number(s). The whole-genome shotgun projects have been deposited at DDBJ/EMBL/GenBank under the accession numbers CP024028 (strain CV1192) and CP024029 (strain CV1197).

ACKNOWLEDGMENTS

This work was supported by the FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) (grant 2016/11547-9) and the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) (grant 303189/2015-0). Genome sequencing was provided by MicrobesNG (http://www.microbesng.uk), which is supported by the BBSRC (grant BB/L024209/1).

REFERENCES

1. DeMoss JA, Jackson RW, Chalmers JH. 1967. Genetic control of the structure and activity of an enzyme aggregate in the tryptophan pathway of Neurospora crassa. Genetics 56:413–424.

2. Hoshino T, Kondo T, Uchiyama T, Ogasawara N. 1987. Biosynthesis of violacein: a novel rearrangement in tryptophan metabolism with a 1,2-shift of the indole ring. Agric Biol Chem 51:965–268. https://doi.org/10.1080/00021369.1987.10868084.

3. Sneath P, Bhagwan Singh R, Whelan J, Edwards D. 1953. Fatal infection by Chromobacterium violaceum. Lancet 265:276–277. https://doi.org/10.1016/S0140-6736(53)91132-5.

4. Yang CH, Li YH. 2011. Chromobacterium violaceum infection: a clinical review of an important but neglected infection. J Chin Med Assoc 74:435–441. https://doi.org/10.1016/j.jcma.2011.08.013.

5. Durán N, Justo GZ, Durán M, Brocchi M, Cordi L, Tasic L, Castro GR, Nakazato G. 2016. Advances in Chromobacterium violaceum and properties of violacein—its main secondary metabolite: a review. Biotechnol Adv 34:1030–1045. https://doi.org/10.1016/j.biotechadv.2016.06.003.

6. da Costa Vasconcelos FN, Padilla G, Spira B. 2016. Chromobacterium violaceum adaptation to low-phosphate conditions. Arch Microbiol 198:269–277. https://doi.org/10.1007/s00203-016-1188-6.

7. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

8. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Skrhotkin 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.

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

10. Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, Wishart DS. 2016. PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res 44:W16–W21. https://doi.org/10.1093/nar/gkw387.

11. Azevedo JSN, Silva-Rocha R, Silva A, Peixe Carepo MS, Cruz Schneider MP. 2008. Gene expression of the arsenic resistance operon in Chromobacterium violaceum ATCC 12472. Can J Microbiol 54:137–142. https://doi.org/10.1139/w07-013.

12. Seyler P, Boaventura G. 2001. Trace elements in the mainstream Amazon River, p 307–327. In McClain ME, Victoria RL, Richey JE (ed), The biogeochemistry of the Amazon Basin. Oxford University Press, New York, NY.