



Draft Genome Sequences of Three *Chromobacterium subtsugae* Isolates from Wild and Cultivated Cranberry Bogs in Southeastern Massachusetts

Kristin Vöing,^a Alisha Harrison,^b ^(D)Scott D. Soby^b

Fachbereich Biologie, Universität Münster, Münster, Germany^a; Biomedical Sciences Program, College of Health Sciences and College of Veterinary Medicine, Midwestern University, Glendale, Arizona, USA^b

Chromobacterium subtsugae was isolated from cranberry bogs in Massachusetts. While it is unknown what environmental role these bacteria play in bog soils, they hold potential as biological control agents against the larvae of insect pests. Potential virulence genes were identified, including the violacein synthesis pathway, siderophores, and several chitinases.

Received 23 July 2015 Accepted 30 July 2015 Published 10 September 2015

Citation Vöing K, Harrison A, Soby SD. 2015. Draft genome sequences of three *Chromobacterium subtsugae* isolates from wild and cultivated cranberry bogs in southeastern Massachusetts. Genome Announc 3(5):e00998-15. doi:10.1128/genomeA.00998-15.

Copyright © 2015 Vöing et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to Scott D. Soby, ssobyx@midwestern.edu.

hromobacterium subtsugae strains were isolated from cultivated (MWU3525) and wild (MWU2576, MWU2920) cranberry bog soils in Massachusetts, and tentatively identified as C. subtsugae by phenotype and by 16S rRNA sequences (1-3). Their genomes were sequenced at the University of Arizona Genetics Core using a 454 GS FLX Titanium following manufacturer protocols. Libraries were generated using the NEBNext Quick DNA Library Prep Master Mix set for 454, and MID tagged using the Library Prep Kit Rapid Library MID adapters kit. The emPCR Amplification Method manual, Lib-L LV, XL+ (June 2013), was followed for bulk emPCR of each sample. Three samples were loaded into each region of a sequencing PTP plate divided with a 2-region gasket. Roughly 670,000 beads were loaded per sample. Roche Newbler Software v2.9 was used for signal processing, sample demultiplexing, and partial assembly. Assembly data for all three isolates are given in Table 1. Isolate genomes were compared to each other and to reference genomes of C. violaceum (ATCC 12472), C. haemolyticum (T124), C. vaccinii (MWU205), and C. subtsugae (F49) using the Genome-to-Genome Distance Calculator (GGDC) provided online by the DSMZ. GGDC mimics in vitro DNA-DNA hybridization by dividing scaffold sequences into fragments approximately the same size as would be expected in vitro, and pairing up homologous segments (4-6). MWU2576, MWU2920, and MWU3525 total genomes were 87.6 to

95.3% homologous by this method, 90 to 96% homologous to the *C. subtsugae* reference genome, confirming them as a member of this species. In contrast, they were 30% homologous to the type species/isolate of the genus, *C. violaceum* ATCC 12472.

Ab initio gene prediction was performed on the assembly using RAST (http://rast.nmpdr.org/). A number of potential virulence factor genes were found that may contribute to larval toxicity, including production of the pigment violacein, siderophores, hydrogen cyanide, and secreted chitinases (7). MWU2576 contained 16 probable chitinase genes, including only one probable chitinase A gene, and six endochitinases. MWU2920 contained 14 probable chitinase genes, including three probable chitinase A genes, and two endochitinases. This isolate was unusual in that it also contained ten genes for chitinase family 18 proteins. MWU3525 contained 17 probable chitinase genes, including nine probable chitinase A genes, and seven endochitinases. In contrast to *C. vaccinii* (8, 9), none of the isolates contained putative hydrolase transmembrane family proteins.

Nucleotide sequence accession numbers. This wholegenome shotgun project has been deposited at DDBJ/EMBL/ GenBank under the accession numbers listed in Table 1. The versions described in this paper are versions LCWQ01000000, LCWP01000000, and LCWO01000000.

TABLE 1 Accession numbers and genome sequencing statistics

Isolate	Accession no.	No. of reads per sample	Coverage	No. of scaffolds/large scaffolds	No. of nucleotides (nt) on large scaffolds	N_{50}	Maximum scaffold length (nt)	G+C fraction (%)	Estimated genome size (MB)
MWU2576	LCWQ0000000	245,078	618.6×	82/61	4,803,580	175,428	394,904	64.76	5.4
MWU2920	LCWP0000000	243,888	$625.8 \times$	86/50	4,665,268	222,142	339,870	64.86	5.1
MWU3525	LCWO0000000	224,323	609.2×	103/63	4,700,397	181,598	397,271	64.83	5.5

ACKNOWLEDGMENTS

This research was supported by the Office of Research and Sponsored Programs, College of Health Sciences and Biomedical Sciences Program, Midwestern University.

Microbial samples were taken with the kind assistance of Frank Caruso of the University of Massachusetts Cranberry Station, East Wareham, MA, USA, and the Cape Cod National Seashore.

REFERENCES

- Martin PAW, Blackburn M. 2008. Characterization of the insecticidal activity of *Chromobacterium subtsugae*. Biopestic Int 4:102–109.
- Martin PA, Gundersen-Rindal D, Blackburn M, Buyer J. 2007. Chromobacterium subtsugae sp. nov., a betaproteobacterium toxic to Colorado potato beetle and other insect pests. Int J Syst Evol Microbiol 57:993–999. http://dx.doi.org/10.1099/ijs.0.64611-0.
- 3. Martin PAW, Shropshire ADS, Gundersen-Rindal D, Blackburn M. 2007. *Chromobacterium subtsugae* sp. nov. and use for control of insect pests. U.S. Patent 7,244,607.
- Auch AF, Klenk H-P, Göker M. 2010. Standard operating procedure for calculating genome-to-genome distances based on high-scoring segment pairs. Stand Genomic Sci 2:142–148. http://dx.doi.org/10.4056/ sigs.541628.

- Auch AF, von Jan M, Klenk H-P, Göker M. 2010. Digital DNA-DNA hybridization for microbial species delineation by means of genome-togenome sequence comparison. Stand Genomic Sci 2:117–134. http:// dx.doi.org/10.4056/sigs.531120.
- Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. 2013. Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14:60. http://dx.doi.org/ 10.1186/1471-2105-14-60.
- Frederiksen RF, Yoshimura Y, Storgaard BG, Paspaliari DK, Petersen BO, Chen K, Larsen T, Duus JØ, Ingmer H, Bovin NV, Westerlind U, Blixt O, Palcic MM, Leisner JJ. 2015. A diverse range of bacterial and eukaryotic chitinases hydrolyzes the LacNAc (Galbeta1-4GlcNAc) and LacdiNAc (GalNAcbeta1-4GlcNAc) motifs found on vertebrate and insect cells. J Biol Chem 290:5354–5366. http://dx.doi.org/10.1074/ jbc.M114.607291.
- Soby SD, Gadagkar SR, Contreras C, Caruso FL. 2013. Chromobacterium vaccinii sp. nov., isolated from native and cultivated cranberry (Vaccinium macrocarpon Ait.) bogs and irrigation ponds. Int J Syst Evol Microbiol 63:1840–1846. http://dx.doi.org/10.1099/ijs.0.045161-0.
- Vöing K, Harrison A, Soby SD. 2015. Draft genome sequence of *Chromobacterium vaccinii*, a potential biocontrol agent against mosquito (*Aedes aegypti*) larvae. Genome Announc 3(3):e00477-15. http://dx.doi.org/ 10.1128/genomeA.00477-15.