



Draft Genome Sequence of *Chromobacterium subtsugae* MWU12-2387 Isolated from a Wild Cranberry Bog in Truro, Massachusetts

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ABSTRACT *Chromobacterium subtsugae* MWU12-2387 was isolated from the rhizosphere of cranberry plants. While it is unknown what environmental role these bacteria play in bog soils, they hold potential as biological control agents against nematodes and insect pests. Potential virulence genes were identified, including the violacein synthesis pathway, siderophores, and several chitinases.

Chromobacterium subtsugae strain MWU12-2387 was isolated from the roots of wild cranberry plants in Truro, MA, and tentatively identified as *C. subtsugae* by phenotype and by 16S rRNA sequence (1–3). *C. subtsugae* has insecticidal properties, most likely due to multiple virulence mechanisms (4). Its genome was sequenced at the Arizona State University CLAS Genomics Core facility using an Illumina MiSeq. Genomic DNA was sheared to approximately 600-bp fragments using the Covaris M220 ultrasonicator, and Illumina libraries were generated on an Apollo 384 liquid handler (Wafergen) using a Kapa Biosystems library preparation kit (catalog no. KK8201). DNA fragments were end-repaired and A-tailed as described in the Kapa protocol. Combined indexes/adapters (catalog no. 520999; Bioo) were ligated onto each sample and multiplexed into one lane. Adapter-ligated molecules were cleaned using AMPure beads (catalog no. A63883; Agencourt Bioscience/Beckman Coulter, Inc.) and amplified with Kapa HIFI enzyme. Libraries were analyzed on an Agilent Bioanalyzer and quantified by quantitative PCR (qPCR) (catalog no. KK4835; Kapa library quantification kit) before multiplex pooling and sequencing in a 2 × 300 paired-end (PE) flow cell on the MiSeq platform (Illumina). Adapters were computationally segregated and trimmed in the Illumina BaseSpace pipeline. The Velvet assembly tool (BaseSpace) was used for signal processing and partial sequence assembly. The sequence is 64.8% G + C and consists of 4,788,922 bp distributed over 243 scaffolds, 129 of which are larger than 1 kbp. The largest contig is 184,525 bp, the N_{50} is 89,418 bp, and the N_{75} is 42,761 bp, with a sequence coverage of 45.75×. The isolate MWU12-2387 genome sequence was compared to reference genomes of *Chromobacterium violaceum* (ATCC 12472), *Chromobacterium haemolyticum* (T124), *Chromobacterium vaccinii* (MWU205), *Chromobacterium pseudoviolaceum* (LMG 3953), *Chromobacterium aquaticum* (CC-SEYA-1), 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 by pairing up homologous segments (5–7). The MWU12-2387 genome was 90% homologous to a *C. subtsugae* reference genome, confirming it as a member of this species.

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

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contribute to insect toxicity, including production of the pigment violacein (8), homologs of *Mycobacterium* virulence operons (9), nonribosomal peptide synthesis siderophores, hydrogen cyanide (10), type III secretion system-associated effectors, chitin binding protein, and secreted chitinases (11, 12). MWU2387 contains 15 probable chitinase genes, including four probable chitinase A genes and 10 endochitinases.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number [MQZZ00000000](https://doi.org/10.1093/nar/gkz000). The version described in this paper is version MQZZ01000000.

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REFERENCES

- Martin PAW, 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. <https://doi.org/10.1099/ijs.0.64611-0>.
- Martin PAW, Shropshire ADS, Gundersen-Rindal D, Blackburn M. 2007. *Chromobacterium subtsugae* sp. nov. and use for control of insect pests. US patent 7,244,607.
- 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. <https://doi.org/10.1128/genomeA.00998-15>.
- Martin PAW, Blackburn M. 2008. Characterization of the insecticidal activity of *Chromobacterium subtsugae*. *Biopestic, Int* 4:102–109.
- 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. <https://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-to-genome sequence comparison. *Stand Genomic Sci* 2:117–134. <https://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. <https://doi.org/10.1186/1471-2105-14-60>.
- Durán N, Justo GZ, Ferreira CV, Melo PS, Cordi L, Martins D. 2007. Violacein: properties and biological activities. *Biotechnol Appl Biochem* 48:127–133. <https://doi.org/10.1042/BA20070115>.
- Forrellad MA, Klepp LI, Gioffré A, Sabio y García J, Morbidoni HR, de la Paz Santangelo M, Cataldi AA, Bigi F. 2013. Virulence factors of the *Mycobacterium tuberculosis* complex. *Virulence* 4:3–66. <https://doi.org/10.4161/viru.22329>.
- Rodgers PB, Knowles CJ. 1978. Cyanide production and degradation during growth of *Chromobacterium violaceum*. *J Gen Microbiol* 108: 261–267. <https://doi.org/10.1099/00221287-108-2-261>.
- 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 (Gal β 1-4GlcNAc) and LacdiNAc (GalNAc β 1-4GlcNAc) motifs found on vertebrate and insect cells. *J Biol Chem* 290:5354–5366. <https://doi.org/10.1074/jbc.M114.607291>.
- Chernin LS, Winson MK, Thompson JM, Haran S, Bycroft BW, Chet I, Williams P, Stewart G. 1998. Chitinolytic activity in *Chromobacterium violaceum*: substrate analysis and regulation by quorum sensing. *J Bacteriol* 180:4435–4441.