

Draft Genome Sequence of Gephyronic Acid Producer *Cystobacter violaceus* Strain Cb vi76

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A draft genome sequence of *Cystobacter violaceus* strain Cb vi76, which produces the eukaryotic protein synthesis inhibitor gephyronic acid, has been obtained. The genome contains numerous predicted secondary metabolite clusters, including the gephyronic acid biosynthetic pathway. This genome will contribute to the investigation of secondary metabolism in other *Cystobacter* strains.

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Myxobacteria are an excellent source of structurally diverse, bioactive natural products (1, 2). Isolated from a soil sample with plant residues near Mitgamr, Egypt, in 2007, *Cystobacter violaceus* strain Cb vi76, DSM 14727, produces the cytotoxic polyketide natural product gephyronic acid, which is a potent and selective inhibitor of eukaryotic protein synthesis (3–6). Herein we present a draft genome sequence of the strain collected in our efforts to determine the biosynthetic pathway for gephyronic acid.

C. violaceus genomic DNA was sequenced using a Roche 454 GS FLX sequencer with a combination of shotgun sequencing and 3-kb paired-end sequencing at the Genomics and Bioinformatics Core Facility at the University of Notre Dame. Shotgun Titanium sequencing yielded 552,177 reads, which were assembled using the Roche Newbler Assembler version 2.3 into 431 contigs comprising 12,505,879 bp in total (13× coverage). An additional 2× coverage was supplied utilizing paired-end sequences that were mapped to the shotgun genome sequence to fill gaps and orient contigs. Based on this assembly, we generated the *C. violaceus* Cb vi76 draft genome sequence consisting of 12,570,057 bp distributed among 83 scaffolds with a GC content of 68.9%, respectively totaling an approximate 15× coverage of the genome.

The *C. violaceus* genome was analyzed and annotated using RAST version 4.0 (7) and the NCBI Prokaryotic Genomes Annotation Pipeline (PGAP) (http://www.ncbi.nlm.nih.gov/genome/annotation_prok/). Analysis of the unclosed draft genome sequence for *C. violaceus* provided an estimated genome size of 12.57 Mbp. The 431 contigs contain 8,201 putative coding sequences (CDS). Genes are evenly distributed between the forward (51.2%) and reverse (48.8%) strands. The average length of the CDSs is 1,065 bp, and 50.1% of the CDSs encode proteins whose functions are unknown. In addition to the identified protein functionalities, single 5S, 16S, and 23S rRNA genes were annotated utilizing RNAmmer (8). The search server, tRNAscan-SE, annotated 79 tRNA genes representing all 20 common amino acids (9).

Additional genome analysis with antiSMASH version 1.1.0 predicted several secondary metabolite biosynthetic gene clusters, including biosynthetic pathways for 5 lantibiotics, 2 bacteriocins, 6 terpenes, 4 polyketides, 7 nonribosomal peptides, and 8 polyketide/nonribosomal peptide hybrids (10, 11). All predicted secondary metabolite gene clusters for polyketide synthase and/or nonribosomal peptide synthetase are of unknown structure, except the gephyronic acid (6). Further inspection of the proposed gene clusters revealed that several are in fact gene cluster fragments, befitting of the draft quality of the *C. violaceus* genome sequence.

The breadth of proposed biosynthetic enzymes with unknown cognate natural products as well as the known gephyronic acid biosynthetic pathway exemplify the potential access to natural products intrinsic to myxobacteria such as *C. violaceus*. We believe the draft genome sequence will help facilitate future work for further investigation of secondary metabolism in myxobacteria.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession number [JPMI000000000](https://www.ncbi.nlm.nih.gov/nuccore/JPMI000000000). The version described in this paper is the first version, JPMI01000000.

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REFERENCES

- Wenzel SC, Müller R. 2009. The impact of genomics on the exploitation of the myxobacterial secondary metabolome. *Nat. Prod. Rep.* 26:1385–1407. <http://dx.doi.org/10.1039/b817073h>.
- Schäberle TF, Lohr F, Schmitz A, König GM. 2014. Antibiotics from myxobacteria. *Nat. Prod. Rep.* 31:953–972. <http://dx.doi.org/10.1039/c4np00011k>.
- Sasse F, Steinmetz H, Höfle G, Reichenbach H. 1995. Gephyronic acid,

- a novel inhibitor of eukaryotic protein synthesis from *Archangium gephyra* (myxobacteria): production, isolation, physico-chemical and biological properties, and mechanism of action. *J. Antibiot.* 48:21–25. <http://dx.doi.org/10.7164/antibiotics.48.21>.
4. Nicolas L, Anderl T, Sasse F, Steinmetz H, Jansen R, Höfle G, Laschat S, Taylor RE. 2011. Gephyronic acid, a missing link between polyketide inhibitors of eukaryotic protein synthesis (part I): structural revision and stereochemical assignment of gephyronic acid. *Angew. Chem. Int. Ed.* 50:938–941. <http://dx.doi.org/10.1002/anie.201005530>.
 5. Anderl T, Nicolas L, Münkemer J, Baro A, Sasse F, Steinmetz H, Jansen R, Höfle G, Taylor RE, Laschat S. 2011. Gephyronic acid, a missing link between polyketide inhibitors of eukaryotic protein synthesis (part II): total synthesis of gephyronic acid. *Angew. Chem. Int. Ed.* 50:942–955. <http://dx.doi.org/10.1002/anie.201005605>.
 6. Young J, Stevens DC, Carmichael R, Tan J, Rachid S, Boddy CN, Müller R, Taylor RE. 2013. Elucidation of gephyronic acid biosynthetic pathway revealed unexpected SAM-dependent methylations. *J. Nat. Prod.* 76: 2269–2276. <http://dx.doi.org/10.1021/np400629v>.
 7. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards 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. <http://dx.doi.org/10.1186/1471-2164-9-75>.
 8. Lagesen K, Hallin P, Rødland EA, Staerfeldt HH, Rognes T, Ussery DW. 2007. RNAMmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res.* 35:3100–3108. <http://dx.doi.org/10.1093/nar/gkm160>.
 9. Lowe TM, Eddy SR. 1997. tRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* 25:955–964. <http://dx.doi.org/10.1093/nar/25.5.0955>.
 10. Medema MH, Blin K, Cimermancic P, de Jager V, Zakrzewski P, Fischbach MA, Weber T, Takano E, Breitling R. 2011. antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic Acids Res.* 39:W339–W346. <http://dx.doi.org/10.1093/nar/gkr466>.
 11. Boddy CN. 2014. Bioinformatics tools for genome mining of polyketide and non-ribosomal peptides. *J. Ind. Microbiol. Biotechnol.* 41:443–450. <http://dx.doi.org/10.1007/s10295-013-1368-1>.