AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements

Draft Genome Sequence of *Streptomyces vitaminophilus* ATCC 31673, a Producer of Pyrrolomycin Antibiotics, Some of Which Contain a Nitro Group

Kristina M. Mahan,[a] Dawn M. Klingeman,[a] Robert L. Hettich,[b] Ronald J. Parry,[c] David E. Graham[a]

Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA[a]; Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA[b]; Department of Chemistry and Department of Biochemistry and Cell Biology, Rice University, Houston, Texas, USA[c]

Streptomyces vitaminophilus **produces pyrrolomycins, which are halogenated polyketide antibiotics. Some of the pyrrolomycins contain a rare nitro group located on the pyrrole ring. The 6.5-Mbp genome encodes 5,941 predicted protein-coding sequences in 39 contigs with a 71.9% G+C content.**

Received 16 November 2015 **Accepted** 27 November 2015 **Published** 21 January 2016

Citation Mahan KM, Klingeman DM, Hettich RL, Parry RJ, Graham DE. 2016. Draft genome sequence of *Streptomyces vitaminophilus* ATCC 31673, a producer of pyrrolomycin antibiotics, some of which contain a nitro group. Genome Announc 4(1):e01582-15. doi:10.1128/genomeA.01582-15.

Address correspondence to David E. Graham, grahamde@ornl.gov.

Streptomyces vitaminophilus (formerly *Actinosporangium vitaminophilum*) is one of several species of *Streptomyces* that are known to produce a family of halogenated antibiotics called the pyrrolomycins (1–6). These compounds exhibit potent antibiotic activity against Gram-positive bacteria, and they inhibit substance P-induced release of myeloperoxidase from human polymorphonuclear leukocytes (7). The most unusual structural feature of the pyrrolomycins is the presence of a nitro group located on the pyrrole ring in some of the antibiotics (5, 8). Natural products that contain nitro groups are uncommon, and relatively little is known about the biochemistry of nitro group formation (9). Although the pyrrolomycin biosynthetic gene clusters have been cloned from *Streptomyces vitaminophilus* and *Streptomyces* sp. UC 11065, sequence analysis of the gene clusters did not reveal the mechanism for nitro group formation in these antibiotics (10).

An Illumina TruSeq paired-end library was prepared with an insert size of approximately 584 bp and sequenced using an Illumina MiSeq instrument (390-fold coverage). Pacific Biosciences single-molecule, real-time DNA sequencing was performed by the University of Maryland School of Medicine Genomics Resource Center using P4-C2 chemistry (44-fold coverage). The genome sequence was assembled from trimmed and corrected Illumina data and preassembled Pacific Biosciences reads using the SPAdes genome assembler version 3.6.1 (11) and PBJelly software version 18.5.24 (12). The assembled genome sequence was polished using merged paired-end Illumina data with Bowtie2 version 2.2.5 (13) and Pilon software version 1.13 (14).

The draft genome sequence of *Streptomyces vitaminophilus* ATCC 31673 included 6,549,812 bp with a G+C content of 71.9%. The assembled genome comprises 39 contigs with an N_{50} of 249,406 bp and an L_{50} of 8 contigs. Coding DNA sequences (CDSs) were identified and annotated by the NCBI Prokaryotic Genome Annotation Pipeline. The genome was predicted to contain 5,941 CDSs, 56 tRNAs, two 16S rRNAs, two 23S rRNAs, and four 5S rRNAs.

A 227-kbp contig includes the entire 56-kbp pyrrolomycin biosynthetic gene cluster previously deposited to GenBank (accession number EF140901.1) (10). There are no recognizable homologs of nitric oxide synthases or previously characterized *N*-oxygenases; however, the genome encodes 15 cytochrome P450 homologs, two assimilatory nitrate reductases, and one nitrite reductase, which could be involved in bionitration.

Previous studies have shown that nitric oxide synthase inhibitors do not adversely affect nitrated pyrrolomycin biosynthesis in *Streptomyces fumanus*, suggesting a completely novel bionitration reaction (15). AntiSMASH version 3.0 (16) predicted 27 biosynthetic gene clusters, including genes for nonribosomal peptide synthases, type I, II, and III polyketide synthases, pyrrolomycins, siderophores, terpenes, lantipeptides, and even a lassopeptide. *Streptomyces vitaminophilus* ATCC 31673 produces the pyrrolomycins A–D, of which pyrrolomycins A and B contain a nitro group. Few enzymes involved in nitro group formation have been identified. The availability of the *Streptomyces vitaminophilus* genome and pyrrolomycin biosynthetic gene cluster sequences will facilitate the future identification of bionitration enzymes and add to the knowledge about nitro group formation.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number LLZU00000000. The version described in this paper is the first version, LLZU01000000.

ACKNOWLEDGMENTS

This work was supported by the Strategic Environmental Research and Development Program (SERDP) under project WP-2332. Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract no. DE-AC05-00OR22725.

FUNDING INFORMATION

The Strategic Environmental Research and Development Program (SERDP) provided funding to Robert L. Hettich, Ronald J. Parry, and David E. Graham under grant number WP-2332.

REFERENCES

1. **Shomura T, Amano S, Yoshida J, Ezaki N, Ito T, Niida T**. 1983. *Actinosporangium vitaminophilum* sp. nov. Int J Syst Bacteriol **33:**557–564. http://dx.doi.org/10.1099/00207713-33-3-557.
2. **Goodfellow M, Williams ST, Alderson G**. 1986. Transfer of *Actinosporangium violaceum* Krasil'nikov and Yuan, *Actinosporangium vitaminophilum* Shomura et al. and *Actinopycnidium caeruleum* Krasil'nikov to the genus *Streptomyces*, with amended descriptions of the species. Syst Appl Microbiol **8:**61–64. http://dx.doi.org/10.1016/S0723-2020(86)80149-7.
3. **Conder GA, Zielinski RJ, Johnson SS, Kuo MT, Cox DL, Marshall VP, Haber CL, Diroma PJ, Nelson SJ, Conklin RD, Lee BL, Geary TG, Rothwell JT, Sangster NC**. 1992. Anthelmintic activity of dioxapyrrolomycin. J Antibiot (Tokyo) **45:**977–983. http://dx.doi.org/10.7164/antibiotics.45.977.
4. **Ezaki N, Koyama M, Shomura T, Tsuruoka T, Inouye S**. 1983. Pyrrolomycins C, D and E, new members of pyrrolomycins. J Antibiot (Tokyo) **36:**1263–1267. http://dx.doi.org/10.7164/antibiotics.36.1263.
5. **Ezaki N, Shomura T, Koyama M, Niwa T, Kojima M, Inouye S, Ito T, Niida T**. 1981. New chlorinated nitro-pyrrole antibiotics, pyrrolomycin A and B (SF-2080 A and B). J Antibiot (Tokyo) **34:**1363–1365. http://dx.doi.org/10.7164/antibiotics.34.1363.
6. **Koyama M, Kodama Y, Tsuruoka T, Ezaki N, Niwa T, Inouye S**. 1981. Structure and synthesis of pyrrolomycin A, a chlorinated nitro-pyrrole antibiotic. J Antibiot (Tokyo) **34:**1569–1576. http://dx.doi.org/10.7164/antibiotics.34.1569.
7. **Masuda K, Suzuki K, Ishida-Okawara A, Mizuno S, Hotta K, Miyadoh S, Hara O, Koyama M**. 1991. Pyrrolomycin group antibiotics inhibit substance P-induced release of myeloperoxidase from human polymorphonuclear leukocytes. J Antibiot (Tokyo) **44:**533–540. http://dx.doi.org/10.7164/antibiotics.44.533.
8. **Carter GT, Nietsche JA, Goodman JJ, Torrey MJ, Dunne TS, Siegel MM, Borders DB**. 1989. Direct biochemical nitration in the biosynthesis of dioxapyrrolomycin. A unique mechanism for the introduction of nitro groups in microbial products. J Chem Soc Chem Commun **17:**1271–1273. http://dx.doi.org/10.1039/C39890001271.
9. **Parry R, Nishino S, Spain J**. 2011. Naturally-occurring nitro compounds. Nat Prod Rep **28:**152–167. http://dx.doi.org/10.1039/C0NP00024H.
10. **Zhang X, Parry RJ**. 2007. Cloning and characterization of the pyrrolomycin biosynthetic gene clusters from *Actinosporangium vitaminophilum* ATCC 31673 and *Streptomyces* sp. strain UC 11065. Antimicrob Agents Chemother **51:**946–957. http://dx.doi.org/10.1128/AAC.01214-06.
11. **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. http://dx.doi.org/10.1089/cmb.2012.0021.
12. **English AC, Richards S, Han Y, Wang M, Vee V, Qu J, Qin X, Muzny DM, Reid JG, Worley KC, Gibbs RA**. 2012. Mind the gap: upgrading genomes with Pacific Biosciences RS long-read sequencing technology. PLoS One **7:**e47768. http://dx.doi.org/10.1371/journal.pone.0047768.
13. **Langmead B, Salzberg SL**. 2012. Fast gapped-read alignment with bowtie 2. Nat Methods **9:**357–U354. http://dx.doi.org/10.1038/nmeth.1923.
14. **Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM**. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One **9:**e112963. http://dx.doi.org/10.1371/journal.pone.0112963.
15. **Ratnayake AS, Haltli B, Feng X, Bernan VS, Singh MP, He H, Carter GT**. 2008. Investigating the biosynthetic origin of the nitro group in pyrrolomycins. J Nat Prod **71:**1923–1926. http://dx.doi.org/10.1021/np800401h.
16. **Weber T, Blin K, Duddela S, 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. http://dx.doi.org/10.1093/nar/gkv437.