



Draft Genome Sequence of *Streptomyces* sp. Strain JV178, a Producer of Clifednamide-Type Polycyclic Tetramate Macrolactams

Yunci Qi^a, John M. D'Alessandro^a,  Joshua A. V. Blodgett^a

^aDepartment of Biology, Washington University in St. Louis, St. Louis, Missouri, USA

ABSTRACT Here, we report the draft genome sequence of *Streptomyces* sp. JV178, a strain originating from Connecticut (USA) garden soil. This strain produces the polycyclic tetramate macrolactam compounds clifednamides A and B. The draft genome contains 10.65 Mb, 9,045 predicted protein coding sequences, and several natural product biosynthetic loci.

Members of the *Streptomyces* genus of Gram-positive filamentous bacteria produce diverse secondary metabolites, including two-thirds of clinical antibiotics (1). *Streptomyces* genomes have been shown to contain a rich repertoire of small-molecule-encoding biosynthetic loci (2). A high proportion of sequenced *Streptomyces* strains encode the biosynthesis of polycyclic tetramate macrolactams (PTMs) (3). *Streptomyces* sp. strain JV178 was isolated from a Connecticut (USA) garden soil sample during a screen to discover new PTM compounds (4). We have sequenced its genome to further understand clifednamide-type PTM biogenesis.

Strain JV178 was grown in Trypticase soy broth supplemented with 0.6% glycine at 28°C. Genomic DNA was extracted by phenol-chloroform extraction as described elsewhere (5). Sequencing was performed on a MiSeq platform (2 × 301-bp reads [Illumina, Inc., San Diego, CA, USA]) on a paired-end library prepared using a high-throughput library preparation kit (Kapa Biosystems). Adapter sequences and low-quality reads (quality score < 0.05) were removed using CLC Genomics Workbench (CLC Bio-Qiagen, Aarhus, Denmark). The trimmed reads totaled 3,064 Mb, corresponding to approximately 287-fold coverage. *De novo* genome assembly was performed in CLC Genomics Workbench, and resulting contigs having less than 200 bp or 3× average coverage were discarded. The resulting draft genome contains 10,650,097 bp in 759 contigs (N_{50} , 540,451 bp), with a G+C content of 71.0%. Gene prediction and annotation by the Rapid Annotations using Subsystems Technology version 2.0 pipeline (6, 7) predicted 9,045 protein coding sequences and 126 RNA genes.

Automated secondary metabolism analysis using antiSMASH version 4.0 (8) and PRISM version 3.0 (9) predicted 39 biosynthetic gene clusters. Six of these matched known clusters for concanamycin (10), coronafacic acid (11), marineosins (12), melanin, ectoine, and anticapsin (13). Using this sequence, the clifednamide biosynthetic cluster was also found (14), along with clusters predicted to encode five terpenes, three polyketides, three nonribosomal peptides, eight hybrid polyketide/nonribosomal peptides, four siderophores, five lantipeptides, a single lasso peptide, and three bacteriocins. Multilocus sequence alignment using *atpD*, *gyrB*, *recA*, *rpoB*, and *trpB* concatenates (15) identified the closest sequenced relative of strain JV178 as *Streptomyces torulosus* strain NRRL B-3889, consistent with prior 16s rRNA gene analysis (3). These results place this strain in the *S. scabiei* clade (16), which contains several plant-associated *Streptomyces* species, including the potato pathogen *S. scabiei* 87.22 (17). While we failed to detect homologs of known pathogenicity loci, such as *txtAB* (18), eight genes were

Received 7 November 2017 Accepted 14 November 2017 Published 4 January 2018

Citation Qi Y, D'Alessandro JM, Blodgett JAV. 2018. Draft genome sequence of *Streptomyces* sp. strain JV178, a producer of clifednamide-type polycyclic tetramate macrolactams. Genome Announc 6:e01401-17. <https://doi.org/10.1128/genomeA.01401-17>.

Copyright © 2018 Qi et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Joshua A. V. Blodgett, jblodgett@wustl.edu.

predicted to encode the plant-growth hormone auxin. The absence of recognizable pathogenicity loci and the predicted ability to produce the plant modulators concanamycin (10), coronafacic acid (11), and auxin suggest a plant-associated but non-pathogenic lifestyle. These observations suggest potential roles for the clifednamides, whose targets are still unknown.

Accession number(s). The draft genome sequence of *Streptomyces* sp. strain JV178 was deposited in DDBJ/ENA/GenBank under the accession number [PEKU00000000](https://doi.org/10.1093/nar/gkx437). The version described in this paper is the first version, PEKU01000000.

ACKNOWLEDGMENTS

The genome of *Streptomyces* sp. strain JV178 was sequenced with the help of Chris Shaffer and the McDonnell Genome Institute at Washington University in St. Louis. We also thank Michael Guzman (WUSTL) for assistance and advice with the assembly.

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sector.

REFERENCES

- Challis GL, Hopwood DA. 2003. Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by *Streptomyces* species. *Proc Natl Acad Sci U S A* 100:14555–14561. <https://doi.org/10.1073/pnas.1934677100>.
- Nett M, Ikeda H, Moore BS. 2009. Genomic basis for natural product biosynthetic diversity in the actinomycetes. *Nat Prod Rep* 26:1362–1384. <https://doi.org/10.1039/b817069j>.
- Blodgett JA, Oh DC, Cao S, Currie CR, Kolter R, Clardy J. 2010. Common biosynthetic origins for polycyclic tetramate macrolactams from phylogenetically diverse bacteria. *Proc Natl Acad Sci U S A* 107:11692–11697. <https://doi.org/10.1073/pnas.1001513107>.
- Cao S, Blodgett JAV, Clardy J. 2010. Targeted discovery of polycyclic tetramate macrolactams from an environmental *Streptomyces* strain. *Org Lett* 12:4652–4654. <https://doi.org/10.1021/ol1020064>.
- Blodgett JAV, Zhang JK, Metcalf WW. 2005. Molecular cloning, sequence analysis, and heterologous expression of the phosphinothricin tripeptide biosynthetic gene cluster from *Streptomyces viridochromogenes* DSM 40736. *Antimicrob Agents Chemother* 49:230–240. <https://doi.org/10.1128/AAC.49.1.230-240.2005>.
- 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. <https://doi.org/10.1186/1471-2164-9-75>.
- Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res* 42:D206–D214. <https://doi.org/10.1093/nar/gkt1226>.
- Weber T, Blin K, Duddela S, Krug D, Kim HU, Brucoleri 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. <https://doi.org/10.1093/nar/gkv437>.
- Skinninger MA, Merwin NJ, Johnston CW, Magarvey NA. 2017. PRISM 3: expanded prediction of natural product chemical structures from microbial genomes. *Nucleic Acids Res* 45:W49–W54.
- Haydock SF, Appleyard AN, Mironenko T, Lester J, Scott N, Leadlay PF. 2005. Organization of the biosynthetic gene cluster for the macrolide concanamycin A in *Streptomyces neyagawaensis* ATCC 27449. *Microbiology* 151:3161–3169. <https://doi.org/10.1099/mic.0.28194-0>.
- Bignell DRD, Seipke RF, Huguet-Tapia JC, Chambers AH, Parry RJ, Loria R. 2010. *Streptomyces scabies* 87–22 contains a coronafacic acid-like biosynthetic cluster that contributes to plant–microbe interactions. *Mol Plant Microbe Interact* 23:161–175. <https://doi.org/10.1094/MPMI-23-2-0161>.
- Salem SM, Kancharla P, Florova G, Gupta S, Lu W, Reynolds KA. 2014. Elucidation of final steps of the marineosins biosynthetic pathway through identification and characterization of the corresponding gene cluster. *J Am Chem Soc* 136:4565–4574. <https://doi.org/10.1021/ja411544w>.
- Mahlstedt SA, Walsh CT. 2010. Investigation of anticapsin biosynthesis reveals a four-enzyme pathway to tetrahydrotyrosine in *Bacillus subtilis*. *Biochemistry* 49:912–923. <https://doi.org/10.1021/bi9021186>.
- Qi Y, Ding E, Blodgett J. 2017. Native and engineered clifednamide biosynthesis in multiple *Streptomyces* spp. *bioRxiv*. <https://doi.org/10.1101/197616>.
- Rong X, Huang Y. 2010. Taxonomic evaluation of the *Streptomyces griseus* clade using multilocus sequence analysis and DNA–DNA hybridization, with proposal to combine 29 species and three subspecies as 11 genomic species. *Int J Syst Evol Microbiol* 60:696–703. <https://doi.org/10.1099/ijs.0.012419-0>.
- Goodfellow M. 2012. Phylum XXVI. *Actinobacteria* phyl. nov., p 33. In Goodfellow M, Kämpfer P, Busse H-J, Trujillo ME, Suzuki K, Wolfgang L, Whitman WB (ed), *Bergey's manual of systematic bacteriology*. Springer, New York, NY.
- Lerat S, Simao-Beunoir AM, Beaulieu C. 2009. Genetic and physiological determinants of *Streptomyces scabies* pathogenicity. *Mol Plant Pathol* 10:579–585. <https://doi.org/10.1111/j.1364-3703.2009.00561.x>.
- Healy FG, Wach M, Krasnoff SB, Gibson DM, Loria R. 2000. The *txtAB* genes of the plant pathogen *Streptomyces acidiscabies* encode a peptide synthetase required for phytotoxin thaxtomin A production and pathogenicity. *Mol Microbiol* 38:794–804. <https://doi.org/10.1046/j.1365-2958.2000.02170.x>.