# Draft Genome Sequence of the Novonestmycin-Producing Strain *Streptomyces* sp. Z26, Isolated from Potato Rhizosphere in Morocco

Anina Buchmann,[a] Carolina Cano-Prieto,[a,b] Ahmed Nafis,[c] Mustapha Barakate,[c] Mohamed Baz,[c] Lahcen Hassani,[c] Nico Ortlieb,[b,d,e] Timo H. J. Niedermeyer,[b,d,e] Harald Gross[a,b]

[a]Department of Pharmaceutical Biology, Pharmaceutical Institute, University of Tübingen, Tübingen, Germany
[b]German Centre for Infection Research (DZIF), Partner Site Tübingen, Tübingen, Germany
[c]Laboratory of Biology and Biotechnology of Microorganisms, Faculty of Sciences Semlalia, Cadi Ayyad University, Marrakech, Morocco
[d]Department of Microbiology and Biotechnology, Interfaculty Institute of Microbiology and Infection Medicine, University of Tübingen, Tübingen, Germany
[e]Department of Pharmaceutical Biology/Pharmacognosy, Institute of Pharmacy, University of Halle-Wittenberg, Halle (Saale), Germany

**ABSTRACT** *Streptomyces* sp. strain Z26 exhibited antifungal activity and turned out to be a producer of the secondary metabolites novonestmycin A and B. The 6.5-Mb draft genome gives insight into the complete secondary metabolite production capacity and builds the basis to find and locate the biosynthetic gene cluster encoding the novonestmycins.

As part of our ongoing efforts to investigate bioactive natural products from *Actinobacteria* (1–13), we isolated the strain *Streptomyces* sp. Z26 from potato rhizosphere in Morocco (14). It exhibited antibacterial as well as antifungal bioactivity (14), and a chemical analysis revealed that the strain produces the antifungal and cytotoxic natural products novonestmycins A and B (14, 15). In order to investigate the complete biosynthetic capacity for secondary metabolism and to locate, analyze, and prove the biosynthetic gene cluster of the novonestmycins, the sequencing of this strain was initiated.

Strain Z26 was cultivated for 4 days in 50 ml Trypticase soy broth (TSB) at 30°C under agitation (200 rpm). Genomic DNA (gDNA) extraction was performed using the Qiagen genomic-tip 100/G kit following the manufacturer's protocol.

The genome of Z26 was sequenced using a combined Illumina/PacBio sequencing approach. Upon Nextera XT paired-end library preparation, gDNA was first subjected to $2 \times 125$-bp paired-end sequencing with the Illumina HiSeq 2500 system. FASTQ sequence files were generated using the Illumina Casava pipeline v1.8.3. The initial quality assessment was based on data passing the Illumina chastity filtering. Subsequently, reads containing the PhiX control signal were removed. In addition, reads containing (partial) adapters were clipped (up to a minimum read length of 50 bp). The second quality assessment was based on the remaining reads using the FastQC quality control tool v0.10.0. The final quality statistics included 3,393,302 reads and an average quality score (Phred) of 37. A *de novo* assembly was performed using CLC Genomics Workbench v7.0.4 to yield contigs.

In addition, a 10-kb genomic sublibrary of strain Z26 was produced and sequenced with PacBio single-molecule real-time (SMRT) technology. The data collected were processed and filtered using the SMRT Analysis software suite v2.3.0. The continuous long read (CLR) data were filtered by read length (>35 bp), subread length (>35 bp), and read quality (>0.75). The final quality statistics included 159,783 reads with an average read length of 5,122 bp and a maximum read length of 38,306 bp. The Illumina-based contigs were aligned against the PacBio CLR reads using BLASR v1 (16).

Based on the alignment, contigs were placed into superscaffolds using the SSPACE-LongRead scaffolder v7.0.4 (17). The gapped regions within the superscaffolds were closed using GapFiller v1.10 (18). Software parameter settings were kept at the defaults, unless stated otherwise.

The final genome comprises three scaffolds with a total size of 6,501,581 bp and a G+C content of 73.4%. The assembled contigs were annotated with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) pipeline (19), yielding a total of 5,335 coding genes. Using autoMLST (http://automlst.ziemertlab.com), the overall genome analysis did not support the initial taxonomic classification of strain Z26 as *S. phytohabitans* (14); according to the average nucleotide identity (ANI) analysis, the closest related type strain species is *S. pini* NRRL-B-24728 (RefSeq assembly accession number GCF_900114215; ANI, 81.9%), while a multilocus sequence analysis revealed *S. oceani* SCSIO02100$^T$ (RefSeq assembly accession number GCF_001751245) as the closest related species. Further bioinformatic analyses using antiSMASH v4.0.2 (20) revealed that strain Z26 possesses, besides the putative novonestmycin gene cluster, 20 orphan natural product biosynthetic gene clusters.

**Data availability.** This whole-genome sequencing (WGS) project has been deposited at DDBJ/ENA/GenBank under the accession number RCHV00000000. Raw sequencing data sets have been registered in the NCBI SRA database under the accession number SRP167030.

## REFERENCES

1. Iftime D, Kulik A, Härtner T, Rohrer S, Niedermeyer THJ, Stegmann E, Weber T, Wohlleben W. 2016. Identification and activation of novel biosynthetic gene clusters by genome mining in the kirromycin producer *Streptomyces collinus* Tü 365. J Ind Microbiol Biotechnol 43:277–291. https://doi.org/10.1007/s10295-015-1685-7.

2. Spohn M, Edenhart S, Alanjary M, Ziemert N, Wibberg D, Kalinowski J, Niedermeyer THJ, Stegmann E, Wohlleben W. 2018. Identification of a novel aminopolycarboxylic acid siderophore gene cluster encoding the biosynthesis of ethylenediaminesuccinic acid hydroxyarginine (EDHA). Metallomics 10:722–734. https://doi.org/10.1039/c8mt00009c.

3. Zeyhle P, Bauer JS, Kalinowski J, Shin-Ya K, Gross H, Heide L. 2014. Genome-based discovery of a novel membrane-bound 1,6-dihydroxyphenazine prenyltransferase from a marine actinomycete. PLoS One 9:e99122. https://doi.org/10.1371/journal.pone.0099122.

4. Spohn M, Kirchner N, Kulik A, Jochim A, Wolf F, Muenzer P, Borst O, Gross H, Wohlleben W, Stegmann E. 2014. Overproduction of ristomycin A by activation of a silent gene cluster in *Amycolatopsis japonicum* MG417-CF17. Antimicrob Agents Chemother 58:6185–6196. https://doi.org/10.1128/AAC.03512-14.

5. Zeyhle P, Bauer JS, Steimle M, Leipoldt F, Rösch M, Kalinowski J, Gross H, Heide L. 2014. A membrane-bound prenyltransferase catalyzes the O-prenylation of 1,6-dihydroxyphenazine in the marine bacterium *Streptomyces* sp. CNQ-509. Chembiochem 15:2385–2392. https://doi.org/10.1002/cbic.201402394.

6. Buchmann A, Eitel M, Koch P, Schwarz PN, Stegmann E, Wohlleben W, Wolanski M, Krawiec M, Zakrzewska-Czerwińska J, Méndez C, Botas A, Núñez LE, Morís F, Cortés J, Gross H. 2016. High-quality draft genome sequence of the Actinobacterium *Nocardia terpenica* IFM 0406, producer of the immunosuppressant brasilicardins, using Illumina and PacBio technologies. Genome Announc 4:e01391-16. https://doi.org/10.1128/genomeA.01391-16.

7. Wolf F, Bauer JS, Bendel TM, Kulik A, Kalinowski J, Gross H, Kaysser L. 2017. Biosynthesis of the ß-lactone proteasome inhibitors belactosin and

cystargolide. Angew Chem Int Ed Engl 56:6665–6668. https://doi.org/10.1002/anie.201612076.

8. Jiao J, Paterson J, Busche T, Rückert C, Kalinowski J, Harwani D, Gross H. 2018. Draft genome sequence of *Streptomyces* sp. strain DH-12, a soilborne isolate from the Thar Desert with broad-spectrum antibacterial activity. Genome Announc 6:e00108-18. https://doi.org/10.1128/genomeA.00108-18.

9. Schwarz PN, Buchmann A, Roller L, Kulik A, Gross H, Wohlleben W, Stegmann E. 2018. The immunosuppressant brasilicardin: determination of the biosynthetic gene cluster in the heterologous host *Amycolatopsis japonicum*. Biotechnol J 13:1700527. https://doi.org/10.1002/biot.201700527.

10. Aziz S, Mast Y, Wohlleben W, Gross H. 2018. Draft genome sequence of the pristinamycin-producing strain *Streptomyces* sp. SW4, isolated from soil in Nusa Kambangan, Indonesia. Microbiol Resour Announc 7:e00912-18. https://doi.org/10.1128/MRA.00912-18.

11. Ortlieb N, Keilhofer N, Schrey SD, Gross H, Niedermeyer THJ. 2018. Draft genome sequence of the xanthocidin-producing strain *Streptomyces* sp. AcE210, isolated from a root nodule of *Alnus glutinosa* (L.). Microbiol Resour Announc 7:e01190-18. https://doi.org/10.1128/MRA.01190-18.

12. Baz M, Lahbabi D, Samri S, Val F, Hamelin G, Madore I, Bouarab K, Beaulieu C, Ennaji MM, Barakate M. 2012. Control potato soft rot caused by *Pectobacterium carotovorum* and *Pectobacterium atrosepticum* by Moroccan actinobacteria isolates. World J Microbiol Biotechnol 28:303–311. https://doi.org/10.1007/s11274-011-0820-5.

13. Samri SE, Baz M, Jamjari A, Aboussaid H, El Messoussi S, El Meziane A, Barakate M. 2015. Preliminary assessment of insecticidal activity of Moroccan Actinobacteria isolates against Mediterranean fruit fly (*Ceratitis capitata*). Afr J Biotechnol 14:859–866. https://doi.org/10.5897/AJB2014.14357.

14. Nafis A, Oubaha B, Elhidar N, Ortlieb N, Kulik A, Niedermeyer T, Hassani L, Barakate M. 2018. Novel production of two nonpolyenic antifungal macrolide derivatives by *Streptomyces* Z26 isolated from Moroccan rhi-

zospheric soil. Online J Biol Sci 18:176–185. https://doi.org/10.3844/ojbsci.2018.176.185.

15. Wan Z, Fang W, Shi L, Wang K, Zhang Y, Zhang Z, Wu Z, Yang Z, Gu Y. 2015. Novonestmycins A and B, two new 32-membered bioactive macrolides from *Streptomyces phytohabitans* HBERC-20821. J Antibiot 68: 185–190. https://doi.org/10.1038/ja.2014.123.

16. Chaisson MJ, Tesler G. 2012. Mapping single molecule sequencing reads using basic local alignment with successive refinement (BLASR): application and theory. BMC Bioinformatics 13:238. https://doi.org/10.1186/1471-2105-13-238.

17. Boetzer M, Pirovano W. 2014. SSPACE-LongRead: scaffolding bacterial draft genomes using long read sequence information. BMC Bioinformatics 15:211. https://doi.org/10.1186/1471-2105-15-211.

18. Boetzer M, Pirovano W. 2012. Toward almost closed genomes with Gap-Filler. Genome Biol 13:R56. https://doi.org/10.1186/gb-2012-13-6-r56.

19. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44: 6614–6624. https://doi.org/10.1093/nar/gkw569.

20. Blin K, Wolf T, Chevrette MG, Lu X, Schwalen CJ, Kautsar SA, Suarez Duran HG, de los Santos ELC, Kim HU, Nave M, Dickschat JS, Mitchell DA, Shelest E, Breitling R, Takano E, Lee SY, Weber T, Medema MH. 2017. antiSMASH 4.0—improvements in chemistry prediction and gene boundary identification. Nucleic Acids Res 45:W36–W41. https://doi.org/10.1093/nar/gkx319.