



Draft Genome Sequence of the Pristinamycin-Producing Strain *Streptomyces* sp. SW4, Isolated from Soil in Nusa Kambangan, Indonesia

Saefuddin Aziz,^{a,b} Yvonne Mast,^{c,d} Wolfgang Wohlleben,^{c,d}  Harald Gross^{a,d}

^aDepartment of Pharmaceutical Biology, Institute of Pharmaceutical Sciences, University of Tübingen, Tübingen, Germany

^bMicrobiology Department, Biology Faculty, Jenderal Soedirman University, Purwokerto, Indonesia

^cDepartment of Microbiology and Biotechnology, Interfaculty Institute of Microbiology and Infection Medicine (IMIT), University of Tübingen, Tübingen, Germany

^dGerman Centre for Infection Research (DZIF), Tübingen, Germany

ABSTRACT *Streptomyces* sp. strain SW4 exhibited broad-spectrum antibacterial activity toward Gram-positive and Gram-negative pathogens. The 7.5-Mb draft genome sequence gives insight into the complete secondary metabolite production capacity and reveals genes putatively responsible for its antibacterial activity.

Indonesia is recognized as a biodiversity hot spot with a high density of macroorganisms and microorganisms (1). Therefore, we hypothesized that Indonesia is a promising source for novel soil bacteria, particularly actinobacterial strains, which are historically known to be prolific antibiotic-producing strains and still represent an important source of chemical diversity and a reservoir to mine for novel chemical structures (2, 3). The discovery of bioactive natural products from Indonesian actinobacteria (4–9) supports this hypothesis.

Accordingly, as part of our ongoing screening program, strain *Streptomyces* sp. SW4 was isolated from soil of the Indonesian island Nusa Kambangan. It exhibited pronounced antibacterial properties in disk diffusion tests, conducted according to EUCAST guidelines (<http://www.eucast.org>), particularly toward *Escherichia coli*, *Bacillus subtilis*, *Streptococcus pneumoniae*, and *Pseudomonas fluorescens*. In order to clarify the active principle for its antibiotic activity and to determine the overall biosynthetic potential of SW4 to produce natural products, we aimed to obtain the whole-genome sequence of this strain.

The strain *Streptomyces* sp. SW4 was cultivated for 2 days in R5 medium (10) at 30°C and 180 rpm. Genomic DNA of SW4 was extracted and purified using the Genomic-tip 100/G kit (Qiagen). The isolation procedure was carried out following the standard protocol provided by the manufacturer. For cell lysis, 1.0 ml achromopeptidase was added to the cell lysate. Subsequently, a paired-end library was constructed and subjected to sequencing using a PacBio RS II sequencing platform. *De novo* assembly was performed utilizing Hierarchical Genome Assembly Process 3 (HGAP3), whose protocol relies on PreAssembler v1 for filtering, PreAssembler v2 and Assemble-Unitig v1 for assembly, BLASR v1 (11) for mapping, and Quiver v1 for consensus polishing using the only unambiguously mapped reads option. HGAP3 settings were kept at the defaults, except for the genome size estimate parameter, which was set to 8.0 Mbp. Overall, 169,628 filtered reads (N_{50} , 5,290 bp) were assembled to a 7,475,027-nucleotide draft genome at 82.6-fold coverage. The resulting draft genome sequence consists of 4 contigs in total, with a G+C content of 73.3%. The assembled contigs were annotated with the PGAP (12), yielding a total of 6,764

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Address correspondence to Harald Gross, harald.gross@uni-tuebingen.de.

predicted protein-coding sequences. The closest related type strain based on the 16S rRNA gene sequence (1,511 bp) is *Streptomyces leeuwenhoekii* (LN831790), with 99.0% sequence identity.

A combined manual and automated analysis for secondary metabolism, using antiSMASH 4.0 (13), predicted 18 biosynthetic gene clusters. Seven of these matched known clusters for the antibiotic pristinamycin (14, 15), specifically, the terpenoids hopene, albaflavenone, and isorenieratene (16–18), the siderophore desferrioxamine (19), the pigment melanin (20), and the osmolyte ectoine (21). The production of pristinamycins was experimentally confirmed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and high-resolution mass spectrometry (HR-MS) analysis (22). The remaining clusters were predicted to encode 1 terpene, 2 siderophores, 1 nonribosomal peptide, 4 polyketides, 1 indole, and 2 bacteriocins.

Thus, the genome sequence of strain SW4 provides the first insights into its antibiotic capabilities and builds the basis for further genome-driven isolation of bioactive natural products from strain SW4.

Data availability. This whole-genome sequencing (WGS) project and the partial 16S rRNA gene sequence have been deposited at DDBJ/ENA/GenBank under the accession numbers [QKWM00000000](#) and [MH517391](#), respectively.

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REFERENCES

- Myers N, Mittermeier RA, Mittermeier CG, da Fonseca G, Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403:853–858. <https://doi.org/10.1038/35002501>.
- Genilloud O. 2017. Actinomycetes: still a source of novel antibiotics. *Nat Prod Rep* 34:1203–1232. <https://doi.org/10.1039/C7NP00026J>.
- Jose PA, Jha B. 2016. New dimensions of research on actinomycetes: quest for next generation antibiotics. *Front Microbiol* 7:1295. <https://doi.org/10.3389/fmicb.2016.01295>.
- Fotso S, Mahmud T, Zabriskie TM, Santosa DA, Sulastri, Proteau PJ. 2008. Angucyclinones from an Indonesian *Streptomyces* sp. *J Nat Prod* 71:61–65. <https://doi.org/10.1021/np0704102>.
- Fotso S, Zabriskie TM, Proteau PJ, Flatt PM, Santosa DA, Sulastri, Mahmud T. 2009. Limazepines A-F, pyrrolo[1,4]benzodiazepine antibiotics from an Indonesian *Micrococcus* sp. *J Nat Prod* 72:690–695. <https://doi.org/10.1021/np800827w>.
- Fotso S, Santosa DA, Saraswati R, Yang J, Mahmud T, Zabriskie TM, Proteau PJ. 2010. Modified phenazines from an Indonesian *Streptomyces* sp. *J Nat Prod* 73:472–475. <https://doi.org/10.1021/np9005647>.
- Sheng Y, Fotso S, Serril JD, Shahab S, Santosa DA, Ishmael JE, Proteau PJ, Zabriskie TM, Mahmud T. 2015. Succinylated apoptolidins from *Amycolatopsis* sp. *ICBB* 8242. *Org Lett* 17:2526–2529. <https://doi.org/10.1021/acs.orglett.5b01055>.
- Sheng Y, Lam PW, Shahab S, Santosa DA, Proteau PJ, Zabriskie TM, Mahmud T. 2015. Identification of elaiophyllin skeletal variants from the Indonesian *Streptomyces* sp. *ICBB* 9297. *J Nat Prod* 78:2768–2775. <https://doi.org/10.1021/acs.jnatprod.5b00752>.
- Handayani I, Ratnakomala S, Lisdiyanti P, Fahrurrozi, Alanjary M, Wohlleben W, Mast Y. 2018. Complete genome sequence of *Streptomyces* sp. strain BSE7F, a Bali mangrove sediment actinobacterium with antimicrobial activities. *Genome Announc* 6:e00618-18. <https://doi.org/10.1128/genomeA.00618-18>.
- Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA. 2000. *Practical Streptomyces genetics*. The John Innes Foundation, Norwich, England.
- 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>.
- 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>.
- 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>.
- Mast Y, Weber T, Götz M, Ort-Winklbauer R, Gondran A, Wohlleben W, Schinko E. 2011. Characterization of the “pristinamycin supercluster” of *Streptomyces pristinaespiralis*. *Microbiol Biotechnol* 4:192–206. <https://doi.org/10.1111/j.1751-7915.2010.00213.x>.
- Mast Y, Wohlleben W. 2014. Streptogramins—two are better than one! *Int J Med Microbiol* 304:44–50. <https://doi.org/10.1016/j.ijmm.2013.08.008>.
- Ghimire GP, Koirala N, Sohng JK. 2015. Activation of cryptic *hop* genes from *Streptomyces peucetius* ATCC 27952 involved in hopanoid biosynthesis. *J Microbiol Biotechnol* 25:658–661. <https://doi.org/10.4014/jmb.1408.08058>.
- Zhao B, Lin X, Lei L, Lamb DC, Kelly SL, Waterman MR, Cane DE. 2008. Biosynthesis of the sesquiterpene antibiotic albaflavenone in *Streptomyces coelicolor* A3(2). *J Biol Chem* 283:8183–8189. <https://doi.org/10.1074/jbc.M710421200>.
- Krügel H, Krubasik P, Weber K, Saluz HP, Sandmann G. 1999. Functional analysis of genes from *Streptomyces griseus* involved in the synthesis of isorenieratene, a carotenoid with aromatic end groups, revealed a novel

- type of carotenoid desaturase. *Biochim Biophys Acta* 1439:57–64. [https://doi.org/10.1016/S1388-1981\(99\)00075-X](https://doi.org/10.1016/S1388-1981(99)00075-X).
19. Barona-Gómez F, Wong U, Giannakopoulos AE, Derrick PJ, Challis GL. 2004. Identification of a cluster of genes that directs desferrioxamine biosynthesis in *Streptomyces coelicolor* M145. *J Am Chem Soc* 126: 16282–16283. <https://doi.org/10.1021/ja045774k>.
 20. Ōmura S, Ikeda H, Ishikawa J, Hanamoto A, Takahashi C, Shinose M, Takahashi Y, Horikawa H, Nakazawa H, Osonoe T, Kikuchi H, Shiba T, Sakaki Y, Hattori M. 2001. Genome sequence of an industrial microorganism *Streptomyces avermitilis*: deducing the ability of producing secondary metabolites. *Proc Natl Acad Sci U S A* 98:12215–12220. <https://doi.org/10.1073/pnas.211433198>.
 21. Bursy J, Kuhlmann AU, Pittelkow M, Hartmann H, Jebbar M, Pierik AJ, Bremer E. 2008. Synthesis and uptake of the compatible solutes ectoine and 5-hydroxyectoine by *Streptomyces coelicolor* A3(2) in response to salt and heat stresses. *Appl Environ Microbiol* 74:7286–7296. <https://doi.org/10.1128/AEM.00768-08>.
 22. Boison J, Lee S, Gedir R. 2009. Analytical determination of virginiamycin drug residues in edible porcine tissues by LC-MS with confirmation by LC-MS/MS. *J AOAC Int* 92:329–339.