





Draft Genome Sequences of *Algoriphagus* sp. Strain PAP.12 and *Roseivirga* sp. Strain PAP.19, Isolated from Marine Samples from Papua, Indonesia

Riyanti,^a © Celine M. Zumkeller,^b © Marius Spohn,^{b,f} © Sanja Mihajlovic,^b © Oliver Schwengers,^{c,d} © Alexander Goesmann,^{c,d} Nur A. Choironi,^e © Till F. Schäberle,^{b,d,f} © Harwoko Harwoko^e

^aFaculty of Fisheries and Marine Science, Jenderal Soedirman University, Purwokerto, Indonesia

Riyanti and Celine M. Zumkeller contributed equally to this work. Author order was determined both alphabetically and in order of increasing seniority.

ABSTRACT Algoriphagus sp. strain PAP.12 (EXT111900) and *Roseivirga* sp. strain PAP.19 (EXT111901) were isolated from marine samples. Here, we report their draft genome sequences, 5.032 Mbp and 4.583 Mbp in size, respectively, and rate their specialized metabolite production capacity. Taxonomic ranks established by genome-based analysis indicate that *Algoriphagus* sp. strain PAP.12 represents a candidate new species.

The phylum *Bacteroidetes* is a proliferating but underexplored bioresource for natural product discovery (1, 2). The bacteria colonize diverse habitats and are among the most abundant groups of bacteria within marine ecosystems (3). In an effort to access new *Bacteroidetes* strains, strains PAP.12 and PAP.19 were isolated from Cenderawasih Bay National Park (Papua, Indonesia). The strains were deposited in the Fraunhofer strain collection (4) under the identifiers EXT111900 and EXT111901.

EXT111900 was retrieved from the upper layer (5 to 10 cm) of marine sediment (1°47'45.6"S, 134°25'12.0"E). EXT111901 was isolated from a sponge (1°48'03.6"S, 134° 06'03.6"E) by cutting using a diving knife while latex gloves were worn. The samples were put into sterile bags, brought to the surface to be kept at ambient seawater temperature, and processed within 2 hours after sampling, as follows. Sponge samples were cut into small pieces using a sterile scalpel, and all samples were rinsed with sterile seawater before being plated on artificial seawater (1.5% agar, 0.01% KBr, 2.3% NaCl, 1.1% MgCl₂·6H₂O, 0.1% CaCl₂·2H₂O, 0.1% KCl, 0.004% SrCl₂·6 H₂O, 0.4% Na₂SO₄, 0.02% NaHCO₃, and 0.003% H₃BO₃ in H₂O) with Escherichia coli prey and cycloheximide. Bacterial colonies were isolated, and sequencing samples were prepared by growing the strains aerobically for 24 h at 30°C in marine broth (product number CP73.1; Carl Roth GmbH). Cell pellets were resuspended in buffer ATL (Qiagen) containing RNase A. ZR BashingBead lysis tubes (Zymo Research) were used for cell disruption. DNA was isolated using QIAmp 96 DNA QIAcube highthroughput (HT) kits with the addition of proteinase K (Qiagen). Libraries for short-read sequencing were prepared using the Illumina DNA preparation tagmentation kit with 500 ng DNA as input and 5 cycles of indexing PCR. Library quality was evaluated (Agilent 2100 Bioanalyzer), and libraries were sequenced on an Illumina NovaSeq system using a NovaSeq 6000 SP v1 sequencing kit with 2×150 bp read length and a depth of 4.0 to 5.0 million reads per sample. For sequence processing and analysis, software tools were run with default settings unless otherwise stated. The sequencing was demultiplexed (Illumina

Editor Frank J. Stewart, Montana State University

Copyright © 2023 Riyanti et al. This is an openaccess article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Till F. Schäberle, till.schaeberle@ime.fraunhofer.de, or Harwoko Harwoko, harwoko.unsoed@gmail.com.

The authors declare no conflict of interest.

Received 6 December 2022 **Accepted** 22 February 2023 **Published** 16 March 2023

^bBranch for Bioresources, Fraunhofer Institute for Molecular Biology and Applied Ecology, Giessen, Germany

^cBioinformatics and Systems Biology, Justus Liebig University Giessen, Giessen, Germany

dGerman Center for Infection Research, Partner Site Giessen-Marburg-Langen, Giessen, Germany

eDepartment of Pharmacy, Faculty of Health Sciences, Jenderal Soedirman University, Purwokerto, Indonesia

fInstitute for Insect Biotechnology, Justus Liebig University Giessen, Giessen, Germany

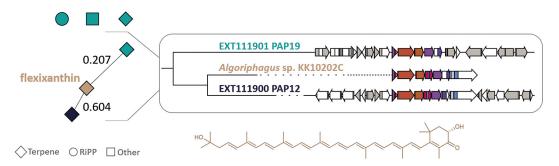


FIG 1 BiG-SCAPE network (left) of strains EXT111901 (turquoise) and EXT111900 (dark blue). BGCs sharing similarity with the flexixanthin MIBiG cluster were aligned (right). EXT111901 carries two terpene BGCs, a ribosomally synthesized and posttranslationally modified peptide (RiPP) BGC, and a homoserine lactone (Other) BGC. Terpene BGCs of EXT111901 and EXT111900 each cluster with the flexixanthin BGC0000650 of *Algoriphagus* sp. strain KK10202C (similarity index values of 0.207 and 0.604, respectively). A difference is the fusion-type lycopene β-cyclase (pink open reading frame [ORF]) present in EXT111900 and absent in EXT111901. The structure of flexixanthin is depicted at the bottom.

bcl2fastq v2.19.0.316), quality checked (Fastp v0.20.1 [5], with additional parameters – detect_adapter_for_pe, –cut_by_quality5, –cut_by_quality3, –low_complexity_filter, – length_required 21, and –correction), and visualized (MultiQC v1.7 [6]). Paired-end reads were quality filtered (Fastp v0.20.1 [5]), assembled (Unicycler v0.4.8 [7]), and quality checked (CheckM v1.0.18 [8]), and genomes were annotated using Bakta v1.5.1 (9). The genome of strain EXT111900 consists of 5,032,044 bp in 101 contigs (coverage, 405×; N_{50} , 235,524 bp), with a GC content of 39.4%, revealing completeness of 99.81% and contamination of 0.19%. The genome contains 4,286 protein-coding genes, 41 tRNAs, 1 transfer-messenger RNA (tmRNA), 3 rRNAs, and 3 noncoding RNAs (ncRNAs). The genome of strain EXT111901 consists of 4,582,827 bp in 6 contigs (coverage, 362×; N_{50} , 3,058,286 bp), with a GC content of 42.1%, revealing completeness of 99.81% and no contamination. The genome contains 3,944 protein-coding genes, 47 tRNAs, 1 tmRNAs, 6 rRNAs, and 3 ncRNAs.

Taxonomic ranks were established by using the Type Strain Genome Server (TYGS) (10) and autoMLST (11) and by determining the average nucleotide identity (ANI) (12). *Roseivirga pacifica* DY53 (13) represents the closest related type strain of EXT111901. Digital DNA-DNA hybridization (dDDH) values 86.6% (d0), 70.8% (d4) and 86.7% (d6) and an ANI of 96.65%, all exceeding the species delineation threshold, support affiliation of EXT111901 with the species *R. pacifica*. The closest related type strain for EXT111900 is *Algoriphagus zhangzhouensis* DSM 25035 (14). The dDDH values are 71.8% (d0), 35.8% (d4), and 62.9% (d6), and the ANI is 88.84%. Accordingly, EXT111900 possibly represents a candidate new *Algoriphagus* species.

Prediction of biosynthetic gene clusters (BGCs) was performed using antiSMASH v6.0 (15). Their grouping into gene cluster families (GCFs) together with the Minimum Information about a Biosynthetic Gene cluster (MIBiG) (16) reference clusters using BiGSCAPE (17) with a similarity index cutoff value of 0.6 allowed BGC similarity determinations. EXT111900 carries one BGC clustering with BGC0000650, encoding the carotenoid flexixanthin (18). Although less similar, the same match was detected for EXT111901 (Fig. 1). A difference is the presence of a lycopene β -cyclase in EXT111900 and its absence in EXT111901.

Data availability. The whole-genome shotgun projects have been deposited in GenBank under BioProject accession number PRJNA904539 with BioSample accession numbers SAMN31845353 (EXT111900) and SAMN31845354 (EXT111901). Raw sequencing reads can be obtained from the Sequence Read Archive (SRA) (SRA accession number SRP421610). The draft genome sequences have been deposited in GenBank under the accession numbers JAPPSQ0000000000 (EXT111900) and JAPPSP0000000000 (EXT111901).

ACKNOWLEDGMENTS

We acknowledge the financial support of the Ministry of Education, Culture, Research, and Technology, Republic of Indonesia (grant 111/E5/PG.02.00/2022), the Hessian State Ministry of Higher Education, Research, and the Arts (HMWK) via the LOEWE Center for

April 2023 Volume 12 Issue 4 10.1128/mra.01264-22

Downloaded from https://journals.asm.org/journal/mra on 26 April 2023 by 140.213.160.202.

Insect Biotechnology and Bioresources, and the Federal Ministry of Education and Research (BMBF) via a German Center for Infection Research (DZIF) sequencing grant.

Sequencing was performed by the Institute for Medical Microbiology (part of the NGS Competence Center Tübingen [NCCT], Tübingen, Germany), while data management, including storage of raw data for this project, was performed by the Quantitative Biology Center (QBiC) (Tübingen, Germany). We acknowledge provision of computing resources and technical support by the Bioinformatics Core Facility (BCF) at Justus Liebig University Giessen.

REFERENCES

- Brinkmann S, Kurz M, Patras MA, Hartwig C, Marner M, Leis B, Billion A, Kleiner Y, Bauer A, Toti L, Pöverlein C, Hammann PE, Vilcinskas A, Glaeser J, Spohn M, Schäberle TF. 2022. Genomic and chemical decryption of the *Bacter-oidetes* phylum for its potential to biosynthesize natural products. Microbiol Spectr 10:e02479-21. https://doi.org/10.1128/spectrum.02479-21.
- Brinkmann S, Spohn MS, Schäberle TF. 2022. Bioactive natural products from *Bacteroidetes*. Nat Prod Rep 39:1045–1065. https://doi.org/10.1039/ d1np00072a.
- Fernández-Gómez B, Richter M, Schüler M, Pinhassi J, Acinas SG, González JM, Pedrós-Alió C. 2013. Ecology of marine *Bacteroidetes*: a comparative genomics approach. ISME J 7:1026–1037. https://doi.org/10.1038/ismej.2012.169.
- 4. Fox JL. 2014. Fraunhofer to mine Sanofi microbial collection. Nat Biotechnol 32:305. https://doi.org/10.1038/nbt0414-305a.
- Chen S, Zhou Y, Chen Y, Gu J. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884–i890. https://doi.org/10.1093/ bioinformatics/bty560.
- Ewels P, Magnusson M, Lundin S, Käller M. 2016. MultiQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics 32:3047–3048. https://doi.org/10.1093/bioinformatics/btw354.
- 7. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595. https://doi.org/10.1371/journal.pcbi.1005595.
- Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25:1043–1055. https://doi.org/10.1101/gr.186072.114.
- Schwengers O, Jelonek L, Dieckmann MA, Beyvers S, Blom J, Goesmann A. 2021. Bakta: rapid and standardized annotation of bacterial genomes via alignment-free sequence identification. Microbial Genomics 7:e000685. https://doi.org/10.1099/mgen.0.000685.
- Meier-Kolthoff JP, Göker M. 2019. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10: 2182. https://doi.org/10.1038/s41467-019-10210-3.

- Alanjary M, Steinke K, Ziemert N. 2019. AutoMLST: an automated web server for generating multi-locus species trees highlighting natural product potential. Nucleic Acids Res 47:W276–W282. https://doi.org/10.1093/ nar/gkz282.
- Lee I, Ouk Kim Y, Park S-C, Chun J. 2016. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 66:1100–1103. https://doi.org/10.1099/ijsem.0.000760.
- Huo Y-Y, Xu L, Wang C-S, Yang J-Y, You H, Oren A, Xu X-W. 2013. Fabibacter pacificus sp. nov., a moderately halophilic bacterium isolated from seawater. Int J Syst Evol Microbiol 63:3710–3714. https://doi.org/10.1099/ijs.0.051276-0.
- 14. Yang C, Li Y, Guo Q, Lai Q, Zheng T, Tian Y. 2013. *Algoriphagus zhangzhouensis* sp. nov., isolated from mangrove sediment. Int J Syst Evol Microbiol 63:1621–1626. https://doi.org/10.1099/ijs.0.044271-0.
- Blin K, Shaw S, Kloosterman AM, Charlop-Powers Z, van Wezel GP, Medema MH, Weber T. 2021. antiSMASH 6.0: improving cluster detection and comparison capabilities. Nucleic Acids Res 49:W29–W35. https://doi.org/10.1093/ nar/gkab335.
- 16. Kautsar SA, Blin K, Shaw S, Navarro-Muñoz JC, Terlouw BR, van der Hooft JJJ, van Santen JA, Tracanna V, Suarez Duran HG, Pascal Andreu V, Selem-Mojica N, Alanjary M, Robinson SL, Lund G, Epstein SC, Sisto AC, Charkoudian LK, Collemare J, Linington RG, Weber T, Medema MH. 2020. MIBiG 2.0: a repository for biosynthetic gene clusters of known function. Nucleic Acids Res 48:D454–D458. https://doi.org/10.1093/nar/gkz882.
- Navarro-Muñoz JC, Selem-Mojica N, Mullowney MW, Kautsar SA, Tryon JH, Parkinson El, De Los Santos ELC, Yeong M, Cruz-Morales P, Abubucker S, Roeters A, Lokhorst W, Fernandez-Guerra A, Cappelini LTD, Goering AW, Thomson RJ, Metcalf WW, Kelleher NL, Barona-Gomez F, Medema MH. 2020. A computational framework to explore large-scale biosynthetic diversity. Nat Chem Biol 16:60–68. https://doi.org/10.1038/s41589-019-0400-9.
- 18. Tao L, Yao H, Kasai H, Misawa N, Cheng Q. 2006. A carotenoid synthesis gene cluster from *Algoriphagus* sp. KK10202C with a novel fusion-type lycopene β -cyclase gene. Mol Genet Genomics 276:79–86. https://doi.org/10.1007/s00438-006-0121-0.