

Genome sequence of the marine alphaproteobacterium *Lentilitoribacter* sp. EG35 isolated from the temperate octocoral *Eunicella gazella*

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ABSTRACT We report the genome sequence of *Lentilitoribacter* sp. strain EG35 isolated from the octocoral *Eunicella gazella* sampled off the coast of Portugal. We reveal the coding potential for the biosynthesis of polyhydroxyalkanoates — biodegradable polyesters that may serve bioplastics production, diverse homoserine lactone-like communication signals, and four putatively novel natural products.

KEYWORDS bacterial evolution, corals, host-microbe interactions, *Rhizobiaceae*, symbiosis

The microbial communities of octocorals are unique in taxonomic composition and presumably benefit their host through nutrient provision and cycling, antioxidant production, and chemical defense (1–3). *Lentilitoribacter* (*Rhizobiaceae*, *Hyphomicrobiales*) is a marine alphaproteobacterial genus currently with only one valid species, *Lentilitoribacter donghaensis* (4) and one RefSeq genome (5, 6) available. It has so far been cultured from seawater (4), marine sponges (5), and octocorals (7), yet little is known about its role in association with marine invertebrates.

We report the genome of *Lentilitoribacter* sp. strain EG35 isolated from a healthy *Eunicella gazella* specimen, sampled by SCUBA diving at 18 m depth in the Atlantic off the coast of Portugal (Pedra da Greta: 36.979778, –7.98911) on 21 April 2021. A microbial cell suspension was retrieved from 1 g of coral soft tissue by mortar-and-pestle homogenization in 9 mL sterile Ca²⁺ and Mg²⁺-free artificial seawater as described earlier (8). Serial dilutions were plated on Marine Agar (MA) and incubated for 7 days at 24°C. Single colonies were streaked until purity on MA plates, and genomic DNA was extracted from a pure culture, freshly grown in Marine Broth using the Wizard Genomic DNA Purification kit (Promega, USA). Strain EG35 was identified by Sanger sequencing of the 16S rRNA gene amplified from genomic DNA using primers F27 (5′-AGAGTTTGATCMTGGCTCAG-3′) and R1492 (5′-TACGGY TACCTGTTACGACTT-3′) and the SILVA/SINA (v1.2.12) database for taxonomic classification. The same DNA sample was used for genome sequencing at GENOINSEQ (Biocant, Cantanhede, Portugal). The genome library was prepared with the Nextera XT DNA Library Preparation Kit, and the genome was paired-end sequenced (average read length, 257/258 bp) on an Illumina MiSeq sequencer with the MiSeq reagent Kit v3 (600 cycles). Default parameters were used for all software unless otherwise specified. Raw reads were imported to KBase (9) and quality-checked using FastQC v0.12.1. Low-quality reads were removed using Trimmomatic v0.36 (10) prior to genome assembly using SPAdes v3.15.3 (11). Contigs below 500 bp were removed. Genome completeness and contamination were assessed with CheckM v1.0.18 (12), and genome taxonomy was confirmed with GTDB-Tk v2.3.2 (13). Contigs were annotated using the NCBI Prokaryotic Genome Annotation Pipeline

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TABLE 1 General features of the *Lentilitoribacter* sp. EG35 genome reported in this study

Genome size (bp)	GC- content (%)	Genome coverage (x)	Number of contigs	Contig N50 (bp)	Number of reads	Average Read length (bp)	Estimate (%) of:		Number of:		Counts of:					Counts of CDs in ^b				
							Completeness (%)	Contamination (%)	Coding sequences ^{a,b}	Genes ^a	rRNA ^a	tRNA ^a	ncRNA ^a	COGs ^b		Pfam ^b				
														accession number	number	accession number	number			
4,063,774	44.5	243.4	45	307,952	2x1,714,668	257/258	99.92	0.00	3,935 ^a	3,977	42	3	35	4	3,131	7,130	JBFNG000	SRR29864	PRJNA11354	SAMN424839
										3,912 ^b							00000	805	83	28

^aAnnotation performed by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP): https://www.ncbi.nlm.nih.gov/genome/annotation_prok/
^bThe Melange pipeline (<https://github.com/sandragodinhosilva/melange>) was used to perform Protein family (Pfam) and Clusters of Orthologous Groups of Proteins (COG)-based annotations of the *Lentilitoribacter* EG35 genome. The corresponding data are available on Zenodo under <https://doi.org/10.5281/zenodo.13856673>

(PGAP v.6.7) (14) and our in-house Melange pipeline (<https://github.com/sandragodinho-silva/melange>). AntiSMASH v7.0 (15) was used to identify secondary metabolite biosynthetic gene clusters (BGCs).

The general features of the genome are shown in Table 1. Strain EG35 shared 98.39% average nucleotide identity (ANI)—calculated with FastANI v0.1.3 (16)—with *Lentilitoribacter* sp. Alg239-R112 ([GCF_900537175.1](https://doi.org/10.1093/gbe/abz005)), its closest relative with a sequenced genome as determined by phylogenomics inference. Strain Alg239-R112 was isolated from the marine sponge *Spongia* sp (5). at the same sampling location where strain EG35 was retrieved.

The EG35 genome encodes the poly[(R)-3-hydroxyalkanoate] polymerase subunit *phaC* (EC 2.3.1.304) crucial for the biosynthesis of polyhydroxyalkanoates (PHAs), biodegradable polyesters that can be used for bioplastics production (17), and two other genes involved in PHA metabolism, the PHA synthesis regulator *phaR*, and the poly(3-hydroxybutyrate) depolymerase *phaZ* (EC 3.1.1.75). The strain's potential to produce PHAs was confirmed *via* Nile Red staining (18). Strain EG35 harbors six BGCs coding for homoserine lactone signaling molecules, including one sharing 100% similarity to the BGC of kolossin, a peptide likely involved in interspecies communication (19). Moreover, four putatively novel BGCs coding for a terpene, arylpolyene, betalactone, and a ribosomally synthesized and post-translationally modified peptide are present.

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Tina Keller-Costa, Conceptualization, Data curation, Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review and editing | Selene Madureira, Formal analysis, Investigation, Visualization | Ana S. Fernandes, Formal analysis, Investigation | Lydia Kozma, Formal analysis, Investigation | Jorge M.S. Gonçalves, Funding acquisition, Resources | Cristina Barroso, Data curation, Formal analysis | Conceição Egas, Data curation, Formal analysis, Funding acquisition, Resources | Rodrigo Costa, Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing – review and editing

DATA AVAILABILITY STATEMENT

The genome sequence of *Lentilitoribacter* sp. strain EG35 has been deposited at DDBJ/ENA/GenBank under the BioProject [PRJNA1135483](#) and the Whole Genome Shotgun accession number [JBFNGQ000000000](#). The antiSMASH and Melange results of BGC, COG and Pfam annotations are available on Zenodo under <https://doi.org/10.5281/zenodo.13856673>. Further details can be found in Table 1.

REFERENCES

- Keller-Costa T, Lago-Lestón A, Saraiva JP, Toscan R, Silva SG, Gonçalves J, Cox CJ, Kyrpides N, Nunes da Rocha U, Costa R. 2021. Metagenomic insights into the taxonomy, function, and dysbiosis of prokaryotic communities in octocorals. *Microbiome* 9:72. <https://doi.org/10.1186/s40168-021-01031-y>
- Keller-Costa T, Kozma L, Silva SG, Toscan R, Gonçalves J, Lago-Lestón A, Kyrpides NC, Nunes da Rocha U, Costa R. 2022. Metagenomics-resolved genomics provides novel insights into chitin turnover, metabolic specialization, and niche partitioning in the octocoral microbiome. *Microbiome* 10:151. <https://doi.org/10.1186/s40168-022-01343-7>
- van de Water JAJM, Allemand D, Ferrier-Pagès C. 2018. Host-microbe interactions in octocoral holobionts - recent advances and perspectives. *Microbiome* 6:64. <https://doi.org/10.1186/s40168-018-0431-6>
- Park S, Lee J-S, Lee K-C, Yoon J-H. 2013. *Lentilitoribacter donghaensis* gen. nov., sp. nov., a slowly-growing alphaproteobacterium isolated from coastal seawater. *Antonie Van Leeuwenhoek* 103:457–464. <https://doi.org/10.1007/s10482-012-9825-9>
- Karimi E, Costa R. 2020. Isolation and genome sequencing of 14 *Spongia* sp. bacterial associates expands the taxonomic and functional breadth of the cultivatable marine sponge microbiome. *Microbiology*. <https://doi.org/10.1101/2020.03.20.000216>
- Almeida JF, Marques M, Oliveira V, Egas C, Mil-Homens D, Viana R, Cleary DFR, Huang YM, Fialho AM, Teixeira MC, Gomes NCM, Costa R, Keller-Costa T. 2023. Marine sponge and octocoral-associated bacteria show versatile secondary metabolite biosynthesis potential and antimicrobial activities against human pathogens. *Mar Drugs* 21:34. <https://doi.org/10.3390/md21010034>
- Kozma L. 2021. Molecular assessment of unculturable and culturable bacterial symbionts of temperate gorgonian corals. In *Master's thesis in Life Sciences Engineering*. EPFL - École Polytechnique Fédérale de Lausanne, Switzerland.
- Keller-Costa T, Eriksson D, Gonçalves JMS, Gomes NCM, Lago-Lestón A, Costa R. 2017. The gorgonian coral *Eunicella labiata* hosts a distinct prokaryotic consortium amenable to cultivation. *FEMS Microbiol Ecol* 93:1–19. <https://doi.org/10.1093/femsec/fix143>
- Arkin AP, Cottingham RW, Henry CS, Harris RL, Stevens RL, Maslov S, Dehal P, Ware D, Perez F, Canon S, et al. 2018. KBase: the United States department of energy systems biology knowledgebase. *Nat Biotechnol* 36:566–569. <https://doi.org/10.1038/nbt.4163>
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>
- 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. <https://doi.org/10.1089/cmb.2012.0021>
- 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>
- Chaumeil P-A, Mussig AJ, Hugenholtz P, Parks DH. 2019. GTDB-Tk: a toolkit to classify genomes with the genome taxonomy database. *Bioinformatics* 36:1925–1927. <https://doi.org/10.1093/bioinformatics/btz848>
- 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, Shaw S, Augustijn HE, Reitz ZL, Biermann F, Alanjary M, Fetter A, Terlouw BR, Metcalf WW, Helfrich EJN, van Wezel GP, Medema MH, Weber T. 2023. antiSMASH 7.0: new and improved predictions for detection, regulation, chemical structures and visualisation. *Nucleic Acids Res* 51:W46–W50. <https://doi.org/10.1093/nar/gkad344>
- Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S. 2018. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nat Commun* 9:5114. <https://doi.org/10.1038/s41467-018-07641-9>
- Muneer F, Rasul I, Azeem F, Siddique MH, Zubair M, Nadeem H. 2020. Microbial polyhydroxyalkanoates (PHAs): efficient replacement of synthetic polymers. *J Polym Environ* 28:2301–2323. <https://doi.org/10.1007/s10924-020-01772-1>

18. Elain A, Le Fellic M, Corre YM, Le Grand A, Le Tilly V, Audic JL, Bruzaud S. 2015. Rapid and qualitative fluorescence-based method for the assessment of PHA production in marine bacteria during batch culture. *World J Microbiol Biotechnol* 31:1555–1563. <https://doi.org/10.1007/s11274-015-1904-4>
19. Bode HB, Brachmann AO, Jadhav KB, Seyfarth L, Dauth C, Fuchs SW, Kaiser M, Waterfield NR, Sack H, Heinemann SH, Arndt HD. 2015. Structure elucidation and activity of kolossin A, the D-/L-pentadecapeptide product of A giant nonribosomal peptide synthetase. *Angew Chem Int Ed Engl* 54:10352–10355. <https://doi.org/10.1002/anie.201502835>