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GENOME SEQUENCES

High-Quality Draft Genome Sequences of Two Deltaproteobacterial Endosymbionts, Delta1a and Delta1b, from the Uncultured Sva0081 Clade, Assembled from Metagenomes of the Gutless Marine Worm *Olavius algarvensis*

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ABSTRACT Here, we present high-quality metagenome-assembled genome sequences of two closely related deltaproteobacterial endosymbionts from the gutless marine worm *Olavius algarvensis* (Annelida). The first is an improved draft genome sequence of the previously described sulfate-reducing symbiont Delta1. The second is from a closely related, recently discovered symbiont of *O. algarvensis*.

Olavius algarvensis lacks a digestive and excretory system and lives in obligate association with bacterial endosymbionts. These symbionts provide the host with nutrition through carbon fixation and recycle its waste products (1–4). The described symbionts of *O. algarvensis* are two *Deltaproteobacteria* (Delta1 and Delta4) and two *Gammaproteobacteria* (Gamma1 ["*Candidatus* Thiosymbion algarvensis"] and Gamma3 [5]), whose draft genome sequences have been published (1), as well as a spirochete. We report an improved draft genome sequence of the Delta1 symbiont and a draft genome sequence of a newly discovered deltaproteobacterial symbiont, closely related to the Delta1 symbiont. Due to their close phylogenetic relationship, we named these symbionts Delta1a (formerly Delta1) and Delta1b. Both symbionts belong to the widespread Sva0081 clade of uncultured, free-living deltaproteobacterial sulfate reducers (6).

O. algarvensis specimens were collected in Sant'Andrea (Elba, Italy; 42°48′26.00″N, 10° 8′28.00″E) in June 2014 as described previously (3), and were stored in RNAlater (Ambion Life Technologies) at 4°C. DNA was individually extracted from 2 worms using the AllPrep DNA/RNA kit (Qiagen). Two separate Illumina TruSeq DNA libraries were prepared at the Max Planck Institute for Plant Breeding Genome Center and sequenced on the Illumina HiSeq 2000 platform (2 × 100 bp), resulting in 28,259,255 and 33,422,854 raw read pairs from Delta1a and Delta1b libraries, respectively. Raw reads were quality controlled using bbduk (minimum [min.] kmer, 11; min. length, 36 bp; min. phred quality, 2) (BBMap toolkit v36.86; https://sourceforge.net/projects/bbmap/) and BayesHammer implemented in SPAdes v3.9.1 (7, 8). Clean reads (26,440,546 and 31,821,934 sequence pairs for Delta1a and Delta1b libraries, respectively) were *de novo* assembled with MEGAHIT v1.0.6 (preset, –meta) (9). Draft symbiont bins were obtained using MetaBAT v0.26.3 (10) and were further refined using Bandage v0.08.1 (11). Completeness and contamination were estimated with CheckM v1.0.5 (12). Enveomics tools (13) was used to calculate average nucleotide and amino acid identities. Genome statistics were calculated with QUAST v4.6.1 (14). Genome contents were annotated and analyzed using RASTtk (15–17) and Pathway Tools v21.0 (18). Default parameters were used for all software unless otherwise noted.

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Two metagenome-assembled genomes (MAGs) were identified as Delta1a and Delta1b based on their 16S rRNA sequences. Delta1a contains the identical 16S rRNA sequence as that contained by Delta1 from the prior study (1). Delta1a and Delta1b share 98.8% identical 16S rRNA sequences, while their average nucleotide and amino acid identities of 82.0% and 78.1%, respectively, clearly separate them as individual species. The Delta1a draft genome has 10.62 Mb (N_{50}, 10.5 kb; G+C content, 49.9%) and greater completeness (96.0%), more gene features (13,141 protein coding sequences [CDSs]), and only slightly increased contamination (2.4%) than the former Delta1 draft genome (11.22 Mb, 83.7% completeness, 10,680 CDSs, and 1.6% contamination). The Delta1b draft genome has 9.89 Mb (N_{50}, 44.4 kb; G+C content, 48.2%), 96.0% completeness, and 0.8% contamination and contains 10,871 CDSs. Both MAGs conform to the Genomic Standards Consortium Minimum Information about a Metagenome-Assembled Genome (GSC MIMAG) standard for high-quality draft genomes (19). The functions carried by their genomes indicate that both symbionts are sulfate reducers capable of carbon monoxide and hydrogen oxidation. Both encode various transporters for the uptake of waste compounds produced by the host, such as short-chain fatty acids, urea, and glycine betaine, highlighting their proposed role in host waste disposal and recycling.

Data availability. The MAGs and raw sequences of Delta1a and Delta1b endosymbionts have been deposited in the European Nucleotide Archive under accession no. PRJEB28157, using the data brokerage service of the German Federation for Biological Data (GFBio) (20), in compliance with the Minimal Information about Any (x) Sequence (MIxS) standard (21).

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REFERENCES

1. Woyke T, Teeling H, Ivanova NN, Huntemann M, Richter M, Gloeckner FO, Boffelli D, Anderson IJ, Barry KW, Shapiro HJ, Szeto E, Kyrpides NC, Mussmann M, Amann R, Bergin C, Ruehland C, Rubin EM, Dubilier N. 2006. Symbiosis insights through metagenomic analysis of a microbial consortium. Nature 443:950–955. https://doi.org/10.1038/nature05192.
2. Dubilier N, Mülders C, Ferdelman T, de Beer D, Pernthaler A, Klein M, Wagner M, Erséus C, Thiermann F, Krieger J, Giere O, Amann R. 2001. Endosymbiotic sulphate-reducing and sulphide-oxidizing bacteria in an oligochaete worm. Nature 411:298–302. https://doi.org/10.1038/35077067.
3. Kleiner M, Wentrup C, Lott C, Teeling H, Wetzel S, Young J, Chang YJ, Shah M, VerBerkmoes NC, Zarzycki J, Fuchs G, Markert S, Hempel K, Voigt B, Becher D, Liebeke M, Lalk M, Albrecht D, Hecker M, Schweder T, Dubilier N. 2012. Metaproteomics of a gutless marine worm and its symbiotic microbial community reveal unusual pathways for carbon and energy use. Proc Natl Acad Sci U S A 109:E1173–E1182. https://doi.org/10.1073/pnas.1121198109.
4. Wippler J, Kleiner M, Lott C, Gruhl A, Abraham PE, Giannone RJ, Young JC, Hettich RL, Dubilier N. 2016. Transcriptomic and proteomic insights into innate immunity and adaptations to a symbiotic lifestyle in the gutless marine worm *Olavius algarvensis*. BMC Genomics 17:942. https://doi.org/10.1186/s12864-016-3293-y.
5. Ruehland C, Blazejak A, Lott C, Loy A, Erséus C, Dubilier N. 2008. Multiple bacterial symbionts in two species of co-occurring gutless oligochaete worms from Mediterranean sea grass sediments. Environ Microbiol 10:3404–3416. https://doi.org/10.1111/j.1462-2920.2008.01728.x.
6. Dyksma S. 2016. Identification and activity of bacteria consuming key intermediates of carbon and sulfur cycling in coastal sands. PhD thesis. University of Bremen, Bremen, Germany.
7. 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.
8. Nikolenko SI, Korobeynikov AI, Alekseyev MA. 2013. BayesHammer: Bayesian clustering for error correction in single-cell sequencing. BMC Genomics 14:S7. https://doi.org/10.1186/1471-2164-14-S1-S7.
9. Li D, Luo R, Liu CM, Leung CM, Ting HF, Sadakane K, Yamashita H, Lam TW. 2016. MEGAHIT v1.0: a fast and scalable metagenome assembler driven by advanced methodologies and community practices. Methods 102:3–11. https://doi.org/10.1016/j.ymeth.2016.02.020.
10. Kang DD, Froula J, Egan R, Wang Z. 2015. MetaBAT, an efficient tool for accurately reconstructing single genomes from complex microbial communities. PeerJ 3:e1165. https://doi.org/10.7717/peerj.1165.
11. Wick RR, Schultz MB, Zobel J, Holt KE. 2015. Bandage: interactive visualization of *de novo* genome assemblies. Bioinformatics 31:3350–3352. https://doi.org/10.1093/bioinformatics/btv383.
12. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckmM: 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.
13. Rodriguez-R LM, Konstantinidis KT. 2016. The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. PeerJ Preprints 4:e1900v1. https://doi.org/10.7287/peerj.preprints.1900v1.
14. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086.
15. 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.

16. 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.
17. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. *RASTtk*: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5:8365. https://doi.org/10.1038/srep08365.
18. Karp PD, Latendresse M, Paley SM, Krummenacker M, Ong QD, Billington R, Kothari A, Weaver D, Lee T, Subhraveti P, Spaulding A, Fulcher C, Keseler IM, Caspi R. 2016. Pathway tools version 19.0 update: software for pathway/genome informatics and systems biology. Brief Bioinform 17:877–890. https://doi.org/10.1093/bib/bbv079.
19. Bowers RM, Kyrpides NC, Stepanauskas R, Harmon-Smith M, Doud D, Reddy T, Schulz F, Jarett J, Rivers AR, Eloe-Fadrosh EA, Tringe SG, Ivanova NN, Copeland A, Clum A, Becraft ED, Malmstrom RR, Birren B, Podar M, Bork P, Weinstock GM, Garrity GM, Dodsworth JA, Yooseph S, Sutton G, Glöckner FO, Gilbert JA, Nelson WC, Hallam SJ, Jungbluth SP, Ettema TJG, Tighe S, Konstantinidis KT, Liu WT, Baker BJ, Rattei T, Eisen JA, Hedlund B, McMahon KD, Fierer N, Knight R, Finn R, Cochrane G, Karsch-Mizrachi I, Tyson GW, Rinke C, The Genome Standards Consortium, Lapidus A, Meyer F, Yilmaz P, Parks DH, Eren AM, Schriml L, Banfield JF, Hugenholtz P, Woyke T. 2017. Minimum information about a single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea. Nat Biotechnol 35:725–731. https://doi.org/10.1038/nbt.3893.
20. Diepenbroek M, Glöckner F, Grobe P, Güntsch A, Huber R, König-Ries B, Kostadinov I, Nieschulze J, Seeger B, Tolksdorf R, Triebel D. 2014. Towards an integrated biodiversity and ecological research data management and archiving platform: the German Federation for the Curation of Biological Data (GFBio), p 1711–1724. *In* Plödereder E, Grunske L, Schneider E, Ull D (ed). Informatik 2014—big data Komplexität meistern. GI-edition: Lecture Notes in Informatics (LNI)—Proceedings. GI ed, vol. 232. Köllen Verlag, Bonn, Germany.
21. Yilmaz P, Kottmann R, Field D, Knight R, Cole JR, Amaral-Zettler L, Gilbert JA, Karsch-Mizrachi I, Johnston A, Cochrane G, Vaughan R, Hunter C, Park J, Morrison N, Rocca-Serra P, Sterk P, Arumugam M, Bailey M, Baumgartner L, Birren BW, Blaser MJ, Bonazzi V, Booth T, Bork P, Bushman FD, Buttigieg PL, Chain PS, Charlson E, Costello EK, Huot-Creasy H, Dawyndt P, DeSantis T, Fierer N, Fuhrman JA, Gallery RE, Gevers D, Gibbs RA, San Gil I, Gonzalez A, Gordon JI, Guralnick R, Hankeln W, Highlander S, Hugenholtz P, Jansson J, Kau AL, Kelley ST, Kennedy J, Knights D, Koren O, Kuczynski J, Kyrpides N, Larsen R, Lauber CL, Legg T, Ley RE, Lozupone CA, Ludwig W, Lyons D, Maguire E, Methé BA, Meyer F, Muegge B, Nakielny S, Nelson KE, Nemergut D, Neufeld JD, Newbold LK, Oliver AE, Pace NR, Palanisamy G, Peplies J, Petrosino J, Proctor L, Pruesse E, Quast C, Raes J, Ratnasingham S, Ravel J, Relman DA, Assunta-Sansone S, Schloss PD, Schriml L, Sinha R, Smith MI, Sodergren E, Spo A, Stombaugh J, Tiedje JM, Ward DV, Weinstock GM, Wendel D, White O, Whiteley A, Wilke A, Wortman JR, Yatsunenko T, Glöckner FO. 2011. Minimum information about a marker gene sequence (MIMARKS) and minimum information about any (x) sequence (MIxS) specifications. Nat Biotechnol 29:415–420. https://doi.org/10.1038/nbt.1823.