



Complete Genome Sequence of *Leisingera aquamixtae* R2C4, Isolated from a Self-Regenerating Biocathode Consortium

Lina Bird,^a  Brian J. Eddie,^b Anthony P. Malanoski,^b Princess Pinamang,^{c,d} Sarah M. Glaven^b

^aNational Research Council, Washington, DC, USA

^bCenter for Bio/Molecular Science and Engineering, Naval Research Laboratory, Washington, DC, USA

^cDepartment of Biology, Prairie View Agricultural and Mechanical University, Prairie View, Texas, USA

^dThe Washington Center for Internships and Academic Seminars, Washington, DC, USA

ABSTRACT Here, we present the complete genome sequence of *Leisingera aquamixtae* R2C4, isolated from the electroautotrophic microbial consortium biocathode MCL (*Marinobacter-Chromatiaceae-Labrenzia*). As an isolate of a current-producing system, the genome sequence of *L. aquamixtae* will yield insights regarding electrode-associated microorganisms and communities. A dark pigment is also observed during cultivation.

Leisingera aquamixtae R2C4 was isolated from the biocathode MCL (*Marinobacter-Chromatiaceae-Labrenzia*), a self-regenerating microbial consortium that uses electrons supplied by the cathode to drive CO₂ fixation and O₂ reduction. Biocathode MCL was enriched from Atlantic Ocean seawater at Rutgers University Marine Field Station, Tuckerton, NJ (1), and metagenomic sequencing showed a low-diversity community of *Gammaproteobacteria* and *Alphaproteobacteria* (2). Isolation followed enrichment of biofilm scrapings in an iron sulfide gradient tube (3) and was carried out by streaking the turbid layer from the tube onto an artificial seawater agar plate supplemented with 5 mM sodium acetate. The resulting colonies grew well on half-strength Zobell's marine agar plates (4). One isolate, designated R2C4 and initially identified as a *Phaeobacter* sp. (5), produced a dark pigment. Several *Phaeobacter* spp. have since been reclassified as *Leisingera* spp. (6), which led us to identify R2C4 as a *Leisingera* sp.

Leisingera spp. are aerobic and moderately halophilic members of the *Rhodobacteraceae* family with the ability to utilize methyl halides as a carbon source (7) and to produce antimicrobial compounds thought to aid in symbiotic relationships (6). There are seven named species of the genus *Leisingera* (6–12), including two (*L. caerulea* and *L. aquimarina*) that were isolated from cathodes (13, 14). Analysis of the genome of *Leisingera aquamixtae* R2C4 will support our efforts to use multiomics in characterizing biocathodes, help identify potential extracellular electron transfer (EET) proteins and pathways, and determine R2C4's role in electroactive microbial communities.

Genome sequencing was performed using the Pacific Biosciences RS II sequencing platform (DNA Link USA, Inc., San Diego, CA). Genomic DNA was extracted from 2 ml Difco marine broth using the Wizard genomic DNA purification kit (Promega). One microgram of DNA was used to prepare a 10-kb insert library and sequenced using two single-molecule real-time (SMRT) sequencing cells and P4-C2 chemistry. Default parameters were used for all software, unless otherwise noted. Filtering and preassembly were performed with SMRT Analysis v2.3.0 HGAP.2 (PacBio). This resulted in 17,730 filtered and preassembled sequence reads with a mean length of 7,272 bp, an *N*₅₀ value of 8,071 bp, and 75× genome coverage. *De novo* assembly (via SMRTpipe HGAP.2 and SMRTpipe Celera assembler) and consensus polishing (SMRTpipe Quiver) yielded one closed circular genome and nine smaller contigs, of which four are closed circular DNA

Citation Bird L, Eddie BJ, Malanoski AP, Pinamang P, Glaven SM. 2019. Complete genome sequence of *Leisingera aquamixtae* R2C4, isolated from a self-regenerating biocathode consortium. *Microbiol Resour Announc* 8:e00833-19. <https://doi.org/10.1128/MRA.00833-19>.

Editor J. Cameron Thrash, University of Southern California

Copyright © 2019 Bird et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Lina Bird, lina.bird.ctr@nrl.navy.mil.

Received 12 July 2019

Accepted 10 August 2019

Published 5 September 2019

TABLE 1 Sequence characteristics of assembled contigs

| Accession no. | Length (bp) | GC content (%) | No. of CDS ^a | Circularization, coverage (×) |
|---------------|-------------|----------------|-------------------------|-------------------------------|
| CP041155 | 13,809 | 66.8 | 13 | No, low |
| CP041156 | 175,590 | 66.01 | 167 | Yes |
| CP041157 | 121,606 | 58.22 | 170 | Yes |
| CP041158 | 59,812 | 68.7 | 55 | Yes |
| CP041159 | 3,820,802 | 64.52 | 3,694 | Yes |
| CP041160 | 6,320 | 65.9 | 7 | No, <30 |
| CP041161 | 14,269 | 68.5 | 11 | No, <30 |
| CP041162 | 26,129 | 65.5 | 21 | No, <30 |
| CP041163 | 32,785 | 67.8 | 29 | No, <30 |
| CP041164 | 108,983 | 65.2 | 86 | Yes |

^a CDS, coding sequences.

and five, with low sequence coverage (<30×), are not closed, as summarized in Table 1. Annotation was performed using NCBI's Prokaryotic Genome Annotation Pipeline. The genome is predicted to contain 4,424,497 bp, 4,093 protein-coding genes, 56 tRNA-encoding genes, and 4 rRNA operons. BLAST alignments of the 16S rRNA genes (locus tags R2C4_03015, R2C4_13205, R2C4_16735, and R2C4_16910) showed 100% identity to *Leisingera aquaemixtae* SSK6-1 (formerly *Phaeobacter aquaemixtae* SSK6) (15). Digital DNA-DNA hybridization (16) confirmed that R2C4 belongs to *L. aquaemixtae*, showing 77.2% identity (74.2 to 80% confidence interval [CI]) to *L. aquaemixtae* CECT8399. An NCBI BLAST search of the relevant genes showed that R2C4 contains a predicted indigoidine synthesis operon, *igiRBCDFE*, which shares 96% to 98% nucleotide identity with the operon in *L. aquaemixtae* CECT8399 (11). The dark color of R2C4 colonies suggests that indigoidine, a blue pigment with antimicrobial properties, is produced.

Data availability. The complete sequence of *Leisingera aquaemixtae* R2C4 is available under NCBI BioProject number [PRJNA480465](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA480465), with the annotated sequences available in GenBank under accession numbers [CP041155](https://www.ncbi.nlm.nih.gov/nuccore/CP041155) to [CP041164](https://www.ncbi.nlm.nih.gov/nuccore/CP041164). Raw sequencing reads are deposited in the Sequence Read Archive under accession number [SRR965965](https://www.ncbi.nlm.nih.gov/sra/SRR965965).

ACKNOWLEDGMENTS

This work was funded through the NRL Base 6.1 program and the office of Naval Research NRL HBCU/MI Internship Program.

REFERENCES

- Strycharz-Glaven SM, Glaven RH, Wang Z, Zhou J, Vora GJ, Tender LM. 2013. Electrochemical investigation of a microbial solar cell reveals a nonphotosynthetic biocathode catalyst. *Appl Environ Microbiol* 79: 3933–3942. <https://doi.org/10.1128/AEM.00431-13>.
- Wang Z, Leary DH, Malanoski AP, Li RW, Hervey WJt, Eddie BJ, Tender GS, Yanosky SG, Vora GJ, Tender LM, Lin B, Strycharz-Glaven SM. 2015. A previously uncharacterized, nonphotosynthetic member of the Chromatiaceae is the primary CO₂-fixing constituent in a self-regenerating biocathode. *Appl Environ Microbiol* 81:699–712. <https://doi.org/10.1128/AEM.02947-14>.
- Emerson D, Moyer C. 1997. Isolation and characterization of novel iron-oxidizing bacteria that grow at circumneutral pH. *Appl Environ Microbiol* 63:4784–4792.
- Zobell CE. 1941. Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. *J Mar Res* 4:42–75.
- Malanoski AP, Lin B, Eddie BJ, Wang Z, Hervey WJ, Glaven SM. 2018. Relative abundance of “Candidatus Tenderia electrophaga” is linked to cathodic current in an aerobic biocathode community. *Microb Biotechnol* 11:98–111. <https://doi.org/10.1111/1751-7915.12757>.
- Breider S, Scheuner C, Schumann P, Fiebig A, Petersen J, Pradella S, Klenk HP, Brinkhoff T, Goker M. 2014. Genome-scale data suggest reclassifications in the *Leisingera*-*Phaeobacter* cluster including proposals for *Sedimentitalea* gen. nov. and *Pseudophaeobacter* gen. nov. *Front Microbiol* 5:416. <https://doi.org/10.3389/fmicb.2014.00416>.
- Schaefer JK, Goodwin KD, McDonald IR, Murrell JC, Oremland RS. 2002. *Leisingera methylohalidivorans* gen. nov., sp. nov., a marine methylotroph that grows on methyl bromide. *Int J Syst Evol Microbiol* 52: 851–859. <https://doi.org/10.1099/00207713-52-3-851>.
- Breider S, Teshima H, Petersen J, Chertkov O, Dalingault H, Chen A, Pati A, Ivanova N, Lapidus A, Goodwin LA, Chain P, Detter JC, Rohde M, Tindall BJ, Kyrpides NC, Woyke T, Simon M, Goker M, Klenk HP, Brinkhoff T. 2014. Genome sequence and emended description of *Leisingera nanhaiensis* strain DSM 24252^T isolated from marine sediment. *Stand Genomic Sci* 9:687–703. <https://doi.org/10.4056/sigs.3828824>.
- Buddrhuhs N, Chertkov O, Petersen J, Fiebig A, Chen A, Pati A, Ivanova N, Lapidus A, Goodwin LA, Chain P, Detter JC, Gronow S, Kyrpides NC, Woyke T, Goker M, Brinkhoff T, Klenk HP. 2013. Complete genome sequence of the marine methyl-halide oxidizing *Leisingera methylohalidivorans* type strain (DSM 14336^T), a representative of the *Roseobacter* clade. *Stand Genomic Sci* 9:128–141. <https://doi.org/10.4056/sigs.4297965>.
- Riedel T, Teshima H, Petersen J, Fiebig A, Davenport K, Daligault H, Erkkila T, Gu W, Munk C, Xu Y, Chen A, Pati A, Ivanova N, Goodwin LA, Chain P, Detter JC, Rohde M, Gronow S, Kyrpides NC, Woyke T, Goker M, Brinkhoff T, Klenk HP. 2013. Genome sequence of the *Leisingera aquimarina* type strain (DSM 24565^T), a member of the marine *Roseobacter* clade rich in extrachromosomal elements. *Stand Genomic Sci* 8:389–402. <https://doi.org/10.4056/sigs.3858183>.
- Rodrigo-Torres L, Pujalte MJ, Arahal DR. 2016. Draft genome of *Leisingera aquaemixtae* CECT 8399^T, a member of the *Roseobacter* clade

- isolated from a junction of fresh and ocean water in Jeju Island, South Korea. *Genom Data* 7:233–236. <https://doi.org/10.1016/j.gdata.2016.01.010>.
12. Sun F, Wang B, Liu X, Lai Q, Du Y, Li G, Luo J, Shao Z. 2010. *Leisingera nanhaiensis* sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol* 60:275–280. <https://doi.org/10.1099/ijs.0.010439-0>.
 13. Vandecandelaere I, Segaert E, Mollica A, Faimali M, Vandamme P. 2008. *Leisingera aquimarina* sp. nov., isolated from a marine electroactive biofilm, and emended descriptions of *Leisingera methylohalidivorans* Schaefer et al. 2002, *Phaeobacter daeponensis* Yoon et al. 2007 and *Phaeobacter inhibens* Martens et al. 2006. *Int J Syst Evol Microbiol* 58:2788–2793. <https://doi.org/10.1099/ijs.0.65844-0>.
 14. Vandecandelaere I, Segaert E, Mollica A, Faimali M, Vandamme P. 2009. *Phaeobacter caeruleus* sp. nov., a blue-coloured, colony-forming bacterium isolated from a marine electroactive biofilm. *Int J Syst Evol Microbiol* 59:1209–1214. <https://doi.org/10.1099/ijs.0.002642-0>.
 15. Park S, Park DS, Bae KS, Yoon JH. 2014. *Phaeobacter aquaemixtae* sp. nov., isolated from the junction between the ocean and a freshwater spring. *Int J Syst Evol Microbiol* 64:1378–1383. <https://doi.org/10.1099/ijs.0.057646-0>.
 16. Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. 2013. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 14:60. <https://doi.org/10.1186/1471-2105-14-60>.