

Draft Genome Sequences of Seven Bacterial Strains Isolated from a Polymicrobial Culture of Coccolith-Bearing (C-Type) *Emiliana huxleyi* M217

Albert Remus R. Rosana, Fabini D. Orata, Yue Xu, Danielle N. Simkus, Anna R. Bramucci, Yan Boucher, Rebecca J. Case

Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada

A.R.R.R. and F.D.O. contributed equally to this work.

Strains of *Rhodobacteraceae*, *Sphingomonadales*, *Alteromonadales*, and *Bacteroidetes* were isolated from a polymicrobial culture of the coccolith-forming (C-type) haptophyte *Emiliana huxleyi* strain M217. The genomes encode genes for the production of algal growth factors and the consumption of their hosts' metabolic by-products, suggesting that the polymicrobial culture harbors many symbiotic interactions.

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Address correspondence to Rebecca J. Case, rcase@ualberta.ca.

The haptophyte *Emiliana huxleyi* is a coccolithophore, a phytoplankton that has the ability to make calcite disks (coccoliths). Although it is possible to cultivate members of this species axenically, they are closely associated with bacteria in nature, and many commonly used cultures are polymicrobial. The *E. huxleyi* M217 and CCMP1516 strains both contain bacteria and represent isogenic lines isolated from the South Pacific in 1991. M217 can form coccoliths, but CCMP1516 has lost the ability to calcify (1). Bacteria associated with the former were isolated and their genomes sequenced to define its microbiota.

DNA was extracted from single-colony isolates using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. Sequencing libraries from the genomic DNA extracts were prepared using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA). Whole-genome sequencing was performed using the NextSeq 500/550 High Output Kit version 2 (for 300 cycles) and NextSeq sequencing technology (Illumina), generating 150-bp paired-end reads. *De novo* assembly of the reads into contiguous sequences (contigs) was done using the CLC Genomics Workbench version 7.5.2 (CLC bio, Aarhus, Denmark). The draft genomes were then annotated using RAST version 2.0 (2) or PGAP (http://www.ncbi.nlm.nih.gov/genome/annotation_prok). All of the genomes se-

quenced exceeded 90× coverage, and the characteristics of the assemblies obtained are described in Table 1. Species identities were determined by an average nucleotide identity >95% using JSpecies version 1.2.1 (3), and genus identities were determined by an average amino acid identity (AAI) >60% using the AAI calculator (<http://enve-omics.ce.gatech.edu/aai>) (4) with previously sequenced genomes in the GenBank database. This analysis identified one *Bacteroidetes* isolate of the *Sphingobacteriales* order (*Balneola* sp. EhC07), one gammaproteobacterium of the *Alteromonadales* order (*Marinobacter* sp. EhC06), and three alphaproteobacteria from the *Roseobacter* clade (*Jannaschia* sp. EhC01, *Roseovarius indicus* EhC03, and *Sulfitobacter* sp. EhC04). *Rhodobacteraceae* bacterium EhC02 and *Sphingomonadales* bacterium EhC05 could not be attributed to a specific genus and were therefore named after the family and order to which they could be assigned, respectively.

All the roseobacters encode genes for the ability to utilize the algal osmolyte dimethylsulfoniopropionate and to degrade lignin, a likely component of the *E. huxleyi* cell wall (5, 6). All bacteria have the ability to transport siderophores, but only one of them, *Marinobacter* sp. EhC06, has the ability to synthesize them. Like many other algal symbionts, all these bacteria encode a pathway to produce multiple vitamin Bs and the phytohormone auxin (7–9).

TABLE 1 Genome features and GenBank accession numbers of the seven strains isolated from a polymicrobial culture of C-type *Emiliana huxleyi* M217

| Isolate | Accession no. | Genome size (kb) | No. of contigs | N_{50} (kb) | G+C (mol%) |
|---|---|------------------|----------------|---------------|------------|
| <i>Jannaschia</i> sp. EhC01 | LXYJ000000000 | 4,580 | 96 | 98 | 62.7 |
| <i>Rhodobacteraceae</i> bacterium EhC02 | LXYH000000000 | 4,089 | 60 | 161 | 63.5 |
| <i>Roseovarius indicus</i> EhC03 | LXYQ000000000 | 5,512 | 168 | 81 | 64.8 |
| <i>Sulfitobacter</i> sp. EhC04 | LXYI000000000 | 4,853 | 89 | 162 | 61.3 |
| <i>Sphingomonadales</i> bacterium EhC05 | LXYP000000000 | 4,190 | 77 | 165 | 53.7 |
| <i>Marinobacter</i> sp. EhC06 | LXYO000000000 | 4,620 | 38 | 540 | 57.2 |
| <i>Balneola</i> sp. EhC07 | LXYG000000000 | 3,624 | 20 | 316 | 38.1 |

This suggests a well-established symbiosis with their host, involving a production and consumption of a number of metabolites.

Nucleotide sequence accession numbers. The whole-genome shotgun projects have been deposited in DDBJ/ENA/GenBank under the accession numbers listed in [Table 1](#).

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REFERENCES

- Zhang X, Gamarra J, Castro S, Carrasco E, Hernandez A, Mock T, Hadaegh AR, Read BA. 2016. Characterization of the small RNA transcriptome of the marine coccolithophorid, *Emiliana huxleyi*. *PLoS One* 11: e0154279. <http://dx.doi.org/10.1371/journal.pone.0154279>.
- 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. <http://dx.doi.org/10.1186/1471-2164-9-75>.
- Richter M, Rosselló-Móra R. 2009. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA* 106: 19126–19131. <http://dx.doi.org/10.1073/pnas.0906412106>.
- Luo C, Rodriguez-R LM, Konstantinidis KT. 2014. MyTaxa: an advanced taxonomic classifier for genomic and metagenomic sequences. *Nucleic Acids Res* 42:e73. <http://dx.doi.org/10.1093/nar/gku169>.
- Seyedsayamdost MR, Case RJ, Kolter R, Clardy J. 2011. The Jekyll-and-Hyde chemistry of *Phaeobacter gallaeciensis*. *Nat Chem* 3:331–335. <http://dx.doi.org/10.1038/nchem.1002>.
- Labeeuw L, Martone PT, Boucher Y, Case RJ. 2015. Ancient origin of the biosynthesis of lignin precursors. *Biol Direct* 10:23. <http://dx.doi.org/10.1186/s13062-015-0052-y>.
- Labeeuw L, Khey J, Bramucci AR, Atwal H, de la Mata AP, Harynuk J, Case RJ. 2016. Indole-3-acetic acid is produced by *Emiliana huxleyi* coccolith-bearing cells and triggers a physiological response in bald cells. *Front Microbiol* 7:828. <http://dx.doi.org/10.3389/fmicb.2016.00828>.
- Amin SA, Hmelo LR, van Tol HM, Durham BP, Carlson LT, Heal KR, Morales RL, Berthiaume CT, Parker MS, Djunaedi B, Ingalls AE, Parsek MR, Moran MA, Armbrust EV. 2015. Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. *Nature* 522: 98–101. <http://dx.doi.org/10.1038/nature14488>.
- Croft MT, Lawrence AD, Raux-Deery E, Warren MJ, Smith AG. 2005. Algae acquire vitamin B12 through a symbiotic relationship with bacteria. *Nature* 438:90–93. <http://dx.doi.org/10.1038/nature04056>.