Genome Sequence of Strain HIMB55, a Novel Marine Gammaproteobacterium of the OM60/NOR5 Clade

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Strain HIMB55 is a phylogenetically unique member of the OM60/NOR5 clade of the *Gammaproteobacteria* isolated from coastal seawater of Kaneohe Bay on the northeastern shore of Oahu, Hawaii, by extinction culturing in seawater-based oligotrophic medium. Here we present the genome sequence of strain HIMB55, including genes for bacteriochlorophyll-based phototrophy.

Strain HIMB55 was isolated from surface seawater of Kaneohe Bay on the northeastern shore of Oahu, Hawaii, via dilution-to-extinction culturing methods in low-nutrient heterotrophic medium (3). rRNA gene sequencing revealed that HIMB55 belongs to the OM60/NOR5 clade, a monophyletic lineage of marine bacterioplankton first identified via 16S rRNA gene cloning and sequencing from the Sargasso Sea (13), coastal waters off Cape Hatteras, North Carolina (14), and the North Sea (4). Cells of this lineage have been detected in high abundance (>10% of total cell counts) in the surface layer of the coastal ocean (5). A number of cultured strains from the OM60/NOR5 clade have been interrogated via whole genome sequencing, including *Congregibacter litoralis* KT71 (6), two strains isolated from Oregon coastal seawater (16), and one from the Yellow Sea (9). Comparative genomics and physiological experimentation show that members of the OM60/NOR5 clade possess diverse metabolic potential, including bacteriochlorophyll-based anoxygenic phototrophy and chemoheterotrophy (1, 2, 6, 15). Recently, a proteorhodopsin gene was also identified within the genome of OM60/NOR5 strain IMCC3088 (9).

The genome of HIMB55 was shotgun sequenced by the J. Craig Venter Institute as part of the Moore Foundation Microbial Genome Sequencing Project (<http://camera.calit2.net/microgenome>), resulting in 623,303 reads of 454 FLX Titanium sequence data. A draft genome was assembled to 95× coverage from 619,671 reads (260,601,228 bases) of sequence data. Gaps between contigs of the initial draft genome assembly were closed by sequencing PCR amplicons from genomic DNA, resulting in a single contig containing one unclosed gap. Functional annotation was performed with the Integrated Microbial Genomes Expert Review (IMG-ER) pipeline (12). Genes were identified using Prodigal (8) and were translated and used to search the NCBI nonredundant protein database and the UniProt, TIGRFam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Nontranslated genes were predicted using tRNAScanSE (11), RNAmmer (10), and Rfam (7). Additional gene prediction analysis and manual functional annotation were performed within IMG-ER.

The draft genome of HIMB55 comprised 2,736,988 bases and had a G+C content of 52.90%. A total of 2,515 predicted open reading frames and 2,470 predicted protein-coding genes were identified, of which 2,085 have a predicted function. There are predicted single copies of the 5S, 16S, and 23S rRNA genes and 38 predicted tRNAs. A complete glycolysis pathway and tricarboxylic acid (TCA) cycle are predicted from the genome sequence, but the

pentose-phosphate and Entner-Doudoroff pathways are incomplete. The genome contains complete pathways for the synthesis of all essential amino acids and a number of vitamins, including vitamin B₆, para-aminobenzoic acid, folate, lipoic acid, nicotinic acid and nicotinamide, pantothenic acid, coenzyme A, and ubiquinone. Pathways for the synthesis of biotin, hemin, retinal, riboflavin, thiamines, and vitamin B₁₂ are incomplete.

The genome of strain HIMB55 contains genes for bacteriochlorophyll-based phototrophy but does not contain a proteorhodopsin gene comparable to that described for strain IMCC3088 (9). HIMB55 also contains genes that encode two phosphoenolpyruvate carboxylases and a carbonic anhydrase, indicating the potential for autotrophic carbon fixation.

Nucleotide sequence accession numbers. This Whole Genome Shotgun project has been deposited at GenBank under accession number [AGIF00000000](https://www.ncbi.nlm.nih.gov/nuccore/AGIF00000000). The version described in this paper is the second version, AGIF02000001.1.

ACKNOWLEDGMENTS

We thank Steve Ferriera, Justin Johnson, and other JCVI scientists for performing the sequencing and assembly of this genome, which was performed by the J. Craig Venter Institute through support provided by the Gordon and Betty Moore Foundation Marine Microbial Sequencing Project (<http://www.moore.org/marinemicro>). We also thank Maria Kim of the JCVI for her assistance.

Strain preparation, genomic DNA extraction, and annotation were supported by the Center for Microbial Oceanography: Research and Education (NSF Science and Technology Center Award EF-0424599).

REFERENCES

1. Cho J-C, Giovannoni SJ. 2004. Cultivation and growth characteristics of a diverse group of oligotrophic marine *Gammaproteobacteria*. *Appl. Environ. Microbiol.* 70:432–440.
2. Cho J-C, et al. 2007. Polyphyletic photosynthetic reaction centre genes in oligotrophic marine *Gammaproteobacteria*. *Environ. Microbiol.* 9:1456–1463.
3. Connon SA, Giovannoni SJ. 2002. High-throughput methods for culturing microorganisms in very-low-nutrient media yield diverse new marine isolates. *Appl. Environ. Microbiol.* 68:3878–3885.

Received 3 February 2012 Accepted 13 February 2012

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doi:10.1128/JB.00171-12

4. Eilers H, Pernthaler J, Glöckner FO, Amann R. 2000. Culturability and in situ abundance of pelagic bacteria from the North Sea. *Appl. Environ. Microbiol.* **66**:3044–3051.
5. Eilers H, et al. 2001. Isolation of novel pelagic bacteria from the German Bight and their seasonal contributions to surface picoplankton. *Appl. Environ. Microbiol.* **67**:5134–5142.
6. Fuchs BM, et al. 2007. Characterization of a marine gammaproteobacterium capable of aerobic anoxygenic photosynthesis. *Proc. Natl. Acad. Sci. U. S. A.* **104**:2891–2896.
7. Griffiths-Jones S, et al. 2004. Rfam: annotating non-coding RNAs in complete genomes. *Nucleic Acids Res.* **33**:D121–D124.
8. Hyatt D, et al. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* **11**:119.
9. Jang Y, Oh H-M, Kang I, Lee K, Yang S-J, Cho J-C. 2011. Genome sequence of strain IMCC3088, a proteorhodopsin containing marine bacterium belonging to the OM60/NOR5 clade. *J. Bacteriol.* **193**:3415–3416.
10. Lagesen K, et al. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res.* **35**:3100–3108.
11. Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* **25**:955–964.
12. Markowitz VM, Ivanova NN, Chen IMA, Chu K, Kyrpides NC. 2009. IMG ER: a system for microbial genome annotation expert review and curation. *Bioinformatics* **25**:2271–2278.
13. Mullins TD, Britschgi TB, Krest RL, Giovannoni SJ. 1995. Genetic comparisons reveal the same unknown bacterial lineages in Atlantic and Pacific bacterioplankton communities. *Limnol. Oceanogr.* **40**:148–158.
14. Rappé MS, Kemp PF, Giovannoni SJ. 1997. Phylogenetic diversity of marine coastal picoplankton 16S rRNA genes cloned from the continental shelf off Cape Hatteras, North Carolina. *Limnol. Oceanogr.* **42**:811–826.
15. Spring S, Lünsdorf H, Fuchs BM, Tindall BJ. 2009. The photosynthetic apparatus and its regulation in the aerobic gammaproteobacterium *Congregibacter litoralis* gen. nov., sp. nov. *PLoS One* **4**:e4866.
16. Thrash JC, et al. 2010. Genome sequences of strains HTCC2148 and HTCC2080, belonging to the OM60/NOR5 clade of the *Gammaproteobacteria*. *J. Bacteriol.* **192**:3842–3843.