Genome sequence of the novel marine member of the Gammaproteobacteria strain HTCC5015.

Journal of Bacteriology

Genome Sequence of the Novel Marine Member of the *Gammaproteobacteria* Strain HTCC5015

J. Cameron Thrash, Ulrich Stingl, Jang-Cheon Cho, Steve Ferriera, Justin Johnson, Kevin L. Vergin and Stephen J. Giovannoni

J. Bacteriol. **2010, 192(14):3838. DOI: 10.1128/JB.00510-10. Published Ahead of Print 14 May 2010.**

JOURNAL OF BACTERIOLOGY, July 2010, p. 3838–3839 Vol. 192, No. 14
0021-9193/10/$12.00 doi:10.1128/JB.00510-10

GENOME ANNOUNCEMENTS

Genome Sequence of the Novel Marine Member of the *Gammaproteobacteria* Strain HTCC5015▽

J. Cameron Thrash,[1] Ulrich Stingl,[1]# Jang-Cheon Cho,[2] Steve Ferriera,[3] Justin Johnson,[3] Kevin L. Vergin,[1] and Stephen J. Giovannoni[1]*

Department of Microbiology, Oregon State University, Corvallis, Oregon 97331[1]; Division of Biology and Ocean Sciences, Inha University, Incheon 402-751, Republic of Korea[2]; and J. Craig Venter Institute, 9704 Medical Center Drive, Rockville, Maryland 20850[3]

Received 4 May 2010/Accepted 5 May 2010

HTCC5015 is a novel, highly divergent marine member of the *Gammaproteobacteria*, currently without a cultured representative with greater than 89% 16S rRNA gene identity to itself. The organism was isolated from water collected from Hydrostation S south of Bermuda using high-throughput dilution-to-extinction culturing techniques. Here we present the genome sequence of the unique *Gammaproteobacterium* strain HTCC5015.

In our ongoing research to isolate and characterize novel bacterioplankton, HTCC5015 was cultivated using previously described high-throughput culturing (HTC) techniques (1, 7). Water collected from a depth of 10 m at Hydrostation S, 12 miles southeast of Bermuda (see reference 6), served as the inoculum for 0.2-μm-filtered/microwave-sterilized heterotrophic medium (FMHM) containing 10 μM NH_4Cl, 1 μM KH_2PO_4, and 10 μM algal lysate. A novel marine gammaproteobacterium, HTCC5015 is phylogenetically unique, having 89% or less 16S rRNA gene identity to other cultured organisms, and thus probably represents the first organism of a novel order in this class. The closest phylogenetic neighbors include members of *Natronocella*, *Thioalkalivibrio*, and *Thiohalomonas*.

Here we present the genome sequence of HTCC5015. DNA shotgun sequencing was conducted by the J. Craig Venter Institute as part of the Moore Foundation Microbial Genome Sequencing Project (http://www.moore.org/microgenome). A draft, unclosed genome consisting of 62 contigs (ABSJ01000001 to ABSJ01000062) was obtained and annotated with the GenDB annotation application program (3) at the Center for Genome Research and Biocomputing at Oregon State University, similarly to that previously described (4, 5). Autoannotation was completed by merging results from the Glimmer 2.0 modeling software package (2) and contrasting with the basic local alignment search tool for proteins (BLASTp). In addition, the protein set was searched against the KEGG, SwissProt, Clusters of Orthologous Groups (COG), Pfam, and Interpro protein databases to annotate the EC number, gene call, and gene description. GenDB annotations predicted major metabolic pathways and biosynthesis of amino acids, vitamins, and growth factors. Manual BLAST searches were run to confirm autoannotations when necessary. G+C mole percent measurements were computed using the genome sequence. DNA base composition was calculated using the Practical Extraction and Reporting Language (PERL).

The uncompleted draft genome has 2,612,424 bases and is comprised of 2,492 predicted open reading frames (ORFs), with a G+C content of 54.05%. There are predicted single copies of the 5S, 16S, and 23S rRNA genes and 33 predicted tRNAs. There are putative genes for a complete tricarboxylic acid (TCA) cycle, but there is no predicted 6-phosphofructokinase, and thus, the glycolysis pathway is incomplete. Similarly, the genome appears to lack complete pentose-phosphate and Entner-Doudoroff pathways. There are four predicted TonB genes, 41 predicted ABC transporters, and many predicted type II secretion genes. There are also putative genes for the synthesis of all essential amino acids and a number of essential vitamins, including biotin, riboflavin, folate, and thiamine. In addition, there are 15 predicted phage-related genes, including those for an endonuclease and tail, capsid, baseplate, and assembly proteins.

Nucleotide sequence accession number. The draft genome sequence of HTCC5015 is available in GenBank under accession number ABSJ00000000. The GenDB-generated data were also processed to be accessible in the Marine Microbial Genomics database at Oregon State University (http://bioinfo.cgrb.oregonstate.edu/microbes/).

* Corresponding author. Mailing address: Department of Microbiology, Oregon State University, Corvallis, OR 97331. Phone: (541) 737-1835. Fax: (541) 737-0496. E-mail: steve.giovannoni@oregonstate.edu.

Present address: Red Sea Science Center, King Abdullah University of Science and Technology (KAUST), 23955 Thuwal, Kingdom of Saudi Arabia.

▽ Published ahead of print on 14 May 2010.

Sequencing, assembly, and annotation and data analysis were supported by the Gordon and Betty Moore Foundation Marine Microbiology Initiative as part of its Marine Microbial Sequencing Project (www.moore.org/marinemicro) and by an individual investigator award to S.J.G.

We thank Scott Givan at the Oregon State University Center for Genome Research and Biocomputing for his work in developing the genome annotation software.

REFERENCES

1. **Connon, S. A., and S. J. Giovannoni.** 2002. High-throughput methods for culturing microorganisms in very-low-nutrient media yield diverse new marine isolates. Appl. Environ. Microbiol. **68:**3878–3885.
2. **Delcher, A. L., D. Harmon, S. Kasif, O. White, and S. L. Salzberg.** 1999. Improved microbial gene identification with GLIMMER. Nucleic Acids Res. **27:**4636–4641.
3. **Meyer, F., A. Goesmann, A. McHardy, D. Bartels, T. Bekel, J. Clausen, J. Kalinowski, B. Linke, O. Rupp, R. Giegerich, and A. PuEhler.** 2003. GenDB—an open source genome annotation system for prokaryote genomes. Nucleic Acids Res. **31:**2187–2195.
4. **Oh, H.-M., S. J. Giovannoni, S. Ferriera, J. Johnson, and J.-C. Cho.** 2009. Complete genome sequence of *Erythrobacter litoralis* HTCC2594. J. Bacteriol. **191:**2419–2420.
5. **Oh, H.-M., S. J. Giovannoni, K. Lee, S. Ferriera, J. Johnson, and J.-C. Cho.** 2009. Complete genome sequence of *Robiginitalea biformata* HTCC2501. J. Bacteriol. **191:**7144–7145.
6. **Steinberg, D. K., C. A. Carlson, N. R. Bates, R. J. Johnson, A. F. Michaels, and A. H. Knap.** 2001. Overview of the US JGOFS Bermuda Atlantic Time-series Study (BATS): a decade-scale look at ocean biology and biogeochemistry. Deep Sea Res. II **48:**1405–1447.
7. **Stingl, U., H. J. Tripp, and S. J. Giovannoni.** 2007. Improvements of high-throughput culturing yielded novel SAR11 strains and other abundant marine bacteria from the Oregon coast and the Bermuda Atlantic Time Series study site. ISME J. **1:**361–371.