Complete Genome Sequence of *Croceibacter atlanticus* HTCC2559^{T∀}

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Here we announce the complete genome sequence of *Croceibacter atlanticus* HTCC2559^T, which was isolated by high-throughput dilution-to-extinction culturing from the Bermuda Atlantic Time Series station in the Western Sargasso Sea. Strain HTCC2559^T contained genes for carotenoid biosynthesis, flavonoid biosynthesis, and several macromolecule-degrading enzymes. The genome confirmed physiological observations of cultivated *Croceibacter atlanticus* strain HTCC2559^T, which identified it as an obligate chemoheterotroph.

The phylum *Bacteroidetes* comprises 6 to \sim 30% of total bacterial communities in the ocean by fluorescence *in situ* hybridization (8–10). Most marine *Bacteroidetes* are in the family *Flavobacteriaceae*, most of which are aerobic respiratory heterotrophs that form a well-defined clade by 16S rRNA phylogenetic analyses (4). The members of this family are well known for degrading macromolecules, including chitin, DNA, cellulose, starch, and pectin (17), suggesting their environmental roles as detritus decomposers in the ocean (6). Marine *Polaribacter* and *Dokdonia* species in the *Flavobacteriaceae* have also shown to have photoheterotrophic metabolism mediated by proteorhodopsins (11, 12).

Several strains of the family *Flavobacteriaceae* were isolated from the Sargasso Sea and Oregon coast, using high-throughput culturing approaches (7). *Croceibacter atlanticus* HTCC2559^T was cultivated from seawater collected at a depth of 250 m from the Sargasso Sea and was identified as a new genus in the family *Flavobacteriaceae* based on its 16S rRNA gene sequence similarities (6). Strain HTCC2559^T met the minimal standards for genera of the family *Flavobacteriaceae* (3) on the basis of phenotypic characteristics (6).

Here we report the complete genome sequence of *Croceibacter atlanticus* HTCC2559^T. The genome sequencing was initiated by the J. Craig Venter Institute as a part of the Moore Foundation Microbial Genome Sequencing Project and completed in the current announcement. Gaps among contigs were closed by Genotech Co., Ltd. (Daejeon, Korea), using direct sequencing of combinatorial PCR products (16). The HTCC2559^T genome was analyzed with a genome annotation system based on GenDB (14) at Oregon State University and with the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (15, 16).

The HTCC2559^T genome is 2,952,962 bp long, with 33.9 mol% G+C content, and there was no evidence of plasmids. The number of protein-coding genes was 2,715; there were two copies of the 16S-23S-5S rRNA operon and 36 tRNA genes. The HTCC2559^T genome contained genes for a complete tri-

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carboxylic acid cycle, glycolysis, and a pentose phosphate pathway. The genome also contained sets of genes for metabolic enzymes involved in carotenoid biosynthesis and also a serine/ glycine hydroxymethyltransferase, which is often associated with the assimilatory serine cycle (13). The potential for HTCC2559^T to use bacterial type III polyketide synthase (PKS) needs to be confirmed because this organism had a naringenin-chalcone synthase (CHS) or chalcone synthase (EC 2.3.1.74), a key enzyme in flavonoid biosynthesis. CHS initiates the addition of three molecules of malonyl coenzyme A (malonyl-CoA) to a starter CoA ester (e.g., 4-coumaroyl-CoA) (1) and takes part in a few bacterial type III polyketide synthase systems (1, 2, 5, 18).

The complete genome sequence confirmed that strain $HTCC2559^{T}$ is an obligate chemoheterotroph because no genes for phototrophy were found. As expected from physiological characteristics (6), the $HTCC2559^{T}$ genome contained a set of genes coding for enzymes required to degrade high-molecular-weight compounds, including peptidases, metallo-/ serine proteases, pectinase, alginate lyases, and α -amylase.

Nucleotide sequence accession number. The complete genome sequence of HTCC2559 was deposited under GenBank accession no. CP002046. The GenDB-generated data were also processed to be accessed at the Marine Microbial Genomics site at Oregon State University (http://bioinfo.cgrb.oregonstate .edu/microbes/).

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REFERENCES

- Austin, M. B., and J. P. Noel. 2003. The chalcone synthase superfamily of type III polyketide synthases. Nat. Prod. Rep. 20:79–110.
- Bauer, M., M. Kube, H. Teeling, M. Richter, T. Lombardot, E. Allers, C. A. Würdemann, C. Quast, H. Kuhl, F. Knaust, D. Woebkin, K. Bischof, M. Mussmann, J. V. Choudhuri, F. Meyer, R. Reinhardt, R. I. Amann, and F. O.

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Glöckner. 2006. Whole genome analysis of the marine *Bacteroidetes 'Gramella forsetii*' reveals adaptations to degradation of polymeric organic matter. Environ. Microbiol. 8:2201–2213.

- Bernardet, J. F., Y. Nakagawa, and B. Holmes. 2002. Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. Int. J. Syst. Evol. Microbiol. 52:1049– 1070.
- 4. Bowman, J. P. 2006. The marine clade of the family Flavobacteriaceae: the genera Aequorivita, Arenibacter, Cellulophaga, Croceibacter, Formosa, Gelidibacter, Gillisia, Maribacter, Mesonia, Muricauda, Polaribacter, Psychro-flexus, Psychroserpens, Robignitalea, Salegentibacter, Tenacibaculum, Ulvibacter, Vitellibacter, and Zobellia, p. 677–694. In M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, and E. Stackebrandt (ed.), The prokaryotes, 3rd ed., vol. 7. Springer, New York, NY.
- Capuano, V., N. Galleron, P. Pujic, A. Sorokin, and S. D. Ehrlich. 1996. Organization of the *Bacillus subtilis* 168 chromosome between kdg and the attachment site of the SPβ prophage: use of Long Accurate PCR and yeast artificial chromosomes for sequencing. Microbiology 142:3005–3015.
- Cho, J.-C., and S. J. Giovannoni. 2003. Croceibacter atlanticus gen. nov., sp nov., a novel marine bacterium in the family *Flavobacteriaceae*. Syst. Appl. Microbiol. 26:76–83.
- 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.
- Cottrell, M. T., and D. L. Kirchman. 2000. Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. Appl. Environ. Microbiol. 66:1692–1697.
- Eilers, H., J. Pernthaler, F. O. Glöckner, and R. Amann. 2000. Culturability and *in situ* abundance of pelagic bacteria from the North Sea. Appl. Environ. Microbiol. 66:3044–3051.
- Glöckner, F. O., B. M. Fuchs, and R. Amann. 1999. Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence *in situ* hybridization. Appl. Environ. Microbiol. 65:3721–3726.
- Gómez-Consarnau, L., J. M. González, M. Coll-Lladó, P. Gourdon, T. Pascher, R. Neutze, C. Pedrós-Alió, and J. Pinhassi. 2007. Light stimulates

growth of proteorhodopsin-containing marine Flavobacteria. Nature 445: 210–213.

- González, J., B. Fernández-Gómez, A. Fernàndez-Guerra, L. Gómez-Consarnau, O. Sánchez, M. Coll-Lladó, J. Del Campo, L. Escudero, R. Rodríguez-Martínez, L. Alonso-Sáez, M. Latasa, I. Paulsen, O. Nedashkovskaya, I. Lekunberri, J. Pinhassi, and C. Pedrós-Alió. 2008. Genome analysis of the proteorhodopsin-containing marine bacterium *Polaribacter* sp MED152 (Flavobacteria). Proc. Natl. Acad. Sci. U. S. A. 105:8724–8729.
- Lidstrom, M. 2006. Aerobic methylotrophic prokaryotes, p. 618–634. *In S. F.* Martin Dworkin, E. Rosenberg, K.-H. Schleifer, and E. Stackebrandt (ed.), The prokaryotes, 3rd ed., vol. 2. Springer, New York, NY.
- Meyer, F., A. Goesmann, A. C. McHardy, D. Bartels, T. Bekel, J. Clausen, J. Kalinowski, B. Linke, O. Rupp, R. Giegerich, and A. Puhler. 2003. GenDB—an open source genome annotation system for prokaryote genomes. Nucleic Acids Res. 31:2187–2195.
- 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.
- 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.
- Reichenbach, H., and M. Dworkin. 1992. The order Cytophagales, p. 3631– 3675. *In* H. G. T. A. Balows, M. Dworkin, W. Harder, and K.-H. Schleifer, (ed.), The prokaryotes, 2nd ed., vol. 4. Springer, Berlin, Germany.
- Schneiker, S., O. Perlova, O. Kaiser, K. Gerth, A. Alici, M. O. Altmeyer, D. Bartels, T. Bekel, S. Beyer, E. Bode, H. B. Bode, C. J. Bolten, J. V. Choudhuri, S. Doss, Y. A. Elnakady, B. Frank, L. Gaigalat, A. Goesmann, C. Groeger, F. Gross, L. Jelsbak, L. Jelsbak, J. Kalinowski, C. Kegler, T. Knauber, S. Konietzny, M. Kopp, L. Krause, D. Krug, B. Linke, T. Mahmud, R. Martinez-Arias, A. C. McHardy, M. Merai, F. Meyer, S. Mormann, J. Munoz-Dorado, J. Perez, S. Pradella, S. Rachid, G. Raddatz, F. Rosenau, C. Ruckert, F. Sasse, M. Scharfe, S. C. Schuster, G. Suen, A. Treuner-Lange, G. J. Velicer, F.-J. Vorholter, K. J. Weissman, R. D. Welch, S. C. Wenzel, D. E. Whitworth, S. Wilhelm, C. Wittmann, H. Blocker, A. Puhler, and R. Muller. 2007. Complete genome sequence of the myxobacterium *Sorangium cellulosum*. Nat. Biotechnol. 25:1281–1289.