JOURNAL OF BACTERIOLOGY, Dec. 2010, p. 6315–6316 Vol. 192, No. 23
0021-9193/10/$12.00 doi:10.1128/JB.01088-10

Genome Sequence of the Marine Alphaproteobacterium HTCC2150, Assigned to the *Roseobacter* Clade▽

Ilnam Kang,[1] Hyun-Myung Oh,[1] Kevin L. Vergin,[2] Stephen J. Giovannoni,[2] and Jang-Cheon Cho[1]*

Division of Biology and Ocean Sciences, Inha University, Incheon 402-751, Republic of Korea,[1] and
Department of Microbiology, Oregon State University, Corvallis, Oregon 97331[2]

Received 12 September 2010/Accepted 20 September 2010

Here we announce the genome sequence of a marine bacterium, HTCC2150, that was isolated off the Oregon coast using dilution-to-extinction culturing and that is affiliated with the *Roseobacter* clade. The 16S rRNA phylogeny showed that the strain was closely related to members of the RCA clade. The genome sequence suggests that strain HTCC2150 is an organoheterotroph carrying diverse metabolic potential, including a close relationship with phytoplankton.

The *Roseobacter* clade in the order *Rhodobacterales* constitutes major alphaproteobacterial lineages in the pelagic ocean together with the SAR11 and SAR116 groups (2, 8). Owing to the well-developed polyphasic taxonomic approach, many novel genera, including *Oceanicola*, *Maribius*, and *Maritimibacter*, have recently been established, exceeding at least 50 genera in this clade (http://www.bacterio.cict.fr). Physiological and genomic analyses of these genera showed that members of the *Roseobacter* clade are phenotypically diverse and functionally heterogeneous, being associated with, e.g., aerobic anoxygenic photoheterotrophy, aerobic sulfite oxidation, methylotrophy, demethylation of dimethylsulfoniopropionate (DMSP), degradation of aromatic compounds, exopolysaccharide production, and rosette formation (9, 10, 13, 14). All of the validly published genera in this clade form colonies on nutrient agar media, such as marine agar 2216. In our continuing studies using dilution-to-extinction culturing (4, 18), however, many novel isolates belonging to several subclades (OM42, NAC11-7, CHAB-I-V, etc.) in the *Roseobacter* clade did not produce colonies upon initial isolation, which made polyphasic characterization extremely difficult. Here we report the cultivation and draft genome sequence of strain HTCC2150, a new member of the *Roseobacter* clade that did not form colonies on nutrient agar medium.

Strain HTCC2150 was cultivated from surface seawaters off the Oregon coast using previously described dilution-to-extinction culturing with a pristine seawater-based medium (3). The initial extinction cultures did not form colonies on various solid agar plates even after prolonged incubation; thus, DNA extraction for genome sequencing was performed using cultures grown in a seawater-based low-nutrient heterotrophic medium (4). Phylogenetic analysis using 16S rRNA gene sequences showed that strain HTCC2150 was most closely related to members of the RCA clade (7, 17), a recently recognized abundant marine bacterial group, with 95 to 96% sequence similarity, but formed a distinct branch in the *Roseobacter* clade.

Genomic DNA was prepared at Oregon State University, sequenced and assembled at the J. Craig Venter Institute, and analyzed by using the Joint Genome Institute IMG system (11) and the GenDB annotation program (12) at the Center for Genome Research and Biocomputing at Oregon State University. A draft genome consisting of 25 contigs (AAXZ01000001 to AAXZ01000025) was 3,582,902 bp in length with a coding density of 92% and contained 40 tRNA genes, 2 copies of the 5S, 16S, and 23S rRNA genes, and 3,667 protein coding genes.

Analysis of the HTCC2150 genome suggests the overall organoheterotrophic mode of carbon and energy acquisition, although we cannot exclude the possibility of lithotrophic energy metabolism mediated by a sulfur oxidation (*sox*) gene cluster and carbon monoxide dehydrogenase (5, 15). The genome had a complete citric acid cycle, pentose phosphate pathway, and Entner-Doudoroff pathway. DMSP demethylase (encoded by *dmdA*) and DMSP lyase (encoded by *dddP*) encoded in the genome could be helpful for the survival of HTCC2150 in coastal environments (9, 16, 19), since members of the *Roseobacter* clade are well known for their association with organic compounds, such as DMSP, originating from phytoplankton (6). In addition, the predicted quorum-sensing-related genes might also be beneficial for HTCC2150 because the microzones surrounding phytoplankton cells could be densely populated with bacteria (1).

Nucleotide sequence accession number. The draft genome sequence of HTCC2150 was deposited in GenBank under the accession number AAXZ00000000. The GenDB-generated data can also be accessed at Marine Microbial Genomics at Oregon State University (http://bioinfo.cgrb.oregonstate.edu/microbes/).

The initial phase of the sequencing, assembly, and annotation efforts was carried out at the Oregon State University with support from a Gordon and Betty Moore Foundation investigator award (to S.J.G.) and the Gordon and Betty Moore Foundation Marine Microbial Sequencing Project. Subsequent data analysis was conducted at Inha University and was also supported by the 21C Frontier Program of Microbial Genomics and Applications (to J.-C.C.).

REFERENCES

1. **Bowen, J. D., K. D. Stolzenbach, and S. W. Chisholm.** 1993. Simulating bacterial clustering around phytoplankton cells in a turbulent ocean. Limnol. Oceanogr. **38:**36–51.
2. **Buchan, A., J. M. Gonzalez, and M. A. Moran.** 2005. Overview of the marine *Roseobacter* lineage. Appl. Environ. Microbiol. **71:**5665–5677.

---

* Corresponding author. Mailing address: Division of Biology and Ocean Sciences, Inha University, Incheon 402-751, Republic of Korea. Phone: 82-32-860-7711. Fax: 82-32-232-0541. E-mail: chojc@inha.ac.kr.

3. **Cho, J.-C., and S. J. Giovannoni.** 2004. Cultivation and growth characteristics of a diverse group of oligotrophic marine *Gammaproteobacteria*. Appl. Environ. Microbiol. **70:**432–440.
4. **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.
5. **Friedrich, C. G., F. Bardischewsky, D. Rother, A. Quentmeier, and J. Fischer.** 2005. Prokaryotic sulfur oxidation. Curr. Opin. Microbiol. **8:**253–259.
6. **Geng, H., and R. Belas.** 2010. Molecular mechanisms underlying roseobacter-phytoplankton symbioses. Curr. Opin. Biotechnol. **21:**332–338.
7. **Giebel, H.-A., D. Kalhoefer, A. Lemke, S. Thole, R. Gahl-Janssen, M. Simon, and T. Brinkhoff.** 1 July 2010. Distribution of *Roseobacter* RCA and SAR11 lineages in the North Sea and characteristics of an abundant RCA isolate. ISME J. doi:10.1038/ismej.2010.87.
8. **Giovannoni, S., and M. Rappé.** 2000. Evolution, diversity and molecular ecology of marine prokaryotes, p. 47–84. *In* D. L. Kirchman (ed.), Microbial ecology of the oceans. John Wiley & Sons, Inc., New York, NY.
9. **Howard, E. C., S. Sun, E. J. Biers, and M. A. Moran.** 2008. Abundant and diverse bacteria involved in DMSP degradation in marine surface waters. Environ. Microbiol. **10:**2397–2410.
10. **Lee, K., Y.-J. Choo, S. J. Giovannoni, and J.-C. Cho.** 2007. *Maritimibacter alkaliphilus* gen. nov., sp. nov., a genome-sequenced marine bacterium of the *Roseobacter* clade in the order *Rhodobacterales.* Int. J. Syst. Evol. Microbiol. **57:**1653–1658.
11. **Markowitz, V. M., I.-M. A. Chen, K. Palaniappan, K. Chu, E. Szeto, Y. Grechkin, A. Ratner, I. Anderson, A. Lykidis, K. Mavromatis, N. N. Ivanova, and N. C. Kyrpides.** 2010. The integrated microbial genomes system: an expanding comparative analysis resource. Nucleic Acids Res. **38:**D382–D390.
12. **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.
13. **Moran, M. A., R. Belas, M. A. Schell, J. M. Gonzalez, F. Sun, S. Sun, B. J. Binder, J. Edmonds, W. Ye, B. Orcutt, E. C. Howard, C. Meile, W. Palefsky, A. Goesmann, Q. Ren, I. Paulsen, L. E. Ulrich, L. S. Thompson, E. Saunders, and A. Buchan.** 2007. Ecological genomics of marine roseobacters. Appl. Environ. Microbiol. **73:**4559–4569.
14. **Moran, M. A., A. Buchan, J. M. Gonzalez, J. F. Heidelberg, W. B. Whitman, R. P. Kiene, J. R. Henriksen, G. M. King, R. Belas, C. Fuqua, L. Brinkac, M. Lewis, S. Johri, B. Weaver, G. Pai, J. A. Eisen, E. Rahe, W. M. Sheldon, W. Ye, T. R. Miller, J. Carlton, D. A. Rasko, I. T. Paulsen, Q. Ren, S. C. Daugherty, R. T. Deboy, R. J. Dodson, A. S. Durkin, R. Madupu, W. C. Nelson, S. A. Sullivan, M. J. Rosovitz, D. H. Haft, J. Selengut, and N. Ward.** 2004. Genome sequence of *Silicibacter pomeroyi* reveals adaptations to the marine environment. Nature **432:**910–913.
15. **Moran, M. A., and W. L. Miller.** 2007. Resourceful heterotrophs make the most of light in the coastal ocean. Nat. Rev. Microbiol. **5:**792–800.
16. **Newton, R. J., L. E. Griffin, K. M. Bowles, C. Meile, S. Gifford, C. E. Givens, E. C. Howard, E. King, C. A. Oakley, C. R. Reisch, J. M. Rinta-Kanto, S. Sharma, S. Sun, V. Varaljay, M. Vila-Costa, J. R. Westrich, and M. A. Moran.** 2010. Genome characteristics of a generalist marine bacterial lineage. ISME J. **4:**784–798.
17. **Selje, N., M. Simon, and T. Brinkhoff.** 2004. A newly discovered *Roseobacter* cluster in temperate and polar oceans. Nature **427:**445–448.
18. **Song, J., H. M. Oh, and J. C. Cho.** 2009. Improved culturability of SAR11 strains in dilution-to-extinction culturing from the East Sea, West Pacific Ocean. FEMS Microbiol. Lett. **295:**141–147.
19. **Todd, J. D., A. R. J. Curson, C. L. Dupont, P. Nicholson, and A. W. B. Johnston.** 2009. The *dddP* gene, encoding a novel enzyme that converts dimethylsulfoniopropionate into dimethyl sulfide, is widespread in ocean metagenomes and marine bacteria and also occurs in some Ascomycete fungi. Environ. Microbiol. **11:**1376–1385.