

# Complete Genome Sequence of Strain HTCC2503<sup>T</sup> of *Parvularcula bermudensis*, the Type Species of the Order “*Parvularculales*” in the Class *Alphaproteobacteria*<sup>∇</sup>

Hyun-Myung Oh,<sup>1</sup> Innam Kang,<sup>1</sup> Kevin L. Vergin,<sup>2</sup> Dongmin Kang,<sup>1</sup> Kwang-Hyun Rhee,<sup>1</sup> Stephen J. Giovannoni,<sup>2</sup> and Jang-Cheon Cho<sup>1\*</sup>

*Division of Biology and Ocean Sciences, Inha University, Incheon 402-751, Republic of Korea,<sup>1</sup> and Department of Microbiology, Oregon State University, Corvallis, Oregon 97331<sup>2</sup>*

Received 8 October 2010/Accepted 19 October 2010

**The order “*Parvularculales*” represents the seventh order in the class *Alphaproteobacteria*. *Parvularcula bermudensis*, the type species of the order, was isolated from the Sargasso Sea using dilution-to-extinction culturing. We present here the complete genome sequence of *Parvularcula bermudensis* HTCC2503<sup>T</sup>, which contains genes for carotenoid biosynthesis, dimethylsulfoniopropionate demethylase, and transduction-like gene transfer agents.**

The high-throughput culturing (HTC) method, which allows large numbers of microbial isolates to be recovered by dilution-to-extinction in natural seawater media (12), has been successfully applied to the isolation of the first cultured representative of the SAR11 clade (20) and many other novel species from the Oregon Coast (5, 10, 13) and the western Sargasso Sea (4, 6–9, 11, 15, 16). Strain HTCC2503<sup>T</sup> was isolated from the Sargasso Sea by the HTC method and registered as the type strain of *Parvularcula bermudensis* that is the type species of the new order “*Parvularculales*” in the *Alphaproteobacteria* (7). The genus *Parvularcula* contains another species, *P. lutaonensis*, a recently cultured, moderately thermotolerant marine bacterium from a coastal hot spring (1).

Here we report the genome sequence of the type strain, *P. bermudensis* HTCC2503<sup>T</sup>, which was initially determined by shotgun sequencing at the J. Craig Venter Institute as a part of the Moore Foundation Microbial Genome Sequencing Project (<http://www.moore.org/microgenome>) and completed in the present study. The sequence in gaps between contigs and scaffolds was completed using direct sequencing of combinatorial PCR products by Macrogen (Seoul, Republic of Korea). The finished genomic contig of HTCC2503<sup>T</sup> was annotated with the GenDB program (17) as in previous analyses (18, 19). Open reading frames (ORFs) were searched by using KEGG, SwissProt, Clusters of Orthologous Groups (COG), Pfam, and InterPro protein databases according to automated GenDB annotation schemes using the Marine Microbial Genomics database at Oregon State University (<http://bioinfo.cgrb.oregonstate.edu/microbes/>). The completed genome of HTCC2503<sup>T</sup> was screened for noncoding rRNAs and tRNAs using RAST (Rapid Annotation using Subsystem Technology)

(2) before the GenBank-deposited draft genome sequence of HTCC2503<sup>T</sup> was updated.

The circular genome of *P. bermudensis* HTCC2503<sup>T</sup> is comprised of 2,687 ORFs, 2,902,643 bp, and a DNA G+C content of 60.0 mol%. The genome was predicted to contain one 16S-23S-5S rRNA operon with 43 tRNA genes. Strain HTCC2503<sup>T</sup> is predicted to possess complete metabolic pathways, including glycolysis, the pentose phosphate pathway, tricarboxylic acid cycle, and amino acid synthesis. The genome also coded for genes for DMSP demethylase, type IV secretion/conjugal transfer systems, and Ton and Tol transport systems in addition to a plethora of genes for cobalt-zinc-cadmium resistance. As expected from the previous physiological study (7), the *P. bermudensis* genome is predicted to code for genes for carotenoid biosynthesis, β-lactamase, flagella synthesis, and various catalytic enzymes for utilizing carbon compounds. The genome contained CRISPR-associated proteins as well as a CRISPR sequence of 1,583 bp that was identified by CRISPR-Finder (14). A homologue of *Rhodobacter capsulatus* gene transfer agent (GTA) was also found in the genome. This transduction-like GTA has been reported from marine bacterioplankton, including members of the *Roseobacter* clade and dilution-to-extinction cultures such as strains HTCC2506, HTCC2594, HTCC2503, and HTCC 2633 (3, 21).

**Nucleotide sequence accession number.** The complete genome sequence of *P. bermudensis* HTCC2503<sup>T</sup> was deposited in GenBank under accession number CP002156. The GenDB-generated data were also processed to be accessible at the Marine Microbial Genomics database at Oregon State University.

The initial phase of genome sequencing and analyses was supported by a Gordon and Betty Moore Foundation investigator award (to S.J.G) and the Gordon and Betty Moore Foundation Marine Microbial Sequencing Project. The completion of the genome sequence and subsequent data analyses were performed at Inha University and supported by the 21C Frontier Program of Microbial Genomics and Applications (to J.-C.C).

\* 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.

<sup>∇</sup> Published ahead of print on 29 October 2010.

## REFERENCES

1. Arun, A. B., W.-M. Chen, W.-A. Lai, J.-H. Chou, P. D. Rekha, F.-T. Shen, S. Singh, and C.-C. Young. 2009. *Parvularcula lutaonensis* sp. nov., a moderately thermotolerant marine bacterium isolated from a coastal hot spring. *Int. J. Syst. Evol. Microbiol.* **59**:998–1001.
2. Aziz, R., D. Bartels, A. Best, M. DeJongh, T. Disz, R. Edwards, K. Formsma, S. Gerdes, E. Glass, M. Kubal, F. Meyer, G. Olsen, R. Olson, A. Osterman, R. Overbeek, L. McNeil, D. Paarmann, T. Paczian, B. Parrello, G. Pusch, C. Reich, R. Stevens, O. Vassieva, V. Vonstein, A. Wilke, and O. Zagnitko. 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* **9**:75.
3. Biers, E. J., K. Wang, C. Pennington, R. Belas, F. Chen, and M. A. Moran. 2008. Occurrence and expression of gene transfer agent genes in marine bacterioplankton. *Appl. Environ. Microbiol.* **74**:2933–2939.
4. 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.
5. 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.
6. Cho, J. C., and S. J. Giovannoni. 2003. *Fulvimarina pelagi* gen. nov., sp. nov., a marine bacterium that forms a deep evolutionary lineage of descent in the order “*Rhizobiales*”. *Int. J. Syst. Evol. Microbiol.* **53**:1853–1859.
7. Cho, J. C., and S. J. Giovannoni. 2003. *Parvularcula bermudensis* gen. nov., sp. nov., a marine bacterium that forms a deep branch in the *Alphaproteobacteria*. *Int. J. Syst. Evol. Microbiol.* **53**:1031–1036.
8. Cho, J. C., and S. J. Giovannoni. 2006. *Pelagibaca bermudensis* gen. nov., sp. nov., a novel marine bacterium within the *Roseobacter* clade in the order *Rhodobacterales*. *Int. J. Syst. Evol. Microbiol.* **56**:855–859.
9. Cho, J. C., and S. J. Giovannoni. 2004. *Robiginitalea biformata* gen. nov., sp. nov., a novel marine bacterium in the family *Flavobacteriaceae* with a higher G+C content. *Int. J. Syst. Evol. Microbiol.* **54**:1101–1106.
10. Cho, J. C., K. L. Vergin, R. M. Morris, and S. J. Giovannoni. 2004. *Lentisphaera araneosa* gen. nov., sp. nov., a transparent exopolymer producing marine bacterium, and the description of a novel bacterial phylum, *Lentisphaerae*. *Environ. Microbiol.* **6**:611–621.
11. Choi, D. H., J. C. Cho, B. D. Lanoil, S. J. Giovannoni, and B. C. Cho. 2007. *Maribius salinus* gen. nov., sp. nov., isolated from a solar saltern and *Maribius pelagius* sp. nov., cultured from the Sargasso Sea, belonging to the *Roseobacter* clade. *Int. J. Syst. Evol. Microbiol.* **57**:270–275.
12. 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.
13. Giovannoni, S. J., D. H. Hayakawa, H. J. Tripp, U. Stingl, S. A. Givan, J. C. Cho, H. M. Oh, J. B. Kitner, K. L. Vergin, and M. S. Rappé. 2008. The small genome of an abundant coastal ocean methylotroph. *Environ. Microbiol.* **10**:1771–1782.
14. Grissa, I., G. Vergnaud, and C. Pourcel. 2007. CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats. *Nucleic Acids Res.* **35**:W52–W57.
15. 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.
16. Lee, K., Y. J. Choo, S. J. Giovannoni, and J. C. Cho. 2007. *Ruegeria pelagia* sp. nov., isolated from the Sargasso Sea, Atlantic Ocean. *Int. J. Syst. Evol. Microbiol.* **57**:1815–1818.
17. Meyer, F., A. Goesmann, A. C. McHardy, D. Bartels, T. Bekel, J. Clausen, J. Kalinowski, B. Linke, O. Rupp, and R. Giegerich. 2003. GenDB—an open source genome annotation system for prokaryote genomes. *Nucleic Acids Res.* **31**:2187–2195.
18. Oh, H.-M., S. J. Giovannoni, S. Ferreira, J. Johnson, and J.-C. Cho. 2009. Complete genome sequence of *Erythrobacter litoralis* HTCC2594. *J. Bacteriol.* **191**:2419–2420.
19. Oh, H.-M., S. J. Giovannoni, K. Lee, S. Ferreira, J. Johnson, and J.-C. Cho. 2009. Complete genome sequence of *Robiginitalea biformata* HTCC2501. *J. Bacteriol.* **191**:7144–7145.
20. Rappé, M. S., S. A. Connon, K. L. Vergin, and S. J. Giovannoni. 2002. Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. *Nature* **418**:630–633.
21. Zhao, Y., K. Wang, C. Budinoff, A. Buchan, A. Lang, N. Jiao, and F. Chen. 2008. Gene transfer agent (GTA) genes reveal diverse and dynamic *Roseobacter* and *Rhodobacter* populations in the Chesapeake Bay. *ISME J.* **3**:364–373.