

A Genomic and Proteomic Characterization of the First Cultured Oligotrophic Marine Gammaproteobacteria from the SAR92 Clade

Brett L. Mellbye, Sarah M. Sowell, Uli Stingl, Kevin L. Vergin, and Stephen J. Giovannoni

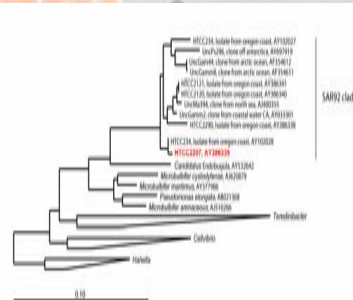
Department of Microbiology, 220 Nash Hall, Oregon State University Corvallis, OR 97331 USA

E-mail: mellbye@onid.orst.edu

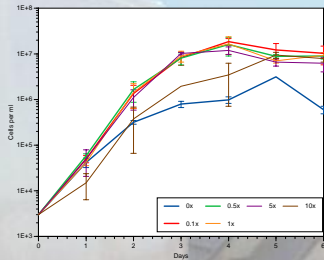
http://mcb.science.oregonstate.edu/giovannoni



16S rRNA Phylogeny



Carbon Concentration Effects



Abstract

Fluorescent *in situ* hybridization and RNA blotting analysis of environmental samples from the Oregon coast suggest that the SAR92 clade is found predominantly in coastal surface water. Strain HTCC2207 is the first isolate cultured from this clade. It was isolated from the Oregon coast by extinction culturing in autoclaved seawater and characterized by genome sequencing and proteomic analysis by liquid chromatography/tandem mass spectrometry. Culture experiments and the genome sequence indicate that HTCC2207 is a motile, aerobic, heterotrophic cell. Optimal growth rate (3.15 d⁻¹) occurred at 16 °C in natural seawater amended with nitrogen, phosphorus, vitamins, and a mixture of organic carbon compounds yielding a maximum cell density of 1.85 × 10⁷ cells per ml. In contrast, the maximum cell density in unamended seawater was 1.01 × 10⁶ cells per ml. Initial sequencing has so far revealed an unclosed genome of 2,619,777 base pairs coding for 2390 open reading frames. The G+C content is 49.10 mol %. Proteomic analyses identified 146 expressed proteins including a sulfate adenylyltransferase, biopolymer transporter, flagellin modifier, ABC-type transporter, efflux transporter, urease, and pilus assembly pathway proteins.

Introduction

Epifluorescence and direct viable counting methods suggests that between 0.01 to 0.1% of all microbial cells from marine environments will form colonies on standard agar plates typically used for isolating bacteria. In addition, current standard practices in the characterization of new species require cultivation on complex, high nutrient media that does not support the growth of most oligotrophic marine bacteria. Here we report on a new method of characterization of environmental isolates that utilizes harvested biomass for complete genome sequencing and genomic analysis, proteomic analysis, fatty acid analysis, and analysis of growth conditions. HTCC 2207, a model oligotrophic *Gammaproteobacterium*, was chosen to be sequenced and characterized.

HTCC 2207 Unclosed Genome Structure

Contigs	6
Genome Size	Approx. 2,619,777 base pairs
ORFs	Approx. 2390
Mol G+C %	49.10

Proteomic Results

Gene/ORF Name	Probable Function
sat	sulfate adenylyltransferase, sulfate metabolism
motA/tolQ/exbB	proton channel family, biopolymer transport
gspE	General secretion pathway protein
fucP	glucose/galactose transporter family protein
C7_0286	putative nitrate transporter
fliM	Flagellin modification protein, motility confirmed by genome
C7_0396	phosphate ABC transporter, permease protein
ureC	urease, alpha subunit
ttG2	toluene tolerance, putative ABC transporter
pilB	Type 4 fimbrial assembly protein, pilus assembly
Proteome Coverage	146 hits/2390 total ORFs

Metabolic Pathways

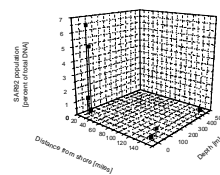
Pathway	Prediction
Glycolysis	+
TCA cycle	+
Glyoxylate shunt	+
Respiration	+
Pentose phosphate cycle	+
Fatty Acid Biosynthesis	+
Cell Wall Biosynthesis	+
Amino Acid Biosynthesis (20)	+
Heme Biosynthesis	+
Ubiquinone	+
Nicotinate and nicotinamide	+
Folate	+
Riboflavin	+
Pantothenate	+
B6	+
Thiamine	-
Biotin	?
B12	-

Metabolic reconstruction predictions made using KEGG

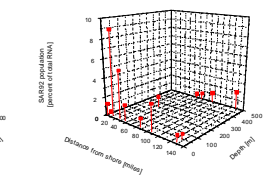
Where does the SAR92 clade play an important role?

RNA Dot Blot and Fluorescent In Situ Hybridization (FISH) analyses of seawater samples indicate that the SAR92 clade predominates in coastal surface waters.

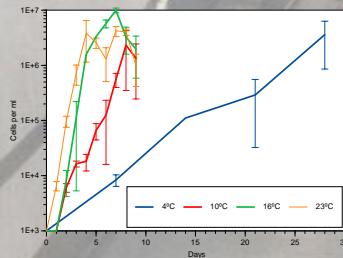
FISH



RNA Dot Blot



Temperature Effects



HTCC 2207



Image courtesy of Michael Nesson, OSU Electron Microscopy Facility

Fatty Acid Profile

Major Fatty Acids	Percentage
C _{16:1} ω7c / C _{16:1} ω6c*	38.01
C _{18:1} ω7c	12.07

*Summed feature that represents two or more fatty acids could not be separated by GLC with the MIDI system.

Conclusions

- HTCC 2207 is characterized as a novel genus and species based on phenotypic and genotypic traits elucidated without traditional culturing.
- HTCC 2207 is a model oligotroph because it possesses the ability to grow at low carbon concentrations.
- The SAR92 clade, which includes HTCC 2207, predominates in Oregon coastal waters where it may reach up to 7% of the total bacterioplankton.