

Polyphasic taxonomy of marine bacteria from the SAR11 group Ia: *Pelagibacter ubiquis* (strain HTCC1062) & *Pelagibacter bermudensis* (strain HTCC7211)

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Abstract

Marine bacteria from the SAR11 clade (class *Alphaproteobacteria*), specifically strains HTCC1062 and HTCC7211, were characterized according to a polyphasic taxonomic approach. Maximum cell densities and growth rates at various temperatures, salinities, and pH's were analyzed. Strains HTCC1062 and HTCC7211 were observed as having different growth optimums. On the basis of experimental data obtained in this study, two separate novel species are proposed, *Pelagibacter ubiquis* gen. nov., sp. nov. (HTCC1062), & *Pelagibacter bermudensis* sp. nov. (HTCC7211).

Introduction

SAR11 is the most abundant clade of marine bacteria in the oceans, often comprising 30% of the euphotic zone (Tripp et al., 2007). Despite their abundance, very few microbial cells from marine environments have been cultured and classified. These cells are difficult to grow in laboratories because of their non-colony forming properties and oligotrophic preferences (Tripp et al., 2007). Growth of SAR11 bacteria in artificial seawater medium using high-throughput culturing methods is a recent advancement.

In this study, two strains of marine bacteria from the SAR11 group Ia were isolated using high-throughput culturing methods (Connon & Giovannoni, 2002) and characterized by polyphasic approaches (Vandamme et al., 1996). Polyphasic taxonomic analyses indicated that these marine bacteria represented separate species; therefore, it is proposed that they should be classified as *Pelagibacter ubiquis* gen. nov., sp. nov. (HTCC1062), and *Pelagibacter bermudensis* sp. nov. (HTCC7211).

Materials and Methods

Cells from both strains were grown to a density of 10^4 cells/mL in low-nutrient heterotrophic medium (autoclaved seawater amended with 1.0 μ M NH_4Cl , 0.1 μ M

KH_2PO_4 , vitamin mix at a 10^{-4} dilution stock, 1X mixed carbons (0.001% (w/v) D-glucose, D-ribose, succinic acid, pyruvic acid, glycerol, N-acetyl D-glucosamine, and 0.002% (v/v) ethanol)). They were transferred to autoclaved artificial seawater medium (macronutrients ($(\text{NH}_4)_2\text{SO}_4$, NaH_2PO_4), buffer (NaHCO_3), Turk's Island Salt Mix ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, KCl), NaCl, & trace metals ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, Na_2SeO_3 , & $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$)) (Moore et al., 2007).

Temperature, pH, and salinity experiments were conducted in triplicates. For the temperature experiments, 1 mL of inoculum was pipetted into a liter of autoclaved ASW amended with nutrients (100 μ M pyruvate, 5 μ M glycine, 5 μ M methionine, 2 μ M FeCl_3 , and 1X vitamin mix). The ASW was partitioned into individual flasks and stored under various temperature conditions (4°C, 8°C, 12°C, 16°C, 20°C, 23°C, 25°C, & 30°C).

For the pH experiment, autoclaved ASW medium was amended with nutrients (as per above) and partitioned into flasks. The pH levels in the flasks were adjusted with 0.1M HCl and 0.1M NaOH. The flasks were labeled accordingly (pH value: 5, 5.5,

6, 6.5, 7, 7.5, 7.8, 8, 8.5, & 9), inoculated with cells, and then incubated at 16°C.

For the salinity experiments, flasks were prepared that contained ASW medium adjusted to the following salt concentrations: 0%, 0.5%, 1%, 1.0%, 1.5%, 2.0%, 2.2%, 2.5%, 2.8%, 3.0%, 3.5%, 4.0%, and 4.5% NaCl. The flasks were inoculated with cells and then incubated at 16°C.

Cell growth was examined at 1 day intervals until cultures reached stationary phase. Cell densities were measured using the Guava Easy-Cyte Flow Cytometer and Cytosoft Data Analysis and Acquisition software. 200µL samples were pipetted from each flask onto 96-well micro-plates and stained with SYBR Green nucleic acid stain for fifteen minutes prior to analysis. Data was outputted to Excel and plotted.

16S rRNA gene sequences of both strains were obtained as described previously (Cho & Giovannoni, 2003a) and used for phylogenetic analyses. Average nucleotide identity (ANI) of conserved genes between strains was computed by blast and MUMmer algorithms in the Jspecies software program.

Results

The optimum temperature is 16°C for strain HTCC1062 and 23°C for strain HTCC7211. The optimum salinity (% NaCl) is 1.5% for HTCC1062 and 2% for HTCC7211. The optimum pH is 6.5 for HTCC1062 and 8 for HTCC7211. Genomic data shows that there is greater than 98.4% 16S rRNA gene sequence similarity between strains HTCC1062 and HTCC7211. According to the Jspecies program, the strains show less than 95-96% ANI.

Discussion

Experimental results from analysis of growth conditions and genome structure serve show that there are significant differences in phenotypic and genotypic traits between strains HTCC1062 and HTCC7211. Genomic comparisons show

that these two strains share a 16S rRNA gene sequence similarity of 98.9%. Based on 16S rRNA gene sequence similarity (>98.4%), we could not say whether these strains belong to the same species and therefore further genomic comparisons were performed (Stackebrandt and Ebers, 2006). Jspecies software was used to measure the average nucleotide identity for all conserved genes between our strains. ANI value ranging from 95-96% corresponds to a DNA-DNA hybridization value of 70% (Richter & Rossello-Mora, 2009). Our strains have an ANI (ANIB76.73, ANIM 82.61) below 95%, which indicates they are different species.

The growth characteristics of HTCC1062 and HTCC7211 were investigated at different temperatures, salinities, and pH levels. Strain HTCC1062 and strain HTCC7211 have different temperature, pH, and salinity optimums. Strain HTCC7211 shows a higher temperature optimum than strain HTCC1062. Growth curves were generated for the effects of temperature, salinity, and pH on the average growth rate of HTCC1062 and HTCC7211. Specific growth rate at each temperature, pH, and salinity level was calculated from each growth curve during exponential phase.

Consequently, on the basis of both genotypic and phenotypic distinction, we propose that these are two different species. Therefore, strain HTCC1062 should be named *Pelagibacter ubiquis* gen. nov., sp. nov., and strain HTCC7211 should be named *Pelagibacter bermudensis* sp. nov.

References

Cho, J. C. and Giovannoni, S. J., 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.

Connon, S. A. and Giovannoni, S. J., 2002,
High-throughput methods for
culturing microorganisms in very-
low-nutrient media yield diverse new
marine isolates. *Appl. Environ.
Microbiology*, v.68, pgs.3878-3885.

Moore, L.R., Coe, A., Zinser, E.R., Saito,
M.A., Sullivan, M.B., et al., 2007,
Culturing the marine cyanobacterium
Prochlorococcus. *Limnol Oceanogr
Methods*, v.5, pgs.353–362.

Richter, M. and Rosselló-Móra, R., 2009,
Shifting the genomic gold standard
for the prokaryotic species
definition. *Proc Natl Acad Sci USA*,
106(45):19126-31,
<http://www.imedea.uib.es/jspecies/about.html> (December 31, 2011).

Stackebrandt E. and Ebers, J., 2006,
Taxonomic parameters revisited:
tarnished gold standards. *Microbio.
Today*, v.33, pgs.152–155.

Tripp, H. J., Kitner, J. B., Schwalbach, M.
S., Dacey, J. W. H., Wilhelm, L. J.,
and Giovannoni, S. J., 2008, SAR11
marine bacteria require exogenous
reduced sulphur for growth. *Nature*,
v.452, pgs.741-744.

Vandamme, P., Pot, B., Gillis, M., De Vos,
P., Kersters, K. & Swings, J., 1996,
Polyphasic taxonomy, a consensus
approach to bacterial systematics.
Microbiol Rev, v.60, pgs.407–438.