

S3-4**Oligotrophic Colony-Formers in the Yellow Sea and East Sea**

Kiyoung Lee, Hana Kim, Seung-Jo Yang, and Jang-Cheon Cho*

Division of Biology and Ocean Sciences, Inha University

Among the fascinating methodologies applied in the environmental microbiology, nearly all modern methods used today have been developed by marine microbiologists or in the field of marine microbiology. The revolutionary 16S rRNA gene approaches since 1990 have revealed that the most abundant marine microbes (so called SAR11)[1] are beautifully small, obligately oligotrophic [2], and comprising at least 20% of the total microbes in the surface water column [3]. The environmental metagenomic approaches using BAC or fosmid clones[4-6] and shotgun sequencing [7-9] have continuously changed our views about light-utilizing mechanisms in the upper pelagic ocean. Some of mysterious clones for oligotrophic aerobic photoheterotrophs are now in laboratory cultures, proving the power of molecular guesses about the function of marine microbes. For example, obligately oligotrophic members of the SAR11 (*Pelagibacter ubique*) [10] and OM60 clades [11,12] are proven to be functionally homologous for solar energy utilization. These findings indicated that the power of molecular community structure analyses can be better proved when the uncultured microbes are brought in laboratory cultures.

The majorities of marine bacterioplankton community have known to be oligotrophs, most of which do not have cultured representatives. The extensive cultivation studies, however, have also revealed that many of marine oligotrophic bacteria such as oligotrophic marine *Gammaproteobacteria* group can form microcolonies on oligotrophic agar media [11]. To understand what kinds of culturable oligotrophic bacteria are abundant in the ocean and to characterize them by polyphasic taxonomy, a dilution-to-extinction culturing followed by colony observation was applied in this study. Seawater samples were collected from the surface of Goseong area, the East Sea, and Incheon and Deokjeok islands area, the Yellow sea. Either serial-dilution plating onto marine agar 2216 and marine 1/10R2A media or dilution-to-extinction culturing followed by microcolonies observation was employed throughout the study. The 16S rDNA sequences obtained from a total of 703 colonies were aligned in the ARB database and phylogenetic analyses were carried out. Approximately 70% of the isolates were affiliated with the order *Rhodobacterales* in the *Alphaproteobacteria*, the family *Flavobacteriaceae* in the

Bacteroidetes, and several unidentified gammaproteobacterial groups. In this study, at least 35 novel genera were identified by phylogenetic analyses.

In the high throughput culturing based on dilution-to-extinction [13], two different seawater samples were diluted to 5 cells per ml in a filtered and autoclaved seawater medium. After screening 768 inocula for cellular growth with DAPI-stained microscopy, 124 extinction cultures grown in the seawater based medium were spread on marine agar 2216 (MA) and marine 1/10 R2A (M-1/10R2A). A total of 57 strains formed colonies onto either MA or M-1/10R2A. Among the 57 isolates, 33 strains grew only on M-1/10R2A and 2 strains grew only on MA, while 22 strains grew on both media. Most of isolates formed very small colonies, which can be visualized by naked eye after 2 weeks. The isolates were generally mesophilic, chemoheterotrophic or photoheterotrophic, and oligotrophic. The isolates were grouped into nineteen 16 rDNA-RFLP groups and almost full-length 16S rDNA sequences were obtained. Based on phylogenetic analyses in ARB database, these isolates formed 11 distinct phyletic lines. The marine isolates were affiliated with four bacterial classes or phyla, the classes *Gammaproteobacteria* (84.2%), *Alphaproteobacteria* (8.8%), *Betaproteobacteria* (5.3%), and the phylum *Verrucomicrobia* (1.8%). Isolates corresponding to previously uncultured *gammaproteobacterial* gene clusters, including MBAE14, CHAB-III-7, ARKICE-74 and ZD0117 were phylogenetically identified.

Conclusively, diverse groups of the *Gammaproteobacteria* might be major constituents of marine oligotrophic bacteria that have a colony forming ability. We are now characterizing these novel oligotrophic bacteria using polyphasic approaches, which might give a new idea of classification of marine oligotrophic microorganisms. Some of the isolates were thought to have molecular functions for utilizing solar energy. An autecological approach using these isolates will bring us more comprehensive understanding about carbon cycling in the upper pelagic ocean. [Supported by the 21C Frontier Program of Microbial Genomics and Applications, MOST, Korea]

References

1. Giovannoni, SJ, Britschgi, TB, Moyer, CL, and Field, KG. (1990) Genetic diversity in Sargasso Sea bacterioplankton. *Nature* 345: 60-63
2. Rappé, MS, Connon, SA, Vergin, KL, and Giovannoni, SJ. (2002) Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. *Nature* 418: 630-633
3. Morris, RM, Rappé, MS, Connon, SA, Vergin, KL, Siebold, WA, and et al. (2002) SAR11 clade dominates ocean surface bacterioplankton communities. *Nature* 420: 806-810
4. Béjà, O, Aravind, L, Koonin, EV, Suzuki, MT, Hadd, A, and et al. (2000) Bacterial rhodopsin: Evidence for a new type of phototrophy in the sea. *Science* 289: 1902-1906
5. Béjà, O, Suzuki, MT, Heidelberg, JF, Nelson, WC, Preston, CM, and et al. (2002) Unsuspected

- diversity among marine aerobic anoxygenic phototrophs. *Nature* 415: 630-633
6. DeLong, EF, Preston, CM, Mincer, T, Rich, V, Hallam, SJ, and et al. (2006) Community genomics among stratified microbial assemblages in the ocean's interior. *Science* 311: 496-503
 7. Venter, JC, Remington, K, Heidelberg, JF, Halpern, AL, Rusch, D, and et al. (2004) Environmental genome shotgun sequencing of the Sargasso Sea. *Science* 304: 66-74
 8. Rusch, DB, Halpern, AL, Sutton, G, Heidelberg, KB, Williamson, S, and et al. (2007) The Sorcerer II Global Ocean Sampling Expedition: Northwest Atlantic through Eastern Tropical Pacific. *PLoS Biol* 5: e77
 9. Yooseph, S, Sutton, G, Rusch, DB, Halpern, AL, Williamson, SJ, and et al. (2007) The Sorcerer II Global Ocean Sampling Expedition: Expanding the Universe of Protein Families. *PLoS Biol* 5: e16
 10. Giovannoni, SJ, Bibbs, L, Cho, J-C, Stapels, MD, Desiderio, R, and et al. (2005) Proteorhodopsin in the ubiquitous marine bacterium SAR11. *Nature* 438: 82-85
 11. Cho, J-C, and Giovannoni, SJ. (2004) Cultivation and growth characteristics of a diverse group of oligotrophic marine *Gammaproteobacteria*. *Appl. Environ. Microbiol.* 70: 432-440.
 12. Cho, J-C, Stapels, MD, Morris, RM, Vergin, KL, Schwalbach, MS, and et al. (2007) Polyphyletic photosynthetic reaction center genes in oligotrophic marine *Gammaproteobacteria*. *Environ. Microbiol.* Published as ahead of print (March 07, 2007)
 13. Cannon, SA, and Giovannoni, SJ. (2002) High-throughput methods for culturing microorganisms in very-low-nutrient media yield diverse new marine isolates. *Appl. Environ. Microbiol.* 68: 3878-3885