Western Pacific Air-Sea Interaction Study,
Eds. M. Uematsu, Y. Yokouchi, Y. W. Watanabe, S. Takeda, and Y. Yamanaka, pp. 199–202.

doi:10.5047/w-pass.a03.003

Photoheterotrophic Process in Surface Seawater Environments

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Keywords: Photoheterotrophy; AAPB; Bacteria; Photosynthesis

Introduction

It has been recently clarified that non-cyanobacterial phototrophic bacteria have been widely distributed in surface seawater environments, contributing to food web structures and biogeochemical cycles (Kolber *et al*. 2000). Aerobic anoxygenic phototrophic bacteria (AAPB) were firstly isolated from seaweeds and seasand in Japanese coastal waters in 1979 (Shiba *et al*. 1979). These bacteria were the newly-named genus *Erythrobacter* and genus *Roseobacter*. The seasonal distribution of bacteriochlorophyll-containing bacteria in Otsuchi Bay in 1995 was reported (Shiba 1995). The maximum percentage of these bacteria was 57% of the total viable count. In 2000, Kolber and colleagues reported a wide distribution of AAPB in oceanic waters by means of the direct measurement of BChl-*a* variable fluorescence. AAPB abundance was 11% of the total bacteria, and the BChl-*a* concentration was 5–10% of total Chl-*a* concentration, implying their significant role in pelagic ecosystems. AAPB require organic carbon for their growth and have little capability of carbon fixation. However, ATP production by phototrophy make them highly efficient in carbon assimilation, and thus their growth is largely facilitated under light conditions. We are now aware of their ubiquitous distribution in the ocean. The questions are how do they fluctuate in time and space, and what are the factors that control their dynamics.

In this study, we report on the AAPB distribution and their temporal variation in Otsuchi Bay, and we also report the fraction of actively growing AAPB in various environments.

Methods

Otsuchi Bay monitoring

Seawater samples were collected in Otsuchi Bay in May and November 2007, February, March, April and May, 2008. The number of AAPB was counted using an infrared epifluorescence microscope after fixation and filtration. Also, a mesocosm experiment was performed in May 2008. Seawater sieved through a 300-μm nylon mesh to remove large zooplankton stored in a 200-L tank. Inorganic nutrients (nitrate, phosphate, silicate) were added to the tank. On day 5, the tank was covered. The

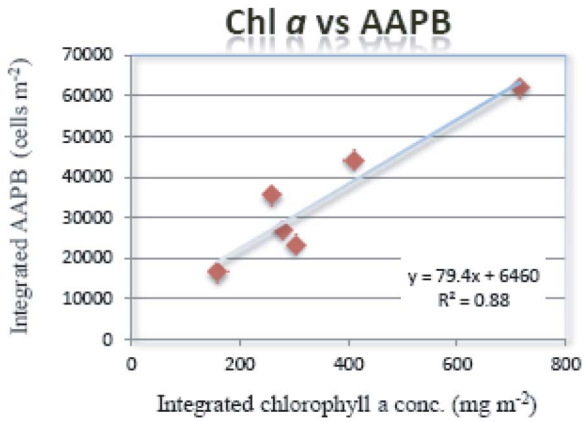

Fig. 1. Correlation between Chl *a* concentration and AAPB abundance.

experiment was then continued for 9 days in the dark. Subsamples were collected everyday to measure Chl-*a* concentration, POC, PON, DOC, inorganic nutrients, and bacterial abundance.

AAPB abundance and their actively growing fraction

Seawater was collected in the South Pacific in 2004, Saromako Lagoon in 2007, and Sagami Bay in 2008. Bromodeoxyuridine (BrdU) was added to the water and incubated for 5 h on deck at the in situ temperature to label actively growing bacteria (Hamasaki *et al*. 2007). After the incubation, seawater was filtered onto a 0.2-μm-pore-size membrane filter. The filters were subjected to DNA extraction, BrdU magnetic beads immunocapture, and real-time PCR, to quantify copy numbers of the pufM gene coding one of the core proteins of the photosynthetic reaction center.

Results and Discussion

Otsuchi Bay monitoring

Abundance of AAPB was higher at the chlorophyll maximum layer (11–13 m) than at the surface (1 m) in May, 2007. Also, the depth integrated number of AAPB was positively correlated to the integrated Chl-*a* concentration during a bloom period from February to May in 2008 (Fig. 1). These results suggest that the supply of organic matter can control the abundance of AAPB in a mesotrophic seawater environment.

During the 15-day mesocosm experiment, the AAPB abundance ranged from 9.0×10^2 to 3.8×10^4 cells mL^{-1}, or from 0.09 to 3.21% of the total bacterial abundance, showing a drastic increase in response to the phytoplankton bloom. Interestingly, the increase of AAPB abundance was 3 days faster than the increase of the total bacterial abundance, implying that their rapid growth was probably due to the supply of light energy.

AAPB abundance and their actively growing fraction

AAPB abundance ranged from 3.5×10 to 2.3×10^5 cells mL^{-1}, while total bacterial abundance ranged from 1.6×10^5 to 1.4×10^7 cells mL^{-1}. The percentage of AAPB was less than 0.1% of the total bacteria in the South Pacific, whereas it was more than 2% in some stations in the Southern Ocean and Saromako Lagoon

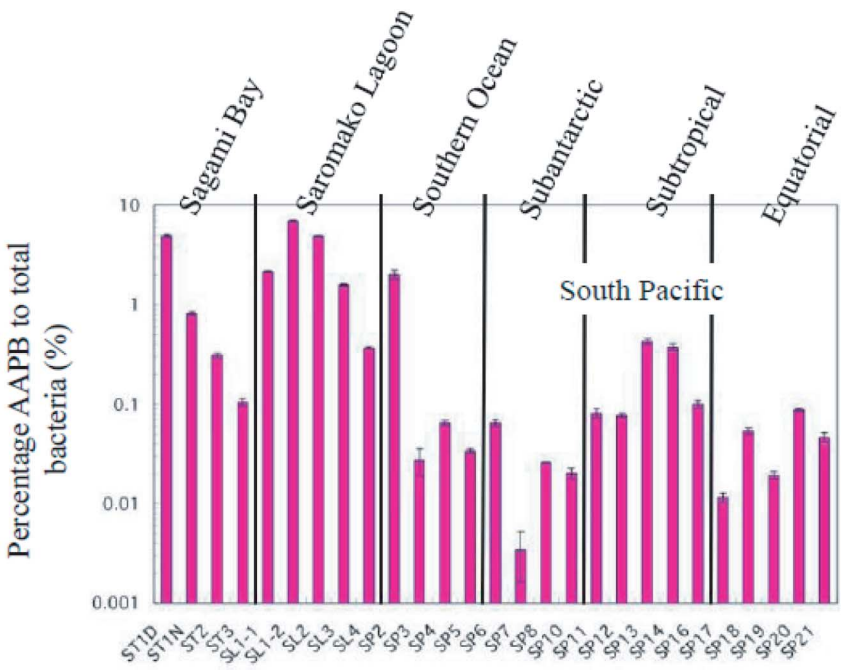

Fig. 2. Percentage AAPB to total bacterial abundance.

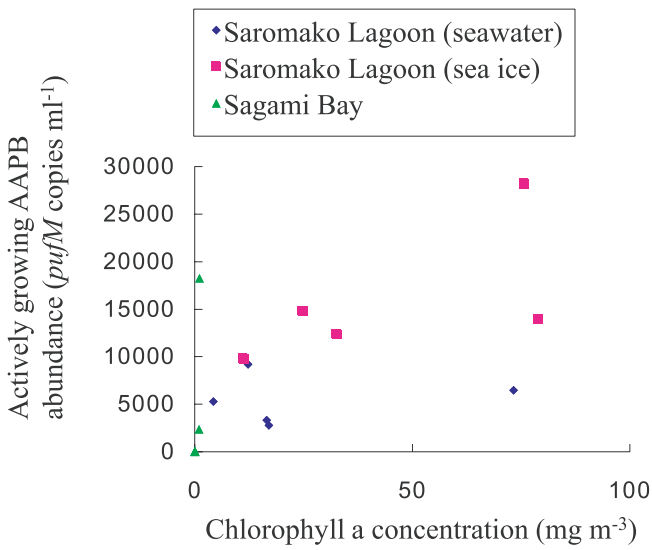

Fig. 3. Relationship between actively growing AAPB abundance and Chl *a* concentration.

(Fig. 2). We found abundant AAPB in low-temperature environments, suggesting the presence of cold-adapted populations, or phylotypes of AAPB. Analysis of BrdU-labeled fractions of AAPB revealed that actively growing AAPB were more abundant at 0 m than at 40- and 100-m depths of sampling in Sagami Bay. Also, they were more abundant in sea ice than in the surrounding seawater in winter in Saromako Lagoon. Actively growing AAPB abundance showed a strong positive correlation to Chl-*a* concentration (Fig. 3). Although the correlation between AAPB abundance and Chl-*a* concentration had already been suggested (Sieracki *et al.* 2006), the relationship between the growth of AAPB and Chl-*a* concentration is shown for the first time in this study. The combination of BrdU labeling techniques with real-time PCR quantification of an AAPB functional gene has revealed a factor possibly controlling their growth and abundance in seawater environments. Our study shows that such an approach can be a powerful tool for studying the dynamics of AAPB in natural environments.

Acknowledgements

We are grateful to the PI, the researchers, the captains, and the crews of the R/V Hakuho-Maru and the staff of the Otsuchi International Coastal Research Center, Atmosphere and Ocean Research Institute for their help in sampling.

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