Distinct distribution pattern of abundance and diversity of aerobic anoxygenic phototrophic bacteria in the global ocean

Nianzhi Jiao,[1]* Yao Zhang,[1] Yonghui Zeng,[1] Ning Hong,[1] Rulong Liu,[1] Feng Chen[2] and Pinxian Wang[3]

[1]*National Key Laboratory of Marine Environmental Sciences, Xiamen University, Xiamen 361005, China.*
[2]*Center of Marine Biotechnology, University of Maryland, Baltimore, MD 21202, USA.*
[3]*National Key Laboratory of Marine Geological Sciences, Tongji University, Shanghai 200092, China.*

Summary

Aerobic anoxygenic phototrophic bacteria (AAPB) are an important bacterial group with capability of harvesting light energy, and appear to have a particular role in the ocean's carbon cycling. Yet the significance of AAPB relative to total bacteria (AAPB%) in different marine regimes are still controversial, and variation trend of genetic diversity of AAPB along environmental gradients remains unclear. Here we present the first comprehensive observation of the global distribution of AAPB in the Pacific, Atlantic and Indian oceans, revealing a general pattern of high abundance of AAPB and AAPB% in coastal waters than oceanic waters. The Indian Ocean contained relatively high AAPB% compared with the other two oceans, corresponding to the high primary production in this region. Both abundance of AAPB and AAPB% were positively correlated with the concentration of chlorophyll *a*, while the diversity of AAPB decreased with increasing chlorophyll *a* values. Our results suggest that AAPB abundance and diversity follow opposite trends from oligotrophic to eutrophic regimes in the ocean.

Introduction

Aerobic anoxygenic phototrophic bacteria (AAPB) represent a group of newly recognized bacteria that can utilize light energy and play a particular role in carbon cycling in the ocean (Kolber *et al.*, 2000; 2001). The percentage of

AAPB in total bacteria (AAPB%) is a key to evaluate the significance of such a role of AAPB in marine ecosystems. As the bacterial chlorophyll *a* (BChl. *a*)-based phototrophic function in AAPB is a supplement to their normal heterotrophic diet of dissolved organic carbon (DOC) (Beatty, 2002; Suyama *et al.*, 2002; Koblizek *et al.*, 2003), it is thus expected to make AAPB more competent when starved of organic carbon electron donors in respiration, and provides AAPB with a selective advantage in oligotrophic environments (Kolber *et al.*, 2000; 2001; Beatty, 2002). However, current literature data on the relative abundance of AAPB in marine environments are still controversial. High AAPB% in oceanic water was reported by certain research groups (Kolber *et al.*, 2001; Cottrell *et al.*, 2006; Masin *et al.*, 2006), while other researchers found relatively high AAPB% in coastal waters (Sieracki *et al.*, 2006). Most recently, an approximate estimation based on *puf*M-*puf*L-*bch*X gene to *rec*A gene copy numbers from global ocean sampling (GOS) data also showed the highest AAPB% occurring at coastal sites (Yutin *et al.*, 2007); and the metagenomic analysis of the GOS data indicated great variability in AAPB genetic diversity with specific AAPB assemblages adapted to open ocean or coastal areas respectively (Yutin *et al.*, 2007). However, as the GOS samples were in the 0.1–0.8 μm size range, and AAPB are usually larger than other bacteria (Yurkov and Beatty, 1998; Sieracki *et al.*, 2006), the diversity of AAPB and relative significance of AAPB drawn from the GOS data may have been underestimated (Yutin *et al.*, 2007). In addition, variation trend of overall diversity of AAPB from oligotrophic to eutrophic regimes remains unknown. Therefore, in order to better understand the distribution pattern of abundance and diversity of AAPB across different environmental provinces, a larger-scale systematic survey covering the world oceans would be desired.

During 2005 and 2006, a global cruise (R/V Ocean No.1) in memorial of the 600-year anniversary of the Admiral Zheng He's expedition voyages (1405–1433) to the occident provided an opportunity to collect samples from the Pacific, Atlantic and Indian oceans. Together with other cruises to the West Pacific marginal seas including the East and South China Seas, the sampling sites in the present study covered tropical, subtropical and temperate
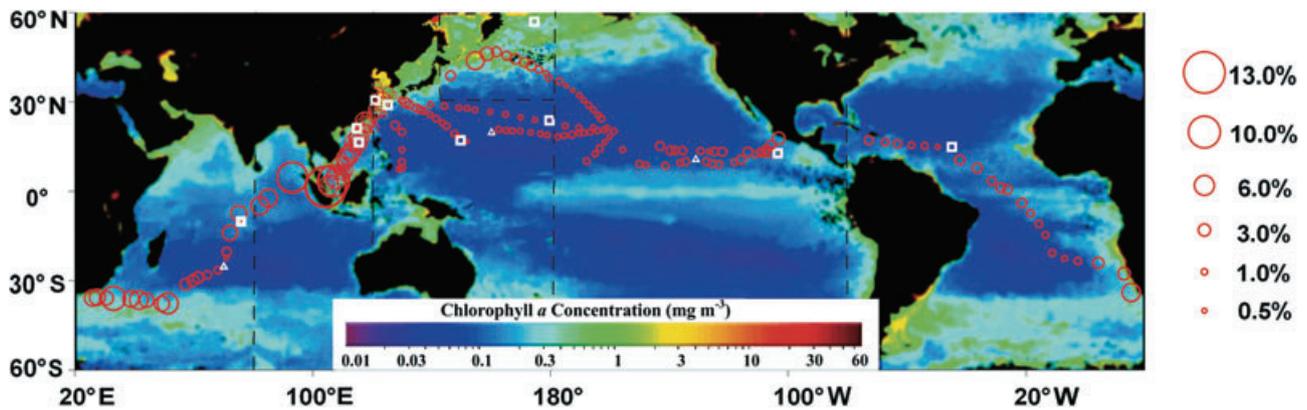
Fig. 1. A global distribution pattern of the percentage of AAPB in total bacteria (AAPB%). Red symbol at each sampling station is proportional in diameter to the value of AAPB%. Stations marked with white square are the sites where the diversity of AAPB was analysed (10 stations). Stations labelled with white triangle are the sites where the depth profile was taken. Samples were collected from various cruises. Most of the samples were taken from a global expedition (April 2005–January 2006) aboard R/V Ocean No.1. Samples were also collected from the Northern Pacific Ocean aboard R/V Revelle, USA (the Vertigo cruise, July–August 2005), R/V Ocean No.1 (October–December 2003 and May and November 2005). Cruises to the West Pacific marginal seas were conducted seasonally between 2003 and 2005 (R/V DONG-FANG-HONG No.2; and R/V YAN-PING No.2). A mosaic base map of Chl. *a* remote sensed images (Aqua-MODIS) of spring 2005 (120°E–180°E, 60°S–60°N), summer 2005 (180°W–80°W, 60°S–60°N), autumn 2005 (80°W–80°E, 60°S–60°N) and winter 2006 (80°E–120°E, 60°S–60°N) was employed as the backgrounds corresponding to each leg of the global cruise. In addition, Chl. *a* image of the area of 140°E–180°E, 30°N–60°N in summer 2005 was added as background for the Vertigo cruise. The black dashed lines showed the boundaries of different images that comprise the whole.

zones, and included coastal, shelf and oceanic waters (Fig. 1). Time-series observation-based infrared epifluorescence microscopy (TIREM) protocol was employed for accurate enumeration of AAPB (Jiao *et al.*, 2006). Genetic diversity of AAPB was examined in representative environments by analysing the *puf*M gene (photosynthetic reaction centre small-subunit gene), which encodes the M subunit in the photosynthetic reaction centre (Beja *et al.*, 2002; Yutin *et al.*, 2007).

Results and discussion

Global distribution pattern of AAPB abundance and its association with autotrophs

In surface water among the three oceans, the Indian Ocean had the highest AAPB% (3.79 ± 1.72%) followed by the Atlantic (1.57 ± 0.68%) and the Pacific (1.08 ± 0.74%) (Fig. 1). This pattern is similar to that of the biomass of cyanobacteria especially *Synechococcus* whose biomass was 4.43, 2.57 and 1.81 mg C m^{-3} in the Indian, Atlantic and Pacific Oceans respectively. The distinguished high AAPB% in the Indian Ocean is also consistent with significantly higher primary production in the Indian Ocean than in the other two oceans (Koblentz-Mishke *et al.*, 1970; Mackey *et al.*, 1995; Beaufort *et al.*, 1997; Gregg *et al.*, 2003; Perez *et al.*, 2005). Within the Indian Ocean, high values of AAPB% occurred at the sites with high concentration of chlorophyll *a* (Chl. *a*) . In general, the coastal/shelf waters of the marginal seas had higher AAPB% (4.46 ± 2.41% with the maximum of 13.51%) than oceanic waters (1.52 ± 1.30%) (Fig. 1).

In addition to the surface distribution, vertical distribution of AAPB was also investigated at selected sampling sites (representative cases shown in Fig. 2). Unlike heterotrophic bacteria (non-AAPB), AAPB were confined to the euphotic zone. In general, AAPB followed Chl. *a* concentration and carbon biomass of cyanobacteria (*Prochlorococcus* and *Synechococcus*) along the depth profile regardless of stratification (Fig. 2A–C). One exception was the extremely oligotrophic Western North Pacific Gyre (WNPG) (Schlitzer, 2004) where AAPB were present at shallower depths than cyanobacteria (Fig. 2D). In the WNPG, as the phytoplankton (mainly cyanobacteria) did not thrive in the upper 100 m layer, AAPB only maintained a minimum abundance throughout the euphotic water column, with a weak maximum occurring at near surface probably benefiting from light (Fig. 2D). These observations suggest that AAPB are associated with phytoplankton.

Indeed, both AAPB abundance and AAPB% were correlated significantly to Chl. *a* concentration in all the oceans as well as in the Western Pacific marginal seas in all seasons (Fig. 3; Table 1). In contrast, correlations between non-AAPB and Chl. *a* were less significant in most cases, even no correlation in some cases of the coastal waters. More interestingly, AAPB were correlated significantly with the biomass of cyanobacteria, while less or no significant correlation existing between non-AAPB and cyanobacterial biomass (Table 2). It is still unclear why AAPB have a closer relationship with phytoplankton than non-AAPB. As Chl. *a* concentration can be an indicator of phytoplankton biomass (Leavitt *et al.*, 1999; Malej
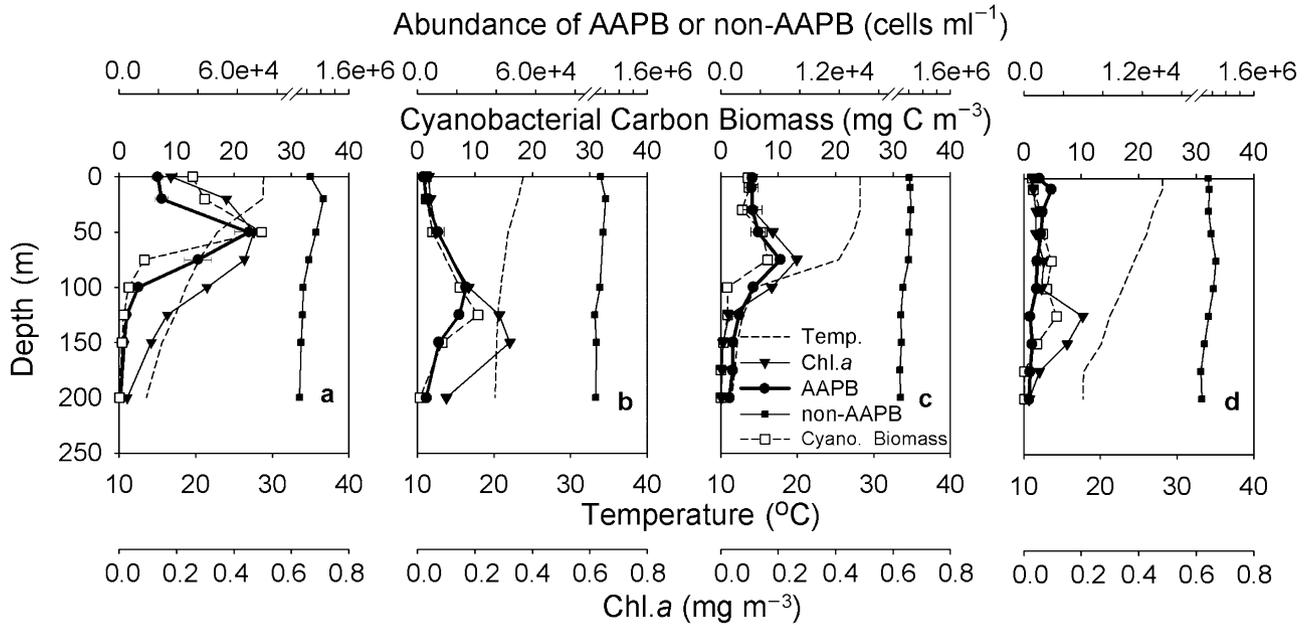
Fig. 2. Depth profiles of AAPB abundance (black line with closed circle), non-AAPB abundance (line with closed square), Chl. *a* concentration (line with closed triangle), biomass of cyanobacteria (doted line with open square) and temperature (doted line) at representative locations: (A) Central Indian Ocean (75.85°E, 10.01°S); (B) Southern Indian Ocean (70.04°E, 25.32°S) (oligotrophic); (C) Tropical Eastern North Pacific (131.06°W, 10.71°N); and (D) Western North Pacific Gyre (160.21°E, 19.66°N) (extremely oligotrophic). Error bars indicate standard deviation of triplicate measurements.

et al., 2003) and phytoplankton can release highly labile DOC (Chen and Wangersky, 1996; Malej *et al.*, 2003) supporting bacterial growth (Nishii *et al.*, 2001), the closer tie between AAPB and phytoplankton suggests that AAPB could rely on the labile DOC to a greater extent than non-AAPB. Thus, the high abundance of AAPB and even higher AAPB% in coastal waters is likely due to high availability and high percentage of labile DOC there (Søndergaard and Middelboe, 1995). On the other hand, the close correlation between AAPB and phytoplankton could

also be partially a result of similar dependence of both organisms on light. Further studies are needed in order to fully understand the trophic mechanism of AAPB.

The global distribution trend of AAPB with respect to trophic regimes revealed in this study is in agreement with results from other relatively large-scale investigations, such as that by Schwalbach and Fuhrman (2005) based on scattered sampling over the oceans, and that by Sieracki and colleagues (2006) from the coast of the Gulf of Maine to the Sargasso Sea ranging from 31.85N/64W to

Table 1. Correlation[a] coefficients among AAPB%, AAPB abundance or non-AAPB abundance and chlorophyll *a* (Chl. *a*) in the western Pacific marginal seas.

	East China Sea	South China Sea				
Items	Summer	Spring	Autumn	Winter	Summer	All seasons
AAPB% versus Chl. *a*						
r	0.68	0.65	0.63	0.64	0.75	0.56
p	< 0.05	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
n	9	42	33	38	25	138
AAPB versus Chl. *a*						
r	0.82	0.66	0.60	0.72	0.76	0.51
p	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
n	9	42	33	38	25	138
Non-AAPB versus Chl. *a*						
r	0.33	0.32	0.04	0.39	0.59	0.31
p	–	< 0.05	–	< 0.05	< 0.01	< 0.01
n	9	42	33	38	25	138

a. The fitting function used is: $y = ae^{bx}$.
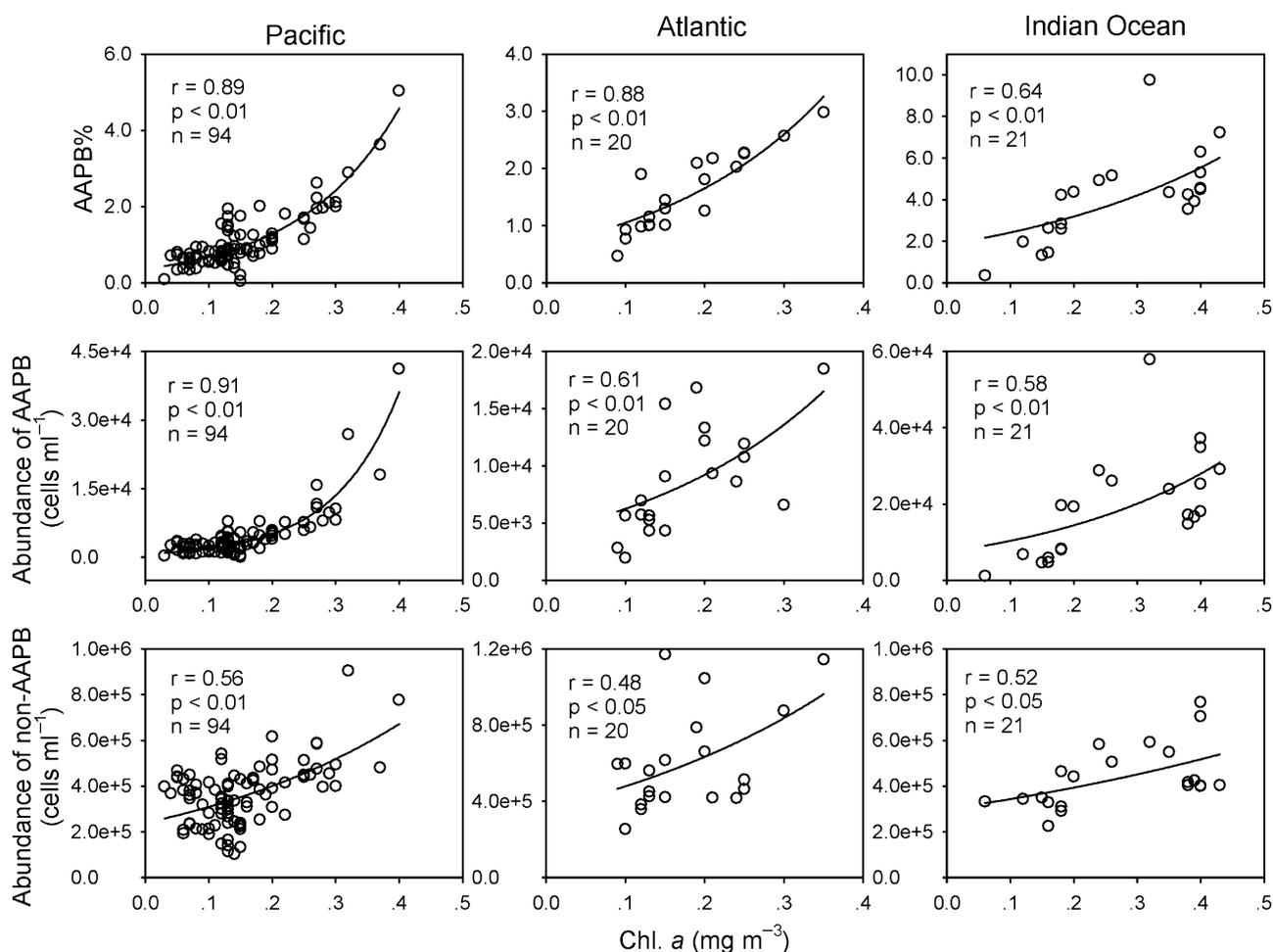–, data not shown when $P > 0.05$.

Fig. 3. Correlations between AAPB%, AAPB abundance or non-AAPB abundance and chlorophyll *a* (Chl. *a*) in surface water of the Pacific, Atlantic and Indian oceans (the fitting function used is: $y = ae^{bx}$).

44.085°N/68°W, as well as that by Yutin and colleagues (2007) estimated on *puf*M-*puf*L-*bch*X gene to *rec*A gene copy numbers from the GOS data set. It is likely that field observations may yield opposite distribution trend of AAPB between eutrophic and oligotrophic sites if investigation coverage is not large enough, such as the cases of

the Atlantic Bights (ranging from 36°N/73°W to 38°N/74.43°W; Cottrell *et al.*, 2006) and Baltic Sea (ranging from 54.44°N/19.042°W to 55.3°N/18°W; Masin *et al.*, 2006). Abundance of AAPB and AAPB% are also influenced by factors beyond light and substrate, such as difference in community structures and interactions

Table 2. Correlation[a] coefficients among AAPB%, AAPB abundance or non-AAPB abundance and carbon biomass of cyanobacteria (*Synechococcus* and *Prochlorococcus*) in the Pacific, Atlantic, Indian oceans.

Items	Pacific, Atlantic and Indian oceans	Indian ocean
AAPB% versus carbon biomass of cyanobacteria		
r	0.66	0.78
p	< 0.01	< 0.01
n	135	21
AAPB versus carbon biomass of cyanobacteria		
r	0.74	0.79
p	< 0.01	< 0.01
n	135	21
Non-AAPB versus carbon biomass of cyanobacteria		
r	0.40	0.50
p	< 0.01	< 0.05
n	135	21

a. The fitting function used is: $y = ae^{bx}$.

among the members (Sieracki *et al.*, 2006). On top of that, data by different means may also result in differences in conclusions; for example, the *puf*M gene to *rec*A gene ratio-based approach can only give approximate estimation of the relative significance of AAPB; the infrared fluorescence microscopy could suffer positive errors in oceanic waters where cyanobacteria are abundant and could be mistaken as AAPB (Zhang and Jiao, 2003). In the present study, the TIREM protocol (Jiao *et al.*, 2006) was employed on board the cruises to ensure accurate enumeration of AAPB throughout the investigations.

Decreasing AAPB diversity with increasing Chl. a gradient and its implications

To better understand the population structure of AAPB in different environments, we analysed 10 clone libraries (total 500 clones) of the AAPB's diagnostic gene *puf*M (Beja *et al.*, 2002; Schwalbach and Fuhrman, 2005) from 10 different sites of global ocean surface water (Fig. 1). Total 208 distinct *puf*M sequences were obtained based on the cut-off of 1% sequence difference. The *puf*M gene phylogeny showed that AAPB are highly diverse and represented by the bacteria from alpha-, beta- and gammaproteobacterial classes (Fig. 4). About a quarter of *puf*M sequences from our samples clustered with well-known genera *Roseobacter* and *Erythrobacter* of alphaproteobacteria. The *Roseobacter*-like and *Erythrobacter*-like clones were distributed across the eutrophic and oligotrophic marine regions. However, about 10% of distinct sequences recovered from South China Sea and North Central Pacific formed a unique cluster within the *Erythrobacter* group but had no their culture counterpart. Moreover, a large portion (93/208) of the environmental *puf*M sequences was closely related to gammaproteobacteria. It is noteworthy that these gammaproteobacteria-like clones were from both oligotrophic and eutrophic waters. Gammaproteobacterial AAPB were dominant in the high-Chl. *a* regions (Fig. 5), consistent with that common gammaproteobacteria are well known for their adaptability and opportunism (Bouvier and del Giorgio, 2002; Bernhard *et al.*, 2005). Cho and colleagues (2007) also found that a bacterial group – OM60 clade containing gammaproteobacterial AAPB – were abundant in coastal oceans.

The recent GOS efforts revealed that AAPB compositions vary between different oceanic regions with specific assemblages adapted to oligotrophic and eutrophic environments respectively (Yutin *et al.*, 2007). However, the limited sampling number (total 41 samples in GOS phase I from the surface waters of North Atlantic and Eastern North Pacific; Rusch *et al.*, 2007) and the relatively narrow size fractionation (0.1–0.8 μm pore size) (Yutin *et al.*, 2007) leave rooms for further recognition of diversity of AAPB and its variation trends with environmental gradients. Covering Atlantic, Indian and Pacific oceans, and with sampling range of 0.22–200 μm, our global ocean survey on AAPB diversity showed that *Roseobacter*-, *Erythrobacter*- and *Gammaproteobacteria*-related AAPB were all widely distributed over the major ocean regimes. The revealed great diversity in AAPB is reflected not only by newly found abundant gammaproteobacteria-like *puf*M sequences (Hu *et al.*, 2006) but also isolates of *Erythrobacter* AAPB spread over the oceans (Koblizek *et al.*, 2003), and even a group of gamma-AAPB strains from the North Sea (Fuchs *et al.*, 2007) and the coast of Oregon, USA (Cho *et al.*, 2007). More importantly, we found that the genetic diversity of AAPB tended to decrease with increasing Chl. *a* gradient. This was evident when a significant inverse correlation between diversity of AAPB (measured by the Shannon index) and the concentration of Chl. *a* was found (Fig. 5). The inverse relationship between AAPB diversity and Chl. *a* is in contrast to the positive correlation between abundance of AAPB and Chl. *a* discussed above.

It has been proposed that all proteobacteria have descended from a common purple photosynthetic bacterial ancestor (Xiong *et al.*, 2000). In evolution, some of them may have lost their photosynthetic genes, while others have retained these genes (Beatty, 2002). The polyphyletic feature of *puf*M genes in gamma-AAPB cultures revealed by Cho and colleagues (2007) seemingly supports such possibility. Assuming the above theory is applicable to AAPB, along such an evolution direction, AAPB species could have further lost their photosynthetic genes and gradually become heterotrophic bacteria as the ocean became richer and richer in DOC. The decreasing trend in diversity of AAPB along increasing Chl. *a* gradient observed in this study provides somewhat a reflecting evidence for the above proposed evolution (Xiong *et al.*, 2000; Beatty, 2002). In the present ocean, river run-offs transport terrestrial organic matters into coastal waters, resulting in high availability of carbon source there. In such environments, AAPB species would be more readily to loose their photosynthetic genes and autotrophic lifestyle, resulting lower diversity than in oligotrophic environments. Phototrophic mechanisms in AAPB and their relationship with eutrophication in the ocean are still poorly understood, but remain as an interesting research area for future work.

Closing remarks

In the present study, our global ocean survey revealed that AAPB are able to maintain high diversity but low abundance in oligotrophic waters, and become more abundant but less diverse in eutrophic waters. The oligotrophic ocean (such as the Western North Pacific Gyre)
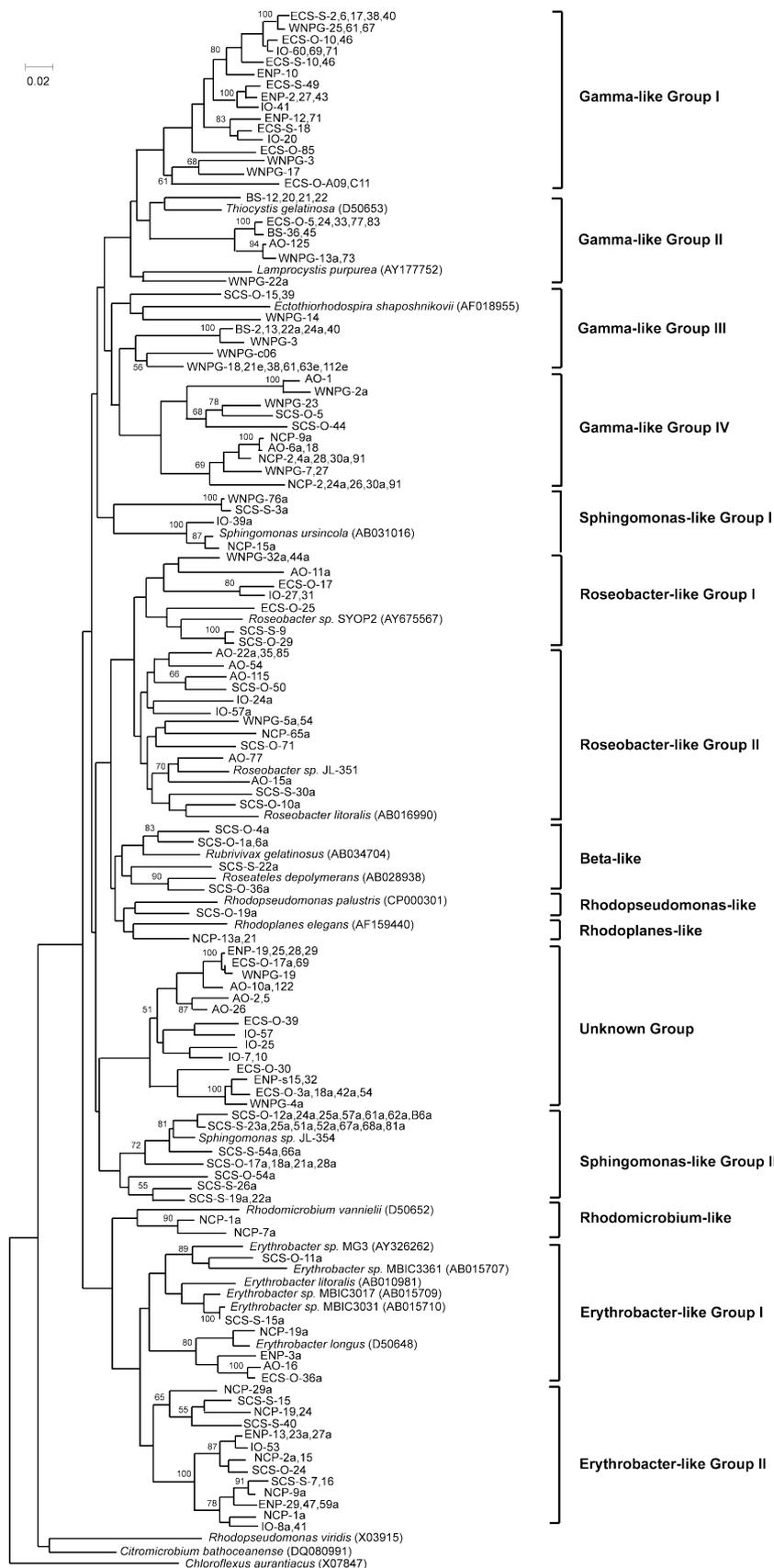
Fig. 4. Phylogenetic analysis of partial *puf*M gene sequences from global oceans. The phylogenetic tree was constructed using the neighbour-joining algorithm. Bootstrap values (100 replicates) greater than 50% are indicated above branches. All *puf*M nucleotide sequences obtained in this work that show >1% difference and reference sequences from cultures were included. Sequences with < 3% difference were combined into a single group. Scale bar represents the 2% nucleotide substitution percentage. *Chloroflexus aurantiacus* was used as outgroup.
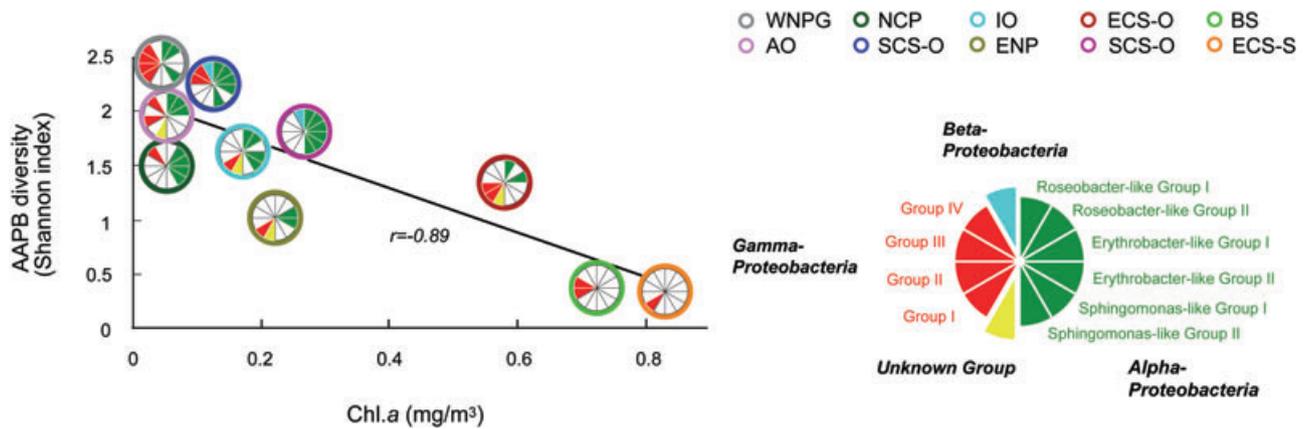
Fig. 5. Inverse correlation between AAPB genetic diversity (by Shannon index, see *Experimental procedures*) and Chl. *a* concentration in global surface oceans. The 10 investigated sites are: Western North Pacific Gyre (WNPG, 149.69°E 17.16°N); Atlantic Ocean (AO, 45.06°W 14.83°N); North Central Pacific (NCP, 179.7°E 23.7°N); Open water of South China Sea (SCS-O, 113.5°E 18.4°N); Indian Ocean (IO, 75.83°E, 10.01°S); Eastern North Pacific (ENP, 103.6°W 12.8°N); Shelf water of South China Sea (SCS-S, 114.8°E 21.2°N); Open water of East China Sea (ECS-O, 126.3°E 29°N); Bering Sea (BS, 174.5°E 56.9°N); Shelf water of East China Sea (ECS-S, 123.5°E 30.5°E) (refer to the white squares in the map of Fig. 1). All AAPB clones are sorted into 12 major groups according to the phylogenetic analysis of *puf*M gene sequences (Fig. 4). Groups accounting for less than 5% of total clones in the clone library were treated as minor group and were not shown in this figure. The aside plate illustration with 12 rooms shows the locations of the 12 major subgroups of AAPB. Closed/open rooms indicate the presence/absence of the corresponding group.

may maintain diverse AAPB species but not necessarily high abundance as previously expected (Kolber *et al.*, 2001; Beatty, 2002). The close tie between AAPB and phytoplankton we observed in variety of marine environments provided evidence for the dependence of abundance of AAPB on availability of organic substrates, which seemed to be more decisive than light in distribution pattern of abundance of AAPB in the ocean. The opposite conclusions about geographic distribution pattern of AAPB along environmental gradients in the literature (Kolber *et al.*, 2001; Schwalbach and Fuhrman, 2005; Cottrell *et al.*, 2006; Masin *et al.*, 2006; Sieracki *et al.*, 2006; Yutin *et al.*, 2007) are likely due to investigation scale, methodology and particular reasons case by case. Further studies on multiple controlling mechanisms of AAPB are necessary. The decreasing diversity of AAPB with eutrophic gradient observed in the present study is somewhat consistent with the proposed evolution direction of phototrophic bacteria (Xiong *et al.*, 2000; Beatty, 2002). The revealed great diversity of AAPB suggests that many new AAPB species can be isolated from variety of marine environments for in-depth study towards a better understanding of AAPB's role in marine ecosystems. The contrasting variation trends of abundance and diversity of AAPB along environmental gradients are of great interests for future studies.

Experimental procedures

Determination of AAPB abundance by TIREM

The TIREM protocol is as described by Jiao and colleagues (2006). Briefly, subsamples for bacterial analysis were col-

lected using 100 ml brown polypropylene bottles. Immediately after sampling, aliquots of 20 ml of seawater were fixed with paraformaldehyde (2%, final concentration) and stained with 4′6-diamidino-2-phenylindole (DAPI) (5 μg ml⁻¹, final concentration) and then filtered onto 0.2 μm pore-size black polycarbonate (PC) membranes (Whatman) for microscopic observation. The filters were mounted on slides, and a small drop of immersion oil and a coverslip added. The slides were frozen before analysis. Infrared fluorescence from BChl. *a* was the diagnostic signal of AAPB. Cells were viewed with an infrared-sensitive charge-coupled device (CCD) camera (SPOT Diagnostic Instruments) on an epifluorescence microscope with a 50 W mercury lamp (Carl Zeiss Light Microscopy AXIOSKOP 40). DAPI-stained images (DAPI image), cyanobacterial images (Cyano image) and infrared images (IR images) were acquired for each microscopic view field using a 100× oil immersion objective. All images were captured using automatic exposure with a gain limit of 8. Time-series images of DAPI, Cyano and IR images were obtained for 10–15 min at intervals of 60 s from the start of the exposure. Images analysis was conducted to get three dynamic curves of IR, Cyano and DAPI counts from each microscopic view field. The accurate estimation of AAPB was calculated from the formula: [AAPB counts] = [plateau count of infrared-positive cells] − [plateau count of cyanobacterial cells] (Jiao *et al.*, 2006). Thirty microscopic fields of each sample were viewed (at least 300 cells could be detected each sample), and the means were multiplied by appropriate factors to yield cell concentrations in the original samples.

Determination of picoplankton abundance and environmental variables

Abundances of total heterotrophic bacteria, *Synechococcus*, *Prochlorococcus*, were determined by flow cytometry (Jiao *et al.*, 2002) on an Epics Altra II (Beckman Coulter, USA) flow

cytometer, equipped with a 306C-5 argon laser (Coherent, USA). Abundance of non-AAPB was obtained by subtracting abundance of AAPB from that of total heterotrophic bacteria. Carbon biomass was calculated using carbon conversion factors of 250, 53 fg C cell^{-1} for *Synechococcus* and *Prochlorococcus* respectively (Kana and Glibert, 1987; Campbell *et al.*, 1994).

Seawater temperature was measured with a SeaBird CTD (SBE 9/11 plus, SeaBird, USA). Water samples for Chl. *a* analysis were immediately filtered through GF/F filter (Whatman, 47 mm) under dim condition and stored at −20°C for later analysis. The Chl. *a* was determined fluorometrically (Parsons *et al.*, 1984) with a Turner- Designs-Model 10 fluorometer (Sigma, USA)

AAPB diversity revealed by puf*M gene sequence analysis*

Surface water samples (3–5 l) were pre-filtered through 200 μm mesh and subsequently filtered onto a 0.22 μm poresize filter (Pall, Gelman Sciences). Filter samples were stored in liquid nitrogen until analysis. Community DNA was extracted using the previously reported protocol (Schwalbach and Fuhrman, 2005). The partial *pufM* gene sequences were amplified on a T3 thermocycler (Biometra, Germany) using the primers pufMF (TAC GGS AAC CTG TWC TAC) and pufMR (CCA TSG TCC AGC GCC AGA A). The reaction mixture and cycle parameters were as previously described (Beja *et al.*, 2002). The PCR products were subsequently cloned into pMD18-T vector (TaKaRa). Fifty clones from each library were randomly selected and the recombinant plasmid DNA was extracted and sequenced on ABI 377A automated sequencer (Applied Biosystems). Sequences obtained and those from cultures deposited in the NCBI database were aligned using the program CLUSTALX 1.81 and edited manually. A common region of *c.* 193 bp length was obtained and used for the phylogenetic tree inference. To obtain a compact tree, *puf*M sequences were combined into groups based on 3% sequence difference prior to tree construction. One sequence from each group was then selected as representative for phylogenetic analysis. The neighbour-joining tree was constructed with the program PHYLIP 3.63 (http://evolution.genetics.washington.edu/phylip.html) with bootstrap value of 100. The nomenclature of clusters in the phylogenetic tree was made according to Yutin and colleagues (2005), using the suffix '-like' to indicate the most possible affiliation. To evaluate the diversity of AAPB in each library, all the *puf*M sequences were grouped through the program DOTUR using the distance 0.05 as species level for functional gene (Schloss and Handelsman, 2005). The Shannon diversity index in each clone library was then calculated using the program PAST (http://folk.uio.no/ohammer/past). The sequences reported in this article have been deposited in the GenBank database (Accession No. AY731094–AY731136, AY640236–AY640252, AY731137–AY731168, AY652818–AY652839, AY652808–AY652817, DQ093226–DQ093270).

Acknowledgements

References

Beatty, J.T. (2002) On the natural selection and evolution of the aerobic phototrophic bacteria. *Photosyn Res* **73:** 109–114.

Beaufort, L., Lancelot, Y., Camberlin, P., Cayre, O., Vincent, E., Bassinot, F., and Labeyrie, L. (1997) Insolation cycles as a major control of equatorial Indian Ocean primary production. *Science* **278:** 1451–1454.

Beja, O., Suzuki, M.T., Heidelberg, J.F., Nelson, W.C., Preston, C.M., Hamada, T., *et al.* (2002) Unsuspected diversity among marine aerobic anoxygenic phototrophs. *Nature* **415:** 630–633.

Bernhard, A.E., Colbert, D., McManus, J., and Field, K.G. (2005) Microbial community dynamics based on 16S rRNA gene profiles in a Pacific Northwest estuary and its tributaries. *FEMS Microbiol Ecol* **52:** 115–128.

Bouvier, T.C., and del Giorgio, P.A. (2002) Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. *Limnol Oceanogr* **47:** 453–470.

Campbell, L., Nolla, H.A., and Vaulot, D. (1994) The importance of *Prochlorococcus* to community structure in the central North Pacific Ocean. *Limnol Oceanogr* **39:** 954–961.

Chen, W.H., and Wangersky, P.J. (1996) Rates of microbial degradation of dissolved organic carbon from phytoplankton cultures. *J Plankon Res* **18:** 1521–1533.

Cho, J., Stapels, M.D., Morris, R.M., Vergin, K.L., Schwalbach, M.S., Givan, S.A., *et al.* (2007) Polyphyletic photosynthetic reaction centre genes in oligotrophic marine *Gammaproteobacteria*. *Environ Microbiol* **9:** 1456–1463.

Cottrell, M.T., Mannino, A., and Kirchman, D.L. (2006) Aerobic anoxygenic phototrophic bacteria in the Mid-Atlantic Bight and the North Pacific Gyre. *Appl Environ Microbiol* **72:** 557–564.

Hu, Y.H., Du, H.L., Jiao, N.Z., and Zeng, Y.H. (2006) Abundant presence of the gamma-like proteobacterial *pufM* gene in oxic seawater. *FEMS Microbiol Lett* **263:** 200–206.

Fuchs, B.M., Spring, S., Teeling, H., Quast, C., Wulf, J., Schattenhofer, M., *et al.* (2007) Characterization of the first marine gammaproteobacterium capable of aerobic anoxygenic photosynthesis. *Proc Natl Acad Sci USA* **104:** 2891–2896.

Gregg, W.W., Conkright, M.E., Ginoux, P., O'Reilly, J.E., and Casey, N.W. (2003) Ocean primary production and climate: global decadal changes. *Geophys Res Lett* **30:** 1809.

Jiao, N.Z., Yang, Y.H., Koshikawa, H., and Watanabe, M. (2002) Influence of hydrographic conditions on picoplankton distribution in the East China Sea. *Aquat Microb Ecol* **30:** 37–48.

Jiao, N.Z., Zhang, Y., and Chen, Y. (2006) Time series observation based InfraRed Epifluorescence Microscopic (TIREM) approach for accurate enumeration of bacteriochlorophyll-containing microbes in marine environments. *J Microbiol Methods* **65:** 442–452.

Kana, T.M., and Glibert, P.M. (1987) Effect of irradiances up to 2000 μE m^{-2} s^{-1} on marine *Synechococcus WH7803*. I. Growth, pigmentation, and cell composition. *Deep Sea Res* **34:** 479–495.

Koblentz-Mishke, O.J., Valkovinsky, V.V., and Kabanova, J.G. (1970) Plankton primary production of the world ocean. In *Scientific Exploration of the South Pacific*. Wooster, W.S. (ed.). Washington, DC, USA: National Academy of Science, pp. 183–193.

Koblizek, M., Beja, O., Bidigare, R.R., Christensen, S., Benitez-Nelson, B., Vetriani, C., *et al.* (2003) Isolation and characterization of *Erythrobacter* sp strains from the upper ocean. *Arch Microbiol* **180:** 327–338.

Kolber, Z.S., Van Dover, C.L., Niederman, R.A., and Falkowski, P.G. (2000) Bacterial photosynthesis in surface waters of the open ocean. *Nature* **407:** 177–179.

Kolber, Z.S., Plumley, F.G., Lang, A.S., Beatty, J.T., Blankenship, R.E., VanDover, C.L., *et al.* (2001) Contribution of aerobic photoheterotrophic bacteria to the carbon cycle in the ocean. *Science* **292:** 2492–2495.

Leavitt, P.R., Findlay, D.L., Hall, R.I., and Smol, J.P. (1999) Algal responses to dissolved organic carbon loss and pH decline during whole-lake acidification: evidence from paleolimnology. *Limnol Oceanogr* **44:** 757–773.

Mackey, D.J., Parslow, J., Higgins, H.W., Griffiths, F.B., and O'Sullivan, J.E. (1995) Plankton productivity and biomass in the western equatorial Pacific: biological and physical controls. *Deep Sea Res Part II Top Stud Oceanogr* **42:** 499–533.

Malej, A., Mozetič, P., Turk, V., Terzič, S., Ahel, M., and Cauwet, G. (2003) Changes in particulate and dissolved organic matter in nutrient-enriched enclosures from an area influenced by mucilage: the northern Adriatic Sea. *J Plankton Res* **25:** 949–966.

Masin, M., Zdun, A., Ston-Egiert, J., Nausch, M., Labrenz, M., Moulisova, V., and Koblizek, M. (2006) Seasonal changes and diversity of aerobic anoxygenic phototrophs in the Baltic Sea. *Aquat Microb Ecol* **45:** 247–254.

Nishii, K., Nakano, S., Tamada, M., Manage, P.M., Nishibe, Y., and Kawabata, Z. (2001) Microbial decomposition of dissolved organic matter in a hypertrophic pond. *Limnology* **2:** 207–212.

Parsons, T.R., Maita, Y., and Lalli, C.M. (1984) *A Manual of Chemical and Biological Methods for Seawater Analysis*. New York, USA: Pergamon Press.

Perez, V., Fernandez, E., Maranon, E., Serret, P., and Garcia-Soto, C. (2005) Seasonal and interannual variability of chlorophyll *a* and primary production in the Equatorial Atlantic: *in situ* and remote sensing observations. *J Plankton Res* **27:** 189–197.

Rusch, D.B., Halpern, A.L., Sutton, G., Heidelberg, K.B., Williamson, S., *et al.* (2007) The Sorcerer II global ocean sampling expedition: Northwest Atlantic through Eastern Tropical Pacific. *PLoS Biol* **5:** e77.

Schlitzer, R. (2004) Export production in the equatorial and North Pacific derived from dissolved oxygen, nutrient and carbon data. *J Oceanogr* **60:** 53–62.

Schloss, P.D., and Handelsman, J. (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Appl Environ Microbiol* **71:** 1501–1506.

Schwalbach, M.S., and Fuhrman, J.A. (2005) Wide-ranging abundances of aerobic anoxygenic phototrophic bacteria in the world ocean revealed by epifluorescence microscopy and quantitative PCR. *Limnol Oceanogr* **50:** 620–628.

Sieracki, M.E., Gilg, I.C., Thier, E.C., Poulton, N.J., and Goericke, R. (2006) Distribution of planktonic aerobic anoxygenic photoheterotrophic bacteria in the northwest Atlantic. *Limnol Oceanogr* **51:** 38–46.

Søndergaard, M., and Middelboe, M. (1995) A cross-system analysis of labile dissolved organic carbon. *Mar Ecol Prog Ser* **118:** 283–294.

Suyama, T., Shigematsu, T., Suzuki, T., Tokiwa, Y., Kanagawa, T., Nagashima, K.V.P., and Hanada, S. (2002) Photosynthetic apparatus in *Roseateles depolymerans* 61A is transcriptionally induced by carbon limitation. *Appl Environ Microbiol* **68:** 1665–1673.

Xiong, J., Fischer, W.M., Inoue, K., Nakahara, M., and Bauer, C.E. (2000) Molecular evidence for the early evolution of photosynthesis. *Science* **289:** 1724–1730.

Yurkov, V.V., and Beatty, J.T. (1998) Aerobic anoxygenic phototrophic bacteria. *Microbiol Mol Biol Rev* **62:** 695–724.

Yutin, N., Suzuki, M.T., and Beja, O. (2005) Novel primers reveal wider diversity among marine aerobic anoxygenic phototrophs. *Appl Environ Microbiol* **71:** 8958–8962.

Yutin, N., Suzuki, M.T., Teeling, H., Weber, M., Venter, J.C., Rusch, D.B., and Beja, O. (2007) Assessing diversity and biogeography of aerobic anoxygenic phototrophic bacteria in surface waters of the Atlantic and Pacific Oceans using the Global Ocean Sampling expedition metagenomes. *Environ Microbiol* **9:** 1464–1475.

Zhang, Y., and Jiao, N.Z. (2003) On the method for quantification of aerobic anoxygenic phototrophic bacteria. *Chin Sci Bull* **48:** 2549–2553.