Community structures of actively growing bacteria shift along a north-south transect in the western North Pacific

Akito Taniguchi1* and Koji Hamasaki2

1Graduate School of Biosphere Science, Hiroshima University, 1-4-4 Kagamiyama, Higashi-Hiroshima, Hiroshima, Japan.
2Ocean Research Institute, The University of Tokyo, 1-15-1 Minamidai, Nakano, Tokyo, Japan.

Summary
Bacterial community structures and their activities in the ocean are tightly coupled with organic matter fluxes and thus control ocean biogeochemical cycles. Bromodeoxyuridine (BrdU), halogenated nucleoside and thymidine analogue, has been recently used to monitor actively growing bacteria (AGB) in natural environments. We labelled DNA of proliferating cells in seawater bacterial assemblages with BrdU and determined community structures of the bacteria that were possible key species in mediating biochemical reactions in the ocean. Surface seawater samples were collected along a north-south transect in the North Pacific in October 2003 and subjected to BrdU magnetic beads immunocapture and PCR-DGGE (BUMP-DGGE) analysis. Change of BrdU-incorporated community structures reflected the change of water masses along a north-south transect from subarctic to subtropical gyres in the North Pacific. We identified 25 bands referred to AGB as BrdU-incorporated phylotypes, belonging to Alphaproteobacteria (5 bands), Betaproteobacteria (1 band), Gammaproteobacteria (4 bands), Cytophaga-Flavobacterium-Bacteroides (CFB) group bacteria (5 bands), Gram-positive bacteria (6 bands), and Cyanobacteria (4 bands). BrdU-incorporated phylotypes belonging to Vibrionales, Alteromonadales and Gram-positive bacteria appeared only at sampling stations in a subtropical gyre, while those belonging to Roseobacter-related bacteria and CFB group bacteria appeared at the stations in both subarctic and subtropical gyres. Our result revealed phylogenetic affiliation of AGB and their dynamic change along with north-south environmental gradients in open oceans. Different species of AGB utilize different amount and kinds of substrates, which can affect the change of organic matter fluxes along transect.

Introduction
A significant ecological role of bacteria in marine environment is now recognized as an important component in the processes of biological production and biogeochemical cycles (Azam, 1998). In the last few decades, bacterial production has been measured by thymidine and/or leucine methods in various marine environments (Fuhrman and Azam, 1980; Kirchman et al., 1985; Cole et al., 1998) and their community composition and distribution have been revealed in detail by culture-independent molecular techniques (DeLong and Karl, 2005; Giovannoni and Stingl, 2005). Recent advances in ecological genomic analysis have been providing powerful tools to access functional genes of as-yet-to-be cultured microorganisms (Tyson et al., 2004; Venter et al., 2004; DeLong, 2005; Rusch et al., 2007). Thus, it has been required to reveal the link between diversity and functions of natural bacterial assemblages in order to understand their roles in biogeochemical cycles.

Several studies have addressed the relationships between diversity and functions of bacterial communities. A combination of microautoradiography and fluorescence in situ hybridization (Micro-FISH) enabled to measure bacterial substrate uptake, specifically identifying phyotypes of every single-cells under the microscope, which revealed spatio-temporal variability of organic matter utilization of major subgroups of marine bacteria (Cottrell and Kirchman, 2000; 2003; Elfantz et al., 2005). Micro-FISH analysis has revealed that Proteobacteria and Cytophaga-Flavobacterium-Bacteroides (CFB) group bacteria are not only abundant but they also account for most of the heterotrophic bacterial production and consumption of various substances in marine environment. Schafer et al. (2001) identified metabolically active bacterial populations in nutrient-enriched seawaters, by...
Environmental characteristics of sampling stations in the western North Pacific.

| Station | Latitude | Longitude | WT (°C) | Sal (PSU) | DO (μM) | NO₂ + NO₃ (μM) | NH₄ (μM) | SiO₂ (μM) | Chl a (μg l⁻¹) | BA (x10⁶ cells ml⁻¹) |
|---------|----------|-----------|---------|-----------|---------|--------------|----------|------------|-----------------|---------------------|
| ST01    | 44°00N   | 155°00E   | 11.8    | 32.96     | 281.2   | 5.4          | 0.60     | 11.3       | 0.73            | 4.0                 |
| ST02    | 47°00N   | 160°00E   | 10.4    | 32.71     | 295.5   | 9.4          | 0.52     | 17.7       | 0.76            | 4.7                 |
| ST03    | 48°30N   | 165°00E   | 9.8     | 32.94     | 290.0   | 9.3          | 0.12     | 17.8       | 0.44            | 4.9                 |
| ST04    | 45°00N   | 165°00E   | 10.2    | 32.99     | 291.1   | 0.36         | 0.10     | 5.1        | 0.47            | 4.8                 |
| ST05    | 44°00N   | 155°00E   | 11.8    | 32.96     | 281.2   | 5.4          | 0.60     | 11.3       | 0.73            | 4.0                 |
| ST06    | 35°00N   | 165°00E   | 25.8    | 34.47     | 212.0   | <0.1⁰         | <0.05¹   | 1.4        | 0.25            | 4.8                 |
| ST07    | 28°00N   | 165°00E   | 28.1    | 35.02     | 209.0   | <0.1⁰         | 0.09     | <0.1⁰      | 0.07            | 5.4                 |

a. Lower than each detection limit.

WT, water temperature; Sal, Salinity; DO, dissolved oxygen; BA, bacterial abundance.

Results

Environmental characteristics

Water temperature and salinity in our studied area increased from north to south, ranging from 9.8°C to 28.1°C and from 32.7 PSU to 35.1 PSU respectively (Table 1). These data showed that four northern stations (from ST01 to ST04) were located in the North Pacific Subarctic Gyre, two southern stations (ST06 and ST07) were located in the North Pacific Subtropical Gyre and ST05 was in a transitional area. Concentrations of dissolved oxygen (DO) and inorganic nutrients (SiO₂, NO₂ and NO₃ and NH₄) at the northern stations were higher than at the southern stations. Dissolved oxygen concentrations varied from 209.0 to 296.1 μM with the maximum values at 48°N (ST03). Nutrient concentrations ranged from <0.1 to 27.5 μM for SiO₂, <0.1 to 17.3 μM for NO₂ plus NO₃ with the maximum value at 48°N (ST03), and from <0.05 to 0.60 μM for NH₄ with the maximum values at 44°N (ST01). Bacterial abundance and chlorophyll a (chl a) concentration ranged from 4.0 × 10⁶ to 5.9 × 10⁶ cells ml⁻¹ and from 0.07 to 1.31 μg l⁻¹ respectively.

© 2007 The Authors

Journal compilation © 2007 Society for Applied Microbiology and Blackwell Publishing Ltd, Environmental Microbiology, 10, 1007–1017
Community structures of total and BrdU-incorporated bacteria

We optimized the procedure of BUMP-DGGE analysis, in which specific immunoseparation of BrdU-labelled DNA from total DNA was confirmed. The optimized procedure gave no PCR amplification products, when seawater samples without BrdU labelling were subjected to BrdU immunocapture in the same conditions as BrdU-labelled samples (data not shown).

The DGGE profiles of PCR-amplified 16S rRNA genes from total DNA clearly represented change of bacterial community structures along a north-south transect. A similar trend was also observed in the profiles from BrdU-incorporated DNA (Fig. 1). The cluster analysis of DGGE profiles (Fig. 2) distinguished bacterial community structures of northern and transitional stations (ST01–05) from those of southern stations (ST06 and ST07). Also, different clusters were formed between total and BrdU-incorporated community structures within either northern or southern stations. The DGGE profiles of total community changed little (less than 10%) after 10 h incubation (see T0 and T10 in Fig. 2).

The DGGE profiles of BrdU-incorporated community showed slight difference between the profiles of 5 h incubation (T5b in Fig. 1) and that of 10 h incubation (T10b in Fig. 1). Some DGGE bands appeared in either community in T5b or T10b. The DGGE bands 12 and 13 were only appeared in T5b but not in T10b, while the band 27 only appeared at ST06 in T10b but not in T5b. Also, the band 8 was found at ST06 in T5b but disappeared in T10b.

Sequencing and phylogenetic analysis

We sequenced partial 16S rRNA genes of 32 bands excised from both total and BrdU-incorporated communities (Fig. 1). The migration position of DGGE bands were compared among all sampling stations and the presence and absence of these 32 phylotypes at each sampling station was determined (Table 2). Twenty-five out of 32 bands were referred to as BrdU-incorporated phylotypes, including five alphaproteobacteria, one betaproteobacterium, four gammaproteobacteria, five CFB group bacteria, six Gram-positive bacteria and four cyanobacteria. Four out of five alphaproteobacteria belonged to *Rhodobacterales*. One betaproteobacterium belonged to *Burkholderiales*. Although the closest match of relative of this betaproteobacterium was an uncultured ‘*Pseudomonas* sp.’ clone in the database, this should be misidentification of the registered clone, and it phylogenetically belonged to *Betaproteobacteria* (Fig. 3). One out of four gammaproteobacteria belonged to *Vibrionales* and others belonged to *Alteromonadales*. As for Gram-positive bacteria, four were high-GC group (three actinobacteria and one unidentified bacterium) and two were low-GC group (*Lactobacillales* bacteria). Also, we found that two phylotypes closely related to *Prochlorococcus* and other two phylotypes closely related to *Synechococcus*. There were five phylotypes (*KH03–30B* and *KH03–7B* of alphaproteobacteria, *KH03–11B* of betaproteobacterium, *KH03–30B* of cyanobacteria, *KH03–24B* of CFB group bacteria) widely distributed from north to south as BrdU-incorporated bacteria. Most of gammaproteobacteria, all Gram-positive bacteria, two
alphaproteobacteria and *Prochlorococcus* were found only at southern and transitional stations. In contrast, five phylotypes (KH03–9B of alphaproteobacteria, KH03–13B, KH03–32B and KH03–38B of CFB group bacteria and KH03-77 of *Synechococcus*) were found only at northern and transitional stations.

**Discussion**

In our previous study, the BUMP-DGGE analysis revealed phylogenetic affiliations of AGB in coastal waters. This approach allows workers to determine phylotypes contributing to bacterial production at a given time and locale. Furthermore, it is also possible to test growth performance of specific bacteria bearing genes important in biogeochemical cycles when the functional genes instead of 16S rRNA gene are amplified after BrdU-labelling and immunocapturing. For example, the protocol should help to address a question whether light-dependent growth of aerobic phototrophic bacteria and proteorhodopsin-containing bacteria contribute to pelagic bacterial production (Schwalbach et al., 2005; Sieracki et al., 2006). In this study, the same method was successfully applied to open ocean waters where bacterial growth rate was lower and thus BrdU incorporation was less than coastal waters. As ambient substrate availability for growth of bacteria should be much lower in the oceanic waters than the coastal ones, we added much lower concentration of BrdU for labelling to oceanic waters (20 nM) than the coastal ones (1 µM). Hence, our methodology was sensitive enough to detect BrdU-incorporated bacteria in nutrient-poor oligotrophic environments such as the North Pacific Subtropical Gyre, although longer labelling time (> 5 h) was required for it than nutrient-rich coastal waters.

To our knowledge, this is the first report on identifying detailed phylotypes of AGB inhabiting oligotrophic open ocean environments. Micro-FISH studies using 3H-thymidine and 3H-leucine showed active growth of SAR11 bacteria in the North Atlantic Ocean (Malmstrom et al., 2004). Also, BrdU immunofluorescence method in combination with 16S rRNA FISH showed that some phylogenetic groups of bacteria such as *Roseobacter* clade, *Alteromonas* clade and SAR86 group are actively growing in the North Sea (Pernthaler et al., 2002). BUMP-DGGE analysis gave more detailed affiliation of AGB. Although several studies using RT-PCR-DGGE have revealed detailed phylotypes of metabolically active bacteria (Moesender et al., 2001; Schafer et al., 2001; Troussellier et al., 2002), it has never been performed in open ocean environments.

Distinct bacterial community structures between the northern and the southern stations in both total and BrdU-
incorporated communities reflected the changes of associated water masses from subarctic to subtropical gyres. Environmental characteristics (Table 1) such as water temperature, salinity and inorganic nutrients clearly indicated the change of water masses. Also, surface seawater (10 m depth) DOC concentrations were 60.0–62.2 μM at ST01–04 (subarctic), 63.9 μM at ST05 (transitional), and 70.9 and 70.8 μM at ST06 and 07 (subtropical) respectively (H. Ogawa, pers. comm.). Dissolved organic matter in the highly productive subarctic area reportedly showed lower carbon concentrations but contained higher labile fractions than those in the oligotrophic subtropical area (Ogawa and Tanoue, 2003). Although it is too early to generalize such distinct patterns of bacterial community structure, change of BrdU-incorporated community structure from one to another gyre could lead to the change of organic matter fluxes owing to variable characteristics of nutrient acquisition by different phylotypes of bacteria. Bacterial productivity measured in the same sampling sites was different between the two gyres (Hamasaki, 2006).

BUMP-DGGE can be a powerful method to address questions in structuring mechanism of bacterial community (e.g. bottom-up versus top-down, growth versus mortality). Cluster analysis of DGGE profiles revealed that total community structures were distinguished from BrdU-incorporated community structures (Fig. 2), suggesting total community was not only structured by the growth of bacteria but also structured by other factors such as grazing and viral lysis in our studied samples. Another possibility is that differences between total and BrdU-incorporated communities may be due to differential growth of various phylotypes of bacteria on fine temporal scales such as daily or half-daily scales. The DGGE bands in total communities reflect major bacteria with high abundance, whereas those in BrdU-incorporated communities reflect bacteria with high growth rate. Bacteria with high growth rates but low abundance due to high mortality

| DGGE band | Band name | Accession number | Phylogenetic group | ST01 | ST02 | ST03 | ST04 | ST05 | ST06 | ST07 |
|-----------|-----------|------------------|-------------------|------|------|------|------|------|------|------|
| 18        | KHO3–30B  | AB307981         | uncultured        | B    | B    | B    | B    | T/B  | T/B  |      |
| 27        | KHO3–9B   | AB307970         | Rodobacterales    | T/B  | T/B  | T/B  | T/B  | T/B  | –    | –    |
| 23        | KHO3–6B   | AB307971         | Rodobacterales    | –    | –    | –    | –    | –    | –    | –    |
| 29        | KHO3–57B  | AB307994         | Rodobacterales    | T    | T    | T    | T/B  | T/B  | –    | –    |

**Table 2.** The phylogenetic group of 16S rRNA gene sequencing of excised DGGE bands and the presence and absence at each samples.

a. Class or Order of the closest isolates. The band matched to no isolates with > 95% similarity represented as ‘uncultured’ for convenience. T, presence of DGGE band in total communities (T0 and T10); B, in BrdU-incorporated comminities (T5b and T10b); T/B, in both communities; –, DGGE band was not detectable.

© 2007 The Authors

**Journal compilation © 2007 Society for Applied Microbiology and Blackwell Publishing Ltd, Environmental Microbiology, 10, 1007–1017**
Fig. 3. A neighbour-joining tree of 16S rRNA gene sequences of actively growing bacteria from members of the Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria retrieved from the coastal (Inland Sea of Japan; Hamasaki et al., 2007) and the oceanic (western North Pacific) sites. The band names of green and blue letters represent phylotypes retrieved from coastal and oceanic environments respectively. Bootstrap values > 50% are indicated. The scale bar represents 2% estimated sequence divergence.
rates or temporally limited growth may not be detected in the total communities but in the BrdU-incorporated communities. We found such phyotypes mostly in subtropical stations. High water temperature might support instantaneous rapid growth but the final yield of their biomass might be limited by low substrate availability in subtropical oligotrophic open oceans. For example, phyotypes closely related to* Alteromonas* and *Vibrio* (KH03–51B, 52B and 22B) were only detected in BrdU-incorporated communities at ST06 and 07. Most of the phyotypes identified as Gram-positive bacteria detected at ST05–07 were only found in BrdU-incorporated communities. Also, no betaproteobacteria were detected in the total communities at ST06 and ST07, although one phyotype was found at all stations in BrdU-incorporated communities. Some rapidly growing bacteria might be subjected to intense grazing by protists or viral lysis and thus prevented from becoming abundant enough to be detected in PCR-DGGE.

We found that bacteria in *Roseobacter* clade appeared as AGB in both subarctic and subtropical stations. Especially, their presence in the subtropical stations suggested their active growth in oligotrophic open oceans. *Roseobacter* clade includes aerobic anoxygenic photosynthetic bacteria (AAPB). This group of bacteria reportedly accounted for up to about 10% of marine prokaryotes (Sieracki et al., 2006) and had more advantages than other bacteria in oligotrophic environments because of their potential for photosynthetic energy acquisition (Kolber et al., 2000; Karl, 2002). As we have no data whether *Roseobacter*-related bacteria found in this study are AAPB or not, more research should be required to reveal the contribution of AAPB to bacterial carbon production in oligotrophic open oceans.

Occurrence of Betaproteobacteria and Gram-positive bacteria was unexpected in this study because they were mostly reported as abundant group in wastewater, soil or subsurface of deep sediments (Hugenholtz et al., 1998). Most DGGE bands related to Gram-positive bacteria were not found in total communities but in BrdU-incorporated communities, suggesting that they are actively growing but relatively minor in abundance. Bouvier and del Giorgio (2007) reportedly showed that Betaproteobacteria and Gram-positive bacteria (*Actinobacteria*) had a potential to rapidly grow in an open ocean pelagic environment but were minor in the assemblages due to high viral mortality. Betaproteobacterium detected in this study (KH03–11B) were closely related (99.5% similarity) to an isolated strain of *Pelomonas saccharophila*, recently reclassified into* Pelomonas saccharophila* (Xie and Yokota, 2005). This bacterium has been reported to fix nitrogen (Barraquio et al., 1986). Identity of nitrogen-fixing microorganisms and their contribution to nitrogen supply to oligotrophic open oceans has been attracted much interest (Capone et al., 1997). *Pelomonas saccharophila*-like betaproteobacterium could be an as-yet-unknown nitrogen-fixing prokaryote in open oceans.

Cyanobacteria were appeared in BrdU-incorporated community at transitional and southern stations (Table 2). Vaulot and colleagues (1995) reported that Prochlorococcus replicated DNA in late afternoon and divided cells in night. If their growth was synchronized by such a day-night cycle, it would depend on the time point of sampling (morning or afternoon) whether these bacteria appear as ‘BrdU-incorporated’ or not. However, we found no correlation between the time points of sampling and BrdU incorporation in this study. BrdU incorporation by Prochlorococcus was detected from the samples collected both in the morning (8–9 AM at the ST06) and afternoon (6–7 PM at the ST07). The incorporation by *Synechococcus* was also detected from the samples collected both in the early morning (3–4 AM at the ST05) and afternoon (5–6 PM at the ST03 and ST04).

We compared AGB phyotypes found in this study with those found in our previous study (Hamasaki et al., 2007), constructing phylogenetic trees representing all AGB ever found by BUMP-DGGE analysis, to see whether common or specific AGB were present between the coastal and the oceanic waters studied in the present and the previous works (Figs 3 and 4). The coastal waters were obtained from nine sampling stations with an environmental gradient from eutrophic to mesotrophic waters (Hamasaki et al., 2007). We have found that the species or strains of AGB found in the coastal sites were basically different from those in the oceanic sites, even though some of them belonged to the same subgroups of bacteria. *Cyanobacteria* and Gram-positive bacteria only appeared in the oceanic sites, while bacteria belonging to *Pseudomonadales*, *Oceanospirillales* and SAR86-cluster only appeared in the coastal sites. Also, AGB belonging to *Roseobacter*-cluster, *Burkholderiales*, *Vibrionales*, *Alteromonadales* and *CFB* were commonly present in both sites. However, the most phyotypes belonging to the same subgroups of bacteria in both sites were not exactly the same as each other if 16S rRNA gene sequences were compared. There were more than 3% differences in those partial 16S rRNA gene sequences (V3 variable regions). *Roseobacter*-related bacteria were previously detected as AGB in the Inland Sea of Japan and North Sea coastal environments using BrdU methodology (Pernthaler et al., 2002; Hamasaki et al., 2007). Also, it has been reported that this group of bacteria are well adapted to nutrient-rich conditions such as coastal waters and algal blooms (Gonzalez et al., 2000; Riemann et al., 2000; Zubkov et al., 2001; Malmstrom et al., 2004; Moran et al., 2004; Grossart et al., 2005). Phyotypes of *Roseobacter*-related AGB found in this study were different with >3% mismatch in partial 16S rRNA gene
Fig. 4. A neighbour-joining tree of 16S rRNA gene sequences of actively growing bacteria from members of the CFB group bacteria, Gram-positive bacteria and Cyanobacteria retrieved from the coastal (Inland Sea of Japan; Hamasaki et al., 2007) and the oceanic (western North Pacific) sites. The band names of green and blue letters represent phylotypes retrieved from coastal and oceanic environments respectively. Bootstrap values > 50% are indicated above the branches. The scale bar represents 2% estimated sequence divergence.
sequences (V3 variable regions) from those found in the Inland Sea of Japan coastal sites. Although the sample coverage is not enough to conclude it, there might be different types of *Roseobacter*-related bacteria adapted to oligotrophic open oceans (Fig. 3). Further investigation should be required to test the hypothesis.

We found that *Roseobacter*-related and CFB group phylotypes were major AGB from subarctic to subtropical transect in the western North Pacific. Also AGB community structures changed along the transect, where phylotypes of *Gammaproteobacteria* and Gram-positive bacteria additionally appeared in subtropical stations. Bacterial phylotypes identified as AGB in this and previous studies are responsible for bacterial production and thus possible key players in marine biogeochemical carbon cycles. These phylotypes should be targeted to monitor their dynamics and ecological functions in future works.

**Experimental procedures**

**Sampling and BrdU labelling**

Surface seawater samples were collected with a clean bucket at seven stations along a north-south transect in the western North Pacific in October 2003 during the KH-03-2 cruise of R/V Hakuho-maru (Table 1). About 35 l of surface seawater samples was pre-filtered through a 200 μm nylon mesh to remove mesozooplankton, and subjected to further procedure within 1 h. Eleven litres of the pre-filtered samples with BrdU (20 nM in final concentration; Sigma-Aldrich, St Louis, MO) was incubated in dark bottles at in situ water temperature for 5 and 10 h. After incubation, bacterial cells were collected with 0.22 μm pore size Sterivex cartridge filters (Millipore, Billerica, MA) with a peristaltic pump. Immediately after filtration, the Sterivex filters were stored at −20°C until further analysis. Chla concentration was measured with Turner-Designs fluorometer after the extraction with N,N-dimethylformamide (Suzuki and Ishimaru, 1990; Welschmeyer, 1994). Bacterial abundance was enumerated by direct counting with the use of epifluorescence microscopy (Olympus BX-51, Olympus, Tokyo, Japan) after staining with 4′,6-diamidino-2-phenylindole (1.0 μg ml⁻¹ in final concentration; Molecular Probes, Eugene, OR) (Porter and Feig, 1980). Nitrate + nitrite, ammonium, phosphate and silicate were measured by continuous flow system (AACS-II, Aichi, Japan) after staining with 4% xanthogenate-SDS DNA extraction and 1 μg of the extracted DNA was used for BrdU immunocapture. The total DNA and immunocaptured BrdU-labelled DNA were used as templates by PCR amplification of 16S rRNA genes using the eubacterial-specific primer 341F-GC with a 40-bp GC clamp (57 mer; 5′-CGC CCG CGC GCG GCG GGC GCG GGC GCG GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC G

© 2007 The Authors
Journal compilation © 2007 Society for Applied Microbiology and Blackwell Publishing Ltd, *Environmental Microbiology*, 10, 1007–1017

**References**

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25: 3389–3402.

Artursson, V., and Jansson, J.K. (2003) Use of bromodeoxyuridine immunocapture to identify active bacteria associated with arbuscular mycorrhizal hyphae. *Appl Environ Microbiol* 69: 6208–6215.
Artursson, V., Finlay, R.D., and Jansson, J.K. (2005) Combined bromodeoxyuridine immunocapture and terminal-restriction fragment length polymorphism analysis highlights differences in the active soil bacterial metagenome due to *Glomus mosseae* inoculation or plant species. *Environ Microbiol* **7**: 1952–1966.

Azam, F. (1998) Microbial control of oceanic carbon flux: the plot thickens. *Science* **280**: 694–696.

Barraquito, W.L., Pader, B.C., Jr, Watanabe, I., and Knowles, R. (1986) Nitrogen fixation by *Pseudomonas saccharophila* Doudoroff ATCC 15946. *J Gen Microbiol* **132**: 237–241.

Bormann, J. (1999) Culture-independent identification of microorganisms that respond to specified stimuli. *Appl Environ Microbiol* **65**: 3398–3400.

Bouvier, T., and del Giorgio, P.A. (2007) Key role of selective viral-induced mortality in determining marine bacterial community composition. *Environ Microbiol* **9**: 287–297.

Capone, D.G., Zehr, J.P., Paerl, H.W., Bergman, B., and Carpenter, E.J. (1997) *Trichodesmium*, a globally significant marine cyanobacterium. *Science* **276**: 1221–1229.

Cole, J.J., Findlay, S., and Pace, M.L. (1998) Bacterial production in fresh and saltwater ecosystem: a cross-system overview. *Mar Ecol Prog Ser* **43**: 1–10.

Cottrell, M.T., and Kirchman, D.L. (2000) Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. *Appl Environ Microbiol* **66**: 1692–1697.

Cottrell, M.T., and Kirchman, D.L. (2003) Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary. *Limnol Oceanogr* **48**: 168–178.

DeLong, E.F. (2005) Microbial community genomics in the ocean. *Nat Rev Microbiol* **3**: 459–469.

DeLong, E.F., and Karl, D.M. (2005) Genomic perspectives in microbial oceanography. *Nature* **437**: 336–342.

Elfantz, H., Malmstrom, R.R., Cottrell, M.T., and Kirchman, D.L. (2005) Assimilation of polysaccharides and glucose by major bacterial groups in the Delaware estuary. *Appl Environ Microbiol* **71**: 7799–7805.

Fuhrman, J.A., and Azam, F. (1980) Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. *Appl Environ Microbiol* **39**: 1085–1095.

Giovannoni, S.J., and Rappe, M.S. (2000) Evolution, diversity, and molecular ecology of marine prokaryotes. In *Microbial Ecology of the Oceans*. Kirchman, D.L. (ed). New York, USA: Wiley-Liss, pp. 47–84.

Giovannoni, S.J., and Stinti, U. (2005) Molecular diversity and ecology of microbial plankton. *Nature* **437**: 343–348.

Giovannoni, S.J., Britschgi, T.B., Moyer, C.L., and Field, K.G. (1990) Genetic diversity in Sargasso Sea bacterioplankton. *Nature* **345**: 60–63.

Gonzalez, J.M., Simo, R., Massana, R., Covert, J.S., Casamayor, E.O., Pedros-Alio, C., and Moran, M.A. (2000) Bacterial community structure associated with a dimethylsulphonio propane-producing North Atlantic algal bloom. *Appl Environ Microbiol* **66**: 4237–4246.

Grossart, H.P., Levold, F., Algalmer, M., Simon, M., and Brinkhoff, T. (2005) Marine diatom species harbour distinct bacterial communities. *Environ Microbiol* **7**: 860–873.

Hamasaki, K. (2006) Comparison of bromodeoxyuridine immunoassay with tritiated thymidine radioassay for measuring bacterial productivity in oceanic waters. *J Oceanogr* **62**: 793–799.

Hamasaki, K., Long, R.A., and Azam, F. (2004) Individual cell growth rates of marine bacteria, measured by bromodeoxyuridine incorporation. *Aquat Microb Ecol* **35**: 217–227.

Hamasaki, K., Taniguchi, A., Tada, Y., Long, R.A., and Azam, F. (2007) Actively growing bacteria in the Inland Sea of Japan, identified by combined bromodeoxyuridine immunocapture and denaturing gradient gel electrophoresis. *Appl Environ Microbiol* **73**: 2787–2798.

Hugenholtz, P., Goebel, B.M., and Pace, N.R. (1998) Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *J Bacteriol* **180**: 4765–4774.

Karl, D.M. (2002) Hidden in a sea of microbes. *Nature* **415**: 590–591.

Kirchman, D.L., Knees, E., and Hodson, R.E. (1985) Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. *Appl Environ Microbiol* **49**: 599–607.

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.

Kumar, S., Tamura, K., and Nei, M. (2004) MEGA3: an integrated software for Molecular Evolutionary Genetics Analysis and Sequence alignment. *Brief Bioinform* **5**: 150–163.

Maidak, B.L., Cole, J.R., Liburn, T.G., Parker, C.T., Jr, Saxman, P.R., Farris, R.J., et al. (2001) The RDP-II (Ribosomal Database Project). *Nucleic Acids Res* **29**: 173–174.

Malmstrom, R.R., Kiene, R.P., and Kirchman, D.L. (2004) Identification and enumeration of bacterial assimilating dimethylsulphonio propane (DMSP) in the North Atlantic and Gulf of Mexico. *Limnol Oceanogr* **49**: 597–606.

Moesander, M.M., Winter, C., and Herndl, G.J. (2001) Horizontal and vertical complexity of attached and free-living bacteria of the eastern Mediterranean Sea, determined by 16S rDNA and 16S rRNA fingerprints. *Limnol Oceanogr* **46**: 95–107.

Moran, M.A., Buchan, A., Gonzalez, J.M., Heidelberg, J.F., Whitman, W.B., Kiene, R.P., et al. (2004) Genome sequence of *Silicibacter pomeroyi* reveals adaptations to the marine environment. *Nature* **432**: 910–913.

Nelson, C.E., and Carlson, C.A. (2005) A nonradioactive assay of bacterial productivity optimized for oligotrophic pelagic environments. *Limnol Oceanogr Methods* **3**: 211–220.

Ogawa, H., and Tanoue, E. (2003) Dissolved organic matter in the oceanic waters. *J Oceanogr* **59**: 129–147.

Pernthaler, A., and Pernthaler, J. (2005) Diurnal variation of cell proliferation in three bacterial taxa from coastal North Sea waters. *Appl Environ Microbiol* **71**: 4638–4644.

Pernthaler, A., Pernthaler, J., Schattenhofer, M., and Amann, R. (2002) Identification of DNA-synthesizing bacterial cells in coastal North Sea plankton. *Appl Environ Microbiol* **68**: 5728–5736.

Porter, K.G., and Feig, Y.S. (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol Oceanogr* **25**: 943–948.

Riemann, L., Steward, G.F., and Azam, F. (2000) Dynamics
of bacterial community composition and activity during a mesocosm diatom bloom. *Appl Environ Microbiol* **66**: 578–587.

Rusch, D.B., Halpern, A.L., Sutton, G., Heidelberg, K.B., Williamson, S., Yooseph, S., *et al.* (2007) The Sorcerer II Global Ocean Sampling expedition: Northwest Atlantic through eastern tropical Pacific. *PLoS Biol* **5**: 398–431.

Schafer, H., and Muyzer, G. (2001) Denaturing gradient gel electrophoresis in marine microbial ecology. In *Methods in Microbiology*. Paul, J.H. (ed). New York, USA: Academic Press, 30: 425–468.

Schafer, H., Bernard, L., Courties, C., Lebaron, P., Servais, P., Pukall, R., *et al.* (2001) Microbial community dynamics in Mediterranean nutrient-enriched seawater mesocosms: changes in the genetic diversity of bacterial populations. *FEMS Microbiol Ecol* **34**: 243–253.

Schmidt, T.M., DeLong, E.F., and Pace, N.R. (1991) Analysis of a marine picoplankton community by 16S rRNA gene cloning and sequencing. *Appl Environ Microbiol* **173**: 4371–4378.

Schwalbach, M.S., Brown, M., and Fuhrman, J.A. (2005) Impact of light on marine bacterioplankton community structure. *Aquat Microb Ecol* **39**: 235–245.

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

Steward, G.F., and Azam, F. (1999) Bromodeoxyuridine as an alternative to 3H-thymidine for measuring bacterial productivity in aquatic samples. *Aquat Microb Ecol* **19**: 57–66.

Suzuki, R., and Ishimaru, T. (1990) An improved method for the determination of phytoplankton chlorophyll using N,N-dimethylformamide. *J Oceanogr* **46**: 190–194.

Thompson, J.D., Higgins, D.G., and Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**: 4673–4680.

Troussellier, M., Schafer, H., Batailler, N., Bernard, L., Courties, C., Lebaron, P., *et al.* (2002) Bacterial activity and genetic richness along an estuarine gradient (Rhone River plume, France). *Aquat Microb Ecol* **28**: 13–24.

Tyson, G.W., Chapman, J., Hugenholtz, P., Allen, E.E., Ram, R.J., Richardson, P.M., *et al.* (2004) Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature* **428**: 37–43.

Urbach, E., Vergin, K.L., and Giovannoni, S.J. (1999) Immunological detection and isolation of DNA from metabolically active and bacteria. *Appl Environ Microbiol* **65**: 1207–1213.

Vaulot, D., Marie, D., Olson, R.J., and Chisholm, S.W. (1995) Growth of *Prochlorococcus*, a photosynthetic prokaryote, in the Equatorial Pacific Ocean. *Science* **268**: 1480–1482.

Venter, J.C., Remington, K., Heidelberg, J.F., Halpern, A.L., Rusch, D., Eisen, J.A., *et al.* (2004) Environmental genomes shotgun sequencing of the Sargasso Sea. *Science* **304**: 66–74.

Walters, S.P., and Field, K.G. (2006) Persistence and growth of fecal *Bacteroidetes* assessed by bromodeoxyuridine immunocapture. *Appl Environ Microbiol* **72**: 4532–4539.

Warnecke, F., Sommaruge, R., Sekar, R., Hofer, J.S., and Perntehler, J. (2005) Abundance, identify, and growth state of *Actinobacteria* in mountain lakes of different UV transparency. *Appl Environ Microbiol* **71**: 5551–5559.

Welschmeyer, N.A. (1994) Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. *Limnol Oceanogr* **39**: 1985–1992.

Xie, C.H., and Yokota, A. (2005) Reclassification of *Alcaligenes latus* strains IAM 12599 and IAM 12664 and *Pseudomonas saccharophila* as *Azohydromonas lata* General nov., comb. nov., *Azohydromonas australica* sp. nov. and *Pelomonas saccharophila* General nov., comb., respectively. *Int J Syst Evol Microbiol* **55**: 2419–2425.

Yin, B., Crowley, D., Sparovek, G., De Melo, W.J., and Borneman, J. (2000) Bacterial functional redundancy along a soil reclamation gradient. *Appl Environ Microbiol* **66**: 4361–4365.

Zubkov, M.V., Fuchs, B.M., Archer, S.D., Kiene, R.P., Aman, R., and Burkil, P.H. (2001) Linking the composition of bacterioplankton to rapid turnover of dissolved dimethylsulphoniopropionate in an algal bloom in the North Sea. *Environ Microbiol* **3**: 304–311.