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Review

Linking Bacterial Community Structure to Carbon Fluxes in Marine Environments

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Microbial oceanography is undergoing a dramatic revolution thanks to the rapid development of novel techniques that allow the examination of microbial diversity and functions via molecular methods, including genomic and metagenomic analyses. During the past decade, studies have revealed previously unknown and surprisingly diverse bacterial communities in marine waters. These studies have radically changed our understanding of spatiotemporal patterns in marine bacterial community composition and the distribution of specific genes. However, our knowledge of the role of individual bacterial subgroups in oceanic food webs and biogeochemical cycles remains limited. To embed the internal dynamics of bacterial communities into marine biogeochemistry models, the characteristic parameters of individual bacterial subgroups (i.e., growth, mortality, and utilization of dissolved organic matter) must be determined. Here, we survey the approaches used to assess variation in and factors controlling bacterial communities in marine environments, emphasizing the importance of quantitative studies that examine growth and grazing parameters of bacterial subgroups.

Keywords:
· Bacterial community,
· biogeochemical cycle,
· carbon flux,
· microbial food webs,
· microbial oceanography.

1. Introduction

Over the past three decades, several studies have examined the abundance, biomass, and production rate of heterotrophic bacteria in various marine environments (Ducklow, 2000; Church, 2008). These studies have revealed that bacteria consume, on average, 50% of primary production in pelagic ecosystems (Azam and Worden, 2004) and play important roles in major biogeochemical processes, such as nutrient cycling (Kirchman, 2000) and organic matter fluxes (Nagata, 2008). In addition, studies have shown that bacterial biomass is comparable to, or even exceeds, phytoplankton biomass in oceanic environments (Fukuda *et al*., 1998), with bacteria representing an important trophic link in marine pelagic food webs (Jürgens and Massana, 2008).

Studies of the role of bacteria in marine food webs and biogeochemical processes have generally treated heterotrophic bacterial communities as an homogeneous pool (Ducklow, 2000). This "black box" approach has the advantage of simplicity: ecosystem models are simplified when entire bacterial communities are treated as a single functional group (i.e., heterotrophic bacteria). However, their predictive power is compromised when the internal dynamics of heterogeneous components exhibiting different physiological and ecological traits are significant. To examine the internal dynamics of bacterial communities and their implications for biogeochemical cycling in marine systems, it is important to understand: 1) the spatiotemporal variation in individual bacterial subgroups; 2) mechanisms underlying community structure controls; and 3) contributions of individual groups to carbon flow in microbial food webs. In this review we discuss recent progress in quantitative studies of bacterial community structure in marine environments, emphasizing the roles of bottom-up and top-down factors in determining phylogenetic compositions of bacteria.

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2. Bacterial Community Analysis in Marine Environments—Brief Survey

Traditionally, microbiologists have examined isolated bacterial species using the agar plate technique originally developed by Robert Koch (1843–1910; Madigan *et al*., 2000). These studies examine the physiology and genetics of bacterial strains isolated on culture media. However, in marine environments, bacteria that are culturable by regular isolation methods represent less than 1% of total cells counted using epifluorescence microscopy (Hobbie *et al*., 1977; Kogure *et al*., 1979). Most marine bacteria are still unculturable and have yet to be tested for taxonomic affiliations and physiological and biochemical properties (Fuhrman and Hagström, 2008).

Advances in culture-independent molecular biology techniques are changing this situation dramatically (Giovannoni *et al*., 1990; DeLong and Karl, 2005; Fuhrman and Hagström, 2008). These new approaches directly examine genetic information from environmental samples (environmental clones) after using polymerase chain reaction (PCR) to amplify target sequences, without isolating or cultivating bacterial species. The taxonomic affiliations of these environmental clones are determined on the basis of small subunit rRNA phylogeny, as originally proposed by Carl Woese (Woese, 1987; Pace, 1997). Molecular analyses of environmental clones obtained from marine environments have revealed many previously unknown bacterial species in coastal and oceanic environments (Fuhrman and Hagström, 2008; Fuhrman, 2009). Sogin *et al*. (2006) used a pyrosequencing technique to analyze more than 100,000 PCR amplicons that span the V6 hypervariable region of the rRNA gene, revealing tremendous numbers of "rare" phylotypes that are present in marine bacterial communities. Studies have also found that *Archaea* are ubiquitous and abundant in marine systems (DeLong, 1992; Fuhrman *et al*., 1992), especially in deep waters (Karner *et al*., 2001; Teira *et al*., 2006) and surface waters of high latitude regions, including the Southern (Church *et al*., 2003) and Arctic Oceans (Kirchman *et al*., 2007). Suzuki and DeLong (2002) discovered that, among the major phylogenetic groups of *Bacteria*, *Proteobacteria* represented 65% of all 16S rRNA genes of marine clones sequenced, whereas *Cytophaga-Flavobacteria-Bacteroides*, Gram-positives, and Cyanobacteria represented 10%, 4%, and 3%, respectively. A notably large discrepancy exists in the phylogenetic compositions of *Proteobacteria* between culturable and unculturable bacteria (or perhaps more correctly "not-yet-cultured" bacteria; Fuhrman and Hagström, 2008). In the GenBank 16S rRNA gene database (Benson *et al*., 2002), *Gammaproteobacteria* are the dominant group in the overall distribution of cultured subgroups, whereas *Alphaproteobacteria* dominate the not-yet-cultured bacteria (Suzuki and DeLong, 2002). Among alphaproteobacterial groups, studies suggest that *Roseobacter* (González and Moran, 1997; Selje *et al*., 2004) and the SAR 11 cluster (Morris *et al*., 2002) are ubiquitous in marine environments. Marine alphaproteobacterial species are now being isolated using the extinction dilution technique (Rappé *et al*., 2002; Giovannoni and Stingl, 2007), revealing unique features that may be linked to their presence in oligotrophic oceans. For example, the genome size of *Pelagibacter ubique* HTCC106, a strain of the SAR11 cluster, is only 1.3M base pairs and encodes the smallest number of predicted open reading frames known for a free-living microorganism (Giovannoni *et al*., 2005). Conceivably, future studies will add new species to the list of the isolates of dominant marine bacteria, although the vast majority of marine clones and species detected using culture-independent techniques remain to be isolated. In short, traditional agar plate determinations of bacterial community compositions have greatly underestimated the diversity of bacterial communities in marine environments.

The use of culture-independent gene sequencing combined with contemporary genomic approaches (e.g., whole-genome shotgun sequencing - Venter *et al*., 2004; pyrosequencing - Dinsdale *et al*., 2008) has started to reveal novel features of microbial community structure (metagenomics or community genomics - Moran, 2008; DeLong, 2009). A powerful feature of these new techniques is the comprehensive genomic information they provide, not only on 16S rRNA phylogeny, but also on functional gene repertoires. At Station ALOHA in the subtropical North Pacific, DeLong *et al*. (2006) used genomic analyses to examine prokaryotic communities distributed throughout the water column. They suggested that sequence variation in microbial community genes reflects the vertical zonation of taxonomic groups as well as the metabolic potentials of prokaryote communities in the oceanic water column. Metagenomic analyses have also led to the discovery of novel metabolic pathways and processes, prominent examples being the findings of proteorhodopsin (Béjà *et al*., 2000) and ammonium oxidation by *Archaea* (Venter *et al*., 2004). We recommend that readers consult Moran (2008) and DeLong (2009) for principles, applications, and the exciting development of metagenomic approaches in the field of microbial oceanography.

3. Quantitative Determinations of Bacterial Community Structure

Although the advancement of culture-independent techniques has substantially improved our knowledge of marine bacterial diversity, little is known of the dynamics and regulation of individual bacterial subgroups. A

fundamental requirement for the study of bacterial community dynamics and the role of bacterial communities in marine biogeochemical cycles is to determine the abundance and biomass of individual bacterial subgroups. Two approaches have been used to examine the relative contributions of different bacterial species or phylogenetic groups to total bacterial abundance. One is PCR-based techniques, including dot-blot hybridization (Stahl *et al*., 1988) and quantitative PCR (Suzuki *et al*., 2001; Ahlgren *et al*., 2006). The frequency of the occurrence of particular phylotypes in DNA fingerprinting (Brown *et al*., 2005) and clone libraries (Pommier *et al*., 2007) also provides insights into variations in community structures among different oceanic regions. Estimates of the relative abundance of phylogenetic groups derived from these techniques may contain errors due to: 1) kinetic biases inherent to PCR amplification; 2) variation in the copy number of 16S rRNA genes among different bacterial species; and 3) differences in DNA extraction efficiency within various bacterial species (Suzuki and Giovannoni, 1996; Nocker *et al*., 2007). Cell morphology and size information cannot be obtained because of nucleic acid extraction. Thus, data obtained using PCR-based approaches (even quantitative PCR) are semi-quantitative, hampering coherent examination of bacterial growth and mortality parameters in terms of cell abundance, biomass, and morphology of individual groups.

The second approach is the fluorescent *in situ* hybridization (FISH) method, which uses fluorescence microscopy to detect individual cells hybridized with fluorescent oligo- or polynucleotide probes (Amann and Fuchs, 2008). Fluorescence emitted from probe-positive cells can be increased using catalyzed reporter deposition (CARD) probes (Teira *et al*., 2004), which help to detect cells with low rRNA content. This approach has its own limitations. Appropriate probes should be carefully chosen or designed to enable complete detection of target groups with minimal inclusion of false positives, a requirement that is not always fully met (Amann and Fuchs, 2008). In addition, variability in the efficiency of hybridization among different subgroups may result in biases in the assessment of community structure (Amann and Fuchs, 2008). Despite these limitations, the single-cell detection of FISH and CARD-FISH is a powerful tool for determining cell abundance, size, and morphology of bacterial subgroups that provides complementary data to study the dynamic nature of bacterial communities. Recent advances in image analysis techniques have substantially improved the sensitivity, precision, and speed of FISH analysis, enabling the analysis of a large number of oceanographic samples in a semi-automated fashion (Cottrell and Kirchman, 2003; Yokokawa, 2004; Posch *et al*., 2009). The development of techniques combining FISH with autoradiography (Cottrell and Kirchman, 2000; Varela *et al*., 2008), nucleoside tracers (Hamasaki *et al*., 2004), Raman spectroscopy (Huang *et al*., 2007), or nanometer-scale secondary ion mass spectrometry (NanoSIMS; Behrens *et al*., 2008) has made it possible to determine the metabolic characteristics of individual bacterial cells. Novel techniques that allow FISH-based visualization of specific genes have also been developed (Zwirglmaier *et al*., 2004; Kenzaka *et al*., 2005; Maruyama *et al*., 2005), although these techniques have yet to be applied to complex natural communities in marine environments.

4. Distributions of Different Phylogenetic Bacterial Groups in Aquatic Systems

Since the first cross-system comparison of bacterial community structure conducted by Glöckner *et al*. (1999), investigators have continued to clarify the biogeographic patterns of bacterial composition in marine and freshwater environments. One notable pattern is the difference in bacterial composition between fresh and saltwater systems. *Alphaproteobacteria*, including the SAR11 cluster, and *Roseobacter* often dominate marine bacterial communities (Morris *et al*., 2002; Selje *et al*., 2004), whereas *Betaproteobacteria* are more abundant in freshwater systems (Glöckner *et al*., 1999), although a recent study reported that *Alphaproteobacteria* were highly abundant in a large freshwater lake (Nishimura and Nagata, 2007). *Gammaproteobacteria* are detected in both freshwater and marine environments, but tend to be more abundant in marine systems (Lebaron *et al*., 2001). On the other hand, *Actinobacteria* appear to be more abundant in freshwater than in marine waters (Warnecke *et al*., 2005). The *Cytophaga-Flavobacter* cluster has been reported to be distributed relatively abundantly in both fresh and marine waters. A drastic shift in community composition has been documented in estuarine environments from the prevalence of *Betaproteobacteria* in freshwater regions to the dominance of *Alphaproteobacteria* in saltwater regions (Fig. 1; Kirchman *et al*., 2005), although this trend was less evident in bacterial communities attached to particles in the Weser Estuary (Selje and Simon, 2003).

These systematic patterns in aquatic bacterial biogeography at the major phylogenetic group level (i.e., division (or phylum) and subdivision; Giovannoni and Stingl, 2005) are quite intriguing, suggesting that some members of individual groups could become competitive and prevalent in different aquatic habitats. Kirchman *et al*. (2005) argued that "the presence of systematic biogeographic patterns for some groups, even those at a high phylogenetic level, indicates that they might function as ecological units with defined roles in mediating biogeochemical processes." Clearly, each major group may consist of diverse subgroups, species, and "ecotypes" (Cohan, 2002; Johnson *et al*., 2006) with quite different

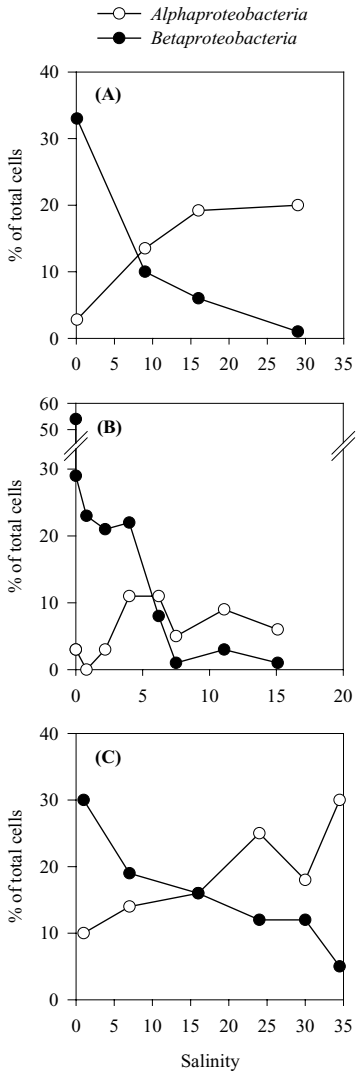

Fig. 1. An example of the systematic shift of the bacterial community structure (division and subdivision levels) along an environmental gradient. The community structures in the estuaries varied from *Betaproteobacteria*-dominated in freshwater regions to *Alphaproteobacteria*-dominated in marine waters. The data were collected from the Atlantic coast of the United States (A - Delaware Bay (Cottrell and Kirchman, 2003), B - Chesapeake Bay (Bouvier and del Giorgio, 2002)), and the coast of the South China Sea (C - Pearl River Estuary (Zhang *et al*., 2006)). Although the relative abundances of the phylogenetic groups were plotted against salinity, we do not mean to imply that salinity *per se* explains this pattern of community change. Several factors, including the source and nature of dissolved organic matter, the extent of pollution, and the abundance of particles, may systematically vary along salinity gradients of estuaries. Mechanisms underlying biogeographic variations in bacterial community structures and diversity in marine systems remain unclear (Kirchman *et al*., 2005; Fuhrman, 2009).

physiological and biochemical traits. For example, *Alphaproteobacteria* consists of diverse groups of bacteria including heterotrophs, carboxydotrophs, and anoxygenic phototrophs (Moran, 2008). Thus, an unequivocal resolution of the mechanisms underlying pattern formations in biogeographic distributions of major phylogenetic groups requires further investigation, including the use of more narrowly targeted probes (Amann and Fuchs, 2008) and detailed genomic analyses (Moran, 2008).

5. Group-Specific Variation in the Use of Dissolved Organic Matter

The combination of FISH and microautoradiography has provided a powerful tool to determine the phylogenetic affiliation of individual cells that take up defined dissolved organic matter (DOM) components (Cottrell and Kirchman, 2000; Teira *et al*., 2006). Cottrell and Kirchman (2000) examined group-specific traits in the use of DOM by Delaware Bay communities and found that the *Cytophaga-Flavobacter* cluster was overrepresented in the portion of the assemblage consuming chitin, *N*-acetylglucosamine, and protein, whereas *Alphaproteobacteria* was the dominant group consuming amino acids. In the North Atlantic Ocean, Malmstrom *et al*. (2005) found that dimethylsulfoniopropionate is mainly taken up (up to 30%) by SAR11 bacteria, which represent a subgroup of the *Alphaproteobacteria*. Alonso-Saez and Gasol (2007) found that concentration-dependent patterns of amino acid uptake differed among different phylogenetic groups of bacteria in Mediterranean coastal waters. These results suggest that distinct phylogenetic groups of bacteria tend to exploit several organic resources with different kinetics, playing different roles in regulating DOM turnover in marine systems.

6. Group-Specific Variation in Bacterial Growth Rates and Community Structure Controls

The growth rates of bulk bacterial communities are related to temperature and the supply of organic and inorganic resources (Church, 2008). Yokokawa and Nagata (2005) tested the hypothesis that the relationship between bacterial growth rates and environmental variables (temperature and substrate supply) varies among different phylogenetic groups in coastal environments of the western North Pacific. They found that a large fraction (62%) of the variation in growth rates of *Alphaproteobacteria* was accounted for by the combination of temperature and chlorophyll *a* concentration (an index of substrate supply; Fig. 2), which was consistent with findings for bulk bacterial communities (Nagata *et al*., 2001). However, the relationship between predictor variables (chlorophyll *a* concentration and temperature) and growth rates of the *Cytophaga-Flavobacter* cluster and

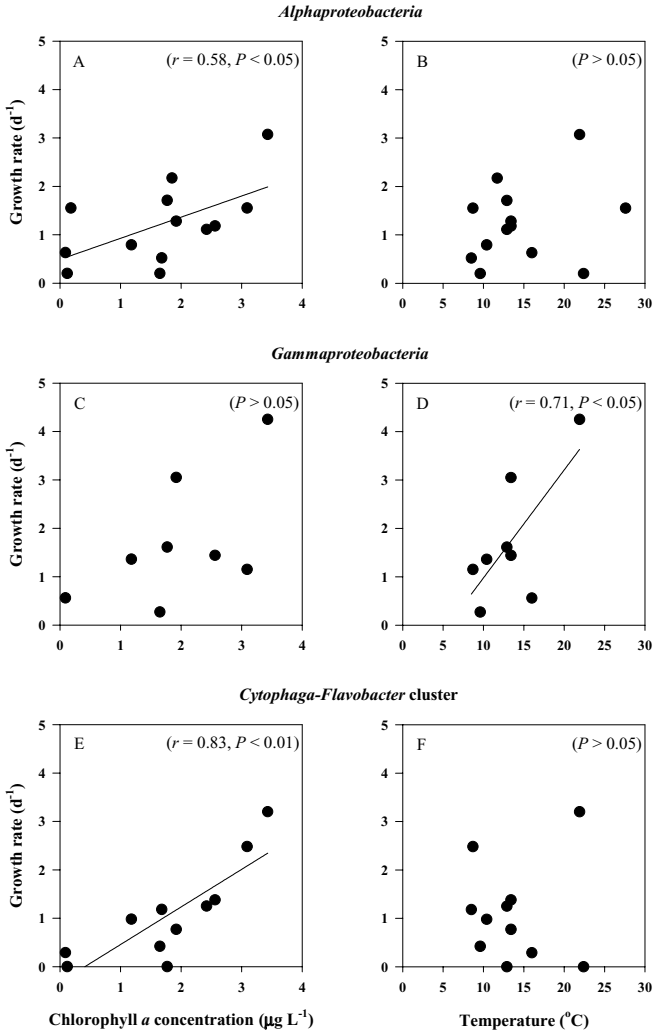

Fig. 2. Relationships between specific growth rates of individual phylogenetic bacterial groups and environmental variables, including chlorophyll *a* concentration (A, C, and E) and water temperature (B, D, and F) (from Yokokawa and Nagata, 2005). To identify the factors accounting for variation in group-specific growth rates, Yokokawa and Nagata (2005) conducted forward stepwise regression analysis using chlorophyll *a* concentration and temperature as independent variables. Temperature and chlorophyll *a* concentration were not significantly correlated ($P > 0.05$). The results showed that the combination of temperature and chlorophyll *a* concentration accounted for 62% of the variation in *Alphaproteobacteria* growth rates. The multiple regression equation is: $GR_{alpha} = 0.612\ (\pm 0.160) \cdot CHL + 0.080\ (\pm 0.029) \cdot T - 0.975\ (\pm 0.607)$, ($r^2 = 0.62$, $P < 0.01$, $n = 13$), where GR_{alpha}, CHL, and T are the growth rate of *Alphaproteobacteria* (day^{-1}), chlorophyll *a* concentration ($\mu g\ L^{-1}$), and temperature (°C), respectively. The relationships between predictor variables and growth rates of *Cytophaga-Flavobacter* cluster (GR_{CF}) and *Gammaproteobacteria* (GR_{gamma}) differed from that of *Alphaproteobacteria*. A significant fraction (73%) of GR_{CF} variation was explained by chlorophyll *a* concentration: $GR_{CF} = 0.798\ (\pm 0.182) \cdot CHL - 0.271\ (\pm 0.404)$, ($r^2 = 0.73$, $P < 0.01$, $n = 9$), but not temperature ($P = 0.28$). In contrast, temperature accounted for a significant fraction of the variation in the growth rate of *Gammaproteobacteria*: $GR_{gamma} = 0.212\ (\pm 0.087) \cdot T - 1.005\ (\pm 1.191)$, ($r^2 = 0.46$, $P = 0.045$, $n = 9$), whereas the stepwise procedure did not select chlorophyll *a* concentration as a significant predictor ($P = 0.11$).

Gammaproteobacteria differed from that of *Alphaproteobacteria* (Fig. 2). Concentrations of chlorophyll *a* alone accounted for a substantial fraction (72%) of growth rate variation in the *Cytophaga-Flavobacter* cluster, whereas temperature was the only variable that predicted the growth rates of *Gammaproteobacteria*. These data support the notion that distinct controls of bacterial growth rates are discernible among different bacterial phylogenetic groups in coastal marine waters.

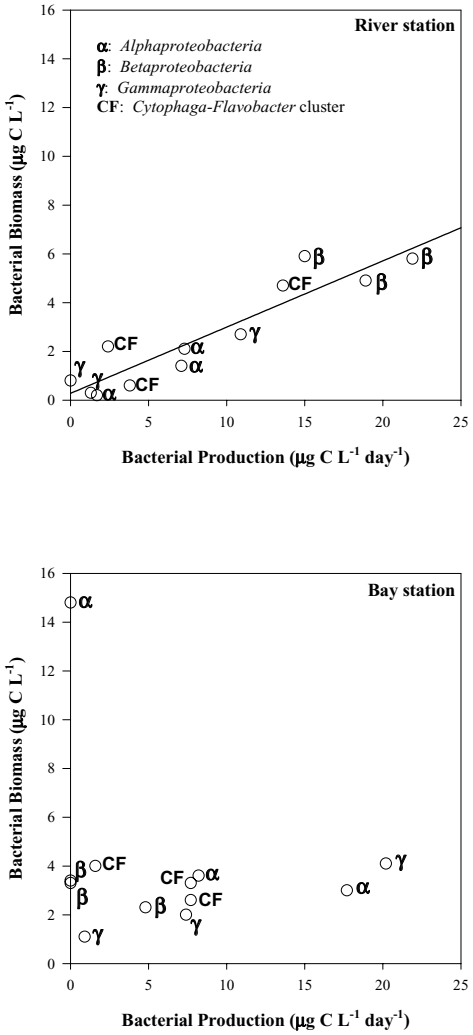

Fig. 3. Relationship between biomass and bacterioplankton production at the River Station (freshwater) and Bay Station (seawater; PSU 26.5–29.8) in the Delaware Estuary (from Yokokawa *et al*., 2004). Individual plots represent the data for individual phylogenetic groups. Data collected in each month were pooled. The linear regression equation for data collected at the River Station is: Biomass = 0.27 (±0.03) × Production + 0.28 (±0.39), $n = 12$, $r^2 = 0.86$, $P < 0.001$ (±standard error). Pearson's correlation between biomass and production was not significant ($P = 0.49$) for the Bay Station data.

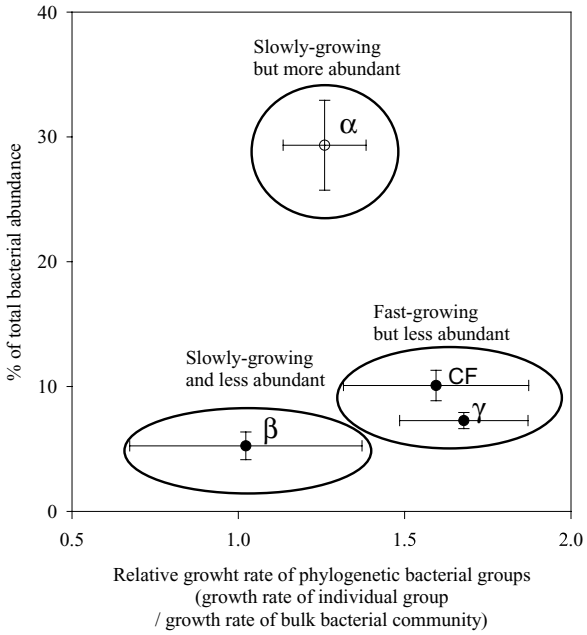

Fig. 4. Relationship between the relative abundance and growth of individual phylogenetic bacterial groups (growth rate of an individual group divided by the growth rate of the bulk bacterial community) in coastal marine environments. Each plot represents the average standard error for the following phylogenetic bacterial groups: *Alphaproteobacteria* (α), *Betaproteobacteria* (β), *Gammaproteobacteria* (γ), and *Cytophaga-Flavobacter* cluster (CF). All data were obtained from Yokokawa *et al*. (2004) and Yokokawa and Nagata (2005).

7. Role of Mortality Factors in Shaping Bacterial Community Structure

A bacterial group that grows faster than others can be expected to dominate the community, if mortality is the same among different groups. Does this scenario of "bottom-up control" of bacterial communities apply to coastal and estuarine bacterial communities? To address this question, Yokokawa *et al*. (2004) conducted dilution culture experiments to estimate the production rate and biomass of individual phylogenetic groups. They examined the production-to-biomass relationship of various phylogenetic groups in different habitats of the Delaware estuary. If communities are largely bottom-up controlled, biomass may linearly increase with increasing production, although this relationship would become less evident as the influence of other factors increases, including mortality (top-down control; Ducklow, 2000). Yokokawa *et al*. (2004) found that biomass increased with increasing production in the freshwater region, but varied little with production in the seawater region (Fig. 3). They suggested that bacterial communities at the freshwater site were more tightly controlled by substrate supply (bottom-up factor), whereas those at the seawater site in the same estuary were relatively strongly controlled by mortality (top-down factor).

Consistent with the above notion, other studies have also suggested the importance of top-down control in shaping bacterial community structure in coastal marine environments. Bacterial phylogenetic groups that exhibit the highest growth rates do not always dominate coastal

marine communities (Zubkov *et al*., 2001; Cottrell and Kirchman, 2004; Alonso-Saez and Gasol, 2007). Indeed, the compilation of data obtained in the Delaware estuary (seawater site; Yokokawa *et al*., 2004) and Otsuchi Bay (Yokokawa and Nagata, 2005) reveals that coastal bacterial communities tend to be dominated by *Alphaproteobacteria* (Fig. 4), although this group generally does not display the highest growth rate; less abundant groups (i.e., *Gammaproteobacteria* and *Cytophaga-Flavobacter* cluster) tend to grow faster than *Alphaproteobacteria* (Fig. 4).

Bacterial mortality in aquatic systems is largely due to protist grazing and viral lysis (Breitbart *et al*., 2008; Jürgens and Massana, 2008). Selective elimination of bacterial subgroups during grazing and lysis can be a fundamental mechanism that explains the top-down control of bacterial community structure. Bacterivorous protists distinguish prey cells on the basis of cell size and morphology (Jürgens and Massana, 2008), although the relationship between bacterial cell traits and phylogenetic affiliations is not well understood. Several studies have examined group-specific responses to grazing pressures in freshwater environments (Pernthaler, 2005). Pernthaler *et al*. (1997) examined the dynamics of individual phylogenetic groups in continuous bacterial cultures. They concluded that *Alphaproteobacteria* outgrew grazing pressure, whereas *Betaproteobacteria* developed inedible filaments with low growth. In the surface waters of Otsuchi Bay, Yokokawa and Nagata (2005) failed to detect group-specific differences in bacterial mortality. The investigators used serial dilution cultures to compare growth and grazing mortality rates among different bacterial groups. Growth rates varied significantly among groups, but no significant differences in grazing mortality rates were found. Thus, grazer discrimination appears to be less pronounced, at least with regard to the major phylogenetic groups in Otsuchi Bay. However, this does not preclude the possibility that grazers selectively eliminate certain bacterial groups that are resolved at finer phylogenetic levels.

Viral lysis has been estimated to account for 10–50% of bacterial mortality in marine coastal environments (Fuhrman, 1999; Breitbart *et al*., 2008). Previous studies have suggested that viruses affect bacterial community structure through the selective elimination of a specific host ("kill-the-winner" hypothesis: Thingstad and Lignell, 1997; Thingstad, 2000). The "kill-the-winner" hypothesis states that more abundant hosts are more susceptible to viral infection because of the increased probability of host–virus encounters. Viral-induced mortality may also depend on the growth rate of host species. In bacteria-virus system cultures, Middelboe (2000) found that burst size increases and latent time decreases with the increasing growth rate of host bacteria. Motegi and Nagata (2007) found that nutrient addition resulted in enhanced viral production in subtropical waters, suggesting that viral production increases with increasing bacterial growth. In experiments conducted in coastal and open waters of the eastern North Pacific, Bouvier and del Giorgio (2007) compared bacterial community structure after incubating seawater cultures for 70 h. Viral abundances were manipulated to be ≤5% of the ambient level. The authors found that the community was dominated by *Alphaproteobacteria* and *Bacteroidetes* in seawater cultures with viruses, which was consistent with community structures in ambient waters. Interestingly, *Betaproteobacteria* and *Actinobacteria* were highly abundant in virus-depleted seawater cultures; these groups were less abundant in the original water samples used in the experiment and were generally less abundant in marine waters (see above). Based on these results, the authors suggested that these "rare" groups (i.e., *Betaproteobacteria* and *Actinobacteria*) in marine waters are in fact competitive in terms of resource exploitation, but their abundances are kept low because of selective elimination by viruses. Although the generality of this finding must be validated by future studies, the data are consistent with the notion that viruses eliminate the host in a growth- or activity-dependent manner.

8. Embedding Bacterial Community Structure into the Carbon Flow Model of Microbial Food Webs

Parameterizing the growth, grazing mortality, and biomass of individual bacterial phylogenetic groups facilitates the construction of microbial food web models that explicitly embed bacterial community structures. Figure 5 shows a preliminary scheme that describes carbon flow within a microbial food web in Otsuchi Bay. The data were obtained from experiments conducted during May 2001 and 2002 (Yokokawa and Nagata, 2005). In this scheme, two major carbon fluxes (DOC–bacteria flux and bacteria–grazer flux) are divided into subfluxes mediated by individual phylogenetic groups of bacteria:

$$F_{DOC \rightarrow bacteria} = \sum(\mu_i \times B_i/GGE_i)$$

$$F_{bacteria \rightarrow grazers} = \sum(m_i \times B_i)$$

where $F_{DOC \rightarrow bacteria}$ and $F_{bacteria \rightarrow grazers}$ represent carbon flow (μg C $L^{-1}day^{-1}$) from DOC to bacteria and from bacteria to grazers, respectively, and μ_i, m_i, B_i, and GGE_i represent growth rate (day^{-1}), grazing mortality rate (day^{-1}), biomass (μg C L^{-1}), and gross growth efficiency (dimensionless), respectively, of the bacterial group. Growth and grazing rates were obtained using dilution cultures (Yokokawa and Nagata, 2005), whereas individual group biomasses were estimated from the cell sizes of individual groups based on the assumption that the cell

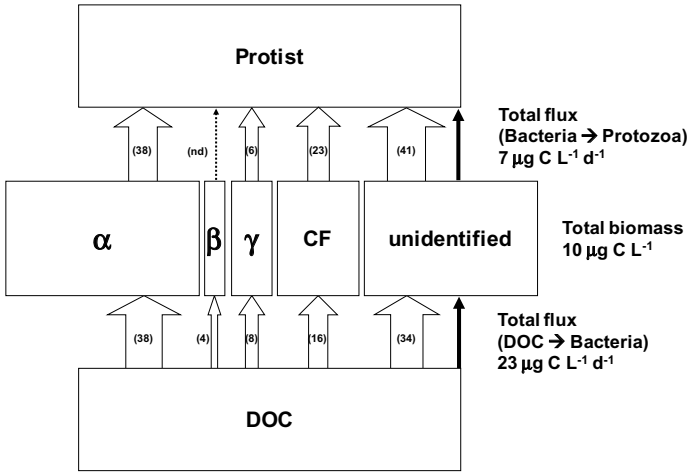

Fig. 5. Bacterial community structure and microbial trophic links in Otsuchi Bay (data obtained from Yokokawa and Nagata, 2005). *Alphaproteobacteria* (α), *Betaproteobacteria* (β), *Gammaproteobacteria* (γ), and the *Cytophaga-Flavobacter* cluster (CF) were examined. "Unidentified" indicates bacteria not detected by the four probes. Values in parentheses indicate the percentage contribution to total fluxes; nd is not determined.

volume to carbon relationship (Norland *et al*., 1987) is identical for all groups. Gross growth efficiency (0.3) was also assumed not to vary among groups. These assumptions stem from the lack of data on group-specific variation in these key factors (i.e., volume to carbon conversion factor and growth efficiency), which should be evaluated in future studies.

A notable feature of the carbon dynamics of the food web model depicted in Fig. 5 is the dominant role of *Alphaproteobacteria*, both as a major DOC consumer and a trophic link that delivers carbon to higher trophic levels. *Alphaproteobacteria* account for 38% of $F_{DOC \rightarrow bacteria}$ (23 μg C $L^{-1}d^{-1}$) and $F_{bacteria \rightarrow grazers}$ (7 μg C $L^{-1}d^{-1}$), suggesting that *Alphaproteobacteria* are not only an abundant bacterioplankton group (Morris *et al*., 2002), but also that they dominate the microbial loop. The second largest contributor to carbon fluxes is the *Cytophaga-Flavobacter* cluster, followed by *Gammaproteobacteria*. *Betaproteobacteria* play a minor role in the microbial food web in Otsuchi Bay. However, a significant fraction of these carbon fluxes (34% of $F_{DOC \rightarrow bacteria}$ and 41% of $F_{bacteria \rightarrow grazers}$) remains to be explained, which reflects the incomplete detection of bacterial cells by the FISH technique used.

We hypothesize that the structure (carbon partitioning) of the microbial loop mediated by multiple groups of bacteria may change, depending on the season and environmental setting. For example, the *Cytophaga-Flavobacter* cluster adapts to degrade polymeric organic matter (Cottrell and Kirchman, 2000), as it is abundant on organic aggregates (Grossart *et al*., 2005). These groups may play more important roles in microbial carbon fluxes during phytoplankton blooms. In future studies, investigation of the factors that affect the relative contributions of different bacterial groups to the major carbon fluxes may help to identify patterns in carbon flow within bacterioplankton communities in marine environments. The key issue is to clarify the characteristic features of growth, mortality, and inorganic and organic resource use of individual bacterial groups. Future studies should also incorporate viruses into the carbon flow model as a major regulator of community structure (Bouvier and del Giorgio, 2007) and carbon flux patterns (Fuhrman, 1999; Miki *et al*., 2008a; Motegi *et al*., 2009).

9. Conclusions and Future Perspectives

Recent studies have clarified the remarkable diversity of marine bacterioplankton, but much remains to be determined regarding the factors that affect bacterial community structure and the role of individual bacterial groups in biogeochemical cycles. Our review has focused on quantitative studies that have examined the growth and grazing parameters of major phylogenetic groups (division and subdivision levels) of bacterioplankton. Growing evidence indicates that growth rates are highly variable among bacterial groups, suggesting that different bacterial groups display different growth responses to bottom-up factors, such as temperature and substrate supply. Clearly, each phylogenetic group may consist of numerous species and ecotypes with different ecological traits (Johnson *et al*., 2006; Fuhrman and Hagström, 2008). Nonetheless, quantitative approaches at the level of the major phylogenetic group appear to have advantages in capturing broad patterns of carbon flows medi-

ated by the prevalent bacterial subgroups. The information obtained by such approaches could also guide further studies on the identification and characterization of the "major players" in carbon fluxes at finer phylogenetic levels, providing clues to clarify which phylogenetic levels it would be most useful to examine in the context of linking carbon cycling to community structure.

This review has presented a preliminary model to depict group-specific carbon flows via the DOM pathway. Although DOM is the major carbon resource for bacteria in surface waters, other modes of carbon flow mediated by carboxydotrophs, methylotrophs, and photoheterotrophs may also play important roles in marine carbon cycles (Béjà and Suzuki, 2008; Moran, 2008). Our model does not explicitly include particle-associated bacteria, but aggregates have been considered "hot spots" of bacteria-mediated material cycling (Simon *et al*., 2002; Nagata, 2008). It is important that these processes be properly incorporated into future models. We emphasize that there is a paucity of data on group-specific mortalities of bacterioplankton communities in marine systems. Despite major progress in analyzing genomic information from marine bacterial communities, quantitative assessment of the dynamics and interactions of individual subgroups of bacterioplankton are still largely constrained by methodological limitations. To achieve the goal of constructing biogeochemical cycle models that embed bacterial community structures, we need a robust modeling framework to facilitate the extraction of essential features of this complex reality, for which novel approaches are now being developed (Follows *et al*., 2007; Miki *et al*., 2008b, 2009). If we are better to understand variations in the biogeochemical state of the oceans, a more comprehensive understanding of the link between carbon fluxes and bacterial community structures is clearly needed.

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