Relative Biomass of Bacteria and Microphytobenthos in Surface Sediments of the Seto Inland Sea of Japan

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Abstract—We examined the community structure and biomass of sediment microorganisms within the Seto Inland Sea of Japan using quinone profiling. Three major quinone species dominated the quinone composition in surface sediments, consisting of plastoquinone-9, ubiquinone-8 and menaquinone-8. This result indicated that benthic microorganisms consisted predominantly of photosynthetic microorganisms, Betaproteobacteria, Gammaproteobacteria, and Deltaproteobacteria. From biomass measurements of sediment bacteria expressed as the respiratory quinone content, sediment bacteria were approximately 5.18 times higher than that of the microphytobenthos, which was expressed by the photosynthetic quinone content. A significant relationship between sediment bacterial biomass and the microphytobenthos biomass was observed, indicating the two may be derived from mutually-shared resource. Thus, studies on primary production of benthic food webs in the Seto Inland Sea and other marine coastal regions should consider the biomass of not only the microphytobenthos but also from sediment bacteria.

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INTRODUCTION

In shallow or coastal areas, the productivity of pelagic and benthic communities is closely related to both the sources and pathways of organic matter (Jahnke et al., 2000; Sarker et al., 2009). The Seto Inland Sea of Japan, which is a representative coastal sea, is well known for high fisheries production (e.g., Takeoka, 2002). Takai et al. (2002) suggested that demersal fish and some benthic animals in the western Seto Inland Sea are more dependent on carbon from benthic primary production, and their data indicated that benthic carbon transport from the 10 to 30 m depth layer is important. Thus, information on the benthic primary production is essential for understanding the spatial and temporal dynamics of higher trophic level production that ultimately lead to fisheries and ecosystem health.

Microphytobenthos and sediment bacteria play an important role in their respective grazing and detritus-based food chains. It is well known that the microphytobenthos provides a large amount of organic carbon to coastal shallow water ecosystems (e.g., MacIntyre et al., 1996). Also, sediment bacterial communities play a significant role in the decomposition of sinking organic matter from the water column, transport of dissolved inorganic matter to phytobenthos and phytoplankton (Blackburn, 1988; Alongi, 1994), and an integral component of benthic food web structure (Moriarty et al., 1985; Findlay et al., 1990). Quantifying the flow of organic matter and energy through benthic microorganisms requires measurement of microbial biomass, and their growth efficiency and production rate. However, accurate measurement of their respective biomass in sediments has proven to be problematic. Most studies regarding to microbial community in sediments have focused upon a single functional group of bacteria such as sulfate-reducing bacteria and nitrifying bacteria, however, studies attempting to clarify transport of sediment organic matter through benthic microorganisms should consider the full microbial community rather than one specific group.

We focused on the use of microbial quinones as microbial biomarkers for quantitative determination of microbial phylogenetic groups. Microbial quinones, which are one of the coenzymes in the electron transport chain of microbial cells, are divided into two groups, respiratory quinone (including ubiquinone and menaquinone) and photosynthetic quinone. In general, one species or genus of bacteria has only one dominant species of respiratory quinone, with microalgae and cyanobacteria having photosynthetic quinones (Hedrick and White, 1986; Hiraishi, 1999). There is a linear relationship between the respiratory and photosynthetic quinone contents and their respective bacterial biomass and photosynthetic microorganism biomass (Saitou et al., 1999; Hiraishi et al., 2003). Hiraishi et al. (2003) reported that one nmol of the total quinone was estimated to be equivalent to $2.5 \times 10^9$ cells which on average included both
bacteria and cyanobacteria. The quinone profile, which is usually defined as the mole fraction of each quinone species, expresses the microbial community structure. Therefore the quantitative analysis of sediment quinone content provides an estimation of the relative biomass of the sediment bacterial community and microphytobenthos.

In this study, we examined the community structure and biomass of sediment microorganisms within the Seto Inland Sea of Japan using quinone profiling. We discuss the dominant phylogenetic groups of sediment microbial community, and the relative biomass of sediment bacteria and microphytobenthos within the Seto Inland Sea.

MATERIALS AND METHODS

Sampling of the sediment

Sampling sites from bays and embayments (nadas) in the Seto Inland Sea of Japan are indicated in Fig. 1. Sediment samples were collected using a Smith-McIntyre Grab and then subsampled by collecting the surface sediment samples up to 2 cm in depth during late summer (from late September to early October, 2008) and up to 1 cm in depth late spring (early May to early June, 2009). One exception was sampling from Stn BP (Fig. 1), in which sediment samples were collected using an Ekman-Berge grab sampler (20 cm × 20 cm). The surface sediment samples were used for determination of median particle diameter, total organic carbon (TOC) and quinone content.

Physico-chemical analysis of the sediment

For determination of median particle diameter, grain size analysis was performed using the dry-sieve technique. For determination of TOC, the sediment
was dried in a drying oven overnight at 60°C, treated with 2N HCl to remove inorganic carbonate, and vacuum-dried. The TOC content of the sediment was measured using an elemental analyzer (NA-1500, Fisons). Sediment samples for analysis of quinone content were stored in a freezer at –20°C until used.

Analysis of quinone content of the sediment

The microbial quinone content of the sediment was determined using a modified method as previously described by Kunihiro et al. (2008, 2011). The types and concentrations of each quinone were determined using a HPLC equipped with an ODS column (Eclipse Plus C18, 3.0 (I.D.) × 150 mm, pore size 3.5 µm, Agilent technologies) and a photodiode array detector (SPD-M20A, Shimadzu). A mixture of 18% isopropyl ether in methanol was used as the mobile phase at a flow rate of 0.5 mL/min. The column oven temperature was maintained at 35°C.

In this study, we refer to the different quinones with the following abbreviations: ubiquinone - UQ-n; menaquinone - MK-n; plastoquinone - PQ-n; and phylloquinone (vitamin K1) - VK1. The number (n) indicates that of the isoprene unit in the side chain of the quinone. Partially hydrogenated MKs were expressed as MK-n(Hx), where x indicates the number of hydrogen atoms saturating the side chain.

A dissimilarity index (D) based on the quinone profiling data was calculated using the following equation (Hiraishi et al., 1991).

\[ D(i,j) = \frac{1}{2} \sum_{k=1}^{n} |f_{ki} - f_{kj}| \]

where \( f_{ki} \) and \( f_{kj} \) are the mole fractions of the k quinone component in the i and j samples, respectively.
Statistical analysis

Analysis with Pearson’s correlation coefficient was performed using the statistical program PASW Statistics for Windows version 18J (IBM Japan).

RESULTS

Microbial quinone compositions

Mean water depth and mean median particle diameter of surface sediments varied between sampling sites (Table 1). Figure 2 shows the microbial quinone composition from surface sediments. Numbers of quinone species observed were from 12 to 21 (avg. 17.7). At Stn S18, the numbers of quinone species were lowest (14 and 12 in summer of 2008 and spring of 2009, respectively) among the samples. The top three dominant quinone species in the surface sediments in order of their mole fraction shows PQ-9 ≥ UQ-8 > MK-8. These three major quinone species accounted for 18.9 to 64.8% (avg. 48.2%) of the total quinone composition in the sediments.

PQ-9, which is contained in photosynthetic microorganisms such as microalgae and cyanobacteria, occupied 7.8 to 54.5% (avg. 19.9%) of the total quinone content in the surface sediment. At Stn BP, PQ-9 contents expressed highest values (43.6 and 54.5% in summer of 2008 and spring of 2009, respectively)
among these samples. UQ-8, which is contained in the members of the class *Gammaproteobacteria* and *Betaproteobacteria*, predominated in the mole fraction of quinone in the sediment. It occupied 4.9 to 26.1% (avg. 15.8%) of the quinone composition of the surface sediment. MK-8, which is contained in members of the class *Deltaproteobacteria* occupied 3.2 to 22.6% (avg. 13.7%) of the quinone composition of the sediment.

To quantitatively evaluate the differences of the microbial community between sampling seasons, we calculated a dissimilarity index using quinone profiles. From a coastal area in Japan, over three years the highest values from the dissimilarity matrix data were 0.29 \((n = 27)\) (Kunihiro *et al.*, data not shown; partly including Kunihiro *et al.*, 2008 and 2011). Dissimilarity values \(\leq 0.29\) suggests some degree of annual variation in the microbial community. Dissimilarity indexes of quinone composition between summer of 2008 and spring of 2009 at Stn S3, S7, S9, S14, S18, S20, S22, and BP were 0.06, 0.14, 0.16, 0.12, 0.36, 0.18, 0.11, and 0.18, respectively. Only the value at Stn S18 failed to show annual variation and therefore change in the microbial community.

**Respiratory quinone contents and photosynthetic quinone contents of surface sediments**

The respiratory and photosynthetic quinone contents in the sediments are shown in Fig. 3. Respiratory and photosynthetic quinone contents in the sediments fluctuated between 0.02 and 5.78 nmol/g-dry sediment and between 0 and 5.79 nmol/g-dry sediment, respectively. At Stn BP, the highest values among the samples were observed in the respiratory quinone contents, 5.78 and 3.85 nmol/g-dry sediment, and in the photosynthetic quinone contents, 5.79 and 4.61 nmol/g-dry sediment.
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**DISCUSSION**

**Dominant microbial community in the surface sediment**

The quinone mole fraction in sediments within the Seto Inland Sea were dominated by PQ-9, UQ-8 and MK-8 (Fig. 2). This result indicated that the surface sediments within the Seto Inland Sea were dominated by photosynthetic microorganisms, Betaproteobacteria, Gammaproteobacteria, and Deltaproteobacteria. We inferred that the PQ-9 originated from microphytobenthos and partial deposition of cyanobacteria or other eukaryal phytoplankton. Urakawa et al. (1999) and Wang et al. (2010) also reported that UQ-8-containing bacteria were predominant in the microbial community structures.
of sediments. UQ-8 has been observed in mainly the class *Gammaproteobacteria* and the class *Betaproteobacteria* within the marine environment. Sulfur-oxidizing bacteria and nitrifying bacteria such as *Beggiatoa* spp. and *Nitrobacter* spp. (Collins and Jones, 1981; Yokota et al., 1992; Yun et al., 2004) also possess UQ-8. MK-8 has been detected in some within the class *Deltaproteobacteria* such as *Desulfuromonas* spp., *Pelobacter* spp., and *Myxococcus* spp. (Yokota et al., 1992; Gray and Herwig, 1996; Li et al., 2009). Members of *Desulfuromonadales* can obtain energy for anaerobic respiration utilizing a variety of compounds as electron acceptors such as sulfate and nitrate (Smith et al., 2007). These facts suggest that photosynthetic and chemoheterotrophic microorganisms were dominant in the sediment microbial community within the Seto Inland Sea.

**Relative biomass of sediment bacteria and microphytobenthos**

The respiratory quinone contents in the sediments were approximately 5.18 ± 2.57 (mean ± SD) times higher than the photosynthetic quinone contents, indicating that the bacterial biomass was 5.18 times higher than the microphytobenthos biomass in the surface sediment within the Seto Inland Sea. This result suggests that the bacterial biomass in the sediment contributed a significant fraction to the benthic production.

The bacterial biomass displayed a linear relationship with organic matter contents in the sediment (Fig. 4a), suggesting that sediment bacterial biomass is dependent on the sediment organic matter content, and that they accounted for a constant ratio in the organic matter content of the sediments from bays and embayments (nadas) in our study. In addition, changes in almost all microbial community structures in the sediment in this study fall within annual fluctuations of change in the microbial community. Resource availability of bacteria plays a key role in their structure and abundance (Fischer et al., 2002; Luna et al., 2004). Thus, from sites of this study, it is possible that organic matter qualities of loading from the water column were similar.

The sediment bacterial biomass also displayed a linear relationship with the microphytobenthos biomass (Fig. 4b). It is well known that heterotrophic bacteria utilize extracellular material from the microphytobenthos, and in-turn the microphytobenthos utilize dissolved inorganic matter mineralized by the sediment bacteria. Thus, our findings indicated a close interaction between surface sediment bacterial and microphytobenthos biomass within the Seto Inland Sea. Further research is needed to assess the production rates of bacteria and microphytobenthos and to clarify the material transport from microorganisms to higher trophic organisms.

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