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ORIGINAL ARTICLE

Increase in *Alphaproteobacteria* in association with a polychaete, *Capitella* sp. I, in the organically enriched sediment

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We conducted bioremediation experiments on the organically enriched sediment on the sea floor just below a fish farm, introducing artificially mass-cultured colonies of deposit-feeding polychaete, *Capitella* sp. I. To clarify the association between the *Capitella* and bacteria on the efficient decomposition of the organic matter in the sediment in the experiments, we tried to identify the bacteria that increased in the microbial community in the sediment with dense patches of the *Capitella*. The relationship between TOC and quinone content of the sediment as an indicator of the bacterial abundance was not clear, while a significant positive correlation was found between *Capitella* biomass and quinone content of the sediment. In particular, ubiquinone-10, which is present in members of the class *Alphaproteobacteria*, increased in the sediment with dense patches of the *Capitella*. We performed denaturing gradient gel electrophoresis (DGGE) analyses to identify the alphaproteobacterial species in the sediment with dense patches of the worm, using two DGGE fragments obtained from the sediment samples and one fragment from the worm body. The sequences of these DGGE fragments were closely related to the specific members of the *Roseobacter* clade. In the associated system with the *Capitella* and the bacteria in the organically enriched sediment, the decomposition of the organic matter may proceed rapidly. It is very likely that the *Capitella* works as a promoter of bacteria in the organically enriched sediment, and feeds the increased bacteria as one of the main foods, while the bacteria decompose the organic matter in the sediment with the assistance of the *Capitella*.

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Introduction

On the sea floor with muddy sediment, deposit-feeding animals tend to dominate in the benthic community (Sanders, 1958; Levinton, 1972; Lopez and Levinton, 1987). They move actively on the sediment surface or burrow into its subsurface layers, feeding and excreting the sedimentary organic and inorganic materials (Fauchald and Jumars, 1979; Lopez and Levinton, 1987). These biological activities have an enormous impact, not only on the reworking of the sediment, but also on the abundance and community structure of microorganisms in the sediment (Dobbs and Guckert, 1988; Steward *et al.*, 1996; Marinelli *et al.*, 2002; Lucas *et al.*, 2003; Papaspyrou *et al.*, 2006). Microbial abundance tends to increase along the inner walls of the burrows (Alongi, 1985; Aller and Aller, 1986; Lucas *et al.*, 2003; Wu *et al.*, 2003; Wada *et al.*, 2006) and around the feces excreted from the animals (Hargrave, 1976; Plante and Wilde, 2001). Various bacteria actively facilitate the decomposition of the organic matter at these sites (Aller *et al.*, 1983; Reichardt, 1988).

Bacteria in the sediment react immediately to fresh and labile organic matter deposited on the sediment (Meyer-Reil, 1983; Gooday and Turley, 1990). Excessive loading of organic matter on the sediment tends to consume an increasing amount of dissolved oxygen in the overlying and interstitial

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water of the sediment, and results in oxygen depletion in the bottom water (Pearson and Rosenberg, 1978; Holmer and Kristensen, 1992). As a result, anaerobic decomposition of the organic matter by sulfate-reducing bacteria often follows production of high levels of hydrogen sulfide in the sediment (Holmer and Kristensen, 1992). As the organic enrichment of the sediment increases, it restricts the occurrence of macro-benthic animals due to the development of reduced conditions. In heavily organically enriched sediments, exclusively several species of deposit-feeding thread-like small polychaetes, such as *Capitella* species and spionids occur exclusively, monopolizing the food resources contained in the sediment (Grassle and Grassle, 1976; Tsutsumi and Kikuchi, 1983; Tsutsumi, 1987, 1990). Therefore, organically enriched sediment appears to be one of the most suitable sites for observing the interaction and association between the deposit-feeding polychaetes and bacteria.

Fish farming has developed in enclosed coastal seas throughout the world since the 1980s (FAO, 1992). However, nowadays, enormous quantities of fish feces and food residues have deposited on the sea floors just below the net pens in many of the fish farms. They are suffering from the deterioration of water quality, due to accelerated consumption of dissolved oxygen from the organically enriched sediment (Gowen and Bradbury, 1987; Wu, 1995; Naylor *et al.*, 2000; Buschmann *et al.*, 2006). On the other hand, to reveal the interaction and association between the deposit-feeding polychaetes and bacteria, the sea floors just below the net pens in the fish farms provide one of the most ideal and accessible sites. There, the *Capitella* species tend to predominate in the macro-benthic communities (Tsutsumi, 1987, 1995; Weston, 1990; Karakassis and Hatziyanni, 2000; Macleod *et al.*, 2007). Tsutsumi and Montani (1993) focused on the rapid increase of *Capitella* species in the organically enriched sediment just below the fish farm during the cold seasons with dissolved oxygen-rich conditions, and its impact on the decomposition of organic matter in the sediment. They proposed a bioremediation technique to treat the organically enriched sediment that placed artificially mass-cultured colonies of *Capitella* sp. I on it in autumn, when dissolved oxygen conditions of the bottom water had recovered.

Based on this idea mentioned above, we have conducted bioremediation experiments to treat the organically enriched sediment deposited just below a fish farm in Amakusa, Kyushu, Western Japan, between 2003 and 2006, introducing the artificially mass-cultured colonies of *Capitella* sp. I on the sea floor in autumn. In the bioremediation experiments, the introduced *Capitella* colonies increased explosively on the organically enriched sediment during the late autumn and winter, and the amount of sedimentary organic matter decreased significantly following the rapid population growth (Tsutsumi *et al.*, 2005a; Kinoshita *et al.*, 2008). Kunihiro *et al.* (2008) found a significant positive correlation between *Capitella* biomass and the quinone content of the sediment as an indicator of the biomass of microorganisms (Saitou *et al.*, 1999; Hiraishi *et al.*, 2003), and a marked increase of ubiquinone-10 (UQ-10) among the quinones when dense patches of the worms were established in the organically enriched sediment. Since UQ-10 is one of the co-enzymes used for aerobic respiration in the respiratory chain by the members of the class *Alphaproteobacteria* (Collins and Jones, 1981), it is very likely that the efficient decomposition of organic matter of the organically enriched sediment was not realized in the bioremediation experiments by the single effect of the feeding activity of *Capitella*, but was caused by the associated activities of *Capitella* with the microorganisms in the sediment (Chareonpanich *et al.*, 1993; Tsutsumi *et al.*, 2002; Kunihiro *et al.*, 2008; Wada *et al.*, 2008). Various biological activities of *Capitella* in the sediment, including burrowing into the sediment, spouting the subsurface sediment on the sediment surface, feeding the subsurface sediment and excreting fecal pellets on the sediment (Tsutsumi *et al.*, 2005b), may act to a create more oxidized environment in the organically enriched sediment that is suitable for increase of aerobic bacteria (Wu *et al.*, 2003; Wada *et al.*, 2005).

This study aims at clarifying the interaction and association between *Capitella* sp. I and bacteria on the decomposition of organic matter in the organically enriched sediment. In the bioremediation experiments on the organically enriched sediment with mass-cultured colonies of *Capitella*, which were conducted in the fish farm in Amakusa, Japan, between June in 2004 and July in 2006, we collected the sediment from the sea floor just below the net pens of the fish farm monthly, monitored the changes of the microbial community to confirm the increase in the members of the class *Alphaproteobacteria* in the sediment with dense patches of *Capitella* colonies, using the quinone profiling method and the polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) technique, and tried to identify the *Alphaproteobacterial* species related with dense patches of *Capitella* colonies by PCR-DGGE of partial 16S rRNA genes. We discuss the ecological implications of the increase in the members of the class *Alphaproteobacteria* with the dense patches of *Capitella* colonies in the organically enriched sediment.

Materials and methods

Study area

Kusuura Bay is an enclosed bay (11.4 km^2 area, 4×3.5 km^2), located between Amakusa Kamishima and Amakusa Shimoshima Islands in Kyushu,

Western Japan (130° 13′E, 32° 23′N). The water depth is approximately 16–20 m at the center of the bay. In 1973, fish farms were established in an area of approximately 400 m in length and 200 m in width in the eastern part of the bay. At the time of this study, 102 net pens 12 m square and 8 m deep were established as fish farms, with approximately 5–20 tons of red sea bream, *Pagrus major*, and yellowtail, *Seriola quinqueradiata*, being cultured in each of the pens.

We set two sampling stations, Stn F beside a fish-farm net pen, and Stn C approximately 400 m away from the fish farms. The mud content of the sediment at Stn F and Stn C was approximately 10% and 60%, respectively. At Stn F, the total amount of reared fish in the net pens changed markedly from month to month (approximately 10–20 tons per net pen) because of their growth and shipping. The total amount of organic matter expended as food for the reared fish per month fluctuated between 0.28 and 2.48 ton-C per month from June 2004 to July 2006 (mean 0.93 ton-C per month), except February 2006 (0.01 ton-C per month). The feeding amount was adjusted to the physiological conditions of the reared fish. Mass-cultured colonies of *Capitella* sp. I were introduced on the organically enriched sediment just below the net pen ($12 \times 12\,m^2$) at Stn F on 5 November 2004 (approximately 9.27 million individuals) and 9 November 2005 (approximately 2.19 million individuals), respectively (Tsutsumi *et al.*, 2005a; Kinoshita *et al.*, 2008).

Sampling of the sediment

We conducted monthly sampling of sediments between June 2004 and July 2006. At Stn F, four sediment core samples were collected with acrylic hand core samplers (45 mm in diameter) by divers from each of five different points just below the four corners and the center of the net pen. One of these four core samples collected in each sampling point was used for determination of density and biomass of the *Capitella* population, and another was used for determination of sediment density, redox potential (ORP), TOC, quinone content and identification of bacteria with DGGE analysis. At Stn C, six sediment core samples were collected in the same manner as Stn F. Three of them were used for the determination of sediment density, ORP and quinone content and identification of bacteria. The remaining three samples were used for the determination of the density of sediment. We also collected a sediment core sample for the determination of TOC with a KK type core sampler (40 mm in diameter), and three grab samples using an Eckman–Birge grab sampler ($20 \times 20\,cm^2$) from a boat. We subsampled 10 sediment samples from each with a core sampler ($5 \times 5 \times 5\,cm^3$) for the quantitative analysis of *Capitella* sp. I.

To identify the alphaproteobacterial species occurring around the body of *Capitella*, one sediment core sample was collected by divers at the center of the net pen at Stn F on 14 December 2006. We collected 10 individuals of live worms from the sediment core sample under a stereoscopic microscope, and kept them in a petri dish with sterilized artificial seawater for several hours. After we confirmed that the worms had excreted feces, we put them in a 1.5-ml tube. The tube with the worms was stored in a freezer at −20 °C until used for DGGE analysis.

Quantitative analysis of Capitella and physico-chemical analysis of the sediment

For determination of the density and biomass of *Capitella* population, the sediment core samples were fixed, and stained in 10% formalin solution with a dye, Rose Bengal. The procedures of the treatment followed Kinoshita *et al.* (2008).

The sediment samples for determination of sediment density, ORP, TOC and quinone content, and DGGE of the sediment were sliced into two layers, the surface layer (up to a depth of 2 cm) and the subsurface layer (from 2 to 4 cm in depth). For measurement of sediment density, the sliced sediment samples in each layer were dried at 110 °C overnight. The sediment density was then calculated by dividing the dry weight by the volume ($33\,cm^3$). ORP of the sediment in each layer was determined with a platinum electrode (RM-20P, TOA DKK). TOC of sediment was determined following Kinoshita *et al.* (2008). We calculated the TOC content of the surface and subsurface layers of the sediment, and expressed as TOC $g\,m^{-2}$, using the sediment density data. At Stn F, ORP and TOC of the sediment were represented by the mean values of the sediment samples collected at five different sites just below the four corners and center of the net pen. At Stn C, they were represented by the mean values of three replicates of the sediment sample.

The remaining sediment samples after determination of ORP and TOC of the sediment were stored in a freezer at −20 °C until used for analysis of quinone content and DGGE. The surface sediment sample collected at Stn F on 8 March 2006 was used for isolating bacteria belonging to the *Alphaproteobacteria*.

Analysis of quinone content of the sediment

The microbial quinone content of the sediment was determined following the procedures adopted by Kunihiro *et al.* (2005, 2008). In this study, we refer to the 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. For example, UQ-10 represents a ubiquinone with 10 isoprenoid units. Partially hydrogenated MKs were expressed as

Table 1 Major marine sediment bacteria and their dominant quinone species

Bacterial division	*Quinone species*[a]	*Marine sediment bacteria*[b]
Cyanobacteria	PQ, VK1	
Alphaproteobacteria	UQ-10	*Azospirillum*, *Beijerinckia*, *Bradyrhizobium*, *Nitrobacter*, *Rhodomicrobium*, *Rhodospirillum*, *Roseobacter*, *Sphingomonas*
Betaproteobacteria	UQ-8	*Nitrosomonas*, *Nitrospira*
Gammaproteobacteria	UQ-8, UQ-9	
	UQ-8	*Beggiatoa*, *Chromatium*, *Ectothiorhodospira*, *Marinomonas*, *Proteus*, *Pseudoalteromonas*, *Pseudomonas*, *Rhodocyclus*, *Shewanella*, *Vibrio*, *Xanthomonas*
Deltaproteobacteria	MK-*n* ($n \leqslant 8$)	
	MK-6	*Desulfomonas*, *Desulfovibrio*
	MK-7	*Desulfobacter*, *Desulfococcus*, *Desulfosarcina*
	MK-8	*Desulfuromonas*, *Myxococcus*
Epsilonproteobacteria	MK-6, MK-7	
	MK-6	*Campylobacter*
Actinobacteria	MK-*n* ($n \geqslant 9$), MK-*n*(Hx)	
		Arthrobacter, *Atopobium*, *Clavibacter*, *Corynebacterium*, *Microbacterium*, *Streptomyces*
Bacteroidetes	MK-*n* ($n \leqslant 8$)	
Flavobacteria	MK-7	*Flavobacterium*
Sphingobacteria	MK-6	*Cytophaga*

Abbreviations: MK, menaquinone; PQ, plastoquinone; UQ, ubiquinone; VK1, vitamin K1.
[a]Dominant quinone species that exist in the main species of the bacterial genus, from the WFCC-MIRCEN World Data Centre for Microorganisms (WDCM) database (1995).
[b]The data were obtained from the references (Akagawa-Matsushita *et al*., 1992; James and Russell, 1996; Urakawa *et al*., 1999; Bissett *et al*., 2006).

MK-*n*(H_x), where *x* indicates the number of hydrogen atoms saturating the side chain. For example, MK-7(H_2) represents an MK with seven isoprenoid units and one of the seven units is hydrogenated with two hydrogen atoms. In general, one species or genus of bacteria has only one dominant species of respiratory quinone, ubiquinone and MK (Hedrick and White, 1986; Hiraishi, 1999). Table 1 summarizes the correspondence between the phylogenetic assignment of bacteria and dominant quinone species.

UQ and MK are key components of the respiratory chain used for aerobic/nitrate and anaerobic/aerobic respirations, respectively (Collins and Jones, 1981; Hedrick and White, 1986; Hiraishi, 1999). The composition ratio of each group in the total quinone content may reflect the energy metabolic characteristics of the microbial community. Therefore, the analysis of UQ/MK ratio can be used to elucidate the respiratory status of the microbial community in the environment (Hedrick and White, 1986).

DNA extraction and PCR amplification

For DNA extraction from the sediment core samples and the sample of living worm bodies, we adopted a modified method based on Isoil kit (Nippon gene, Tokyo, Japan). A total of 0.5 g-wet sediment was put in a 1.5-ml tube prior to the DNA extraction. Samples were centrifuged (8000 × *g* for 10 min) three times with 1 ml of PBS (8 g of NaCl, 0.2 g of KCl, 2.88 g of Na_2HPO_4 and 0.48 g of KH_2PO_4, pH 7.4), and pelletized by centrifugation (8000 × *g* for 3 min). Each pellet was re-suspended in 665 μl of Lysis solution HE and 35 μl of Lysis solution 20S (supplied in the kit), and incubated at 65 °C for 1 h. The sample was frozen at −80 °C for 1 h, and thawed at 65 °C for 15 min. The freeze-thaw cycle was repeated three times to increase DNA extraction efficiency. The following precipitation steps were performed according to the manufacturer's instruction (Nippon gene). Obtained DNA pellets were dried under vacuumed conditions, and re-dissolved in 100 μl of TE buffer (10 mM Tris–HCl, 1 mM EDTA-2Na, pH 8.0). Absorbency of the DNA solution was measured at A_{260} with an ultraviolet–visible spectrophotometer (UV-2450(PC)S, Shimadzu, Kyoto, Japan) to determine the DNA concentration. Final extractions were stored at −20 °C until used for PCR amplification.

Approximately 50 ng of DNA extract was amplified by PCR with a Zymoreactor II AB1820 (ATTO, Tokyo, Japan). PCR amplification targeting bacterial 16S rDNA was performed with the GC-341f (5′- CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC G-CC TAC GGG AGG CAG CAG-3′) and 907r (5′-CCG TCA ATT CCT TT[A/G] AGT TT-3′) (Muyzer *et al*., 1993). The PCR mixture contained 10 μl of 10 × PCR buffer, 200 μM of each dNTP, 3.5 mM of $MgCl_2$, 100 μg ml^{-1} of bovine serum albumin (Roche Applied Science Biochemicals, Tokyo, Japan), 0.25 μM of each primer, 2.5 U AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA) and sterile MilliQ water, for a final volume of 100 μl. The PCR amplification was performed for 35 cycles in a touchdown thermocycling program as follows: after initial denaturation for 9 min at 94 °C, each cycle consisted of denaturation at 94 °C for 1 min, primer annealing at the annealing temperature (T_A) for 1 min and primer extension at 72 °C for 1 min. In the first 15 cycles, T_A was decreased by 2 °C

every 3 cycles, from 65 °C to 55 °C. In the last 20 cycles, T_A was 55 °C. Cycling was followed by a final primer extension at 72 °C for 10 min.

DGGE analysis and sequencing analysis of DGGE bands

The DGGE analysis was performed with a D-code universal mutation detection system (Bio-Rad Laboratories, Hercules, CA, USA). About 500 ng of the amplified PCR product (approximately 25 μl of the PCR product) was loaded onto an 8% (w/v) acrylamide gel (acrylamide/bis solution, 37.5:1, Sigma-Aldrich Japan, Tokyo, Japan) containing a linear chemical gradient ranging from 20% to 70% denaturant (100% denaturant consisted of 7 M urea and 40% (v/v) formamide). Electrophoresis was run at a constant voltage of 200 V for 6 h at 60 °C in 0.5 × TAE buffer (20 mM Tris–HCl, 20 mM sodium acetate, 0.5 mM EDTA-2Na, pH 8.0). The gels were stained with SYBR Green I (Molecular Probes, Carlsbad, OR, USA) at 10 000 × dilution in MilliQ water for 30 min, and then photographed on a UV transilluminator.

The gel photo was scanned, and the intensity of band peaks (not containing band background) was manually measured using WinROOF (MITANI Co., Fukui, Japan). The DGGE band profiles in each lane were constructed based on the intensities of the band peaks to the total intensity in the lane (Supplementary Table 1).

For the sequence analysis of the DGGE bands, the bands were excised from the gel, placed into sterile 0.5 ml tubes and washed in order to exclude the urea using sterilized water. A small piece of the band served as a template for PCR amplification. The amplified products were subjected to a new DGGE step to confirm their electrophoretic mobility. The PCR products of the sequences obtained in this study were purified with an EXOSAP-IT kit (USB Corp., Santa Clara, OH, USA), sequenced using an ABI Prism BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems), and run on an ABI 3100 Genetic Analyzer (Applied Biosystems). The sequences obtained from the DGGE bands were compared with the Ribosomal Database Project data (release 10.23) with the Seqmatch version 3 to retrieve similar sequences and phylogenetically related species.

Cluster analysis based on the quinone profile or DGGE band profile

To evaluate the changes in the microbial community in the sediment, a dissimilarity index (D) of microbial community was calculated using the following equation (Hiraishi *et al*., 1991).

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

In quinone profiles, f_{ki} and f_{kj} are the mole fractions of the k quinone component for the i and j samples, respectively. In the DGGE band profile, f_{ki} and f_{kj} are the fractions of the k DGGE bands intensity for the i and j samples, respectively. Values $\leqslant 0.1$ of dissimilarity of quinones are not recognized as different quinone profiles (97% statistical reliability; Hu *et al*., 2001). Cluster analysis was performed with the StatPartner program (O-Ha Inc., Tokyo, Japan) based on the distance matrix, and a dendrogram was constructed using the between-groups linkage method.

Isolation of bacteria from the fish-farm sediment

We attempted to isolate alphaproteobacterial species from the surface sediment that was collected at Stn F in 8 March 2006. The samples diluted in a 10-fold series with filtrated natural seawater. A total of 0.1 ml of each dilution were spread on Shioi medium plates (Shioi, 1986), and were subsequently incubated at 20 °C under light conditions for 2 weeks. Pink-colored colonies were picked up from one of these plates, and again grown on Shioi's medium. This purification procedure was repeated several times until we obtained a single strain. The DNA extraction and sequencing of the 16S rDNA of the isolated bacteria were performed as described above.

Phylogenetic analysis and sequence accession numbers

The sequences obtained from the DGGE bands of the alphaproteobacterial species, the isolated bacteria and their similar sequences cited from the Ribosomal Database Project data (release 10.23) were aligned. Phylogenetic trees were constructed by the neighbor-joining method using the ClustalX version 2.1 (Thompson *et al*., 1997) and visualized using NJplot (Perrière and Gouy, 1996).

The 16S rDNA sequences of the DGGE bands (bands 6, 7, 8, CB) and the isolated bacteria (KMUT1, 3–7) have been deposited under DDBJ accession numbers from AB583757 to AB583767 and from AB583769 to AB583773.

Statistical analysis

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

Results

Fluctuations of Capitella population, ORP, TOC and quinone content of the sediment

Figure 1 shows fluctuations of the densities of *Capitella* sp. I and three parameters of the environmental conditions of the sediment including ORP, TOC and quinone content at Stn F (inside the fish farm) and Stn C (approximately 400 m apart from the fish farm). At Stn F, the density of *Capitella* sp. I decreased to extremely low levels of <1500 individuals

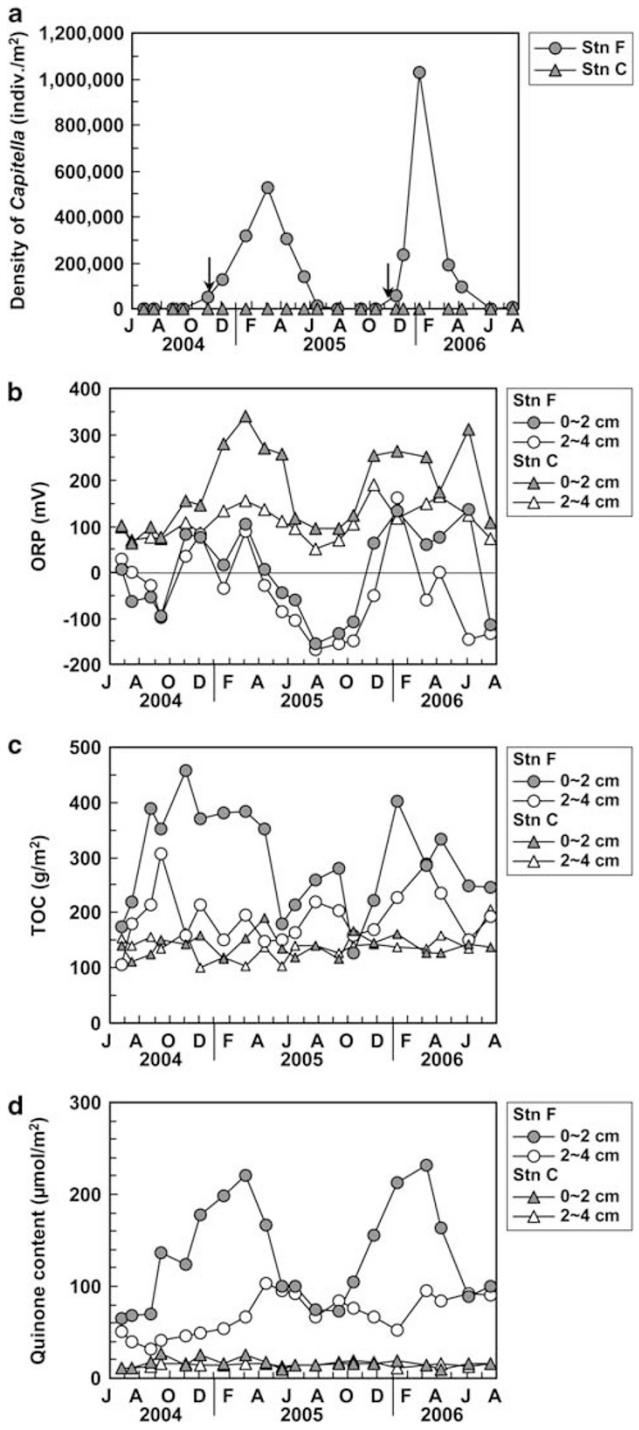

Figure 1 Fluctuations in (**a**) density of *Capitella*, (**b**) ORP, (**c**) TOC and (**d**) quinone content of the surface layer (up to a depth of 2 cm) and the subsurface layer (from 2 cm to 4 cm in depth) of the sediment at Stn F and Stn C. Arrows indicate the release of mass-cultured colonies of *Capitella* sp. I at Stn F. Plots of *Capitella* density and TOC content of the sediment are modified from Kinoshita *et al.* (2008). Plots of ORP and quinone content of the sediment between May 2004 and September 2005 are modified from Kunihiro *et al.* (2008).

per m^2 in the summer, when reduced conditions developed in the organically enriched sediment (−1 to −99 mV between July and September 2004, −44 to −170 mV between May and October 2005 and −115 to −135 mV in July 2006 in the sediment depth of 4 cm; Figure 1b), following the occurrence of hypoxic water in the bottom water (Srithongouthai *et al.*, 2006). The density of *Capitella* sp. I tends to recover naturally during the autumn to winter, when oxygen-rich water is supplied to the bottom layers by the vertical mixing of the water, and consequently oxidized conditions are formed in the surface layer of the sediment (Tsutsumi and Kikuchi, 1983; Tsutsumi, 1987, 1995; Tsutsumi, 1990; Srithongouthai *et al.*, 2006). In this study, ORP of the surface layer of the sediment (up to a depth of 2 cm) at Stn F recovered to +82 to +5 mV between November 2004 and April 2005, and +65 to +135 mV between November 2005 and June 2006. We had attempted to accelerate the recovery of the *Capitella* population by the introduction of mass-cultured colonies and to promote bioremediation of the organically enriched sediment at Stn F since 2003 (Kinoshita *et al.*, 2008; Tsutsumi *et al.*, 2005a), and succeeded to realize an explosive population increase in the autumn to winter of 2004 and 2005. The density of *Capitella* population reached 528 000 individuals per m^2 in March 2005 and 1 028 000 individuals per m^2 in January 2006.

The organic matter content of the sediment is expressed in terms of TOC content of the sediment per square meter, since the water content of the sediment changed markedly in the process of this study. At Stn F, TOC content of the sediment in June was 213 ± 36 g-C m^{-2} (mean ± s.d.) in the surface layer (up to a depth of 2 cm) and 140 ± 31 g-C m^{-2} in the subsurface layer (2–4 cm in depth) during the 3 years between 2004 and 2006. It was only slightly higher than that in the surface layer (133 g-C m^{-2}), and almost equivalent to that in the subsurface layer (143 g-C m^{-2}) at Stn C outside the fish farm, since the sediment at Stn F had been treated with *Capitella* colonies since the cold seasons between the winter in 2003 and early spring in 2004 (Tsutsumi *et al.*, 2005a; Kinoshita *et al.*, 2008). However, TOC content of the surface layer of the sediment increased markedly in the autumn to early winter, and reached 459 g-C m^{-2} by November 2004 and 403 g-C m^{-2} by January 2006, since the organic matter discharged from the net pens, such as feces of reared fish and food residues deposited on the sea floor intensively just below the net pens due to vertical mixing of the water (Tsutsumi *et al.*, 2006). The increase in TOC content of the sediment in the subsurface layer of the sediment was much less distinct than that in the surface layer. We noted a peak 306 g-C m^{-2} in September 2004 and 286 g-C m^{-2} in March 2006. When the sediment was kept relatively oxidic, and dense patches of *Capitella* sp. I of >100 000 individuals per m^2 were formed between the winter and the spring, TOC of the surface layer of the sediment decreased, and returned to the level that was almost equivalent to that in June of the previous year (179 g-C m^{-2} in May

2005 and 248 g-C m^{-2} in June 2006). At Stn C, both layers of the sediment were kept oxidic (+52 to +340 mV), and the TOC contents of the sediment fluctuated in a low range between 101 and 205 g-C m^{-2} throughout the period of this study. Such oxidic conditions of the sediment did not seem to restrict the occurrence of the macro-benthic animals, but *Capitella* sp. I remained at extremely low densities of <320 individuals per m^2 in all seasons. It seems that *Capitella* sp. I does not favor the less organically enriched sediment as a habitat.

Quinone content of the sediment, which indicates the abundance of microorganisms in the sediment, increased markedly in the surface layer at Stn F between the autumn and the spring of the next year, and reached a peak in March (220 μmol m^{-2} in 2005 and 232 μmol m^{-2} in 2006). These two peaks of quinone content in the surface layer of the sediment were delayed from those of TOC content of the sediment approximately for 4 and 2 months, respectively. Figure 2 compares the relationships (a) between TOC and quinone content in the surface layer of the sediment and (b) between biomass of *Capitella* sp. I in the sediment up to a depth of 5 cm and quinone content in the surface layer of the sediment at Stn F. The relationship between TOC and quinone content of the sediment was not clear ($r^2 = 0.267$), while a significant positive correlation was found between biomass of *Capitella* sp. I and quinone content of the sediment (Pearson's correlation coefficient, $r^2 = 0.580$, $P < 0.05$). These results indicate that the abundance of bacteria in the surface layer of the sediment did not change simply depending on the organic matter content of the sediment, but increased in association with *Capitella* colonies in the organically enriched sediment.

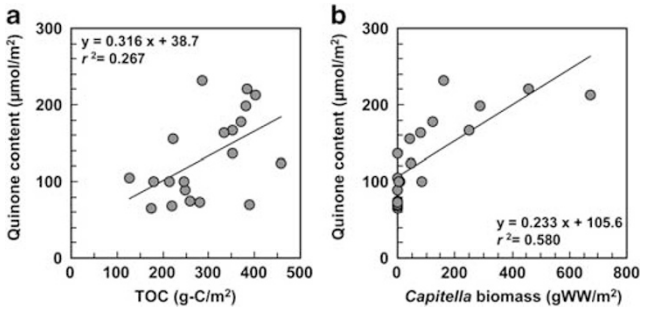

Figure 2 Comparisons (**a**) between TOC and quinone content and (**b**) between *Capitella* biomass and quinone content in the surface layer (from the surface to 2 cm in depth) of the sediment at Stn F.

Changes in the quinone profile of the surface sediment

Figure 3 shows changes in the quinone profiles of the surface layer of the sediment at Stn F and Stn C. At Stn F, UQ-10 predominated in the mole fraction of quinone in the sediment. It occupied 30.5–47.4% in the quinone composition of the sediment throughout the period of this study, and tended to increase from the autumn to winter (47.4% in December 2004 and 42.7% in January 2006), when dense patches of *Capitella* sp. I were established. UQ-8, which is present in the members of the class *Gammaproteobacteria*, fluctuated in the second largest ratio (10.4–17.4%) in the quinone composition of the sediment. MK-7, which is present in the members of the class *Deltaproteobacteria* and class *Flavobacteria*, occupied 5.0–8.7% of the quinone composition of the sediment. The partially hydrogenated MKs, which is present in the members of the phylum *Actinobacteria*, accounted for 2.6–12.3% of the quinone composition of the sediment throughout the period of this study. The photosynthetic quinones (PQ and vitamin K1), which are present in photosynthetic microorganisms, such as

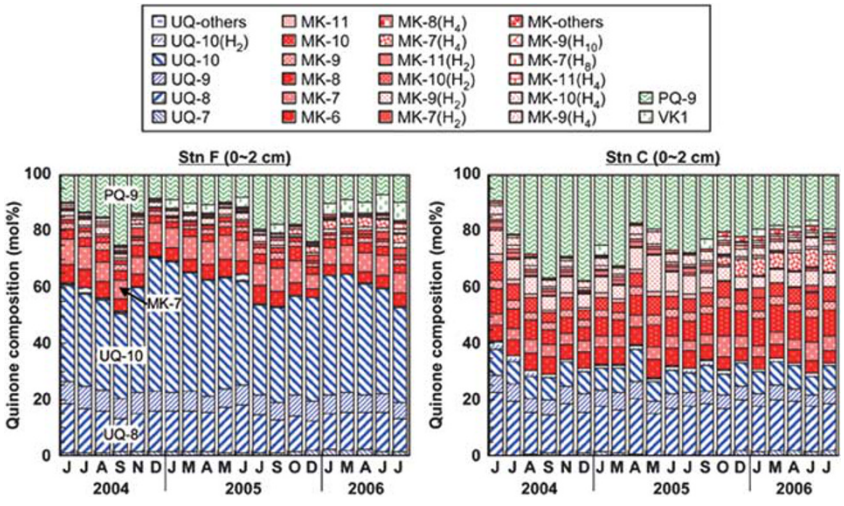

Figure 3 Changes in the mole fractions of quinone of the surface layer (up to a depth of 2 cm) and the subsurface layer (from 2 cm to 4 cm in depth) of the sediment. Plots between May 2004 and September 2005 are modified from Kunihiro *et al.* (2008).

micro-algae and cyanobacteria, fluctuated from 8.3% to 25.3% of the total quinone content of the sediment throughout the period of this study, and tended to increase in the warm seasons. We inferred that the photosynthetic quinones were derived from benthic algae or phytoplankton deposited on the sediment. At Stn C, the photosynthetic quinones predominated in the quinone composition in the surface layer of the sediment throughout the period of this study (9.4–37.3%). The composition of UQ-8 of the sediment fluctuated in a similar range, 13.6–22.4%, with that at Stn F, while the composition of UQ-10 of the sediment did not increase in the cold seasons (6.9–11.5%).

Figure 4 compares the content of each quinone species in the surface layer of the sediment at Stn F between the high-density period (48 000–1 028 000 individuals per m^2, November 2004 to May 2005 and November 2005 to April 2006) and the low-density period (0–13 000 individuals per m^2, June to September in 2004, June to October in 2005 and June to July in 2006) of *Capitella* sp. I. The total quinone content of the sediment in the high-density period of the worm, $175 \pm 42.2\ \mu mol\ m^{-2}$ (mean ± s.d., $n = 10$), was approximately 2.0 times higher than that in the low-density period ($88.0 \pm 22.6\ \mu mol\ m^{-2}$, $n = 10$). In particular, the total UQs content of the sediment increased in the high-density period ($113 \pm 30.6\ \mu mol\ m^{-2}$) to a level approximately 2.3 times higher than that in the low-density period ($50.1 \pm 11.9\ \mu mol\ m^{-2}$), while the mean total MKs content increased 1.7 times in the high-density period ($38.6 \pm 7.5\ \mu mol\ m^{-2}$) to that in the low-density period ($23.2 \pm 5.0\ \mu mol\ m^{-2}$). Among the UQs, the increase of the content of UQ-10 of the sediment was most distinct between the low- and high-density periods of *Capitella* sp. I ($29.9 \pm 7.5\ \mu mol\ m^{-2}$ and $73.5 \pm 21.0\ \mu mol\ m^{-2}$, respectively). Therefore, these results on the increase of UQs content in the surface layer of the sediment with dense patches of *Capitella* sp. I reveal the association of bacteria, in particular, the members of the class *Alphaproteobacteria* containing UQ-10 with *Capitella* colonies in their increases in the organically enriched sediment in the cold seasons.

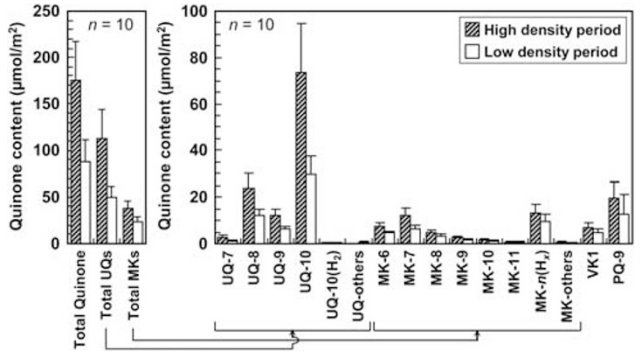

Figure 4 Comparison of the content of each quinone species in the surface layer of the sediment (up to a depth of 2 cm) at Stn F between the high- and low-density periods of *Capitella* population.

Cluster analysis based on the DGGE band pattern and the quinone profile

The composition of major species of the bacterial community was visualized in the surface layer of the sediment at Stn F and Stn C by PCR-DGGE, using the sediment samples collected in July and September in 2004; January, March, June and September in 2005 and January and March in 2006. In all, 18 DGGE bands (band 1–18) were detected on the gel for DGGE analysis in total at both stations (see Supplementary Table 1). Figure 5a shows the results of cluster analysis with the dissimilarity value

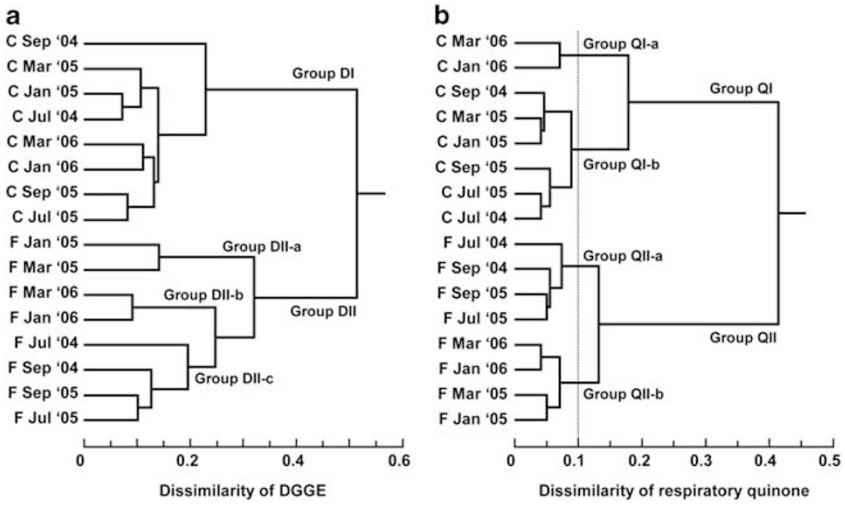

Figure 5 The results of the cluster analysis with the dissimilarity value matrix data calculated from (**a**) the DGGE band profiles of the sediment samples and (**b**) the mole fractions of respiratory quinones of the same sediment samples for DGGE analysis. Abbreviation of each sample indicates the sampling site and period. For example, C Mar '06 represents sampling at Stn C in March 2006.

matrix data calculated from the DGGE band profiles of the sediment samples. The DGGE band profiles were divided clearly into two different major groups, group DI (Stn C) and group DII (Stn F) in the dendrogram with dissimilarity of DGGE. It indicates that the bacteria community composition of the sediment was markedly different between the outside and inside of the fish farm. Group DII was divided further into three groups, group DII-a (January and March in 2005), group DII-b (January and March in 2006) and group DII-c (July 2004 and September in 2004 and July and September in 2005). Group DII-a and group DII-b were characterized by the presence of distinct bands 7 and 8 in the DGGE band profiles, although the bands in group DII-b were relatively thin, and coincided with the periods that dense patches of *Capitella* sp. I were established (192 000–1 028 000 individuals per m^2). In the case of group DII-c, band 7 was formed thinly and band 8 was absent.

Figure 5b shows the results of the cluster analysis with the dissimilarity value matrix data calculated from the mole fractions of respiratory quinones of the same sediment samples for DGGE analysis. The dendrogram on the respiratory quinone profiles was also divided clearly into two different major groups, group QI (Stn C) and group QII (Stn F), and each of them was divided further into two minor groups (group QI-a and QI-b, group QII-a and QII-b). The sediment samples of group QI-a (Stn C in January and March in 2006) were characterized by relatively high mole fractions of the partially saturated MKs in the quinone composition of the sediment in comparison with those of group QI-b (see July and September in 2004, and January, March, July and September in 2005 in Figure 3). The sediment samples of group QII-a were collected at Stn F in the warm seasons (July and September in both of 2004 and 2005), while those of group QII-b were collected at the same station in the cold seasons (January and March in both of 2005 and 2006), when dense patches of *Capitella* sp. I (192 000–1 028 000 individuals per m^2) were established (see Figure 1a). Group QII-b was characterized by the highest levels of UQ-10 mole fraction in the quinone composition of the sediment (42.2–47.4%, see Figure 3), and corresponds to group DII-a and DII-b with distinct bands 7 and 8 in the DGGE band profiles in Figure 5a. Since UQ-10 is one of the co-enzymes used for aerobic respiration in the respiratory chain by the members of the class *Alphaproteobacteria* (Table 1), it is very likely that the bacteria with bands 7 and 8, which increased in the sediment with dense patches of *Capitella* sp. I, are the members of the class *Alphaproteobacteria*.

Phylogenetic affiliation of the DGGE bands and the isolated bacteria from the sediment below the fish farm

Nine distinct bands in the 15 bands observed on the lane in the DGGE gel at Stn F were excised and sequenced, and their phylogenetic affiliations were determined using 16S rRNA gene sequences (Table 2). Three of them, the bacteria with bands 6–8, were identified commonly as the members of the family *Rhodobacteraceae* in the class of *Alphaproteobacteria* on which we are focusing in this study (Figure 6).

To confirm the increase of the bacteria belonging to the family *Rhodobacteraceae* in the organically enriched sediment with dense patches of *Capitella* sp. I at Stn F in the cold seasons, we collected the worms at Stn F on 14 December 2006, and identified the dominant bacteria attached on the body of the worms with 16S rRNA gene sequences. We found one distinct band (band CB) on the DGGE lane with the extract from the body tissues of the worms (Table 2), which was very close genetically to the bacteria in the family *Rhodobacteraceae* with band 7 (the similarity in phylogenetic affiliation between them was 98.6% (556/564)). The bacteria with band CB was notably predominant in the bacterial community around the body of *Capitella* sp. I, because the DGGE profile tends to detect only abundant species, not all species in the complex bacterial community.

Table 2 Sequence analysis of bands excised from DGGE gels with bacterial 16S rDNA

Band	*Highest similarity*	*Homology*	*Phylum/class*	*Dominant quinone*[a]
1	Uncultured bacterium Hg92C2 (EU236396)	567/586 (96.7)	*Flavobacteria*	MK-6, 7
3	Uncultured bacterium 76IIIOMF3 (GU197418)	566/582 (97.2)	*Flavobacteria*	MK-6, 7
5	Uncultured bacterium VHS-B3-86 (DQ394967)	532/547 (97.2)	*Gammaproteobacteria*	UQ-8
6	Alphaproteobacterium MGP-80 (AF530151)	487/501 (97.2)	*Alphaproteobacteria*	UQ-10
7	Rhodobacteraceae bacterium SH4-1 (FJ882053)	565/586 (96.4)	*Alphaproteobacteria*	UQ-10
8	Uncultured bacterium D7_10.4_1 (FJ716886)	558/582 (95.9)	*Alphaproteobacteria*	UQ-10
13	Uncultured actinobacterium 3G1820-55 (DQ431888)	425/434 (97.9)	*Actinobacteria*	MK-*n* ($n \geqslant 9$), MK-*n*(Hx)
14	Uncultured bacterium 096B62 (EU735002)	565/568 (99.5)	*Actinobacteria*	MK-*n* ($n \geqslant 9$), MK-*n*(Hx)
16	Uncultured bacterium Amsterdam-1B-57 (AY592358)	497/524 (94.8)	*Actinobacteria*	MK-*n* ($n \geqslant 9$), MK-*n*(Hx)
CB	Rhodobacteraceae bacterium SH4-1 (FJ882053)	561/563 (99.6)	*Alphaproteobacteria*	UQ-10

Abbreviations: DGGE, denaturing gradient gel electrophoresis; MK, menaquinone; PQ, plastoquinone; UQ, ubiquinone.
Band CB on the DGGE lane was derived from the extract of the body tissues of *Capitella* sp. I.
[a]Dominant quinone species that exist in the main species of the bacterial genus, from the WFCC-MIRCEN World Data Centre for Microorganisms (WDCM) database and references (Collins and Jones, 1981; Yokota *et al.*, 1992).

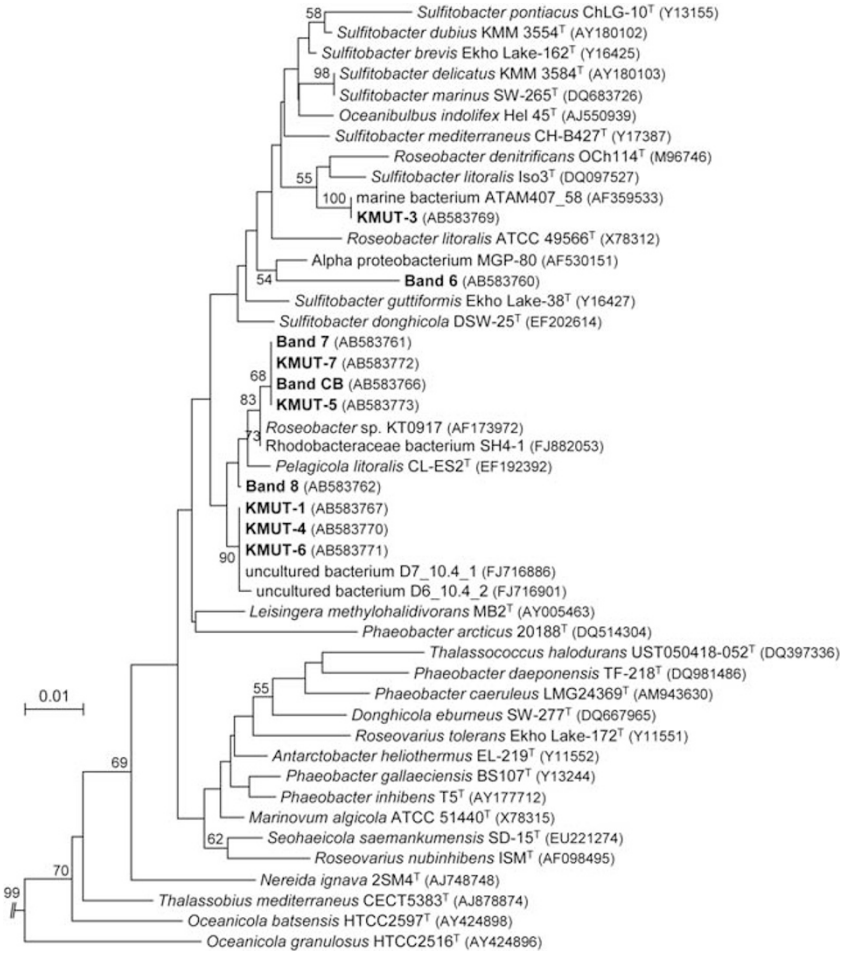

Figure 6 Phylogenetic affiliation of *Roseobacter* clade members described by comparative analysis of 16S rDNA sequences (approximately 500 bp) from the DGGE fragments (band numbers: 6, 7, 8, CB), the isolated bacteria (KMUT-1, 3, 4, 5, 6, 7) and those stored in the public nucleotide database. 'Band' and 'KMUT' indicate sequences of DGGE bands and isolated bacteria, respectively. *Ensifer meliloti* IAM12611^T and *Rickettsia prowazekii*^{T} were used as outgroup (not shown) to define the root of the tree. The scale bar represents 1% estimated difference divergence. The bootstrap values (%) indicate the value of 1000 replicate trees supporting the branching order. Numbers at the nodes represent bootstrap values (only >50% are shown) from 1000 replicates. Accession numbers are given in parentheses.

We attempted to isolate the alphaproteobacterial species from the surface layer of the sediment collected at Stn F on 8 March 2006. We obtained 73 colonies on a Shioi medium plate, chose seven colonies randomly from them, and sequenced their 16S rRNA genes. Six of the chosen seven colonies were identified as the members of the family *Rhodobacteraceae*, which were referred to the strain KMUT-1, 3, 4, 5, 6 and 7 in the phylogenetic tree of the partial *Roseobacter* clade (Figure 6; Supplementary Figure 1; Supplementary Table 2). Although the phylogenetic affiliations of these isolated bacteria were not determined because of the complexity of the community structure in the *Roseobacter* clade, three bacteria named as KMUT-1, 4 and 6 were located close to the *Phaeobacter* group, and the remaining three bacteria (KMUT-3, 5 and 7) were located close to the *Sulfitobacter* group (Supplementary Figure 1). The sequence similarities between band CB and KMUT5 and between band CB and KMUT7 were 99.8% (562/563), and indicated that these bacteria belonged to the same bacterial genus. These results indicated that the bacteria associated with *Capitella* colonies were located at the narrow part of the partial *Roseobacter* clade.

Discussion

In this study, we conducted bioremediation experiments on the organically enriched sediment deposited just below a fish farm (Stn F), introducing artificially mass-cultured colonies of *Capitella* sp. I on the sea floor in early November in 2004 and 2005 (Tsutsumi *et al*., 2005a; Kinoshita *et al*., 2008). The *Capitella* colonies increased rapidly in the cold seasons, and reached a peak density in March 2005 (528 000 individuals per m^2) and January 2006 (1 028 000 individuals per m^2) (Figure 1a). The quinone content of the surface sediment (up to a

depth of 2 cm), which indicates the abundance of microorganisms, also increased rapidly in the organically enriched sediment in the cold seasons in 2004 and 2005 (Figure 1d), although the activities of microorganisms vary depending on the water temperature in general (Ratkowsky *et al.*, 1982; Pomeroy and Wiebe, 2001). Furthermore, the increase in the quinone content of the sediment did not depend on the organic matter content of the sediment, but depended rather on the biomass of *Capitella* population in the sediment (Figure 2). As the biomass of *Capitella* population increases in the sediment, the biological activities of the worms, such as feeding and reworking the sediment, exert a larger impact on the exchange of water at the interface of the sediment and the overlying water, and can contribute to the creation of a more oxidized environment suitable for increase of aerobic bacteria in the sediment (Wu *et al.*, 2003; Wada *et al.*, 2005).

Capitellid polychaetes including *Capitella* sp. I in this study are deposit feeders occurring in the sediment. They secrete a protein-rich mucopolysaccharide to agglutinate sediment grains and create tubes and burrows (Zola, 1967; Fauchald and Jumars, 1979), which is a labile organic matter and exploitable easily as one of the resources for increase of the microorganisms (Aller and Aller, 1986; Arnosti *et al.*, 1994). The organic matter that is fed, digested and assimilated by the worms is used not only for growth and reproduction of the worms, but also is excreted partly as a mucopolysaccharide around the worms, and utilized by the microorganisms; in other words, it is used to stimulate further increase in the microorganisms. Actually, Alongi (1985) and Wu *et al.* (2003) reported that the microorganisms increased and worked actively around the inner walls of the burrows of *Capitella* in the sediment.

It is interesting to note that the *Alphaproteobacteria* consistently dominated in the microbial community in the fish-farm sediment throughout the study period (Figure 3). Previous studies reported that increases in *Alphaproteobacteria* were triggered by an addition of organic matter in seawater (Pinhassi *et al.*, 1999; Cottrell and Kirchman, 2000b; Elifantz *et al.*, 2005, 2007; Malmstrom *et al.*, 2005; Nogales *et al.*, 2007; Yokokawa and Nagata, 2010). The *Alphaproteobacteria* relative to other phylogenetic groups contributes to uptake of low-molecular weight DOM, such as amino acids, protein, glucose and *N*-acetyl-glucosamine in various marine environments (Cottrell and Kirchman, 2000a; Malmstrom *et al.*, 2005; Alonso and Pernthaler, 2006; Elifantz *et al.*, 2007; Yokokawa and Nagata, 2010). The *Alphaproteobacteria*, therefore, has significant roles in decomposing and assimilating of the organic matter in the organically enriched sediment. The increase in the *Alphaproteobacteria* was positively correlated with the dense patches of the *Capitella* under oxidized conditions of the organically enriched sediment (Figures 1b, 2b and 4). This suggests that the *Capitella* activities promote the increase in the *Alphaproteobacteria* by providing an oxygen-rich environment, which is suitable for them in the organically enriched sediment.

In this study, we succeeded in identifying the bacteria that increased around *Capitella* sp. I in the organically enriched sediment as the members of the *Roseobacter* clade in the class of *Alphaproteobacteria* (the majority of them belong to the family *Rhodobacteraceae*) (Figure 6). Band 8 was observed only in the sediment samples during the high-density period of *Capitella* sp. I (192 000–1 028 000 individuals per m^2) (Supplementary Table 1). The increase in the *Rhodobacteraceae* species with band 8 is strongly associated with the establishment of dense patches of *Capitella* sp. I that contributed to decomposition of the organic matter in the sediment. The marine *Roseobacter* clade members are primary colonizers of surfaces in marine environments (Dang and Lovell, 2000; Dang *et al.*, 2008), and relate to degradation of a multitude of organic compounds, sulfur metabolism, production of antibacterial compounds and symbiotic relationships with diverse marine eukaryotes (Moran *et al.*, 2003, 2007; Brinkhoff *et al.*, 2008). The growth of the partial *Roseobacter* clade members might be promoted further by the gradient in the physicochemical properties of the burrow environment (Marinelli *et al.*, 2002) and stimulatory effects of grazing by *Capitella* species and protozoa (Alongi, 1985; Plante and Shriver, 1998).

For a future study on the association of the *Roseobacter* clade members with *Capitella* sp. I, we are trying to make clear the question why *Capitella* stimulates the increase of bacteria around the burrows. Hylleberg (1975) proposed a concept 'bacterial gardening' by which infaunal macrobenthic animals promote the increase of bacteria in the sediment through the arrangement of oxygen-rich environment by their biological activities. The increase of bacteria around the inner walls of the burrows of *Capitella* is one of the typical cases of this gardening. Although the concept of bacterial gardening does not refer to the benefits of the benthic animals, the behaviors of the worms that stimulate the bacterial growth in the sediment will become meaningful for abundance, if they can get some benefits through the increase of bacteria, such as increase of available food resource. Several previous studies have suggested a potential role of the bacteria occurring in the sediment as one of the important food resources for deposit-feeding polychaetes (Haines and Montague, 1979; Findlay and Tenore, 1982; Tsutsumi *et al.*, 2001), and confirmed that $>50\%$ of bacteria are digested and eliminated in their guts in various polychaetes (Cammen, 1980; Grossmann and Reichardt, 1991; Lucas and Bertru, 1997).

From the standpoint of bioremediation of the organically enriched sediment with *Capitella*

colonies, the phenomenon in which the organically enriched sediment is treated very efficiently following the increase of *Capitella* colonies very efficiently (Tsutsumi *et al.*, 2005a; Kinoshita *et al.*, 2008; this study) would become explicable not only empirically but also theoretically, if we assumed mutual interaction between the *Capitella* colonies and the bacteria around the worms in the sediment. If the *Capitella* could exploit the bacteria as a food resource, the stimulation of increase of the aerobic bacteria by arrangement of oxidized environment in the sediment and excretion of labile organic matter would contribute to an increase in the food resource for *Capitella*, since the digestible organic matter for *Capitella* is limited even in the organically enriched sediment. In the associated system with *Capitella* and the bacteria in the organically enriched sediment, the decomposition of the organic matter will proceed very rapidly, as we observed in actual bioremediation experiments (Tsutsumi *et al.*, 2005a; Kinoshita *et al.*, 2008; this study). It is very likely that *Capitella* sp. I works as a promoter of bacteria in the organically enriched sediment, and feeds the increased bacteria as one of the main foods, while the bacteria decompose the organic matter in the sediment with the assistance of *Capitella* for arrangement of the suitable environment. In a future study, we will attempt to clarify the question whether *Capitella* sp. I can utilize the members of the *Roseobacter* clade of the microorganisms as a food resource.

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References

Akagawa-Matsushita M, Itoh T, Katayama Y, Kuraishi H, Yamasato K. (1992). Isoprenoid quinone composition of some marine *Alteromonas*, *Marinomonas*, *Deleya*, *Pseudomonas* and *Shewanella* species. *J Gen Microbiol* **138**: 2275–2281.

Aller JY, Aller RC. (1986). Evidence for localized enhancement of biological activity associated with tube and burrow structures in deep-sea sediments at the HEBBLE site, western North Atlantic. *Deep-Sea Res* **33**: 755–790.

Aller RC, Yingst JY, Ullman WJ. (1983). Comparative biogeochemistry of water in intertidal Onuphis (Polychaeta) and Upogebia (Crustacea) burrows: temporal patterns and causes. *J Mar Res* **41**: 571–604.

Alongi DM. (1985). Microbes, meiofauna, and bacterial productivity on tubes constructed by the polychaete *Capitella capitata*. *Mar Ecol Prog Ser* **23**: 207–208.

Alonso C, Pernthaler J. (2006). *Roseobacter* and SAR11 dominate microbial glucose uptake in coastal North Sea waters. *Environ Microbiol* **8**: 2022–2030.

Arnosti C, Repeta DJ, Blough NV. (1994). Rapid bacterial degradation of polysaccharides in anoxic marine systems. *Geochim Cosmochim Ac* **58**: 2639–2652.

Bissett A, Bowman J, Burke C. (2006). Bacterial diversity in organically-enriched fish farm sediments. *FEMS Micorbial Ecol* **55**: 48–56.

Brinkhoff T, Giebel H-A, Simon M. (2008). Diversity, ecology, and genomics of the *Roseobacter* clade: a short overview. *Arch Microbiol* **189**: 531–539.

Buschmann AH, Riquelme VA, Hernández-González MC, Varela D, Jiménez JE, Henríquez LA *et al.* (2006). A review of the impacts of salmonid farming on marine coastal ecosystems in the southeast Pacific. *ICES J Mas Sci* **63**: 1338–1345.

Cammen LM. (1980). The significance of microbial carbon in the nutrition of the deposit feeding polychaete Nereis succinea. *Mar Biol* **61**: 9–20.

Chareonpanich C, Montani S, Tsutsumi H, Matsuoka S. (1993). Modification of chemical characteristics of organically enriched sediment by *Capitella* sp. I. *Mar Pollut Bull* **26**: 375–379.

Collins MD, Jones D. (1981). Distribution of isoprenoid quinines structural types in bacteria and their taxonomic implications. *Microbiol Rev* **45**: 316–354.

Cottrell MT, Kirchman DL. (2000a). 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 MT, Kirchman DL. (2000b). Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence *in situ* hybridization. *Appl Environ Microbiol* **66**: 5116–5122.

Dang H, Li T, Chen M, Huang G. (2008). Cross-ocean distribution of *Rhodobacterales* bacteria as primary surface colonizers in temperate coastal marine waters. *Appl Environ Microbiol* **74**: 52–60.

Dang H, Lovell CR. (2000). Bacterial primary colonization and early succession on surfaces in marine waters as determined by amplified rRNA gene restriction analysis and sequence analysis of 16S rRNA genes. *Appl Environ Microbiol* **66**: 467–475.

Dobbs FC, Guckert JB. (1988). Microbial food resources of the macrofaunal deposit feeder *Ptychodera bahamensis* (Hemichordata: Enteropneusta). *Mar Ecol Prog Ser* **45**: 127–136.

Elifantz H, Dittel AI, Cottrell MT, Kirchman DL. (2007). Dissolved organic matter assimilation by heterotrophic bacterial groups in the western Arctic Ocean. *Aquat Microb Ecol* **50**: 39–49.

Elifantz H, Malmstrom RR, Cottrell MT, Kirchman DL. (2005). Assimilation of polysaccharides and glucose by major bacterial groups in the Delaware Estuary. *Appl Environ Microbiol* **71**: 7799–7805.

FAO (1992). Aquaculture production, 1984–1990. *FAO Fisheries Circular* **815**: 206. Rome, Italy.

Fauchald K, Jumars PA. (1979). The diet of worms: a study of polychaete feeding guilds. *Oceanogr Mar Biol Ann Rev* **17**: 193–284.

Findlay S, Tenore K. (1982). Nitrogen source for a detritivore: detritus substrate versus associated microbes. *Science* **218**: 371–373.

Gooday AJ, Turley CM. (1990). Responses by benthic organisms to inputs of organic material to the ocean floor: a review. *Philos T Roy Soc A* **331**: 119–138.

Gowen RJ, Bradbury NB. (1987). The ecological impact of salmonid farming in coastal waters: a review. *Oceanogr Mar Biol Ann Rev* **25**: 563–575.

Grassle J, Grassle JF. (1976). Sibling species in the marine pollution indicator *Capitella* (Polychaeta). *Science* **192**: 567–569.

Grossmann S, Reichardt W. (1991). Impact of *Arenicola marina* on bacteria in intertidal sediments. *Mar Ecol Prog Ser* **77**: 85–93.

Haines EB, Montague CL. (1979). Food sources of estuary invertebrates analyzes using $^{13}C/^{12}C$ ratios. *Ecology* **60**: 48–56.

Hargrave BT. (1976). The central role of invertebrate faeces in sediment decomposition. In: Anderson JM, Macfadyen A (eds). *The Role of Terrestrial and Aquatic Organisms in Decomposition Processes*. Blackwell Press: Oxford, pp 301–321.

Hedrick DB, White DC. (1986). Microbial respiratory quinones in the environment. *J Microbiol Methods* **5**: 243–254.

Hiraishi A. (1999). Isoprenoid quinones as biomarkers of microbial populations in the environment. *J Biosci Bioeng* **88**: 449–460.

Hiraishi A, Iwasaki M, Kawagishi T, Yoshida N, Narihiro T, Kato K. (2003). Significance of lipoquinones as quantitative biomarkers of bacterial populations in the environment. *Microbes Environ* **18**: 89–93.

Hiraishi A, Morishima Y, Takeuchi J. (1991). Numerical analysis of lipoquinone patterns in monitoring bacterial community dynamics in wastewater treatment systems. *J Gen Appl Microbiol* **37**: 57–70.

Holmer M, Kristensen E. (1992). Impact of marine fish cage farming on metabolism and sulphate reduction of under-lying sediments. *Mar Ecol Prog Ser* **80**: 191–201.

Hu H-Y, Lim B-R, Goto N, Fujie K. (2001). Analytical precision and repeatability of respiratory quinones for quantitative study of microbial community structure in environmental samples. *J Microbiol Methods* **47**: 17–24.

Hylleberg J. (1975). Selective feeding by *Abarenicola pacifica* with notes on *Abarenicola vagabunda* and a concept of gardening in lugworms. *Ophelia* **14**: 113–137.

James PG, Russell PH. (1996). Phylogenetic analysis of the bacterial communities in marine sediments. *Appl Environ Microbiol* **62**: 4049–4059.

Karakassis I, Hatziyanni E. (2000). Benthic disturbance due to fish farming analyzed under different levels of taxonomic resolution. *Mar Ecol Prog Ser* **203**: 247–253.

Kinoshita K, Tamaki S, Yoshioka M, Srithonguthai S, Kunihiro T, Hama D *et al.* (2008). Bioremediation of organically enriched sediment deposited below fish farms with artificially mass-cultured colonies of a deposit-feeding polychaete *Capitella* sp. I. *Fish Sci* **74**: 77–87.

Kunihiro T, Miyazaki T, Kinoshita K, Satou A, Inoue A, Hama D *et al.* (2005). Microbial community dynamics in organically enriched sediment below fish net pen culture with artificially cultured colonies of the polychaete *Capitella* sp. I. *Bull Soc Sea Water Sci Jpn* **59**: 343–353 (in Japanese).

Kunihiro T, Miyazaki T, Uramoto Y, Kinoshita K, Inoue A, Tamaki S *et al.* (2008). The succession of microbial community in the organic rich fish-farm sediment during bioremediation by introducing artificially mass-cultured colonies of a small polychaete, *Capitella* sp. I. *Mar Pollut Bull* **57**: 68–77.

Levinton JS. (1972). Stability and trophic structure in deposit-feeding and suspension-feeding communities. *Am Nat* **106**: 472–486.

Lopez GR, Levinton JS. (1987). Ecology of deposit-feeding animals in marine sediments. *Q Rev Biol* **62**: 235–260.

Lucas F, Bertru G. (1997). Bacterialysis in the gut of *Nereis diversicolor* (O.F. Müller) and effect of the diet. *J Exp Mar Biol Ecol* **215**: 235–245.

Lucas FS, Bertrus G, Hofle MG. (2003). Characterization of free-living and attached bacteria in sediments colonized by Hediste diversicolor. *Aquat Microb Ecol* **32**: 165–174.

Macleod CK, Moltschaniwskyj NA, Crawford CM, Forbes SE. (2007). Biological recovery from organic enrichment: some systems cope better than others. *Mar Ecol Prog Ser* **342**: 41–53.

Malmstrom RR, Cottrell MT, Elifantz H, Kirchman DL. (2005). Biomass production and assimilation of dissolved organic matter by SAR11 bacteria in the Northwest Atlantic Ocean. *Appl Environ Microbiol* **71**: 2979–2986.

Marinelli RL, Lovell CR, Wakeham SG, Ringelberg DB, White DC. (2002). Experimental investigation of the control of bacterial community composition in macrofaunal burrows. *Mar Ecol Prog Ser* **235**: 1–13.

Meyer-Reil LA. (1983). Benthic response to sedimentation events during autumn to spring at a shallow water station in the Western Kiel Bight. II. Analysis of benthic bacterial populations. *Mar Biol* **77**: 247–256.

Moran MA, González JM, Kiene RP. (2003). Linking a bacterial taxon to sulfur cycling in the sea: studies of the marine *Roseobacter* group. *Geomicrobiol J* **20**: 375–388.

Moran MA, Schell MA, González JM, Sun F, Sun S, Binder BJ *et al.* (2007). Ecological genomics of marine roseobacters. *Appl Environ Microbiol* **73**: 4559–4569.

Muyzer G, Dewaal EC, Uitterlinden AG. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* **59**: 695–700.

Naylor RL, Goldburg RJ, Primavera JH, Kautsky N, Beveridge MCM, Clay J *et al.* (2000). Effect of aquaculture on world Fish supplies. *Nature* **405**: 1017–1024.

Nogales B, Aguiló-Ferretjans MM, Martín-Cardona C, Lalucat J, Bosch R. (2007). Bacterial diversity, composition and dynamics in and around recreational coastal areas. *Environ Microbiol* **9**: 1913–1929.

Papaspyrou S, Gregersen T, Kristensen E, Christensen B, Cox RP. (2006). Microbial reaction rates and bacterial communities in sediment surrounding burrows of two nereidid polyshaetes (*Nereis diversicolor* and *N. virens*). *Mar Biol* **148**: 541–550.

Pearson TH, Rosenberg R. (1978). Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanogr Mar Biol Ann Rev* **16**: 229–311.

Perrière G, Gouy M. (1996). WWW-Query: an on-line retrieval system for biological sequence banks. *Biochimie* **78**: 364–369.

Pinhassi J, Azam F, Hemphälä J, Long RA, Martinez J, Zweifel UL *et al.* (1999). Coupling between bacterioplankton species composition, population dynamics, and organic matter degradation. *Aquat Microb Ecol* **17**: 13–26.

Plante CJ, Shriver AG. (1998). Differential lysis of sedimentary bacteria by *Arenicola marina* L: examination of cell wall structure and exopolymeric capsules as correlates. *J Exp Mar Biol Ecol* **229**: 35–52.

Plante CJ, Wilde SB. (2001). Bacterial recolonization of deposit-feeder egesta: *in situ* regrowth of immigration? *Limnol Oceanogr* **46**: 1171–1181.

Pomeroy LR, Wiebe WJ. (2001). Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. *Aquat Microb Ecol* **23**: 187–204.

Ratkowsky DA, Olley J, McMeekin TA, Ball A. (1982). Relationship between temperature and growth rate of bacterial cultures. *J Bacteriol* **149**: 1–5.

Reichardt W. (1988). Impact of bioturbation by *Arenicola marina* on microbiological parameters in intertidal sediments. *Mar Ecol Prog Ser* **44**: 149–158.

Saitou K, Nagasaki K, Yamakawa H, Hu H-Y, Fujie K. (1999). Linear relation between the amount of respiratory quinones and the microbial biomass in soil. *Soil Sci Plant Nutr* **45**: 775–778.

Sanders HL. (1958). Benthic studies in Buzzards Bay: I. Animal–sediment relationships. *Limnol Oceanogr* **3**: 245–258.

Shioi Y. (1986). Growth characteristics and substrate specificity of aerobic photosynthetic bacterium, *Erythrobacter* sp. (OCh 114). *Plant Cell Physiol* **27**: 567–572.

Srithongouthai S, Endo A, Inoue A, Kinoshita K, Yoshioka M, Sato A *et al.* (2006). Control of dissolved oxygen levels of the water in the net pens for fish farming with a microscopic bubble generating system. *Fish Sci* **72**: 485–493.

Steward CC, Nold SC, Ringelberg DB, White DC, Lovell CR. (1996). Microbial biomass and community structures in the burrows of bromophenol producing and non-producing marine worms and surrounding sediments. *Mar Ecol Prog Ser* **133**: 149–165.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**: 4876–4882.

Tsutsumi H. (1987). Population dynamics of *Capitella capiteta* (Polychaeta; Capitellidae) in an organically polluted cove. *Mar Ecol Prog Ser* **36**: 139–149.

Tsutsumi H. (1990). Population persistence of *Capitella* sp. (Polychaeta; Capitellidae) on a mud flat subject to environmental disturbance by organic enrichment. *Mar Ecol Prog Ser* **63**: 147–156.

Tsutsumi H. (1995). Impact of fish net pen culture on the benthic environment of a cove in south Japan. *Estuar* **18**: 108–115.

Tsutsumi H, Kikuchi T. (1983). Benthic ecology of a small cove with seasonal oxygen depletion caused by organic pollution. *Publ Amakusa Mar Biol Lab* **7**: 17–40.

Tsutsumi H, Kinoshita K, Srithongouthai S, Sato A, Nagata S, Inoue A *et al.* (2005a). Treatment of the organically enriched sediment below the fish farm with the biological activities of the artificially mass-cultured colonies of a small deposit feeding polychaete, *Capitella* sp. I. *Benthos Res* **60**: 17–24.

Tsutsumi H, Montani S. (1993). Utilization of biological activities of capitellied polychaete for treatment of 'Hedoro' (organically enriched sediment) deposited on the marine bottom below fish net pen culture. *Nippon Suisan Gakkaishi* **59**: 1343–1347 (Japanese).

Tsutsumi H, Montani S, Kobe H. (2002). Bioremediation of organic matter loaded on the sediment in outdoor pools with a polychaete, *Capitella* sp. 1. *Fish Sci* **68**: 613–616.

Tsutsumi H, Srithongouthai S, Inoue A, Sato A, Hama D. (2006). Seasonal fluctuations in the flux of particulate organic matter discharged from net pens for fish farming. *Fish Sci* **72**: 119–127.

Tsutsumi H, Taniguchi A, Sakamoto N. (2005b). Feeding and burrowing behaviors of a deposit-feeding capitellid polychaete, *Capitella* sp. I. *Benthos Res* **60**: 51–58.

Tsutsumi H, Wainright S, Montani S, Saga M, Ichihara S, Kogure K. (2001). Exploitation of a chemosynthetic food resource by the polychaete *Capitella* sp. I. *Mar Ecol Prog Ser* **216**: 119–127.

Urakawa H, Kita-Tsukamoto K, Ohwada K. (1999). Microbial diversity in marine sediments from Sagami Bay and Tokyo Bay, Japan, as determined by 16S rRNA gene analysis. *Microbiology* **145**: 3305–3315.

Wada M, Wu S-S, Kogure K, Tsutsumi H. (2005). Shor-term impact of biological activities of a burrowing polychaetes, Capitella sp. I, on bacterial abundance and the chemical characteristics in organically enriched sediment. *Benthos Res* **60**: 59–66.

Wada M, Wu S-S, Tsutsumi H, Tsukamoto K-K, Do H-K, Nomura H *et al.* (2006). Visual assessment of the respiring microorganisms associated with burrow structures of *Capitella* sp. I in seawater soft-agar microcosms. *Plankton Benthos Res* **1**: 54–58.

Wada M, Zhang D, Do H-K, Nishimura M, Tsutsumi H, Kogure K. (2008). Co-inoculation of *Capitella* sp. I with its synergistic bacteria enhances degradation of organic matter in organically enriched sediment below fish farms. *Mar Pollut Bull* **57**: 86–93.

Weston DP. (1990). Quantitative examination of macrobenthic community changes along an organic enrichment gradient. *Mar Ecol Prog Ser* **61**: 233–244.

WFCC-MIRCEN World Data Centre for Microorganisms (WDCM) database. (1995). Available from 〈http://wdcm.nig.ac.jp/wdcm/Quinone.html〉. Final updated on 3 October 1995.

Wu RSS. (1995). The environmental impact of marine fish culture: towards a sustainable future. *Mar Pollut Bull* **31**: 159–166.

Wu SS, Tsutsumi H, Kita-Tsukamoto K, Kogure K, Ohwada K, Wada M. (2003). Visualization of the respiring bacteria in sediments inhabited by *Capitella* sp. I. *Fish Sci* **69**: 170–175.

Yokokawa T, Nagata T. (2010). Linking bacterial community structures to carbon fluxes in marine environments. *J Oceanogr* **66**: 1–12.

Yokota A, Akagawa-Mathusita M, Hiraishi A, Katayama Y, Urakami T, Yamasato K. (1992). Distribution of quinone systems in microorganisms: Gram-negative eubacteria. *Bull JFCC* **8**: 136–171.

Zola H. (1967). Sugar phosphate polymers in polychaete tubes and in mineralized animal tissues. *Comp Biochem Physiol* **21**: 179–183.

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