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Response of bacteria in the deep-sea sediments and the Antarctic soils to carbohydrates: Effects on ectoenzyme activity and bacterial community

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Abstract

The response of bacteria to various carbohydrates in the deep-sea sediments and the Antarctic soils was investigated using cellulose, chitin, and olive oil. It was found that the carbohydrates significantly increased the corresponding specific ectoenzyme activity (β-glucosidase, β-N-acetylglucosaminidase, lipase) in the samples from deep-sea sediments. In the case of Antarctic soil samples, the cellulose or olive oil amendments had minor or no effect on β-glucosidase or lipase activity, except the chitin which stimulated β-N-acetylglucosaminidase production. The responses of the bacteria in the deep-sea sediment sample WP02-3 and the Antarctic soil sample CC-TY2 towards the chitin amendment were further analyzed. Chitin amendments were shown to stimulate the ectoenzyme activity in all the tested sediments and the soils. The bacterial response before and after the carbohydrates amendments were compared by denaturing gradient gel electrophoresis and quantitative competitive polymerase chain reaction. Significant changes were found in the structure and density of the bacterial community in the deep sea sediments as compared to the Antarctic soil sample, where the effects were relatively lower. There was no change in the bacterial population in both studied samples in response to carbohydrates amendments. These data indicate that the bacterial communities in the oligotrophic deep-sea sediments are more dynamic than that in the Antarctic soils as they respond to the nutrient sources efficiently by regulation of ectoenzyme activity and/or changing community structure.

Key words: deep-sea sediments; Antarctic soils; amending; cellulose; chitin; olive oil; ectoenzyme activity
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Introduction

The deep sea is regarded as an extreme environment with commonly high hydrostatic pressure, low nutrient, and predominantly low temperatures below 4°C (Deming, 1998). All organic material has to be transported to the deep sea by sedimentation from the productive upper water column, or lateral transport from the continental shelves (Lochte et al., 2000). During this transport, a large proportion of the labile organic material is lost or occasionally intense pulses of the labile organic matter result from the fall of large whale carcasses (Smith and Baco, 2003). It was assumed that the scarce supply of organic material from the productive zones, rather than high pressures or low temperatures, is the main controlling factor in the deep-sea environments (Boetius and Lochte, 1996a). POM (particle organic matter) reaching the deep sea floor (sediments) is composed of macromolecules with long half-lives. Only small-molecular-mass compounds (< 600 Da) can be directly uptake by the organisms (Boetius and Lochte, 1996b). Higher-molecular-mass compounds, which represent 30%–95% of the total organic matter, must be hydrolyzed prior to uptake (Amon and Benner 1994). Therefore, hydrolyzing of the particular material is a prerequisite for survival of the bacteria. For this purpose, they rely on the extracellular enzymes in this environment as compared to those who live in the upper water column (Boetius and Lochte, 1996a). Determination of the ectoenzymatic activity in the sediments represents a key parameter for understanding the actual role of bacteria in the deep-sea sediments. The soils of the Antarctic, which are also extremely cold temperature environments, contain organic C, emit CO_2 and support communities of heterotrophic soil organisms (Hopkins et al., 2006). It was found that the growth of bacterial microcolonies (colony number and colony areas) from Antarctic soils could best be stimulated with carbohydrates (Bölter, 1993).

Microbial communities may respond to a varying supply of substrates either by physiological adaptation or by changes in the community composition. To study the effect of substrate addition on short-term bacterial population

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dynamics and species composition in seawater, Pinhassi et al. (1999) found that even small addition of organic substrates may trigger a shift in the composition of the microbial community and an accompanying change in the relative abundance of specific hydrolytic ectoenzymes. The microorganisms in some deep-sea sediments such as cold seeps, hydrothermal vents, have attracted intensive investigations (Arakawa et al., 2006; Danovaro et al.,1993; Huber et al., 2002; Knittel et al., 2005; Poremba and Hoppe, 1995; Sheridan et al., 2002). However, a little has been done on the microorganisms in the oligotrophic open ocean deep-sea environments, especially on how they react in terms of food supply.

Both sediments and soils represent some of the most complex microbial habitats on the Earth (Torsvik et al., 1990). The deep-sea and Antarctic microorganisms live and grow permanently in the cold environment, with their adaptive response to low temperature, such as 0–4°C. And some deep-sea microorganisms probably originated in polar regions and disseminate with the global deep ocean circulations (Maruyama et al., 2000). In the present study, the deep sea sediments and the Antarctic soils were amended with different kinds of carbohydrates (cellulose, chitin, olive oil), the specific ectoenzyme activity and the bacterial community in the samples were analyzed accordingly. The objectives of the study were to determine: (1) whether the change of ectoenzyme activity profile depends on the availability of substrates; (2) the short-term impact of carbohydrates introduction on the indigenous bacterial community of the samples; (3) the different responses of bacteria in the deep-sea sediments and the Antarctic soils.

1 Materials and methods

1.1 Sampling sites

The study was carried out using four west Pacific deep-sea sediments and two Antarctic soils samples. The deep-sea sediments were collected by a multi-core sampler during a cruise of HaiYang No. 4 in the west Pacific at the sites of WP02-1 (125°00′00″E, 16°56′09″N, 3000 m in depth), WP02-2 (148°44′08″E, 19°24′01″N, 5080 m in depth), WP02-3 (148°00′00″E, 13°00′00″N, 4500 m in depth) and WP02-4 (141°43′07″E, 09°46′08″N, 2900 m in depth) during May, 2002. The Antarctic soil samples CC-TY2 (58°57′52″W, 62°12′59″S) and CC-TY4 (58°57′52″W, 62°12′59″S) were collected using sterile falcon tubes during the 19th Antarctic expedition of China, in March 2003. All the samples were stored at –20°C during shipping to the laboratory.

1.2 Biochemical parameters of the sediments and soils

The pH and salinity of each sample were measured according to the procedures of Munson et al. (1997). The total organic carbon contents (TOC) of the samples were determined by the method of Gaudette et al. (1974). The carbon, hydrogen and nitrogen contents (elemental) in the samples were measured by elemental analyzer EA1110 (Carloerba, Italy). The lipids were extracted from the dried sediment samples directly by chloroform and methanol elution and were analyzed as described (Bligh and Dyer, 1959). All analyses were performed in triplicates.

Five gram of each sample was transferred into the sterile flask, then amended with 1% (*W/W*, 0.05 g/5 g dry weight sample) different carbohydrates including colloidal chitin (prepared from Sigma chitin, Wang et al., 2003), cellulose (Sigma, USA), or olive oil (Leveking Bio-engineering, China) along with the controls (without the carbohydrates). All the experiments were performed in triplicates. Then the samples were incubated in the dark at 4°C for 15 or 30 days. After incubation, samples were taken out and divided into two equal portions: one for enzyme activity test, the other for DNA extraction and DGGE analysis.

1.3 DNA extraction

DNA was extracted from the samples according to Wang et al. (2004). This procedure employed enzymatic and freeze-thawing based lysis, which was a relatively gentle method that avoided excessive shearing of DNA.

1.4 Bacterial biomass

The total bacterial counts in samples were determined by semi-quantification of the 16S rRNA gene copies by quantitative competitive PCR (QC-PCR) as described earlier (Wang et al., 2004). A plasmid pMD18-T containing a fragment of 16S rRNA gene of a bacteria strain isolated in this study was digested with *Bam*HI. The linear plasmid pMD18-T was used as the standard 16S rRNA gene fragment template to evaluate the efficiency and accuracy of the competitor template DNA. The competitor template was constructed by digestion of pMD18-T with Kpn2I to get a 300-bp deletion and self-ligated. The quantities of pMD18-T and pMD18-TΔ300bp were determined using a spectrophotometer (Ultrospec 2100, Amersham Pharmacia, USA).

The number of culturable bacteria has been determined by the standard plate counts (SPC) method and the most probable number (MPN) technique. Each 0.1 mL (containing 100 mg sediment) of the suspension, which was diluted serially 10^{-1} to 10^{-7}, was spread on the marine agar plates (2216E) or Luria-Bertani (LB) plates, then incubated at 10°C for around 2 weeks. The colony forming units (CFUs) from triplicate plates were averaged and expressed as CFUs/g of wet sediments.

1.5 Ectoenzyme activity assay

The enzyme activities of the cellulase, chitinase and lipase were tested in the samples. β-Glucosidase (3.2.1.21), β-N-acetylglucosaminidase (3.2.1.30) were used to represent the cellulase and chitinase activities, respectively. Enzyme assays were based on the methods reported previously (Boetius and Lochte, 1996a, 1996b; Allison and Vitousek, 2005). The corresponding substrates to enzyme (β-glucosidase, β-N-acetylglucosaminidase, lipase) used in this study were as follows, 50 mmol/L pNP-β-glucopyranoside, 20 mmol/L pNP-β-N-acetylglucosaminide, 50 mmol/L pNP-acetate. According to the temperature for soils enzyme assays

(Allison and Vitousek, 2005) and the optimal temperature for most cold-adapted enzyme, all the reaction was performed at 20°C. The sample and the pNP-substrate (saturating concentrations) were mixed and then incubated for 1–4 hr at 20°C. NaOH (1 mol/L) was added at 20 mmol/L final concentration to terminate the reaction and the samples were centrifuged (3000 × *g*, 5 min). The released pNP was measured by spectrophotometer at λ 405 nm. All analyses were performed in triplicates.

1.6 DGGE and sequence analysis

A 16S rRNA gene fragment (169–194 bp in length) was amplified using a universal primer complementary to position 517–534 (5′-ATTACCGCGGCTGCTGG-3′), and a bacterial primer complementary to position 341–358 (5′-TACGGGAGGCAGCAG-3′) according to Muyzer et al. (1993). For denature gradient gel electrophoresis (DGGE) analysis, a 40-bp GC-rich sequence (GC-clamp): 5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG-3′ was attached to the 5′ end of primer 341F to prevent the complete dissociation of the two DNA strands. The PCR reaction mixture included 25 ng of purified DNA, 50 pmol/L of each primer, 0.25 U Taq DNA polymerase, 200 μmol/L of each deoxyribonucleoside triphosphate, and 10 μL of 10 × PCR buffer (100 mmol/L gelatin, 1% Triton X-100, 15 mmol/L $MgCl_2$).

A hot-start PCR was performed at 95°C for 10 min, and touchdown PCR was performed as follows: the annealing temperature was initially set at 65°C and then decreased by 0.5°C every cycle until reach to 55°C. Twenty additional cycles were carried out at 55°C. Denaturing was carried out at 94°C for 1 min. Primer annealing was performed using the scheme described above for 1 min, and primer extension was performed at 72°C for 3 min. The final extension step was 10 min at 72°C. DGGE was performed with the D Code System (BioRad, USA). The PCR samples were loaded onto 8% (*W/V*) polyacrylamide gels with a 40% to 65% denaturing gradient in 1 × TAE (40 mmol/L Tris, 20 mmol/L acetic acid, and 1 mmol/L EDTA at pH 8.0), where 100% denaturant contains urea (7.0 mol/L) and formamide (40%).

The electrophoresis was run at 60°C for 15 min at 30 V, and subsequently for 6 hr at 180 V. After electrophoresis, the gels were stained for 20 min with ethidium bromide (0.5 μg/mL). DGGE gels were visualized with UV transillumination. To get the sequence information from the bands, DGGE bands were selected and excised from the gel. Slices were placed in 1.5 mL screw-cap polypropylene tubes containing 50 μL sterile deionized water and incubated at 4°C overnight. Soaked DNA liquid of 10 μL was used as the template to re-amplify the 16S rDNA with the primer pair Univer 341F and Eubac 518R. The PCR products were ligated to pMD18-T vector (Takara, Japan) and transformed into *Escherichia coli* XL-Blue. Sequencing was performed with an ABI PEISM 377DNA sequencer (Sangon, China). Sequence was aligned to known sequences in the database using BLAST and compared with their closest relative species.

1.7 Nucleotide sequence accession numbers

The following DGGE band sequence samples have been deposited in GenBank under the indicated accession numbers (from band 1 to band 9 in order): WP02-3-1, AM292543; WP02-3-2, AM292544; WP02-3-3, AM292545; WP02-3-4, AM292546; WP02-3-5, AM292547; WP02-3-6, AM292548; WP02-3-7, AM292549; WP02-3-8, AM292550; WP02-3-9, AM292551.

2 Results

2.1 Sample characterization

All the four deep-sea sediment samples had low total organic carbon (TOC) contents (ranging from 0.11% to 0.29%) and low total organic matter (TOM) (ranging from 0.21% to 0.51%) (Table 1). The Antarctic soils contained higher concentrations of TOC and OM (Table 1). Almost no detectable N existed in the deep-sea and Antarctic samples. All the deep-sea sediments used were from low nutrient open ocean deep-sea environments, whereas the Antarctic soils were carbon rich, nitrogen depleting. The lack of N in the Antarctic soils indicated that the carbon sources in these samples were of plant origin. The Antarctic soils contained much higher lipid concentration than the deep-sea sediments, indicating that the Antarctic soils had higher biomass than the deep-sea sediments.

Bacteria were retrieved from the deep-sea sediment and Antarctic soil samples using marine 2216E or LB agar plates respectively. The number of culturable strains is about 10^4 CFU/g dry soil sample in the west Pacific deep sea sediments, which was almost equal to that of the Antarctic soils (Table 2). The total bacterial population as determined by QC-PCR in the most deep-sea sediment samples was in the range of 10^5–10^6 copies/g dry sediment. The Antarctic soils contained higher bacterial number (10^6–10^7 copies/g), which was in agreement with the result of lipids assay.

Table 1 Sample locations and properties

Origin	Sample	pH	Temperature (°C)	Salinity (‰)	TOC (%)	TOM (%)	Lipid (μg/g dry sediment)	C (%)	H (%)	N (%)
Deep-sea sediments	WP02-1	7.52	1.58	34.50	0.11	0.51	458.91	0.36	0.53	–
	WP02-2	7.51	1.44	34.00	0.29	0.36	169.54	0.24	0.33	–
	WP02-3	7.50	ND	35.00	0.18	0.21	644.35	0.52	1.16	–
	WP02-4	7.55	1.60	34.00	0.23	0.39	159.80	0.24	0.21	–
Antarctic soils	CC-TY2	7.20	< 2.000	ND	0.34	0.59	895.00	1.12	1.26	–
	CC-TY4	7.45	< 2.000	ND	5.30	9.14	2107.00	33.08	4.81	–

ND: not determined; –: not detectable.

Table 2 Bacterial quantity and ectoenzyme activity in the samples

Origin	Sample	CFU (copies/g)	TC (copies/g)	Ectoenzyme activity (mU/(g·hr))		
				Lipase	β-Glucosidase	β-N-acetylglucosaminidase
Deep-sea sediments	WP02-1	$(1.00 \pm 0.20) \times 10^4$	$(6.60 \pm 0.10) \times 10^6$	799.20 ± 11.00	5.55 ± 0.30	116.50 ± 11.00
	WP02-2	$(4.50 \pm 0.50) \times 10^4$	$(6.10 \pm 0.20) \times 10^6$	666.00 ± 33.00	4.35 ± 0.60	66.60 ± 6.00
	WP02-3	$(1.20 \pm 0.30) \times 10^4$	$(1.80 \pm 0.20) \times (10^5–10^6)$	666.00 ± 11.00	5.55 ± 0.30	99.90 ± 6.00
	WP02-4	$(1.45 \pm 0.10) \times 10^4$	$(1.90 \pm 0.30) \times (10^6–10^7)$	799.20 ± 11.00	3.98 ± 0.30	32.50 ± 3.00
Antarctic soils	CC-TY2	$(2.00 \pm 0.30) \times 10^4$	$(6.00 \pm 0.20) \times (10^6–10^7)$	0.00 ± 0.02	4.59 ± 0.30	30.10 ± 3.00
	CC-TY4	$(2.50 \pm 0.20) \times 10^4$	$(1.50 \pm 0.10) \times (10^6–10^7)$	0.00 ± 0.01	5.51 ± 0.60	33.30 ± 6.00

CFU: colony forming units; TC: total cell counts. Values are given as mean ± SE.

2.2 Microbial response to various carbohydrates

2.2.1 Effect of carbohydrates amendments on ectoenzyme activity

The ectoenzyme activities in the sediments and soils were determined as shown in Table 2. The activities of β-N-acetylglucosaminidase varied from 32.5 to 116.5 mU/(g·hr), in the deep-sea sediments, and kept around 30 mU/(g·hr) in the Antarctic soils. The activities of β-glucosidase (3.98–5.55 mU/(g·hr)) were similar in all the samples of two different origin. All the deep-sea sediment samples were shown to contain high lipase activity (666.00–799.20 mU/(g·hr)), while the Antarctic did not have any.

The kinetic ectoenzyme profiles of the samples with the addition of the corresponding carbohydrates (cellulose, chitin, olive oil) were also tested. After incubating the samples at 4°C in the dark for 30 days, the specific ectoenzyme activities of the six samples were measured. As can be seen in Fig. 1, the addition of cellulose and chitin into the deep-sea sediments significantly improved the corresponding ectoenzyme activity. The activities of β-glucosidase, β-N-acetylglucosaminidase in the deep-sea sediments were elevated for 5–10 times, 4–8 times, respectively. The addition of olive oil into the deep-sea sediments only improved lipase activity slightly because of the native high activities (666.00–799.20 mU/(g·hr)). As to the Antarctic soils, adding olive oil or cellulose had little effect on the lipase or β-glucosidase activity, except that the adding of chitin significantly improved the specific β-N-acetylglucosaminidase activity from ca. 50 to 150 mU/(g·hr).

Addition of the colloidal chitin which could serve as both C and N sources stimulated the specific ectoenzyme activities in both the deep-sea sediments and the Antarctic soils (Fig. 1). Therefore, the responses of the samples towards chitin amendments were further analyzed. The activities of the ectoenzymes (β-glucosidase, β-N-acetylglucosaminidase, lipase) were recorded on day 15 and 30 in the deep-sea sediment sample WP02-3 and Antarctic soil sample CC-TY2 after amending chitin. It was shown that the addition of chitin significantly increased all the three ectoenzyme activities in both samples (Fig. 2). After 15 days of chitin amendment into the sediment sample WP02-3, the activities of lipase and β-glucosidase increased 13 to 16 folds, respectively, while the β-N-acetylglucosaminidase activity only increased slightly. After 30 days, the production of

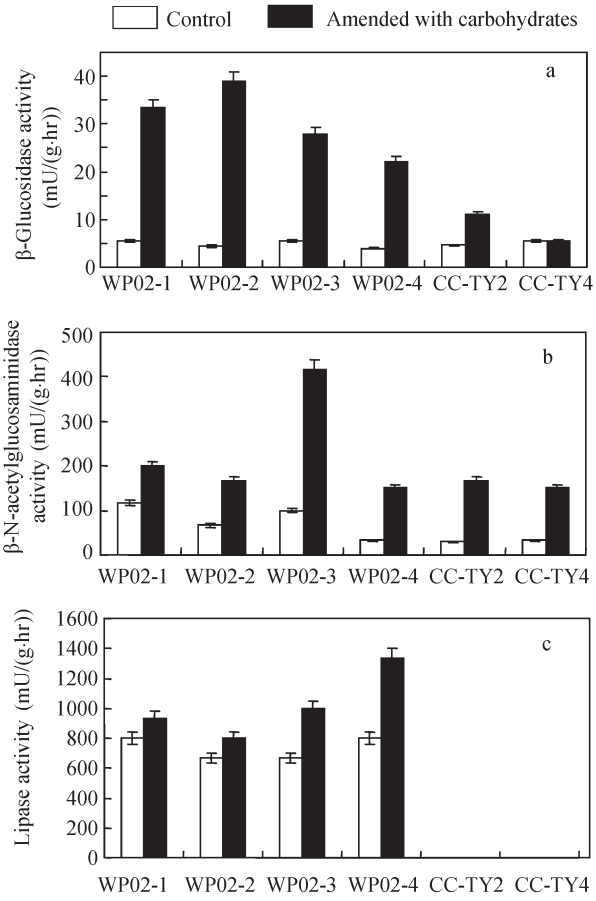

Fig. 1 Stacked bar graphs showing ectoenzyme activities. The samples were amended with the corresponding carbohydrates (cellulose (a), chitin (b), or olive oil (c)) and incubated in the dark for 30 days. Mean values from three to five experiments are shown, bars stand for standard deviations.

β-N-acetylglucosaminidase increased 20 times. The data suggested that amending of chitin stimulated the activity of the microorganisms (including different ectoenzymes) in the deep sea sediments and Antarctic soils. The numbers of bacteria in the samples before and after amending of chitin were determined by QC-PCR method. The bacterial total numbers in the WP02-3 and CC-TY2 samples were stable in the range of 10^5–10^6 copies/g, 10^6–10^7 copies/g, respectively (Fig. 2).

2.2.2 Effect of carbohydrates amendments on the bacterial community

The change of bacterial community structure before and after the amending of carbohydrates (cellulose, chitin,

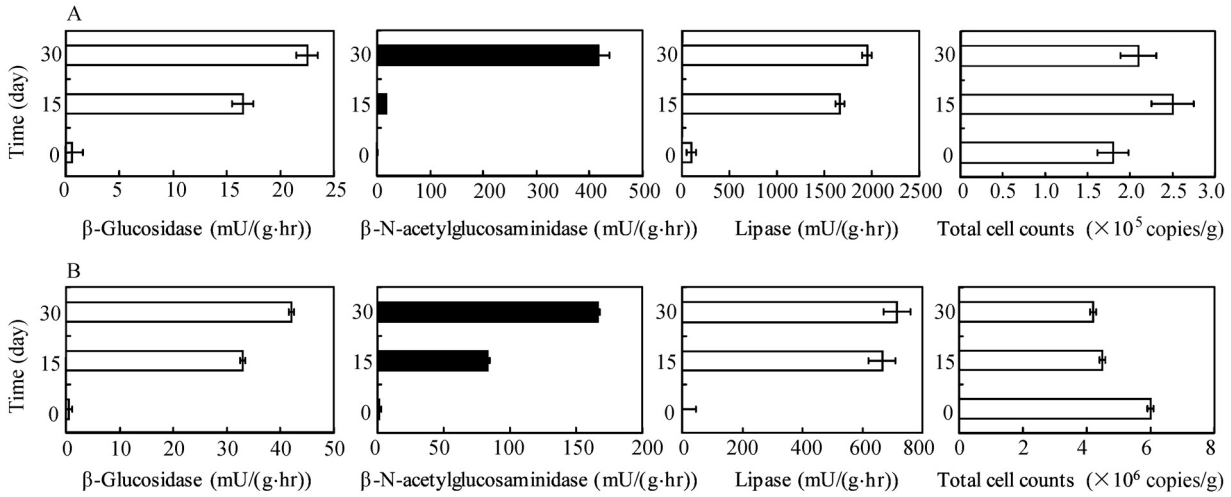

Fig. 2 Profiles of ectoenzyme activities in the deep-sea sediment sample WP02-3 (A) and the Antarctic soil sample CC-TY2 (B) amended with 1% chitin after 15 and 30 days. Bars stand for standard deviations.

olive oil) into the sediments and soils were also checked by 16S rRNA-DGGE method. The bacterial community analysis of the deep-sea sediment WP02-3 was shown here as an example. Figure 3 presents the bacterial community shifts before and after amending of three different carbohydrates (cellulose, chitin, and olive oil) into the sample WP02-3. A total of 9 PCR bands were retrieved from the DGGE gel (Fig. 3, Table 3). No heteroduplex was found among the excised bands after cloning and sequencing analyses. Two α-Proteobacteria, one β-Proteobacteria, and six γ-Proteobacteria clones were detected in the deep-sea sediment of WP02-3. Two sequences were grouped with α-Proteobacteria. Clone WP02-3-7 was most closely related (98% similar) to uncultured α-Proteobacteria KC-IT-H2. Clone WP02-3-8 were similar (98%) to the isolate *Thalassospira lucentensis*, which was isolated under oligotrophic conditions from the Mediterranean Sea (López-López et al., 2002). The other clone WP02-3-6 was grouped to β-Proteobacteria closest with one uncultured clone sequence. Six sequences clustered with the γ-Proteobacteria, which belonged to four genera (*Acinetobacter*, *Alteromonas*, *Pseudomonas*, and *Serratia*) (Table 3). Three clones (WP02-3-2, WP02-3-4, WP02-3-9) contained sequences that clustered with the *Pseudomonas* group, with high 98%–99% similarity.

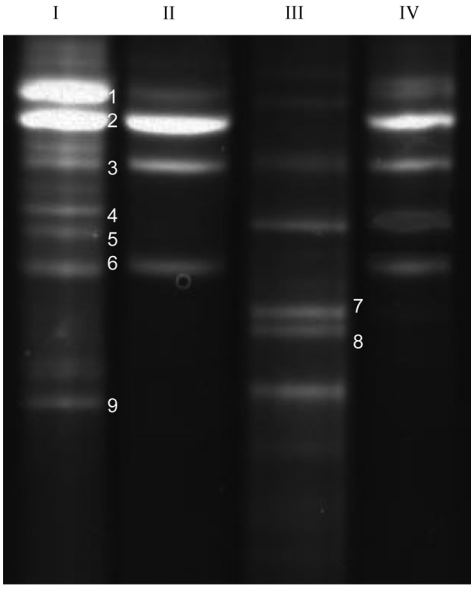

Fig. 3 DGGE profiles of the bacterial community in the sample WP02-3. The bacterial communities in the samples before and after amending with different carbohydrates on day 30 were determined. The bands which were excised from the gel for further cloning and sequencing analysis were numbered consecutively. I: sample without amending; II, III, IV: samples amending with cellulose, oilve oil, and chitin, respectively.

Table 3 DGGE band patterns in the samples and their relationship with known sequences in the database

Bands	Community profiles after amending carbohydrates				Closest related clones (Accession)	Similarity (%)	Phylum
	I	II	III	IV			
WP02-3-1	+	+	–	+	*Serratia marcescens* (U65971)	99	γ-Proteobacteria
WP02-3-2	+	+	–	+	*Pseudomonas* sp. ACP14 (AY464463)	98	γ-Proteobacteria
WP02-3-3	+	+	+	+	*Acinetobacter* sp. Hi9 (AB192395)	99	γ-Proteobacteria
WP02-3-4	+	–	+	–	*Pseudomonas fulgida* (AJ492830)	99	γ-Proteobacteria
WP02-3-5	+	–	–	+	*Alteromonas macleodii* (Y18234)	99	γ-Proteobacteria
WP02-3-6	+	+	–	+	Uncultured bacterium, isolate DGGE5 (AJ520094)	97	β-Proteobacteria
WP02-3-7	–	–	+	–	Uncultured α-Proteobacterium (AF359545)	98	α-Proteobacteria
WP02-3-8	–	–	+	–	*Thalassospira lucentensis* (AJ581988)	99	α-Proteobacteria
WP02-3-9	+	–	–	–	*Pseudonmonas putida* (X93997)	99	γ-Proteobacteria

I: sample without amending; II, III, IV: samples amending with cellulose, oilve oil and chitin, respectively.
+: band present; –: band absent.

Ten DGGE bands which represent nine clones related with γ-Proteobacteria and one closest to β-Proteobacteria were also screened in the Antarctic soil sample CC-TY2 after amending the carbohydrates, which displayed the small variation of bacterial community, mainly in the density of the bands (data not shown).

3 Discussion

The bacterial distribution is largely dependent upon the amounts of utilizable OC in the sediments, which in turn is largely controlled by the sedimentation and degradation rates in the water column (Danovaro et al., 1993). All the environmental parameters showed that the west Pacific sediments are typically oligotrophic. The limitation of organic matter could be one of the main controlling factors to the bacterial survival. Bacteria play important roles in microbial food webs under severe nutrient limitations such as the deep-sea sediments (Lochte et al., 2000). Ectoenzymes of bacteria are important parts in the food uptake process, and many bacterial ectoenzymes were studied (Eggert et al., 2000; King, 1986; Pinhassi et al., 1999; Poremba and Hoppe, l995). The majority of marine bacteria had extremely high protease and lipase activities, owing to its significance in organic matter cycling in the oceans (Martinez et al., 1996). In this study, we studied the response of the bacterial community in the deep-sea sediments and Antarctic soils towards addition of different carbohydrates. We found that the lipase activities in the deep-sea sediments studied were very high, whereas, which were little in the Antarctic soils. Meanwhile, we also found that, 43% (10 of 23) of the psychrotrophic strains isolated from the west Pacific deep-sea sediments showed lipolytic activity (Zeng et al., 2004). Low activities of β-glucosidase and β-N-acetylglucosaminidase were found in the deep-sea sediments. Our data suggested that the low activity of these enzymes might be due to that the degradation of polysaccharides is complicated by the insolubility of the substrates and inaccessibility of the glucosidic bonds. It is interesting to notice that, after amending olive oil or cellulose, the activity of the corresponding ectoenzymes (lipase or β-glucosidase) in the deep-sea sediments increased significantly, while those in the Antarctic soils remained unaffected (lipase) or with little changes (β-glucosidase). The Antarctic soils contained relatively high TOC contents than those in the deep-sea sediments (Table 2). This could explain why amending carbon sources (olive oil or cellulose) into Antarctic soils had little influence on the ectoenzyme activities comparing with those in the deep-sea sediments. Amending chitin could significantly induce the β-N-acetylglucosaminidase activities in both the deep-sea and Antarctic samples. Moreover, it was found that chitin could stimulate all the ectoenzyme activities tested (β-glucosidase, β-N-acetylglucosaminidase, lipase). Previous studies reported that cycling of nitrogen compounds was largely influenced by C/N ratio of OM in the sediments. The deep-sea sediments and Antarctic soils samples are all depleted of nitrogen. Amending of chitin, which could serve as both C and N sources, significantly triggered the benthic microbial loop function.

To investigate if the changeable profiles of ectoenzymes in the sediments and soils were the result of the changed biomass or the quantity of ectoenzyme themselves, the total bacterial counts and structure were further analyzed. It was found that the quantity of bacteria remained relatively stable before and after amending of the carbohydrates as revealed by QC-PCR. The 16S rRNA gene PCR-DGGE was used to reflect the major bacterial compositions in the samples. The PCR primers targeting the V3 region of the 16S rRNA gene were shown to produce the best DGGE profiles among all the primers tested (Yu and Morrison, 2004). Significant changes in the bacterial composition of deep sea sediments were shown before and after the carbohydrates amendments. We have analyzed the bacterial diversity in the open ocean deep-sea sediments by 16S rRNA gene library construction and sequencing, it was found that Proteobacteria division, especially γ-Proteobacteria dominated in the deep-sea open ocean sediments, and Green nonsulfur bacteria and Cytophaga-Flexibacter-Bacteroides bacteria were also detected (Xu et al., 2004). In the present study, it was found that the availability of carbohydrates contributed to their relative abundance especially γ-Proteobacteria. The bacterial communities after amending of cellulose or chitin are very similar, which enriched bands of WP02-3-2, WP02-3-3, WP02-3-6 (Fig. 3). These data suggested that cellulose and chitin stimulated the activity of the same groups of bacteria, and/or that these bacterial groups increased both cellulose and chitinase activities. In the Antarctic soils sample CC-TY2, the change in bacterial community was mainly in the density of the bands but not the structure. It indicated that bacterial types especially dominating species in the Antarctic soils were stable.

In conclusion, the ectoenzyme activity and bacterial community profiles were shown to be influenced significantly after carbohydrates amendments in the deep-sea sediments. While, the ectoenzyme activity of the Antarctic soils was only triggered by the chitin among all the carbohydrates tests. On the other hand, the bacterial community structure was shown to be more sensitive in the deep-sea sediments as compared to the Antarctic soils. In contrast, it may also be concluded that in the bacterial loop of the deep-sea sediments, there was a more sensitive response to organic matter, by regulation of enzyme production and/or changing community structure.

Acknowledgments

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