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Studies of glucosidase activities from surface sediments in mangrove swamp

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ABSTRACT

Four transects including sixteen stations were established in the Fugong mangrove (117°54′-117°55′E, 24°22′-24°24′N) of the Jiulong River Estuary, Fujian, China. Besides geochemical characterization and estimation of bacterial abundances, the distribution of α- and β-glucosidase activity was studied to explore the degradation of carbohydrates which can be expected to occur in high quantities in mangrove systems. The distribution pattern of microbial α-glucosidase and β-glucosidase activities was investigated using a fluorogenic model substrate (FMS) technique in order to allow better understanding of in situ enzyme activities, as well as their relation to bacterial biomass, metabolic activity and environmental factors in mangrove sediments. The results showed that the enzyme activities of α-glucosidase (10.83~100.86 μmol g^{-1} h^{-1}) and β-glucosidase (39.60~222.75 μmol g^{-1} h^{-1}) varied among the different stations, and the enzyme activities of β-glucosidase were higher than those of α-glucosidase at all stations. The extracellular enzyme activities were positively related to organic C, organic matter and bacterial abundance. In addition, the use of the FMS technique to measure extracellular enzyme activities of mangrove sediments could help us to evaluate their catabolic behavior in situ and so lead to a better understanding of the bacterial role in material cycle of mangrove swamp ecosystems.

1. Introduction

Mangrove is an important inter-tidal estuarine wetland, which occurs along the coastlines of many tropical countries, and mangrove forests, which, when adjacent to human activities, maybe very organic rich environments, and accumulate large quantities of anthropogenic pollutants (Tam, 1998). Enzymatic catalysis may potentially play an important role in the flow of material and energy in ecosystems. Some substance transforming processes would not be completed without extracellular enzymes (Münster, 1991), and heterotrophic microbes, especially heterotrophic bacteria, fungi are the primary producers of extracellular enzymes (Chróst, 1989). Some natural high molecular weight substances such as polysaccharide, protein and nucleic acid undergo enzymatic hydrolyzation by extracellular enzymes to form low molecular weight substances, which can then be transferred through the cell membrane and utilized(Chróst, 1989). This key biochemical process results in changes to the composition of organic matter and its biological availability(Hoppe, 1983). On the other hand, those microbes living in the sediment environment would also be fluctuated greatly by some environmental factors such as temperature, oxygen content, nutrients, and dissolved organic matter (DOM).

There is abundant organic matter in mangrove sediments which delivered to the coastal zone are often very fine-grained and can have high concentration of terrestrial organic carbon and other pollutants (Alongi, 1994; Lovelock et al., 2007). In the present study we were particularly interested in the degradation of carbohydrates, which are common structural and storage polymers in aquatic organisms and represent the major form of photosynthetically fixed carbon. From the saccharidases which take part in the carbon cycle and energy flow in mangrove swamps, we have studied α-glucosidase and β-glucosidase. Amylum and cellulose are two important polysaccharides in natural environments, including the mangrove ecosystem, and maltose and inosite are produced during their metabolism. High α-glucosidase and β-glucosidase activity mean high hydrolyzing rate to the relevant substances - maltose and inosite(Rulík and Spácil, 1999). Through the control-induce mechanism, variation in the ingredients of DOM can control the synthesis of extracellular enzymes in the cell, and thus promote bacterial adaptation varying with the environment.

Fluorogenic substrates have been widely used since the early 1980s to assess extracellular enzyme activity in water and sediment (Chróst and Velimirov, 1991). Extracellular enzyme activity can be measured by monitoring the hydrolysis of specific fluorogenic substrates which produce highly fluorescent end products, such as 7-amino-4-methyl-coumarin (AMC) or methylumbelliferone (MUF) which can be easily quantified by fluoro- etry (Hoppe, 1983). The use of fluorogenic substrate analogs have yield new insights into the regulation of sediment carbon flow (Hakulinen et al., 2005; Wittmann et al., 2004).

In this study, we evaluated the physical, chemical and microbiological factors involved in regulation enzymatic reactions in mangrove sediments, and applied the FMS method for assaying α-glucosidase and

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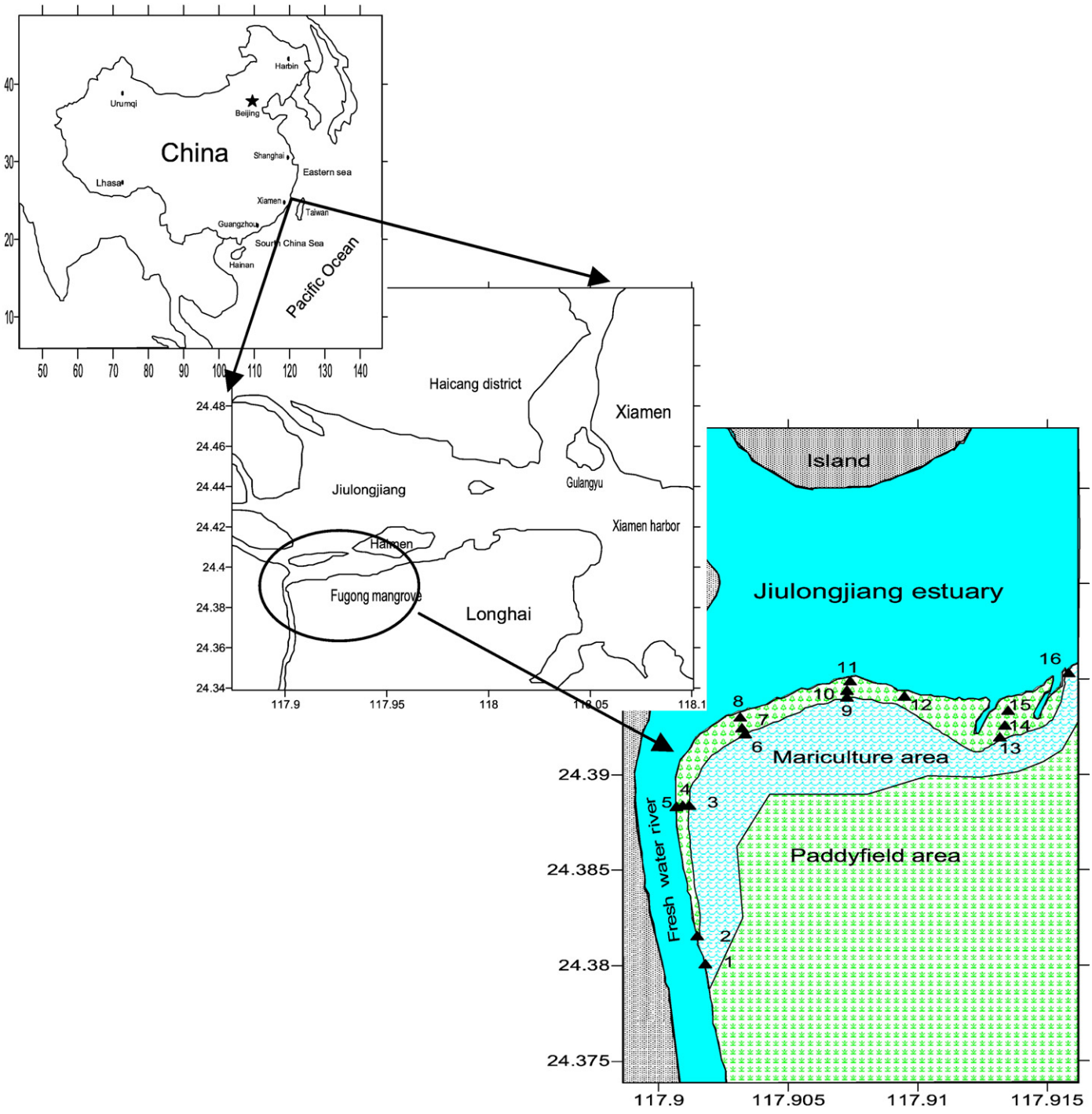

Fig. 1. Geographical location showing sampling stations of the mangrove swamp in this study.

β-glucosidase activities characterized with respect to spatial parameters including, organic carbon, organic matter, electron transport system activity (ETSA).

2. Materials and methods

2.1. Study sites and sampling

The Jiulong River Estuary is one of the largest river/estuary systems in south China, with a length of 285 km and an area of 14741 km^2. It is the major source of freshwater to Xiamen coastal seas. The estuary is characterised by intense agricultural activities and rapid industrial development, and represents a potential major source of pollutants in the form of the Xiamen Economic Special Zone which, since 1986, has resulted in significant stress to Xiamen Harbour and its surrounding environments (Maskaoui et al., 2005), and to a steady increase in organic pollution in Xiamen coastal waters over recent years (Hong et al., 1995). Fugong mangrove (117°54′-117°55′E, 24°22′-24°24′N)(Ge et al., 2005) is located to the south of the Jiulong River Estuary, and is a mangrove nature conservation area of Fugong, Fujian Province of China (Lin et al., 2005). The area of the mangrove community is about 670 km^2 (Chen et al., 1996). On account of its geographical location, it has been affected by ship wastes, and the discharge of industrial, livestock, mariculture and household waste and wastewater.

Table 1
The physical, chemical and biological properties of mangrove sediments

Site	pH	Salt (‰)	Fine sand (%)	Temp (°C)	OC (%)	OM (%)	Bacteria ($\times 10^6$ CFU g^{-1})	ETSA (μgO_2 g^{-1} min^{-1}
1	6.73	10.0	55.00	25.60	1.74	3.00	206.50	47.09
2	6.86	12.0	54.00	24.10	1.60	2.76	143.50	82.48
3	7.06	20.0	49.00	24.20	1.85	3.19	258.50	55.43
4	6.57	19.5	51.00	24.10	2.33	4.01	279.00	39.44
5	6.49	16.0	57.00	24.00	1.85	3.20	500.50	49.93
6	6.36	20.5	70.00	23.40	3.41	5.87	274.00	84.98
7	6.50	24.0	54.00	23.00	1.81	3.13	333.00	57.39
8	6.60	20.0	51.00	22.10	1.59	2.73	205.00	61.66
9	6.73	21.5	48.40	25.30	3.17	5.47	140.00	83.05
10	6.49	29.0	58.40	24.50	1.52	2.62	434.00	103.23
11	6.48	20.0	60.60	22.30	1.74	3.01	383.50	55.02
12	6.65	20.5	49.40	25.80	1.13	1.95	398.00	70.58
13	6.62	18.0	54.00	25.30	1.52	2.61	371.00	91.61
14	6.44	24.0	61.40	24.40	3.88	6.70	450.00	126.79
15	6.65	21.0	55.40	22.30	2.43	4.18	669.00	102.64
16	6.78	23.0	57.00	25.20	1.31	2.26	450.00	100.79

OC, Organic carbon; OM, Organic matter.

Four transects including sixteen stations running across different parts of the tidal coastline/zone were selected (Fig. 1). Station 1 was mud-flats which have not been covered with mangrove forests; Station 2 was located at the start of the mangrove; Transect 1 (stations 3, 4 and 5) was the nearest the river mouth; Transect 2 (stations 6, 7 and 8) was sited at the top of the Jiulong Estuary mangrove; Transect 3 (stations 9, 10 and 11) was at the middle of the mangrove; Station 12 was also mud-flats among the mangroves which were not covered with plants; Transect 4 (station 13, 14 and 15) was a more wide mangrove region along the tidal channel; and Station 16 was at the end of the mangrove swamps and was not covered with plants. The dominant species in this region of mangrove forest is *Kandelia candel* (Chen et al., 1996).

Mangrove swamp surface sediments were sampled in November 2006. The surface sediment samples (0-5 cm) were randomly collected, in triplicate, from an area of around 1 m^2 at the each station during low tide (Guo et al., 2005). We mixed them together as a sample from one station. 500 g sediment samples were collected in every station. Sediment samples were packed on-site into sealed polythene bags, and transported to the laboratory in a box with ice (Al-Sayed et al., 2005) for ectoenzyme activities and microbiological analysis within 24 h of collection.

2.2. Physical, chemical and microbiological factors

Temperature, pH and salinity measurements were made in situ using a digital glass thermometer, a battery operated pH meter, and a refractometer, respectively. Physical and chemical analysis was performed on air-dried and sieved (<2 mm) sediment samples. Sediment organic C and organic matter were determined using the dichromate oxidation method (Andreoni et al., 2004).

The spray-plate method was used to enumerate cultivable heterotrophic bacteria (Kästner et al., 1994). 10 g of each sediment sample was suspended in 90 mL sterilised in situ seawater in a 250 mL conical flask including several glass beads for 1 h on a shaker bed, in order to separate bacteria from sediment particles. 1 mL of the well mixed liquid obtained was then 10-fold serially diluted in 9 mL sterilised in situ seawater in tubes. Appropriate dilutions were plated onto 1% 2216E agar medium (5 g Peptone, 1 g Yeast Extract, 0.01 g $FePO_4$, 1.0% Agar in 1000 mL of 0.45 μm Millipore-filtered seawater, pH 7.6-7.8, autoclaved at 121 °C for 20 min) for heterotrophic bacterial counts (Maeda et al., 2001; Martinez et al., 1996; Zheng et al., 2005). The plates were incubated at 25 °C for 8 days and then the numbers of colony-forming units (CFU) counted (Andreoni et al., 2004); all enumerations were performed with three replicates (Margesin et al., 2000).

2.3. Measurements of in situ extracellular enzyme activity

The stock and the working solutions of enzyme substrates and the fluorogenic standard MUF (Sigma, Chemical) were prepared in ethylene glycol monomethyl ether, due to the low solubility of the compounds in water (Palmroth et al., 2006).

Standards of MUF were prepared in duplicate in sediment slurries separately for each sampling station to take into account possible quenching of the fluorescence by sediment particles. The correlation coefficient of the calibration curve was r=0.998 for the model MUF. Excellent linear correlation (r>0.99) was shown over the concentration range tested.

The activity of microbial extracellular enzymes in the mangrove sediments was determined as follows: A 10 g wet sediment sample was shaken (150 rpm) for 1 hour with 90 mL sterile filtered (0.2 μm) in situ seawater (King, 1986) (6.5 pH and 19.0‰ salinity) in a 250 mL Erlenmeyer flask at 25 °C. 1 mL sediment suspension was transferred to 1.5 mL centrifugation tube and amended with MUF-α-glucosidase and MUF-β-glucosidase (Sigma, Chemical) in the final concentrations 250 μmol/L substrate, which had been tested in advance (Zheng et al., 2002). The samples were incubated in the dark at 25 °C (in situ temperature) with different MUF substrates. After 1 hour of incubation, the slurry with fluorescent substrate was boiled at 100 °C for 10 min to terminate the enzyme reaction, these sediment slurry samples were then cooled rapidly (2 min) in a cold water bath and placed in a clinical centrifuge (King, 1986; Parham and Deng, 2000; Sakami et al., 2005). Every sediment slurry sample in tubes were centrifuged at 2500 ×g for 15 min (Belanger et al., 1997). The fluorescence of the hydrolysed model substrates in the supernatant was measured with a fluorometer immediately at 355 nm excitation and 460 nm emission. The rates at which enzyme substrate was hydrolysed [μmol MUF/1×g sediment×h] were calculated for dry sediment to enable comparison of sediment samples with different moisture contents.

All reagents used were analytical grade (Sigma). Solutions of reagents were made with ultra-pure water from a Millipore-Q water purification system. All glassware was cleaned with detergent, rinsed with Millipore-Q water, and autoclaved at 121 °C for 20 min before use (Parham and Deng, 2000).

2.4. Determination of ETSA

Packard's method was applied to measure phytoplankton ETSA. The main agent used was 2-p-iodophenyl-3-p-nitrophenyle-5-phenyl tetrazolium chloride (INT) (Sigma, Chemical). The measurement procedure was as follows.

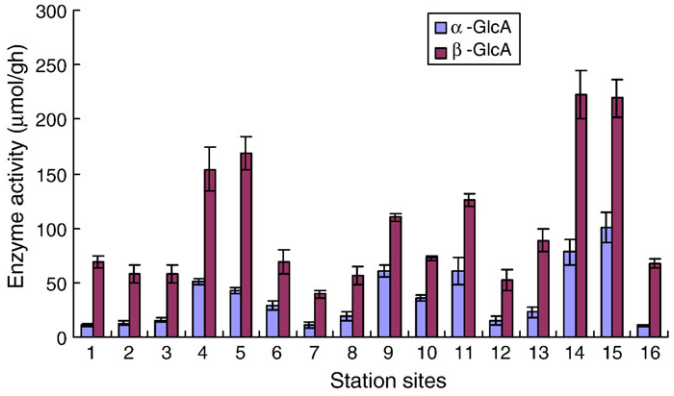

Fig. 2. Comparison of extracellular enzyme activities for α-glucosidase and β-glucosidase at different stations in mangrove sediments.

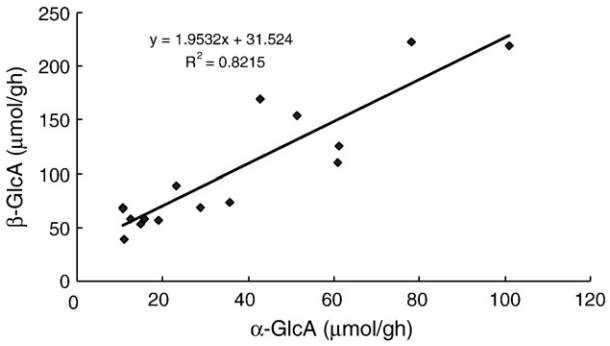

Fig. 3. The relationship between α-GlcA and β-GlcA.

250 μL of sediment suspension (from 2.2 sediment blending) together with 500 μL INT were allowed to interact for 30 min at room temperature (25 °C) in the dark, then 50 μL of formalin was used to stop the reaction. The mixture was centrifuged (10,000 ×g/min, 5 min) in a Hitachi SCR20BC centrifuge. The supernatant was removed, and 500 μL methanol added into the reaction tube. Centrifugation for 5 min at 10,000 ×g with a micro-12 centrifuge was repeated after the mixture was homogenized, and then the reaction tube was put into an ice bath. Absorbance was measured at 495 nm using a spectrophotometer. ETSA in $\mu gO_2/g/min$ was calculated from the following formula (Huang et al., 2005):

$$\mathrm{ETSA}(\mu gO_2 g^{-1} min^{-1}) = (\mathrm{Ab}/15.9) \times \mathrm{V} \times 32/2 \times 1/\mathrm{S.t}$$

where, Ab is absorbance; V is final reaction volume (mL) (methanol volume); S is the sample amount (g or mL); t is the incubation time (min); 32/2 is constant; and 15.9 is the molar absorbance of formazan.

2.5. Data analysis

Unless otherwise specified, all results reported are averages of triplicate determinations. All results are expressed on a sediment dry weight basis. Moisture was determined after drying at 105 °C for 12 h. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 13.0 for windows (SPSS Inc, Chicago, IL, USA).

3. Results

3.1. Physico-chemical and microbiological properties of mangrove sediments

Table 1 summarizes different abiotic and biotic factors characterizing mangrove sediments.

Major variations in mangrove sediment properties have been observed in salinity, organic C and others. The pH values and temperature differed only slightly in all stations. The pH values, ranging from 6.36-7.06, indicated a sub- to moderately-acid mangrove sediment. Differences of pH amounted only 0.7. The sediment pH can provide valuable information on the availability and toxicity of several elements, including Fe, Al, Mn, Cu, Cd and others, to plants and microorganisms. At the same time, it also can provide a suitable condition for sediment extracellular enzymatic activities.

Temperature appeared to be rather similar in the 16 stations. Temperatures of 22.10 °C, 22.30 °C and 22.30 °C were measured at some offshore stations (8, 11 and 15) inshore stations had temperatures of 23.40 °C, 25.30 °C and 25.30 °C (6, 9 and 13). There was no significant difference between sediment particle size and temperature at any of the stations, indicating the same temperature conditions in these areas. In addition, all the stations also showed a salinity variation from 10.0‰ to 29.0‰. Salinity is one of the most variable environmental factors in mangrove sediments. Salinity periodically fluctuates in response to tides and freshwater inputs from rivers and landward discharges (Tam et al., 2002).

The superficial sediment was very rich in OM (mean of 3.54 ± 1.36%). Moreover, the concentration OM fluctuated between 1.95% and 6.70%, in addition, the concentration of OC varied from 1.13% to 3.88%, respectively.

The number of heterotrophs in mangrove sediments significantly varied between 1.40×10^8 and 6.69×10^8 CFU g^{-1} among different stations, Heterotrophic bacterial numbers were the highest at station 15.

Highest ETSA values of 126.79 $\mu gO_2\ g^{-1}\ min^{-1}$ were found at station 14, a station with a high proportion of fine-grained sediment and organic content. Lowest ETSA occurred at station 4, where the values of these parameters were much lower.

3.2. The distribution of α-GlcA and β-GlcA and their relation to extracellular enzyme activities

The mean extracellular enzyme activity of α-glucosidase per g mangrove sediment was around 36.13 μmol $g^{-1}\ h^{-1}$ and ranged from 10.63 to 100.86 μmol $g^{-1}\ h^{-1}$. These values were significantly lower than those for the β-glucosidase, which had mean extracellular enzyme activity of 102.08 μmol $g^{-1}\ h^{-1}$ and ranged from 39.60 μmol $g^{-1}\ h^{-1}$ to 222.75 μmol $g^{-1}\ h^{-1}$. The results indicated that significant spatial variations occurred both between transects and between stations (Fig. 2). The highest values of α-glucosidase were found at stations 14 and 15, in addition, and the same was true for the β-glucosidase in the mangrove sediments. The lowest values of enzyme activities for α-glucosidase and β-glucosidase were found at station 7. Station 12 has similar values like stations 1,2, 3 and 8. The highest extracellular enzyme activities were found at transect 4 (station 13, station14 and station 15) where the density of covering with mangrove plants was highest, which also had a large number of heterotrophic bacteria and a high organic C content.

Fig. 2 also reveals that different enzyme activities of α-glucosidase and β-glucosidase were found at the same transect. In transect 1, there were higher glucosidase activities in the offshore stations 4 and 5 than that in inshore station 3. The same tendency had been found in transect 4 that high glucosidase activities in offshore stations 14 and 15 in comparison to the inshore values at station 13.

The relationship between the enzyme activities of α-glucosidase and β-glucosidase at the 16 stations in the mangrove sediments are shown in Fig. 3. When comparing α-GlcA with β-GlcA, a similar tendency was seen at all stations, and significant positive relationship ($r=0.91$, $P<0.01$) was found between them.

The relationships between various sediment characteristics and the enzyme activities of α-glucosidase and β-glucosidase are indicated in Table 2. No correlation was found between α-GlcA and pH, salinity, particle size, temperature and ETSA at the different stations, and the same results were seen between β-GlcA and these sediment characteristics. However, the α-GlcA was positively related to organic C ($r=0.60$, $P<0.01$), organic matter ($r=0.60$, $P<0.01$) and the number of cultivable heterotrophic bacteria ($r=0.52$, $P<0.05$). The organic C ($r=0.56$, $P<0.05$), organic matter ($r=0.56$, $P<0.05$) and

Table 2
Analysis of relationships between the extracellular enzyme activity and environmental factors

		pH	Salt	Size	Temp	OC	OM	Bacteria	ETSA	α-GlcA	β-GlcA
α-GlcA	r	-0.34	0.27	0.19	-0.35	0.60	0.60	0.52	0.36	1.00	0.91
	p	0.20	0.31	0.48	0.18	0.01**	0.01**	0.04*	0.17		0.00**
β-GlcA	r	-0.33	0.09	0.20	-0.22	0.56	0.56	0.59	0.30	0.91	1.00
	p	0.21	0.74	0.45	0.42	0.03*	0.02*	0.02*	0.26	0.00**	

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Table 3
Comparison of enzymatic activities (α-GlcA and β-GlcA) in water bodies and sediments environment from various studies

Habitat	Enzymatic activities						Reference
	α-GlcA	β-GlcA	phosphatase	LAP[#]	Chitobiase	Lipase	
Water body environment							
Nant Waen, Wales, GB		0.05-0.25[a]					1
River Clywedog, Wales, GB		0.02-0.48[a]					1
Breitenbach, Germany		0.54-0.77[a]					2
Sitka stream, interstitial water	0.002-0.24[a]	0.002-0.25[a]					3
Natural waters, Germany		1.1-291[c]	9.2 – 320[c]	5.8 – 1560[c]			4
Kiel Fjord surface water	0.01[a]	0.005[a]		0.05[a]	0.005[a]		5
Taiwan strait, China		0.031-8.07 [c]summer 0.34-1.89[c] winter					6
Sediment environment							
Oberer Seebach, Austria	5.2-67.4[b]	8.3-179.7[b]					2
Baltic Sea, 3.5 m average		1-55[d]		5-140[d]			7
Gokasho Bay, Aquaculture area, 17 m		13-206[d]		15-137[d]			7
Sitka stream, bed sediments	0.66-3.36[b]	1.02-9.1[b]					3
Deep sea sediments	0.006[b]	0.089[b]		0.05[b]	0.36[b]	0.075[b]	5
Intertidal sediments		23.2[2]			354[b]		8
superficial sediment of Eutrophic lake	15.1 ± 6.2[d]	30.7 ± 11.0[d]					9
Mangrove sediments	10.83-100.86 [b]	39.60-222.75 [b]					This study

[a]μmol l^{-1} h^{-1}; [b]μmol g^{-1} h^{-1}; [c]nmol l^{-1} h^{-1}; [d]nmol g^{-1} h^{-1}; [#]Leucine aminopeptidase 1, (Jones and Lock, 1989); 2, (Marxsen and Fiebig, 1993); 3, (Rulík and Spácil, 1999); 4, (Hendel and Marxsen, 1997); 5, (Boetius, 1995); 6, (Zheng et al., 2002); 7, (Sakami et al., 2005); 8, (King, 1986); 9, (Mallet and Debroas, 2001).

bacterial numbers (r=0.59, P<0.05) showed a similar pattern with β-GlcA at all stations.

4. Discussion

In the mangrove environment, it can be expected that glucosidase activities have a significant importance in degradation in carbohydrates. The extracellular hydrolytic activity for converting high molecular weight organic compounds to smaller molecules is performed predominantly by bacterial extracellular enzymes (Belanger et al., 1997). In order to fully understand their ecological significance, it is necessary to measure extracellular enzyme activity rates in situ (Chróst and Velimirov, 1991). In this study, the use of a fluorogenic model substrate and slurry conditions cause this assay to provide a reasonable estimate of potential in situ extracellular enzyme activities. In addition, use of these fluorometric methods, which are more sensitive than colorimetric assays, will reduce the incubation times necessary for extracellular enzyme activity assays from days to hours. So, this method is most suitable for measuring of extracellular enzyme activity in natural samples (Hendel and Marxsen, 1997).

Potential extracellular enzyme activities may be interpreted as approximate indicators of the relative importance of various organic carbon components to bacterial metabolism (Sinsabaugh and Linkins, 1988). Unfortunately, no related reported about α-glucosidase and β-glucosidase activities with FMS measured have been published at present in mangrove ecosystem. Most published values of EEA measured either in lake ecosystems or in marine ecosystems (Mallet and Debroas, 2001). The enzyme activities presented in this work are compared with published data from other water bodies and sediments in Table 3. From this table, the two glucosidase activities are higher in the mangrove ecosystem than those in the other ecosystems. It would maybe contribute to mangrove's unique features of high primary productivity, abundant detritus, rich organic carbon and anoxic/reduced conditions (Bernard et al., 1996). In the other hand, in order to fully understand the characteristic of extracellular enzyme in mangrove ecosystem, it is also necessary to consider the other enzyme activities, e.g., phosphatase, peptidase from the study area and other estuarine system.

If the production of hydrolytic enzymes is induced by their respective organic substrates, the potential enzyme activities are related to substrate concentration and activity profiles should mirror the distribution of the substrates (Boetius, 1995). In our results, it shows that α-glucosidase presented low values of activity at all the stations in comparison with the β-glucosidase activity tested. It would have been attributed to the idea that OM reaching sediment would be low in easily degradable organic compounds such as starch, leading to a decreased requirement for amylase activity, whereas the other degradable compounds such as cellulose derived from the leaf fall and decaying materials of mangrove plants which are commonly found within this area are more to reach the sediments. It also suggests that cellulose depolymerisation is more active in mangrove sediment. The enzyme activities of α-glucosidase and β-glucosidase were higher in the offshore stations of transects 1 (stations 4 and 5) and 4 (stations 14 and 15), respectively, in comparison to the inshore values in the same transect (station 3 on transect 1 and station 13 on transect 4). This suggested that there was more organic matter offshore than inshore. This organic matter was not only from land (including anthropogenic pollutants), but also from the marine (including ship transport).

Extracellular enzyme activities in the natural environment, including mangrove swamps, are influenced strongly by a wide variety of abiotic and biotic factors including pH, temperature, sediment type, organic and inorganic matter, all types of nutrients, sediment toxicity, physico-chemical properties, microbial number and status (Andreoni et al., 2004; Margesin et al., 2000; Sakami et al., 2005). The chemical and physical properties of a sediment as well as the evaluation of its degree of pollution may help to estimate the impact of pollutants on the quality of the soil under investigation, if they are complemented with the measurement of biological properties (Margesin et al., 2000). The degradation of organic material is a common feature of heterotrophic bacteria via the production of hydrolytic enzymes. Therefore, data related to different environmental factors can be compared with the production of these enzymes. The pH, salinity and temperature optima would tend to reflect the in situ values for α-glucosidase and β-glucosidase activities. α-glucosidase and β-glucosidase would effect on organic carbon cycle in mangrove ecosystem. In our results, none of these investigated sediment parameters (except the organic matter content) had a predominant influence on glucosidase activities, It would maybe attribute to no obvious change value of pH, temperature, salinity, particle size in different stations, also suggest that these are not the limiting factors to α-GlcA and β-GlcA in our study areas. No correlation was found between ETSA and enzyme activities, it would maybe contribution of impact from not only marine bacteria but also the phytoplankton, zooplankton and other organisms in mangrove ecosystem. The absence of the correlation between both parameters also can be an indication that glucosidase and metabolic activities were regulated by different mechanisms.

On the contrary, there was significant correlation between α-GlcA and β-GlcA. In addition, there were also correlations between enzyme activities and organic C, organic matter and bacterial numbers. Microorganisms play the key role in the decomposition processes of organic matter in mangrove ecosystem. The microbial decomposition of the more resistant organic matter will be considerably stimulated by the availability of easily decomposable organic substances. Heterotrophic bacteria are the major producers of the enzymes (Chróst, 1989). They were able to react with stimulation of enzyme production when decomposable organic material became available. The sediment with high organic C stimulated microbial activity and provided a more conductive environment for enzyme synthesis and accumulation in the sediment matrix of the mangrove(Dinesh et al., 1998).This suggests that predominantly active bacteria are closely related with extracellular enzyme activities. It would also be hypothesized, therefore, that the potential for decomposition of particulate organic matter in natural sediments is related to bacterial growth and multiplication. From the results of this investigation, there was a close correlation between availability of organic substrates and the production of microbial hydrolytic enzyme. Thus, the idea of a close correlation between potential enzyme activities and the availability of organic substances in mangrove sediments can be supported. However, there are low correlation coefficients between extracellular enzyme activities and total number of cultivable heterotrophic bacteria although they are significant ($P<0.05$). It would attribute to bacteria may not be the sole source of extracellular glycosidase activities, and invertebrate animals, fungi, and other eukaryotes (diatoms, protozoa etc.) must be considered as possible enzyme producers (Vrba et al., 2004). On the other hand, it also suggests that not all the cells of planktonic bacteria are active. If the other producers of extracellular enzymes may contribute to the total enzyme activity detected in all the samples, they may spoil any correlation between bacterial number and enzyme activity (Vrba et al., 2004). As a direct consequence of our findings, future studies would be carried out in mangrove swamp ecosystem.

5. Conclusions

(1) The use of a fluorogenic model substrate technique is sensitive enough to measure extracellular enzyme activities of α-glucosidase and β-glucosidase which effect on the bacterial role in carbon cycle of mangrove sediments.
(2) A significant positive relationship between the enzyme activities of α-glucosidase and β-glucosidase was found at the 16 stations, and β-glucosidase was more active than α-glucosidase in the mangrove sediments.
(3) The activities of α-glucosidase and β-glucosidase were positively related to organic C, organic matter and bacterial abundance, but no correlation was found between extracellular enzyme activities and the other environmental factors. Future studies will, hopefully, clarify the effect of various environmental factors, including physico-chemical properties and biological characters, on extracellular enzyme activity.

By utilizing the information obtained from enzyme activities of α-glucosidase and β-glucosidase, efforts are being made to determine other extracellular enzyme activities would help us to evaluate their in situ catabolic behavior and lead to a better understanding of the bacterial role in material cycle of mangrove swamp ecosystems.

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References

Al-Sayed, H.A., Ghanem, E.H., Saleh, K.M., 2005. Bacterial community and some physico-chemical characteristics in a subtropical mangrove environment in Bahrain. Marine Pollution Bulletin 50, 147–155.
Alongi, D., 1994. The role of bacteria in nutrient recycling in tropical mangrove and other coastal benthic ecosystems. Hydrobiologia 285, 19–32.
Andreoni, V., Cavalca, L., Rao, M.A., Nocerino, G., Bernasconi, S., Dell'Amico, E., Colombo, M., Gianfreda, L., 2004. Bacterial communities and enzyme activities of PAHs polluted soils. Chemosphere 57, 401–412.
Belanger, C., Desrosiers, B., Lee, K., 1997. Microbial extracellular enzyme activity in marine sediments: extreme pH to terminate reaction and sample storage. Aquatic Microbial Ecology 13, 187–196.
Bernard, D., Pascaline, H., Jeremie, J.J., 1996. Distribution and origin of hydrocarbons in sediments from lagoons with fringing mangrove communities. Marine Pollution Bulletin 32, 734–739.
Boetius, A., 1995. Microbial hydrolytic enzyme activities in deep-sea sediments. Helgolander Meeresunters 11, 177–187.
Chen, X.Y., Lin, P., Lin, Y.M., 1996. Mating systems and spontaneous mutation rates for chlorophyll-deficiency in populations of the mangrove Kandelia candel. Hereditas 125, 47–52.
Chróst, R.J., 1989. Characterization and significance of β-glucosidase activity in lake water. Limnology and Oceanography 34, 660–672.
Chróst, R.J., Velimirov, B., 1991. Measurement of enzyme kinetics in water samples: effect of freezing and soluble stabilizer. Marine Ecology-Progress Series 70, 93–100.
Dinesh, R., Shome, B.R., Shome, R., Bandyopadhyay, A.K., 1998. Soil enzymes in the mangroves: Activities and their relation to relevant soil properties. Current Science 75, 510–512.
Ge, J.P., Cai, B.Y., Ping, W.X., Song, G., Ling, H.Z., Lin, P., 2005. Mating system and population genetic structure of Bruguiera gymnorrhiza (Rhizophoraceae), a viviparous mangrove species in China. Journal of Experimental Marine Biology and Ecology 326, 48–55.
Guo, C.L., Zhou, H.W., Wong, Y.S., Tam, N.F., 2005. Isolation of PAH-degrading bacteria from mangrove sediments and their biodegradation potential. Marine Pollution Bulletin 51, 1054–1061.
Hakulinen, R., Kähkönen, M.A., Salkinoja-Salonen, M., 2005. Vertical distribution of sediment enzyme activities involved in the cycling of carbon, nitrogen, phosphorus and sulphur in three boreal rural lakes. Water Research 39, 2319–2326.
Hendel, B., Marxsen, J., 1997. Measurement of low-level extracellular enzyme activity in natural waters using fluorigenic model substrates. Acta Hydrochimica et Hydrobiologica 25, 253–258.
Hong, H.S., Xu, L., Zhang, L., Chen, J.C., Wong, Y.S., 1995. Environmental fate and chemistry of organic pollutants in the sediments of Xiamen and Viictoria Harbours. Marine Pollution Bulletin 31, 229–236.
Hoppe, H.G., 1983. Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. Marine Ecology-Progress Series 11, 299–308.
Huang, B.Q., Hong, H.S., Xu, X.Z., 2005. Study on respiratory electron transport system (ETS) of phyto-plankton in Taiwan Strait and Xiamen Harbour. Chinese Journal of Oceanology and Limnology 23, 176–182.
Jones, S.E., Lock, M.A., 1989. Hydrolytic extracellular enzyme activity in heterotrophic biofilms from two contrasting streams. Freshwater Biology 22, 289–296.
Kästner, M., Breuer-Jammali, M., Mahro, B., 1994. Enumeration and characterization of the soil microflora from hydrocarbon-contaminated soil sites able to mineralize polycyclic aromatic hydrocarbons (PAH). Applied Microbiology and Biotechnology 41, 267–273.
King, G.M., 1986. Characterization of beta-Glucosidase Activity in Intertidal Marine Sediments. Applied and Environmental Microbiology 51, 373–380.
Lin, X., Huang, Y., Fang, M., Wang, J., Zheng, Z., Su, W., 2005. Cytotoxic and antimicrobial metabolites from marine lignicolous fungi, Diaporthe sp. FEMS Microbiology Letters 251, 53–58.
Lovelock, C.E., Feller, I.C., Ellis, J., Schwarz, A.M., Hancock, N., Nichols, P., Sorrell, B., 2007. Mangrove growth in New Zealand estuaries: the role of nutrient enrichment at sites with contrasting rates of sedimentation. Oecologia 153, 633–641.
Maeda, M., Mizuki, E., Hara, M., Tanaka, R., Akao, T., Yamashita, S., Ohba, M., 2001. Isolation of Bacillus thuringiensis from intertidal brackish sediments in mangroves. Microbiology Research 156, 195–198.
Mallet, C., Debroas, D., 2001. Regulation of b- and a-Glycolytic Activities in the Sediments of a Eutrophic Lake. Microbial Ecology 41, 106–113.
Margesin, R., Zimmerbauer, A., Schinner, F., 2000. Monitoring of bioremediation by soil biological activities. Chemosphere 40, 339–346.
Martinez, J., Smith, D.C., Steward, G.F., Azam, F., 1996. Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. Aquatic Microbial Ecology 10, 223–230.
Marxsen, J., Fiebig, D.M., 1993. Use of perfused cores for evaluating extracellular enzyme activity in stream-bed sediments. FEMS Microbiology Ecology 13, 1–12.
Maskaoui, K., Zhou, J.L., Zheng, T.L., Hong, H., Yu, Z., 2005. Organochlorine micropollutants in the Jiulong River Estuary and Western Xiamen Sea, China. Marine Pollution Bulletin 51, 950–959.
Münster, U., 1991. Extracellular enzyme activity in eutrophic and polyhumic lakes. In: Chróst (Ed.), Spring-Verlag, New York. 60-83 pp.

Palmroth, M.R., Langwaldt, J.H., Aunola, T.A., Goi, A., Munster, U., Puhakka, J.A., Tuhkanen, T.A., 2006. Effect of Modified Fenton's Reaction on Microbial Activity and Removal of PAHs in Creosote Oil Contaminated Soil. Biodegradation 17, 29–39.

Parham, J.A., Deng, S.P., 2000. Detection, quantification and characterization of beta-glucosaminidase activity in soil. Soil Biology & Biochemistry 32, 1183–1190.

Rulík, M., Spácil, R., 1999. Assessment of extracellular enzyme activities of α- and β-glucosidase in sediments of a small lowland stream (sitka stream, czech republic). Acta Universitatis Palackianae Olomucensis Facultas Rerum Naturalium 37, 99–105.

Sakami, T., Yokoyama, H., Ishihi, Y., 2005. Microbial hydrolytic enzyme activity in the sediments of a net cage aquaculture area. Fisheries Science 71, 271–278.

Sinsabaugh, R.L., Linkins, A.E., 1988. Exoenzyme activity associated with lotic epilithon. Freshwater Biology 20, 249–261.

Tam, N.F.Y., 1998. Effects of wastewater discharge on microbial populations and enzyme activities in mangrove soils. Environmental Pollution 102, 233–242.

Tam, N.F.Y., Guo, C.L., Yau, W.Y., Wong, Y.S., 2002. Preliminary study on biodegradation of phenanthrene by bacteria isolated from mangrove sediments in Hong Kong. Marine Pollution Bulletin 45, 316–324.

Vrba, J., Callieri, C., Bittl, T., Simek, K., Bertoni, R., Filandr, P., Hartman, P., Hejzlar, J., Macek, M., Nedoma, J., 2004. Are bacteria the major producers of extracellular glycolytic enzymes in aquatic environments? International Review Hydrobiology 89, 102–117.

Wittmann, C., Kähkönen, M.A., Ilvesniemi, H., Kurola, J., Salkinoja-Salonen, M.S., 2004. Areal activities and stratification of hydrolytic enzymes involved in the biochemical cycles of carbon, nitrogen, sulphur and phosphorus in podsolized boreal forest soils. Soil Biology and Biochemistry 36, 425–433.

Zheng, T.L., Hong, H.S., Wang, F., Maskaoui, K., Su, J., Tian, Y., 2002. The distribution characteristics of bacterial beta-glucosidase activity in Taiwan strait. Marine Pollution Bulletin 45, 168–176.

Zheng, T.L., Su, J.Q., Maskaoui, K., Yu, Z.M., Hu, Z., Xu, J.S., Hong, H.S., 2005. Microbial modulation in the biomass and toxin production of a red-tide causing alga. Marine Pollution Bulletin 51, 1018–1025.