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The distribution characteristics of bacterial β-glucosidase activity in Taiwan strait

Tianling Zheng [a,*], Huasheng Hong [b], Fei Wang [b], Khalid Maskaoui [a], Jianqiang Su [a], Yun Tian [a]

[a] *Institute for Applied and Environmental Microbiology, School of Life Sciences, Xiamen University, Xiamen 361005, China*
[b] *Key Laboratory of Marine Environmental Science of SED, Xiamen 361005, China*

Abstract

Twenty stations were established in the near-shore regions of South Fujian Shoal (116°10′–119°00′ E, 21°20′–24°10′ N) on summer and winter cruises during the period from August 1997 and February to March 1998. The distribution pattern of marine bacterial β-glucosidase activity (β-GlcA) has been investigated by using fluorogenic model substrate (FMS) technique in order to have better understanding of the β-GlcA, as well as its relation to marine bacterial biomass, productivity and environmental factors in Taiwan strait. The results showed that: (1) In summer, the average of β-GlcA at the Southern stations of Taiwan strait was 1.94 nmol/1 h. While in winter, the average of β-GlcA at the Northern stations was 0.86 nmol/1 h and the range of variation (0.34–1.89 nmol/1 h) was much more narrow than that in summer (0.31–8.1 nmol/1 h). (2) According to the carbon conversion factor, the β-GlcA was 0.14 and 0.062 ugc/1 h in summer and winter respectively. These β-GlcA values were higher than the bacterial production of the two seasons respectively. (3) The β-GlcA gradually rises from offshore water to near-shore water. (4) The correlation between the β-GlcA and the bacterial secondary production was not so obvious. (5) The correlation between the section distributions, daily varying of the β-GlcA and the bacterial production was not obvious. (6) In the surface water, the distribution character of free-state β-GlcA from bacteria was equal to that of the total β-GlcA in the whole sea area.

Keywords: Heterotrophic bacteria; Extracellular enzyme; β-glucosidase activity; Taiwan strait

1. Introduction

Enzymatic catalysis plays an important role in the flow of material and energy in marine ecosystem. Some substance transforming processes would not be completed without the extracellular enzyme (Münster, 1991). Heterotrophic microbes, especially heterotrophic bacteria, are the primary producers of extracellular enzyme. Generally, extracellular enzyme refers to the kind of enzyme with hydrolyzation, which is synthesized in the cells and transferred through the cell membrane. Some of them inlay on the membrane, exist in the periplamstic space or break away from the producer completely and diffuse into water or sediment environment (King, 1986; Priest, 1984). Some natural high molecular weight matter such as polysaccharide, protein and nucleic acid undergo enzymatic hydrolyzation by extracellular enzyme to low molecular weight substances, which could then be transferred through cell membrane and be utilized. This key biochemical process makes change of the composition of organic matter and biological availability. It is of great importance to those microbes living in the water environment where the environmental factors such as temperature, content of oxygen, nutrients and dissolved organic matter (DOM) fluctuate greatly (Münster, 1991). High β-glucosidase means high hydrolyzing rate to relevant substance, it can release more utilized dissolved matter (UDOM) from high molecular weight organic matter and provide necessary substrate for bacterial secondary production (Chróst, 1989a,b). In the other hand, through the control-induce mechanism, the ingredient variation of DOM can control the synthesis of extracellular enzyme in the cells, thus promote bacterial adaptation varying to environment. Therefore, as the medium of the interaction between bacteria and organic matter in marine environment, the β-glucosidase reflect the reconstructive degree that bacteria is doing to

* Corresponding author.

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some organic compositions and what this composition contributes to bacterial growth and multiplication. So, the study on the β-glucosidase would be helpful to have a better understanding of the role of heterotrophic bacteria in the carbon cycle of biogeochemical in marine environment. It is indispensable to understanding the structures and functions of marine ecological system. But until now, only a very few studies on this field were carried out in Chinese Seas.

2. Materials and methods

2.1. Study site and sampling

The field studies were carried out during August 1997 and February to March 1998 as a part of a larger interdisciplinary research project in situ in Taiwan strait (21°–27° N, 116.5°–122.3° E), to advance the comprehension parameters of hydrology, chemistry and biology.

Four sections at the south strait of Taiwan bank in summer and five stations at the north strait in winter have been designed as the sampling locations. The water samples were collected from various water layers (0, 10, 20, 30, 40, 50 m) and between an interval time of 3 h and/or 6 h. The station 9701 and 9704 were used to carry out a continuous survey of diel cycle in both seasons (Fig. 1).

2.2. Determination of extracellular enzyme activity in situ

Water samples were taken by using sterile bacteriological samplers in situ and 2 ml sub-samples were distributed to five sterile flasks (two for blanks and three for parallels). 50 μl, 20 mM working solution of MUF-β-D-glucosidase (Sigma Corp.) were added to yield 250 μmol final solution of the substrate in each sample and immediately 50 μl, 2 mM of $HgCl_2$ have been introduced into blanks to stop catalysis of aquatic enzyme. Then enzymatic reaction was stopped with adding $HgCl_2$ into parallels, which would be stored at 4 °C. After incubating for 2–3 h in the dark and in situ temperature, all samples got the same laboratory temperature before being measured for extracellular enzyme activity (EEA) by spectrofluorometer (Hitachi 860). Excitation and emission characteristics of fluorescence should be installed in the instrument (353, 450 nm respectively).

EEA is calculated by the equation (Hoppe, 1993):

$$V = (F - F_b)/(TS)$$

where V is hydrolysis rate of EEA to substrate (μmol/1 h); F is fluorescence intensity of parallels (average value); F_b is fluorescence intensity of blanks (average value); S is fluorescence intensity of stander fluorogenic substrate per μm; T is incubation time (h).

EEA is also measured as an increase in fluorescence as the non-fluorescent 4-methylumbelliferysubstrates were enzymatically hydrolyzed with a release of the highly fluorescent product 4-methylumbelliferone (MUF). The rate of MUF cleaved was equivalent to the rate of substrate hydrolyzed.

The reaction hydrolyzed of extracellular enzyme to substrate is following the Michaelis and Mentden kinetic equation:

$$V = (V_{max}S)/(K_m + S)$$

V is the rate of substrate hydrolyzed; V_{max} is the max of the rate hydrolysis; K_m is the kinetic parameter; S is the concentration of substrate.

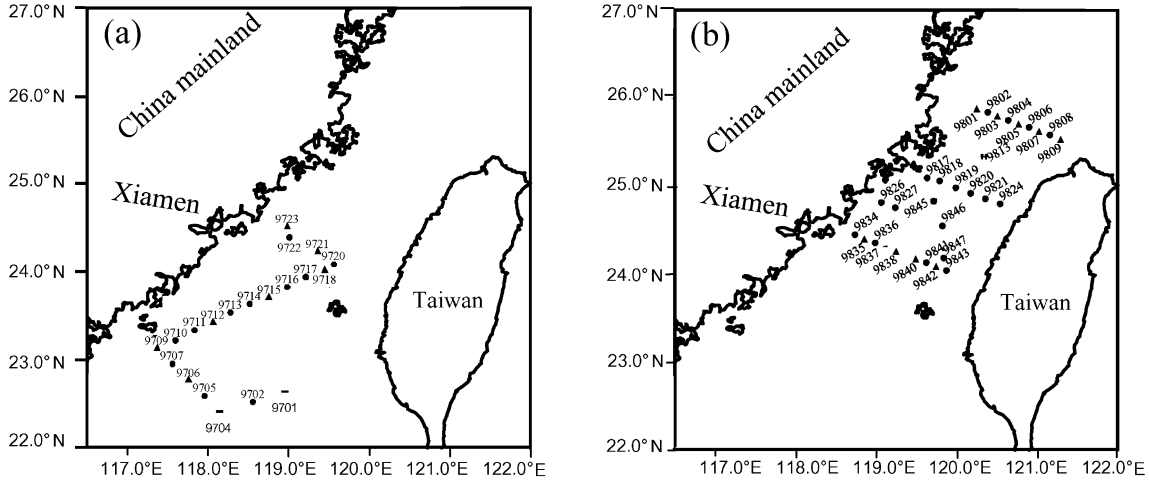

Fig. 1. Stations (a) August 1997 cruise; (b) February to March 1998 cruise.

V is relevant to S, the calculation of the rate of hydrolysis of extracellular enzyme depends on different the model substrate concentration, which were determined by substrate addition method of series concentration.

2.3. Bacterial production

Bacterial production was measured by thymidine incorporation using a modified method described by Azam and Fuhrman (1983). 10 ml water samples were placed in polyethylene bottle, which had been washed with field water samples three times. Each sample, which was assayed in triplicate was then immediately added to 100 μl ^{3}H-Thy at a final concentration of 10 nM. Blanks were treated by addition of formalin. Incubation of samples at laboratory or in situ temperature for 2–3 h in dark was then immediately stopped by addition of formalin. After being stored at a low temperature for 15–30 min, the samples were filtered on 0.22 μm membrane filters under negative pressure of less than 0.07 atm. Membrane filters have been put into Scintillation vials. And after adding 1 ml cellulose acetate 10 min later, 8 ml scintillation liquids were added into. Radioactivity was measured by scintillation counting (Wallac1409). The calculation of bacterial growth rate is as follows:

$$\mathrm{BG} = 1.4 \times 10^{15} \times (U - U_\mathrm{b}) \times 4.5 \times 10^{-13}/(RVT)$$

BG: bacterial growth rate (cell/h l); U: radioactivity of duplicate samples (dpm); U_b: radioactivity of blanks (dpm); R: radioactivity of ^{3}H-Thy (Ci/mmol); V: volume of filtered water sample; T: time of incubation. Bacterial production (μg carbon/1h) = BG × 20 × 10^{-9}; 20: carbon content of single bacteria (fg/ml).

2.4. Bacterial biomass estimates

Bacterial biomass was determined by DAPI (4′6-diamidino-2-phenylindole, Sigma Corp.) staining. 100 ml water samples were taken into plastic bottles, which soaked in acetic acid and washed by field water samples, then the samples were fixed with addition of 5 ml formalin and refrigerated in dark. 0.22 μm nucleopore membrane filters were soaked in Sudan Black B for 4 h and made it black before being used. DAPI were made with sterile water at concentration of 20 μg/ml and refrigerated in dark. We added DAPI to water samples at final concentration of 4 μg/ml and stained for 5 min. Filtered water samples under negative pressure of less than 0.07 atm. At least 10 randomly chosen fields containing 15–30 cells were counted per filter under epifluoroscope microscopy.

The formula of bacterial abundance is

$$\text{Bacterial abundance (cell/ml)} = A\,S1/(S2\,V)$$

A: average number of cells per 10 fields; $S1$: area of field of vision; $S2$: effective filter area; V: volume of sample filtered.

Bacterial biomass (μgc/ml)

= bacterial abundance × 20 × 10^{-9}

20 is carbon content of single bacteria (fg/ml).

3. Result and discussion

3.1. The distribution of β-GlcA at station of Taiwan strait

In summer 1994, the average of β-glucosidase activity (β-GlcA) at the Southern stations of Taiwan strait was 1.94 nmol/1 h. The maximum value (8.1 nmol/1 h) was measured at the station 9704 under 40 m water layer depth. In winter the average of β-GlcA at the Northern stations was 0.86 nmol/1 h, and the range of variation (0.34–1.89 nmol/1 h) was much more narrow than that in summer (0.31–8.1 nmol/1 h). The maximum value was measured at the station 9813 under 10 m water layer depth. According to carbon conversion factor, the β-GlcA was 0.14 μgc/1 h and 0.062 μgc/1 h in summer and in winter respectively. These β-GlcA values are higher than the bacterial productivity in the both seasons respectively (Table 1, Figs. 2–6).

3.2. The vertical distribution of β-GlcA

There were an obvious difference between the vertical distribution of β-GlcA and that of the relevant bacterial biomass and productivity during the two cruises in summer and in winter. The range of variation was larger than the bacterial productivity. The peak value of β-GlcA were presented at 20 m depth water layer and at bottom during the different sampling times. The other stations had the same distribution character and the peak value of β-GlcA appeared at 10–20 m depth water layer and 70 m bottom respectively. This might be explained by the fact that those stations are far from the land and/or the organic matter was mostly originated

Table 1
The average and range of bacterial biomass, productiviy and β-GlcA in the Taiwan strait

Bacterial parameter	Summer	Winter
Biomass (μgc/l)	10.62 (0.69–32.21)	16.24 (5.18–41.26)
Production (μgc/1 h)	0.089 (0.003–0.39)	0.047 (0.002–0.16)
β-GlcA (μgc/1 h)	0.14 (0.023–0.58)	0.062 (0.024–0.14)
(nmol/1 h)	1.94 (0.031-8.07)	0.86 (0.34-1.89)

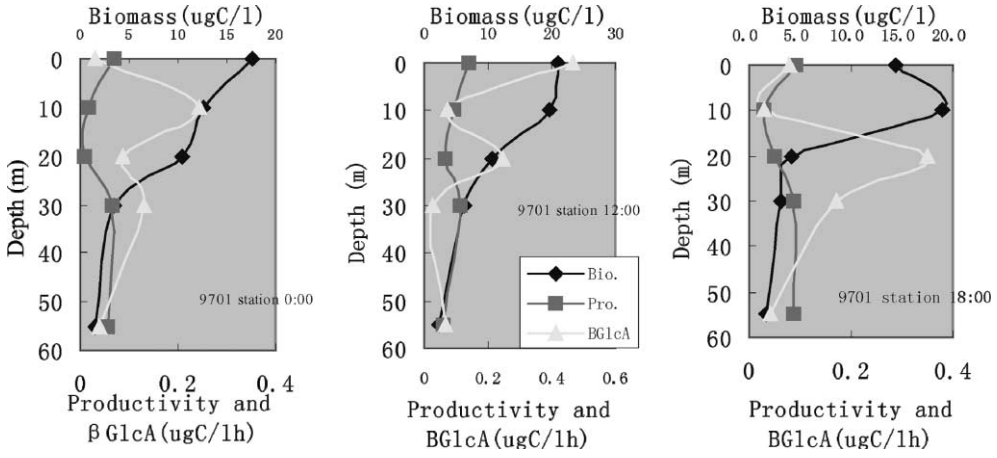

Fig. 2. Vertical distribution of the bacterial β-GlcA, biomass and productivity in station 9701.

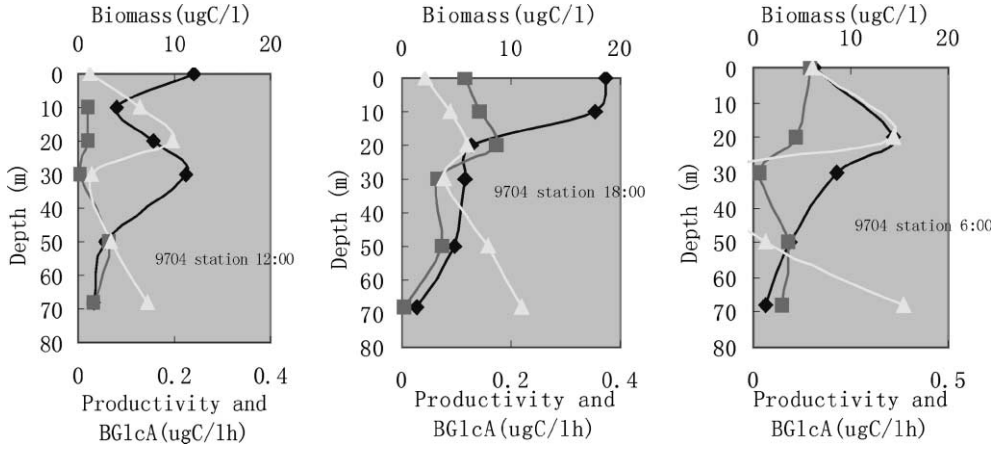

Fig. 3. Vertical distribution of the bacterial β-GlcA, biomass and productivity in station 9704.

from the primary productivity. Consequently, there exist more a high molecular weight DOM, so utilized dissolved organic carbon (UDOC) has a comparatively abundant activity in Taiwan strait.

According to the induction/inhibition theory (Chróst, 1989a,b), Chróst had put forward that the synthesis of the extracellular enzyme is inhibited. So commonly, the activity of exoenzymes is low. But at the bottom, there are a large amount of deposit high molecular weight DOM and UDOC relatively. According to the same theory, when the content of UDOC decrease to a certain level, the inhibition of bacterial enzyme synthesis will be depressed, so bacteria can synthesize a large amount of exoenzymes, which show high activity. At 20 m water layer, exoenzymes showed high activity because there were a high amount of particulate organic matter (POM). That may be caused by a number of factors such as feeding habit of phytoplankton, upwelling and so on (Wu and Chen, 1987; Hong et al., 1991; Zheng et al., 1997; Zheng and Cai, 1993; Chróst, 1989a,b; Jost and Ballin, 1992; Karner and Rassoulzadegan, 1995).

3.3. Diel cycle of the β-GlcA

In the field study and during winter cruises, we found that most of the water samples showed no β-GlcA. To comparing β-GlcA and bacterial biomass at station 9701 with that in station 9704 under different water layer, a larger range of variations could be observed (Fig. 6)

3.4. Horizontal distribution of the β-GlcA

The first section of summer cruises showed that the β-GlcA has gradually been increasing from offshore surface water to near-shore surface water. The maximum value (0.24 μgc/1 h) was determined at station 9709. However, at the station 9723, nearest from the land, the surface water samples showed no β-GlcA. The β-GlcA was low in the surface water samples of winter cruise and six samples of eleven samples have showed no β-GlcA. The maximum value (0.049 μgc/1 h) has emerged at station 9813. There was an obvious difference between the character of horizontal distribution of the β-GlcA and that of bacterial biomass and productivity (Fig. 4).

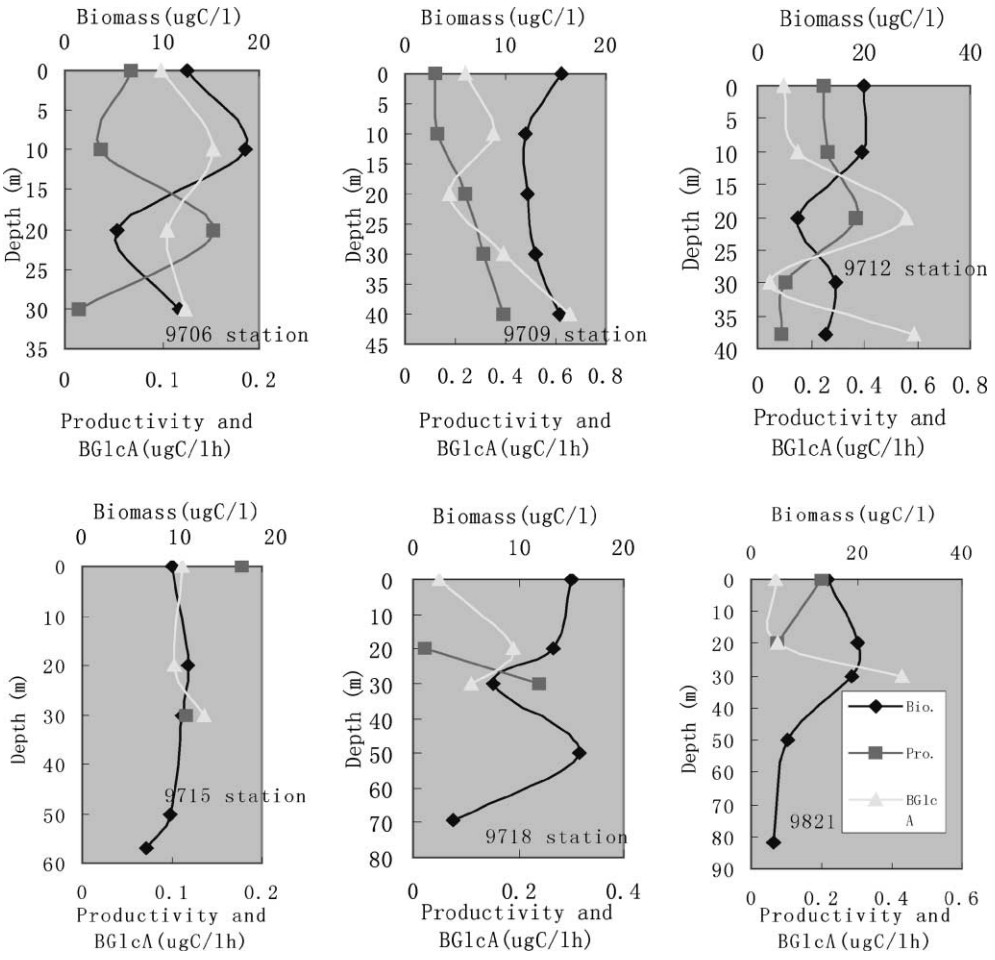

Fig. 4. Vertical distribution of bacterial β-GlcA, biomass and productivity during summer cruise.

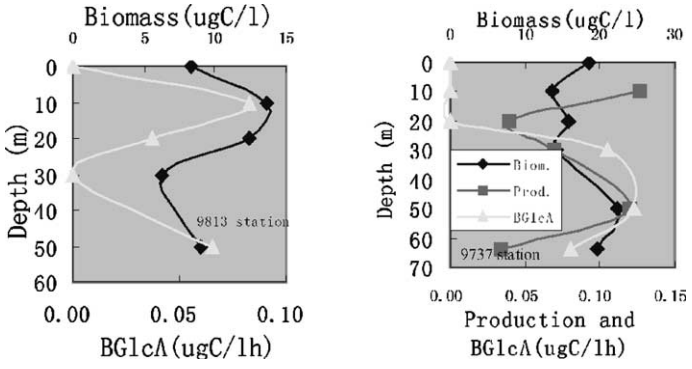

Fig. 5. Vertical distribution of bacterial β-GlcA, biomass and productivity during winter cruises.

In the surface water, the distribution character of free-state β-GlcA from bacterial cell was equal to the total β-GlcA in the whole sea area (Figs. 7 and 8).

3.5. The relationship between bacterial productivity and GlcA

High β-GlcA means a high hydrolyzing rate to relevant substance, it can release more UDOM from high molecular weight organic matter and provide necessary substrate for bacterial secondary production. Chróst found that the amount of free-state β-glucosidase (the fraction <0.22 μm) was very low and on average 3.4% of the total β-GlcA in water. Most of these free-states attached bacterial cells, thus the products of substrate hydrolysis can be rapidly taken up by bacterial cells, exoenzymatic hydrolysis and the uptake of hydrolytic products are tightly coupled processes. So, β-GlcA was

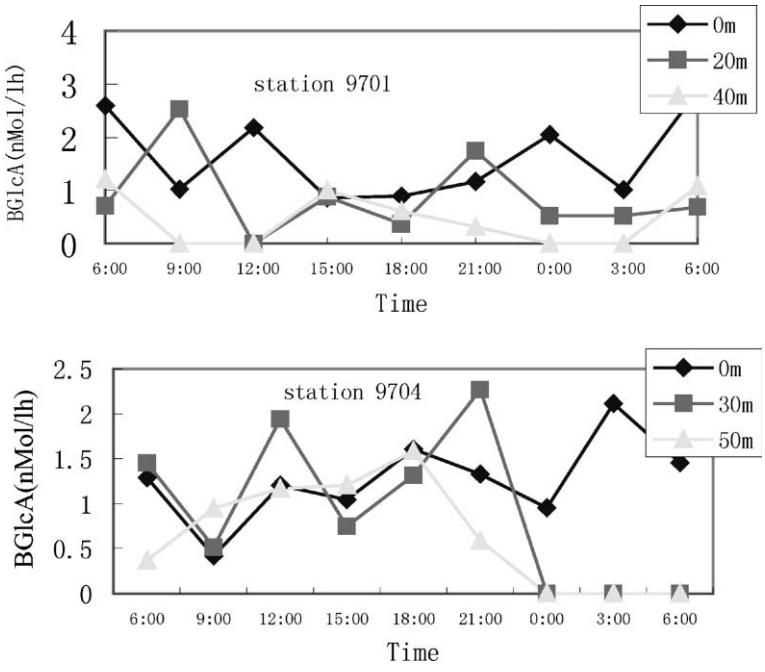

Fig. 6. Diel cycle of β-GlcA in different depths in two stations during the summer cruise.

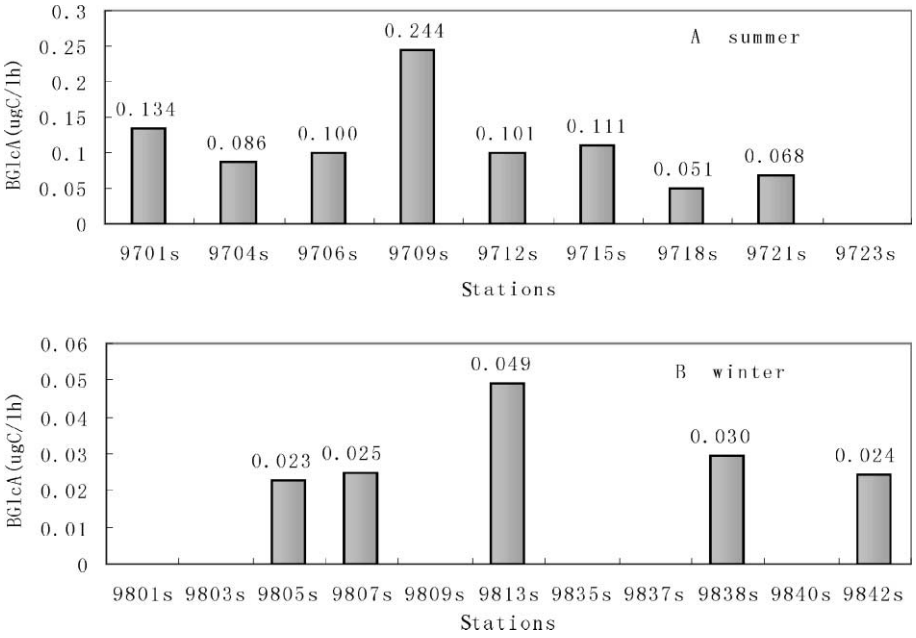

Fig. 7. Horizontal distribution of the β-GlcA: (A) data from the surface water, (B) data from average of 0 and 10 m deep water.

correspond with the second productivity in the distribution of time and space. But in our studies the correlation between the section distribution and daily varying of the β-GlcA and the bacterial production was not obvious enough. (Figs. 2–6). The reason may be related to that DOC produced by substrate hydrolysis, which are not available effectively to bacteria. Through analyzing the character of the fraction at the station 9813, a large amount of free-state β-glucosidase were found, its activity was 56.8% of the total β-GlcA. Free-state β-glucosidase of some samples was more than 100% probably originated from damage of cells during filtration. Karner's studies in mediterranean sea had showed the same results (Karner and Rassoulzadegan, 1995).

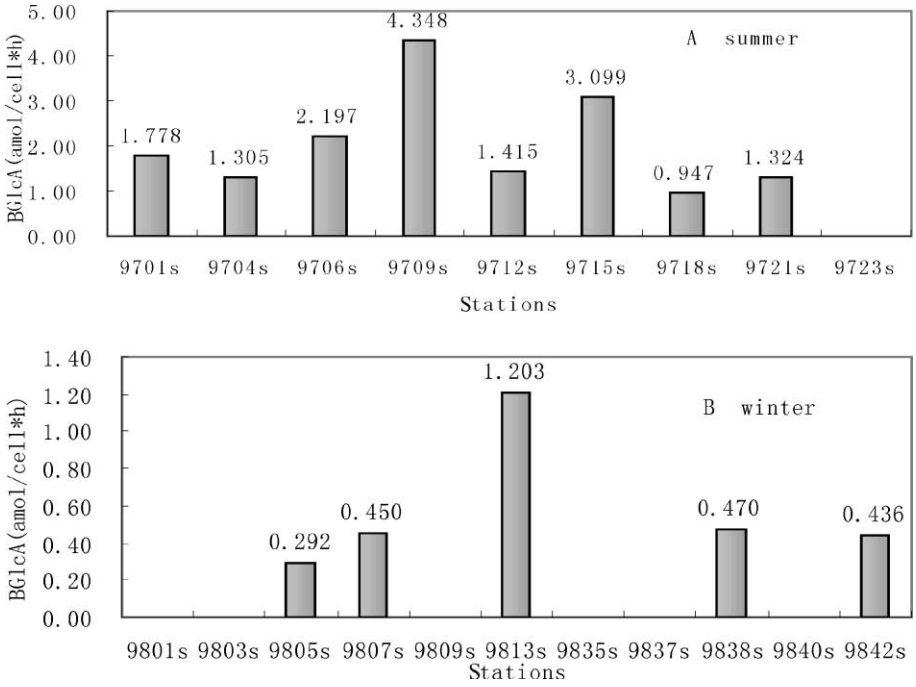

Fig. 8. Horizontal distribution of the specific β-GlcA: (A) data from the surface water, (B) data from average of 0 and 10 m deep water.

3.6. Physi-chemical characteristics in Taiwan strait and the importance of EEA in microbial-loop

Ecosystem dynamics process of Taiwan strait is complicated. Because of the strong change of water dynamical, the biomass and productivity of plankton have put up the asymmetry in spatial distribution (flecky distribution), and had an obvious characteristic of temporal change.

At larger scales, hydrodynamic parameters and therefore the transport of nutrients would be more important in regulating the primary productivity. The distribution of biomass and productivity depended largely on the movement of water mass and upwelling (Zhang and Yang, 1995). In summer, Minjing river was in flush times of water and had plenty nutriment imported from land. So, the biomass and productivity in the area in shore of Northern part were relatively high. Countercurrent and frontal zone are formed in the side near the Northern part of Taiwan Island with infall of intrusion of the Kuroshio water (Chuang et al., 1993; Chuang and Liang, 1994), then the group of plankton clusters together. In the meantime, the result observed by Shiah et al. (1995) indicated that there was an upwelling in the sea area and the primary productivity was high (from 418 to 769 mg/m^2 d). Thus, a ring-like, high biomass area was formed in the Northern part of Taiwan strait, which encircled a relative ''barren'' sea area. In the Southern part, the upwelling brought by wind was formed in the area in shore in Summer, and upwelling brought by is mainly formed in the Southern part of Taiwan Bank (Hong et al., 1991) and the upwelling region greatly tallies with the high biomass area in this season. Although Zhe-Min long-shore currents bring plenty nutriment in winter, the high biomass area could not be formed in shore because of very low water temperature (e.g., the water temperature of surface layer was only 11–12 °C in the Northern part in shore). However, a feeble frontal zone could be formed because of infall of high temperature and low salt water, and being mixed with low temperature and high salt water out of the Northwest corner in Taiwan Island. At the Southern edge in the Southern Taiwan riffle, the upwelling front could be formed because of the existence of an upwelling brought by topography. Thus, the high biomass and productivity area mainly concentrates in the frontal zone.

The former study has indicated that the phosphoric supplement and circle have the most important role in regulating the primary productivity of Taiwan strait than other nutrients (Hong et al., 1991). On the question of nutrimental limit to productivity, two sides should be considered: external import flux and inner circle flux. It was estimated that the import flux of DIP (including river and upwelling etc.), the only accounts for 11% of that needed by photosynthesis of phytoplankton in this sea area. This result showed that in this sea, there must be the fast circle of phosphor in various forms (POP and DOP etc.) whose medium were organisms. According to the correlative formula about DIP of the bottom of the sea area and AOU $[\mathrm{DIP} = 0.003 \times 2\mathrm{AOU} + 0.19(r = 0.69, n = 63)]$, it could be estimated out that 55% of DIP in bottom was from organic oxidation decomposed.

The inner fast circle of nutriment in this sea area could also get further evidences.

Hong Huasheng et al. have used sediment capture implement to estimate out the value of new output is 19.6% in the Southern Sea area. This indicated that the carbon accounting for 80% of primary productivity carries out the circle in the upper layer water.

The study on the bioactivity of DOP indicated that phytoplankton could directly utilize DOP, and DOP had a similar nutrition function with DIP (Hong et al., 1995). In summer, the abundance of phytoplankton was very high in the Southern stations, DIP can hardly be examined, and the content of DOP was also very low. This phenomenon may be relational with the DOP utilization by phytoplankton.

Finally, we want to say that till now the application of the ectoenzyme approach to aquatic microbial ecology is still relatively new. A review of ectoenzyme studied in water and sediments showed that the majority were hydrolases. The most commonly investigated hydrolyses include phosphohydrolytic and proteolytic ectoenzymes and those involved in depolymerization of polysaccharides (Münster and Chróst, 1990). The majority of the organic compounds produced in aquatic environments are of a polymeric mature. In order to make them available for transfer across the microbial cell membranes, they have to undergo depolymerization. The stepwise degradation of polymeric organic matter is obviously mediated by microorganisms, mostly by heterotrophic bacteria and their hydrolytic ectoenzymatic activities are of the utmost importance in the process. The hydrolytic depolymerization of organic matter is a rate-limiting step for microbial uptake of monomeric compounds. The polymers, in this sense, are biologically the most important nutritional sources, and compounds such as proteins, polysaccharides (cellulose, chitin, starch), and organ phosphoric esters predominate in aquatic non-polluted environments.

The coupled ectoenzyme hydrolysis and uptake systems greatly increase the efficiency of DOM utilization by free-living bacteria in aquatic environments. This has a great importance for increasing the range of the microbial loop. Because the major step in the microbial loop is the conversion of UDOM into bacterial biomass and production. Most bacterial production appears to be utilized by protozoan and very small metazoans (Pace, 1988). Heterotrophic bacteria have a very high respiratory potential for oxidation of organic matter, thereby releasing CO_2, PO_4^{-3}, NH_4^+, and other small molecules that are required nutrients for phytoplankton. Therefore, the microbial loop is an important contributor to the steady supply of algal nutrients and promotes a stead state of algal biomass and production in the absence of other sources of nutrients. In instances where the microbial loop does not operate efficiently, phytoplankton is a self-limiting nutrient sink, and their population's crash when nutrients are exhausted (Pomeroy and Wiebe, 1988).

Moreover, the attached bacteria operation on different levels of the aquatic food webs may compete with particle-feeding animals (zooplankton, benthos) by a rapid solubilizing of POM via hyper-production of hydrolytic ectoenzyme.

Thus, the particles become a source of DOM that is not accessible for animals. This is of great importance for energy partitioning between the microbial loop and grazing chains in aquatic environments. When the importance of ectoenzyme in microbial loop formation is considered, it can be concluded that microbial ectoenzyme operate at the molecular leveling aquatic environments, but they affect the function of the whole aquatic ecosystem (Chróst, 1989a,b).

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