DISTRIBUTION CHARACTERISTICS AND AFFECTING FACTORS OF SPRING HETEROTROPHIC BACTERIA IN BOHAI SEA

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Abstract: Distribution characteristics, variation patterns and affecting factors of hetorotrophic bacteria were studied from April to May 1999 in Bohai Sea by standard Acridine Orange epifluorescence microscopy (AO method). The biomass in surface waters showed a small day-night variation, varying from 0.13-2.51 $\mu g \cdot dm^{-3}$ with an average of 0.84 $\mu g \cdot dm^{-3}$. The biomass in bottom waters showed, however, a large variation, changing from 0.15-4.18 $\mu g \cdot dm^{-3}$ with an average of 1.36 $\mu g \cdot dm^{-3}$. The peak values were obtained at 5 and 11 am. The bottom water biomass showed a significant correlation with particulate organic carbon ($r=0.639$, $P<0.05$). Heterotrophic bacteria showed high biomass in nearshore waters and low values in offshore areas with a high biomass zone around Yellow Sea river mouth, which was consistent with the distribution of nutrients. The vertical distribution of heterotrophic bacteria showed biomass in bottom waters was higher than in surface water. The biomass of heterotrophic bacteria in Bohai Sea was similar with that in other marine waters.

Keywords: Bohai Sea; Heterotrophic bacteria; Biomass distribution

Microorganisms were traditionally regarded as decomposer. Recent studies showed, however, they played roles not only as producer in food chains, but decomposer and transformer of nutrients, and restorer of materials and energy. Free-living heterotrophic bacteria can transform most Dissolved Organic Carbon (DOC), produced through photosynthesis, into Particulate Dissolved Carbon (POC) as their body composition, thus allowing this portion of carbon entering higher trophic levels. This process is also termed as secondary production[1]. DOC can be absorbed by bacteria, then via protozoon, uptaken by zooplankton. This material flowing route is called microbial loop[2]. This discovery brought about a breakthrough in marine ecology and marine biogeochemistry study. Microorganisms not only play a dominant role in total biological kingdom, taking up 26-62% of organic and inorganic carbon[3], but also show a high transformation efficiency. They, thus, constitute a loop with special significance in ecosystem energy web[4]. In nearshore waters, only 20% of primary production is uptaken by zooplankton and the rest is utilized by planktonic and benthic heterotrophic bacteria[5]. In natural waters, microorganisms can use a variety of substances as their food sources and thus exhibit a high diversity in both trophic states and biomass distribution. Furthermore, marine microorganisms are closely related to red tides. Through the study of biomass distribution of planktonic bacteria, the water qualities can be judged and red tides preliminarily predicted. Microorganisms, therefore, play an important role in marine ecosystems, especially in upper layer of the systems. The study of the biomass and fluctuation of marine bacteria is of great importance for further understanding the roles of such biota in substance transformation and energy flow processes in food chains. It's also important for exploitation, utilization and conservation of marine resources. Recently, much work has been done in this field and Chinese scientists also did a lot of research. Ye Dezan[9], Shi Junxian [13] and Zheng Guoxing[14] reported consecutively the quantity and biomass distribution characteristics of heterotrophic bacteria in Daya Bay, Yangzi River mouth and its adjacent areas, Xiamen western harbor and Taiwan Straight. This paper reported the distribution, day-night variation and affecting factors of heterotrophic bacteria in Bohai Sea.

1. MATERIALS AND METHODS

1.1. Sampling

The bacteria samples were collected during a cruise of Dongfanghong 2 research vessel within an area of between 37°45'-39°00'N, 118°30'-121°34'E in Bohai Sea. Water temperature was 14.6~16.8°C. One consecutive site and twenty-seven surface sites were selected. For the consecutive site, samples were collected every three hours (nine samples in total); for each surface site, only one sample was collected. Water samples were collected from both surface and bottom layers in sterilized sampling bottles. Two parallel samples were collected.

1.2. Sample test
Bacteria quantity in water samples were tested by Acridine Orange epifluorescence microscopy (AO method)[7].

1.3. Determination of bacteria volume and biomass
Bacteria volume was measured through negative-dying. The samples were observed under Transmission Electron Microscope H-7000 and photos were taken. The width (W) and length (L) were measured accurately for each species. The volume and biomass of bacteria were calculated by the following formula[8][9],

$$\text{Volume } (\mu m^3) = \pi \times W^2 \times (L - W/3)/4$$
$$\text{Biomass } (\mu gC \cdot dm^{-3}) = 2.2 \times 10^{-13} \times N \times V,$$

in which N is total number of bacteria and V, volume of bacteria.

2. RESULTS

2.1. Spatial variation in quantity and biomass of heterotrophic bacteria
Heterotrophic bacteria varied from 5.57-106.72×10^4 individuals per milliliter with an average of 35.68×10^4 individuals per milliliter in surface waters, and 6.29-177.63×10^4 individuals per milliliter with an average of 57.84×10^4 individuals per milliliter in bottom waters. The biomass of heterotrophic bacteria in surface waters ranged from 0.13-2.51 $\mu gC \cdot dm^{-3}$ with an average of 0.84 $\mu gC \cdot dm^{-3}$. The highest biomass was recorded in Station B1 and the lowest in E5. The biomass in bottom waters ranged from 0.15-4.18 $\mu gC \cdot dm^{-3}$ with an average of 1.36 $\mu gC \cdot dm^{-3}$.

The highest biomass was recorded in Station C1 and the lowest also in E5. The biomass in both surface and bottom waters was higher in nearshore and lower in offshore areas (Figures 1 and 2).

2.2. Day-night variation in biomass of heterotrophic bacteria
The day-night variation in biomass of heterotrophic bacteria was analyzed for Station E3. The results did not show any large variation in surface waters. The lowest values of 0.44 and 0.47 $\mu gC \cdot dm^{-3}$ were recorded at 1 am and 14 pm, respectively and the highest values of 0.73 and 0.67 $\mu gC \cdot dm^{-3}$ were recorded at 20 pm and 11 am, respectively. The variation in bottom waters was larger and biomass in daytime was higher than in nighttime. The maximum value of 1.38 $\mu gC \cdot dm^{-3}$ was recorded at 5 am and the minimum of 0.54 $\mu gC \cdot dm^{-3}$ at 23pm.

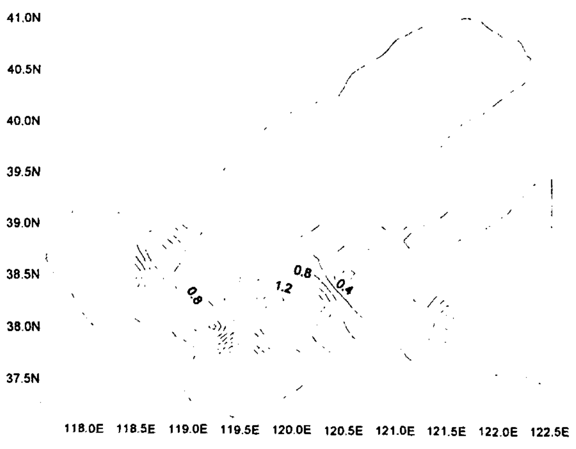

Fig.1 Horizontal distribution in biomass of heterotrophic bacteria

in surface waters (μgC·dm^{-3})

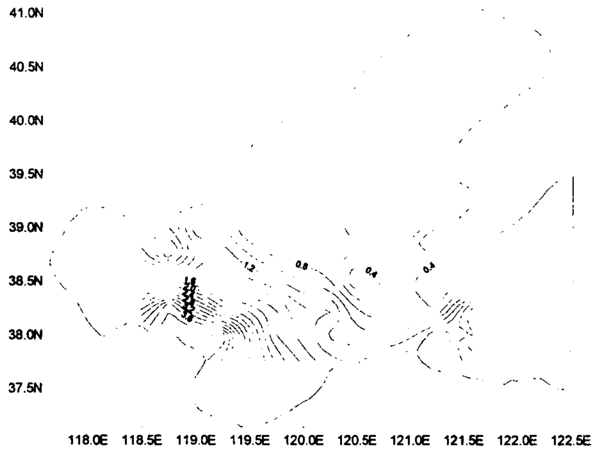

Fig.2 Horizontal distribution in biomass of heterotrophic bacteria in bottom waters (μgC·dm^{-3})

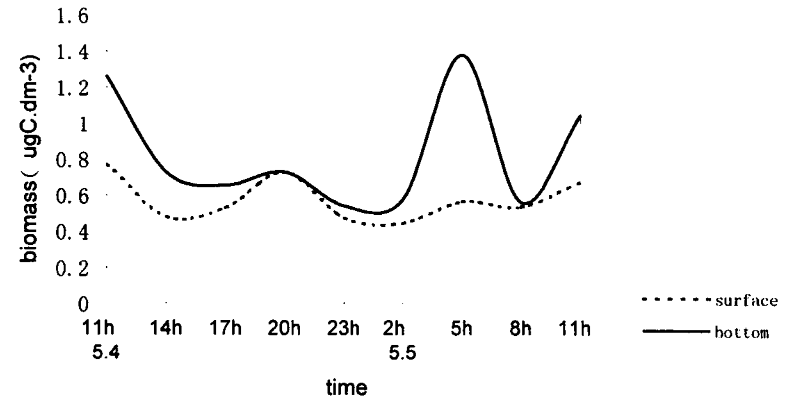

Fig 3. Day-night variation in biomass of heterotrophic bacteria

3. DISCUSSION

3.1. The relationship between heterotrophic bacteria and environmental factors

Nutrients, especially POC, have significant influence on heterotrophic bacteria and hydrologic conditions directly affect the distribution of organic substances. Based on the distribution pattern of heterotrophic bacteria in Bohai Sea, the biomass in surface waters was obviously higher in the vicinity of Bohai Bay and Laizhou Bay. The biomass of heterotrophic bacteria was significantly correlated with that of zooplankton ($r=0.472$, $P<0.05$), which shows that marine microorganisms may constitute a major part of food sources for micro-zooplankton and play an important role in microbial loop. Water temperature is one of the important factors affecting the distribution of bacterial biomass[10]. The biomass shows a positive correlation with water temperature when it is below 14°C[11]. This paper also shows that the biomass of heterotrophic bacteria in surface waters was significantly correlated with water temperature ($r=0.466$, $P<0.05$). Water temperature is, therefore, a limiting factor for the distribution of heterotrophic bacteria, particularly the bacteria in surface waters, in Bohai Sea. The organic pollutants and nutrients were high in these areas because of, for example, mariculture pollution. The biomass of heterotrophic bacteria in surface waters of Bohai Sea was, however, not significantly correlated with these factors. The biomass of heterotrophic bacteria in bottom waters showed high values at Yellow River and Luanhe River mouths. Nutrients, DOC and POC concentrations in these areas were also high because of land transport and mariculture pollution. The heterotrophic bacteria biomass showed a highly significant correlation with DOC concentrations ($r=0.66$, $P<0.01$) and a significant correlation with chlorophyll a contents ($r=0.44$, $P<0.05$). The heterotrophic bacteria in bottom waters of Bohai Sea are, therefore, probably controlled by DOC. The biomass of heterotrophic bacteria in bottom waters was also significantly correlated with POC, PON and POP concentrations (r values was 0.53, 0.55 and 0.52, respectively, $P<0.01$). The biomass in bottom waters was higher than that in surface waters because the organic substances, especially POC, increased in bottom layer through deposition and water-sediment exchange. This is different from the situations in open oceans where upwelling currents significantly affect the bacteria distribution.

3.2. Causes of day-night variation in the biomass of heterotrophic bacteria

During daytime, phytoplankton in surface waters reproduces quickly. Consequently, DOC concentrations increase and this promotes the reproduction of heterotrophic bacteria[12]. This study also showed that the biomass of heterotrophic bacteria in surface waters had a positive correlation with chlorophyll contents ($r=0.668$, $P<0.05$). The biomass of heterotrophic bacteria in bottom waters showed a large day-night variation and had a significant correlation with POC concentrations ($r=0.639$, $P<0.05$). This is because the water-sediment exchange of nutrients and the deposition of organic substances in bottom waters promote the reproduction of bacteria.

3.3. Comparisons of bacterial biomass with other areas and testing methods.

The biomass of heterotrophic bacteria in Bohai Sea was comparable with those in west coast areas of Xiamen (8.8 $\mu gC \cdot dm^{-3}$)[9] and Yangzi River mouth (10^7 individuals$\cdot ml^{-1}$)[13].

AO method is now more commonly used because of its easy operation, no requirements of immediate observation after sampling and stable results. To compare with references, both AO and flat culture methods were employed in this study. The bacteria counts obtained by flat culture method were 2-3 orders lower than those by AO method. With the wide application of AO method, marine bacteria are understood in more details. It is generally considered that marine bacteria consist of two communities, epiphytic and planktonic. Most epiphytic bacteria adhere to organic detritus and mainly use particulates as their carbon sources. Such bacteria can be easily cultured in agar. They were described and identified traditionally and constitute only a small part of the bacteria community. Planktonic bacteria have high density and float in waters. They use DOC as their carbon source and can not be cultured in normal agar. It is the existence of planktonic bacteria that greatly increases the total bacterial count through AO method.

In nearshore marine ecosystems, about 25-60% of carbon fixed through photosynthesis enters food web via bacteria. The ecological efficiency is as high as 50%. The survival and functions of bacteria control the development and complexity of food chains in waters[14][15]. The biomass of heterotrophic bacteria affects its role in the whole ecosystem and it warrants further study.

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