

Abundance of general aerobic heterotrophic bacteria in the Bering Sea and Chukchi Sea and their adaptation to temperature

Chen Haowen(陈皓文), Gao Aiguo(高爱国), Sun Haiqing(孙海青) and Jiao Yutian(矫玉田)

First Institute of Oceanography, State Oceanic Administration, Qingdao 266061, China

Received June 11, 2004

Abstract The abundance of general aerobic heterotrophic bacteria (GAB) from the water and sediment in the Bering Sea and the Chukchi Sea was determined by using petri dish cultivation and counting method. The abundance of GAB among the different sea areas, sampling sites, layers of sediments surveyed and adaptability to differential temperatures was studied. The result obtained showed that: the occurrence percentage of GAB in the surface water was higher than that in sediment, but the abundance was only 0.17% of sediment. The occurrence percentage of GAB in surficial layer of sediment was higher than that in the other layers. The occurrence percentage of GAB in surficial layer of sediment was higher than that in the other layers. The occurrence percentage, abundance and its variation of GAB in the Bering Sea were higher than that in the Chukchi Sea respectively. The average value of the abundance in the all sediment surveyed was $3166.3 \times 10^2 \text{ CFU} \cdot \text{g}^{-1} (\text{wet.})$. The abundance of GAB in sediment showed a trend: roughly higher in the lower latitudinal area than higher latitude. The results from temperature test mean that: the majority of bacteria tested were cold-adapted ones, minority might be mesophilic bacteria. The results indicated that, Arctic ocean bacteria had a stronger adaptability to environmental temperature.

Key words Arctic, marine general aerobic heterotrophic bacteria, abundance, adaptability to temperature.

1 Introduction

In the deep sea with a water temperature lower than 5 °C and the sea bottom, the organic matters are oxidized mainly by the aerobic bacteria (Bender and Heggie 1984; Herbert 1986), which means that the aerobic bacteria in this environment are rather abundant. The arctic sea area is an area where environment is permanently cold, but some research results (Sahm *et al.* 1998) indicate that there are dominant bacteria—eubacteria in the arctic seawater and sediment, and their adaptation to temperature has no substantial difference in comparison with that of the bacterial strains from the temperate zone and warm-temperate zone, which means that many microbes in cold area persistently live in very low temperature, but their respiration and growth activities can quickly achieve an optimum state in the event that the microbes come in a temperatures higher than the on-site temperature. For example, the environmental temperature in the Antarctic area is permanently lower than 0 °C, but it is shown that the optimum temperature for microbial

metabolism is at least 10 °C higher than the local temperature. The real psychrophilic bacteria have been found in the Antarctic area, but the main isolated aerobic bacteria are rather psychrotrophic bacteria than psychrophiles (Delille and Perret 1989; Delille 1993), which indicates that the low temperature is an important factor inhibiting the on-site microbial physiologic and biochemical metabolism.

Some studies on the general aerobic heterotrophic bacteria (GAB) in some arctic sea areas have been made (Sahm *et al.* 1998; Forrari and Hollibargh 1999; Bano and Hallibaugh 2000; Ravensschlag *et al.* 2001), but the study on GAB in the Bering Sea and Chukchi Sea has not been reported yet. In this paper, the abundance of GAB in sediments and seawater in before-mentioned sea areas is studied, which will help analyze and study the microbial condition and function of GAB.

2 Sample collection and analysis

The samples were collected during the first Chinese Arctic Research Expedition from July to September, 1999. Sediment samples were collected at 10 stations in the Bering Sea and at 24 stations in the Chukchi Sea, the sediments from the Bering Sea shelf are fine sand or silt, and the sediments from the Chukchi Sea shelf are mainly silty clay or clayey silt. Two surface water samples were collected at Station W₁(74°50' N, 160°33' W) and Station W₂(77°4' N, 160°33' W) in the Chukchi Sea, respectively, and the surface water temperature was about - 1.4 °C. The locations of sediment sampling sites and their environmental conditions were described by Chen and Gao (2000).

After being collected onto the ship deck by box corer, the sediment samples were immediately sealed up and stored in clean plastic bags for later analysis in the domestic laboratory, and the sediment samples were divided into surface layer sample (upper 0-5 cm), subsurface layer sample (upper 5-10 cm) and mixed layer sample for microbiological assay.

The helicopter was used to collect the surface water samples at stations W₁ and W₂, and the collected water samples were sealed up and stored in clean water sample bottles for microbiological assay in the domestic laboratory.

The different sediment samples were diluted to specified concentrations, respectively, 0.1 cm³ diluted sediment samples were then taken to be smeared on Zobell 2216E plates, and 0.1 cm³ raw water samples were taken to be smeared on above-mentioned solid culture medium plates. The above sample plates were cultured for over 2 weeks at 4 °C, 15 °C, 25 °C and 28 °C temperatures, respectively. The colony forming units (hereafter referred to as CFU) were then observed and counted, and the CFU per g (wet sediment) or per cm³ water were calculated. The abundances of GAB in different sea areas, at different survey sites and in different layers were analyzed and compared with each other.

3 Results and discussions

3.1 Analysis of GAB content condition in the survey area

The data for GAB in the water samples and sediment samples at different survey sites are listed in Table 1.

Table 1 Status of abundance of GAB from surface water and sediment in the Bering Sea and Chukchi Sea

Kinds of samples	No. of samples	Names of stations	longitude (W)	latitude (N)	GAB CFU $\cdot 10^2 \cdot \text{cm}^{-3}$ or $[\text{g}^{-1}(\text{wet})]$		
					Water or surface sediment (upper 0–5cm)	Subsurface layer (5–10cm)	Mixed layer (non-divided)
water	1	W1	160°33′	74°50′	0	/	/
	2	W2	160°33′	77°04′	4.4	/	/
	average	/			2.2	/	/
sediment	1	C1	170°06′	67°30′	150.0	/	/
	2	C2	169°59′	68°00′	/	0	/
	3	C3	170°00′	68°30′	0	/	/
	4	C4	169°59′	69°00′	/	/	30.0
	5	C5	170°00′	69°20′	0	/	/
	6	C7	170°01′	70°00′	/	/	0
	7	C8	172°15′	69°59′	/	0	/
	8	C9	175°00′	70°01′	0	/	/
	9	C10	175°02′	70°30′	/	0	/
	10	C11	173°54′	71°00′	182.0	/	/
	11	C12	172°30′	71°01′	/	200.0	/
	12	C14	167°31′	70°00′	/	0	
20	average				66.4	50.0	10.0
	13	C16	169°53′	66°30′	/	/	1,100.0
	14	C17	169°59′	67°00′	/	/	800.0
	15	C24	170°00′	70°59′	/	/	0
	16	C25	168°53′	71°42′	/	/	0
	17	C26	168°40′	72°00′	/	0	/
	18	C27	168°38′	72°30′	0	/	/
	19	C28	165°03′	73°00′	0	/	/
	C28	165°03′	73°00′	/	0	/	
	21	C29	164°57′	73°26′	0	/	/
	22	C30	160°01′	71°15′	/	/	0
	23	C31	159°34′	71°041′	/	0	/
	24	C32	159°09′	72°06′	0	/	/
	25	C33	158°56′	72°22′	/	0	/
	average				0	0	380.0
	26	B1-9	179°26′	60°15′	/	/	0
	27	B1-10	179°05′	60°25′	/	/	18,000.0
	28	B1-11	178°45′	60°32′	/	/	0
	29	B1-12	178°14′	60°40′	0	/	/
	30	B1-13	177°45′	60°55′	/	0	/
	31	B2-10	178°26′	59°30′	0	/	/
	32	B2-11	178°10′	59°33′	/	70,000.0	/
	33	B2-12	177°51′	59°43′	360.0	/	/
	34	B5-9	175°59′	58°34′	/	/	20000.0
	35	B5-10	175°54′	58°40′	/	/	0
average					120.0	35,000.0	7,600.0

* count after 12 days culture under 4 °C

It can be calculated by using the data in Table 1 that the overall detection rate of

GAB in the sediment samples at all survey sites is 28.6% (10/35), and the overall average abundance of GAB is $3166.3 \times 10^2 \text{ CFU} \cdot \text{g}^{-1}$ (wet) with a variational range of 0 to $70000.0 \times 10^2 \text{ CFU} \cdot \text{g}^{-1}$. Described by sea area, the detection rate of GAB in sediment from the Bering Sea is 40.0%, and the average abundance is $10836.0 \times 10^2 \text{ CFU} \cdot \text{g}^{-1}$ with a variational range of 0 to $70000.0 \times 10^2 \text{ CFU} \cdot \text{g}^{-1}$; the detection rate of GAB in sediment from the Chukchi Sea is 24.0%, and the average abundance is $98.5 \times 10^2 \text{ CFU} \cdot \text{g}^{-1}$ with a variational range of 0 to $1100.0 \times 10^2 \text{ CFU} \cdot \text{g}^{-1}$, which indicates that the detection rate, average abundance and abundance range of GAB in sediment from the Bering Sea are all higher than those from the Chukchi Sea, and the bottom water temperature and salinity in the Bering Sea during that period were higher than those in the Chukchi Sea (Chen and Gao 2000). The above-mentioned detection rates of GAB are all detection results for the 4 °C culture temperature, and the detection results for other 3 culture temperatures show much greater detection rates of GAB, being up to 88.7% (as described below). The detection rate of GAB in the surface water sample is 50.0%, and the average abundance is $2.2 \times 10^2 \text{ CFU} \cdot \text{cm}^{-3}$, which is much less than that in the sediment, the former is only 0.07% of the latter, namely, the abundance of GAB in the sediment is 1439.2 times great as that in the surface water, which is 6 orders of magnitude lower than that determined by using DAPI (4',6'-diamidino-2-phenylindol) dyeing method (Sahm *et al.* 1998).

The GAB abundance distributions in the Bering Sea and the Chukchi Sea are listed in Table 2 and Table 3, respectively.

Table 2 Regional contrast of GAB abundances in Bering Sea

Region		GAB($\text{CFU} \cdot 10^2 \cdot \text{g}^{-1}$)		
		Surface	Subsurface layer	Mixed layer
latitude (N)	61°– 60°	0	0	6000.0
	60°– 59°	180.0	70000.0	/
	59°– 58°	/	/	15000.0
	average	120.0	350000.0	10200.0
longitude (W)	180°– 179°	/	/	6000.0
	179°– 177°	120.0	35000.0	/
	177°– 175°	/	/	15000.0
	average	120.0	35000.0	10200.0

It is shown in Table 2 that for the Bering Sea the GAB abundance in sediment collected in the lower latitudes is greater than that in the higher latitudes, and the GAB abundance in the mixed layer sediment is greater in the eastern Bering Sea than in the western Bering Sea.

It is shown in Table 3 that for the Chukchi Sea the GAB abundance in the surface and mixed layer sediments collected in the lower latitudes is greater than that in the higher latitudes, but that in the subsurface layer sediments is greater in the higher latitudes than in the lower latitudes; and the GAB abundance in the sediments from the Chukchi

Sea has no significant difference in east-west direction.

Table 3. Regional contrast of GAB abundances in Chukchi Sea

Region		GAB(CFU • 10 ² • g ⁻¹)		
		Surface	Subsurface layer	Mixed layer
Latitude (N)	75° – 70°	30.3	33.3	0
	70° – 66.30°	50.0	0	613.3
	average	36.9	28.6	275.7
Longitude (W)	175°	0	0	/
	170°	30.0	100.0	482.5
	165°	0	0	/
	160°	0	0	0
	average	5.0	40.0	386.0

As shown in Tables 2 and 3, the GAB contents in the surface and subsurface layer sediments from the Bering Sea 61°–60°W and the Chukchi Sea 175°W and 165°W are all undetected, and those in the sediments from the Chukchi Sea 160°W are undetected, which means that the sediment environment in the Bering Sea might be more suitable for GAB to survive than that in the Chukchi Sea. The cause for the above phenomena remains to be explored.

3.2 Sensitiveness of GAB to temperature

The environmental temperature is one of important factors to control the microbial survival. With other conditions being the same, an appropriate rise temperature can facilitate and promote the microbial survival and reproduction to result in the increase in microbial abundance. The survey sea area is permanently cold sea area and the microbes in the area live permanently in cold environment, so the microbes can be both psychrophilic bacteria and psychrotrophic bacteria. Once an appropriate rise in temperature occurs, the psychrotrophic bacteria in the moderate temperature (25 °C to 45 °C) conditions become rather active and begin to reproduce. Four replicas of 35 bacterial strains were cultured at 4 °C, 15 °C, 25 °C and 28 °C temperatures separately in order to show their sensitiveness to temperature, and the culture results are listed in Table 4.

Table 4. Response to differential culture temperatures of GAB from Bering Sea and Chukchi Sea

Culture temperature(°C)	Occurence % of GAB
4	28. 6(10/ 35)
15	28. 6(10/ 35)
25	22. 9(8/ 35)
28	8. 6(3/ 35)

* Number of testing samples: 35×4 , time of cultivation: 15d

It is shown in Table 4 that GAB all occurred at the 4 culture temperatures. The occurrence frequencies of GAB at 4 °C and 15 °C temperatures account for 64. 5% (57. 2/ 88. 7), and those at 25 °C and 28 °C temperatures account for 35. 5% (31. 5/ 88. 7), which indicates that most of GAB are favorable for living at lower temperature and about one third of GAB are favorable for living at higher temperature (25 °C to 28 °C). If the experiment can be viewed as an optimum temperature experiment, according to the opinion of Isaksen and Jrgensen (1996), the experiment results indicate that most of the detected bacteria in sediment are psychrophilic bacteria or psychrotrophic bacteria, and the rest might be mostly mesophilic bacteria with a small amount of psychrotrophic bacteria (Delille and Perret 1989). The results also show the interaction between bacteria and temperature, namely, temperature screens bacteria and bacteria select the adaptable temperature. It is shown in the experiment results that the bacteria in the Arctic sea area have considerable adaptability to the environmental temperature.

4 Conclusions

The GAB abundances in 2 surface water samples from the Chukchi Sea and in 35 sediment samples from the Chukchi Sea and Bering Sea were determined. and their differences in sea area, survey site and sediment depth and their sensitiveness to the culture temperature were analyzed. Generally speaking, the detection rate of GAB in water sample is higher than that in sediment sample, but the GAB abundance in water sample is low, being only $2.2 \times 10^2 \text{ CFU} \cdot \text{cm}^{-3}$, and the average abundance of GAB in sediment is $3166.3 \times 10^2 \cdot \text{g}^{-1}$. The detection rate of GAB in the surface layer sediment is higher than that in the subsurface and mixed layer sediments, but the GAB abundance in the surface layer sediment is not necessarily great. The detection rate, abundance and abundance range of GAB in sediment from the Bering Sea are all greater than those from the Chukchi

Sea. The differences of GAB abundance in varied the latitude show a trend that GAB abundance in lower latitudes higher than that in higher latitudes.

It is shown in the temperature experiment results that most of the determined GAB samples are psychrotrophic bacteria, a part of them are inferred to be psychrophilic bacteria, and a small amount of them are mesophilic bacteria; the bacteria in the Arctic sea area have adaptability to the environmental temperature with some variational range, so an appropriate rise in temperature can make their growth, reproduction and metabolic activities be accelerated or enhanced. However, the sustained global warming caused by different factors including the man-made factor may be unfavorable for conserving the biodiversity of psychrophilic bacteria and even psychrotrophic bacteria. The marine bacteria in the Arctic area remain to be further studied.

Acknowledgements The authors would like to thank the Polar Expedition Office of State Oceanic Administration of China and the crew on “Xuelong” expeditionary ship for their support and assistance.

References

- Bano N, Hallibaugh JJ (2000) : Diversity and distribution of DNA sequences with affinity to ammonia-oxidizing bacteria of the subdivision of the class protobacteria in the Arctic ocean, *Appl. Environ. Microbiol.*, 66 (5) : 1960 - 1969.
- Bender ML, Heggie DT (1984) : Fate of organic carbon reaching the deep sea floor : a status report, *Geochim. Cosmochim. Acta.*, 48: 977 - 986.
- Chen HW, Gao AG (2000) : Preliminary study on sediment sulphate reducing bacteria in the survey areas of Bering Sea and Chukchi Sea. *Chinese Journal of Polar Research*, 12(3) : 211 - 218 (in Chinese).
- Delille D (1993) : Seasonal changes in the abundance and composition of marine heterotrophic bacterial communities in an Antarctic coastal area. *Polar Biol.*, 13: 463 - 470.
- Delille D, Perret E (1989) : Influence of temperature on the growth potential of southern polar marine bacteria, *Microb. Ecol.*, 18: 117 - 123.
- Ferrari VC, Hollibaugh JT (1999) : Distribution of microbial assemblages in the central arctic basin studied by PIR/ DGGE: analysis of a large data set. *Hydrobiologia*, 401: 55 - 68.
- Herbert RA (1986) : The ecology and physiology of psychrophilic microorganisms, In. *Microbes in extreme environments*. Ed. By Herbert RA and Codd GA, Academic Press Inc, New York, 1 - 23.

- Isaksen MF, Jrgensen BB(1996) : Adaption of Psychrophilic and Psychrotrophic Sulfate2reducing bacteria to permanently cold marine environments. *Appl. Environ. Microbiol.*, 62(2): 408 - 414.
- Ravenschlag K, Sahm K, Amann R(2001) : Quantitative molecular analysis of the microbial community in marine arctic sediments (Svalbard), *Appl. Environ. Microbiol.*, 67(1): 387 - 395.
- Sahm K, Berninger UG (1998) : Abundance, vertical distribution and community structure of benthic prokaryotes from permanently cold marine sediments (Svalbard, Arctic Ocean), *Mar. Ecol. Prog. Ser.*, 165: 71 - 80.