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Distribution of general aerobic heterotrophic bacteria in sediment core taken from the Canadian basin and the Chukchi Sea

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Abstract The occurrence percentage and abundance of General Aerobic Heterotrophic Bacteria (GAB) were determined by using the method of MPN for 182 subsamples from 10 sediment cores taken from the Canadian basin and the Chukchi Sea at two different culturing temperatures. The results showed that the general occurrence percentage of GAB was quite high, average abundances of GAB at cultured temperatures of 4℃ and 25℃ were 4.46×10^7 and 5.47×10^7 cells · g^{-1} (wt), respectively. The highest abundance of GAB occurred at 20 ~ 22 cm section in the sediment. GAB abundances changed among the section of sediments, but there is a trend: the abundances at the middle or lower sections were lower than those at upper section. Cultivation at 25℃ could improve the occurrence percentage and abundances of GAB, which suggests that the increasing of temperature may change the living circumstances of GAB. The differences of GAB among the latitudes areas indicated that occurrence percentage and abundances of GAB in middle latitude areas were higher than those in the higher or lower latitude areas, and were more obvious at 4℃ than those at 25℃. The GAB abundances in sediment under the shallower water seemed to be lower than those in sediments under the deeper water and this status was more obvious at 25℃ than that at 4℃.

Key words The Canadian Basin, the Chukchi Sea, general aerobic heterotrophic bacteria, spatial distribution, sediment core.

1 Introduction

General Aerobic Heterotrophic Bacteria (GAB) is a kind of bacteria that widely distributed in the Arctic Ocean. They play an important role in the substance transformation, circulation of marine sediments, and in marine sediments geochemical activities.

As early as in 1884, Cęrtes first discovered bacteria in the sediment at the water depth of 5100 m from Talisman; until the beginning of this century, there are still many scholars tirelessly researching the deep-sea sediment microbiology in different habitats, but with some more exciting goals or in a wider geographical area, through accurate observation or using more advanced instrument. There are two reasons: One is that bacteria in marine sediment are involved in various geochemistry and ecological activities; another one is that many bacteria (including new types) may have unique metabolism, and may produce some kind of new bioactive substances.

Today's Arctic is no longer difficult for people to reach as in the past. During past recent decades, many reports about the bacteria in the Arctic Ocean sediment have been brought out[1-7]; but there still been a large number of problems which needed to solve. Based on the study of the GAB distribution in the surface sediments of the research area[7], The 10 sediment cores from the 41 ~ 3850 m water depth in the area of the Canada Basin and the Chukchi Sea were examined by MPN method to determine the occurrence percentage and the abundance of General Aerobic Heterotrophic Bacteria, and geographical distribution of the bacteria are discussed.

2 Material and Methods

2.1 Sampling

The samples were collected from the depth of 41 m to 3580 m in the Canada Basin and the Chukchi Sea (69°29'43"N ~ 80°13'25"N, 146°44'16"W ~ 169°59'37" W) during the second Chinese Arctic Research Expedition from July to September, 2003. The 10 cores basically represent each sedimentary unit and the ecological environment of the research area. Figure 1 shows the specific distribution of the sampling location.

The sediment sampling was taken on deck of RV Xuelong using sediment box-cores and multi-tube cores. After being taken onboard, the 10 high resolution sampling sediments cores were processed according to microbiological standards aseptic condition, that is, removing the floating surface soil of the samples in the sampler, and taking the sample slice in 1cm intervals in 0 ~ 10 cm depth, 2 cm intervals below 10 cm. The total number of the subsamples is 182. All samples were preserved at 4℃ in clean, capped plastic bottles, which had been high temperature sterilized, until analysis in the domestic laboratory.

2.2 Analysis in domestic laboratory

The sediment samples were diluted to ZoBell 2216E liquid culture medium[8] according to MPN. The specific culture temperature was set referring to the research result of the first Chinese Arctic Research Expedition[6]. Based on MPN, 1g from each wet sample was taken quantitatively and diluted to specified concentrations series[9]. After the inoculation, The samples were then cultured in the test tubes for over 3 weeks at 4℃ and 25℃, respectively. During the cultivation, samples were observed at intervals to ensure lasting turbidity, the precipitation or bacteria film in tubes as positive for GAB.

According to the number of positive tubes and the rate of sample dilution, GAB abundance calculating was based on GB/T4789.3-2003[10], and the abundance as well as the occurrence percentage of GAB was in each core or each sediment layer was statistical analyzed (cells per gram of wet sediment weight, hereafter referred as cell · g^{-1}). The distribution of GAB was discussed referring to the information of sediment lithology and geochemistry[10-11].

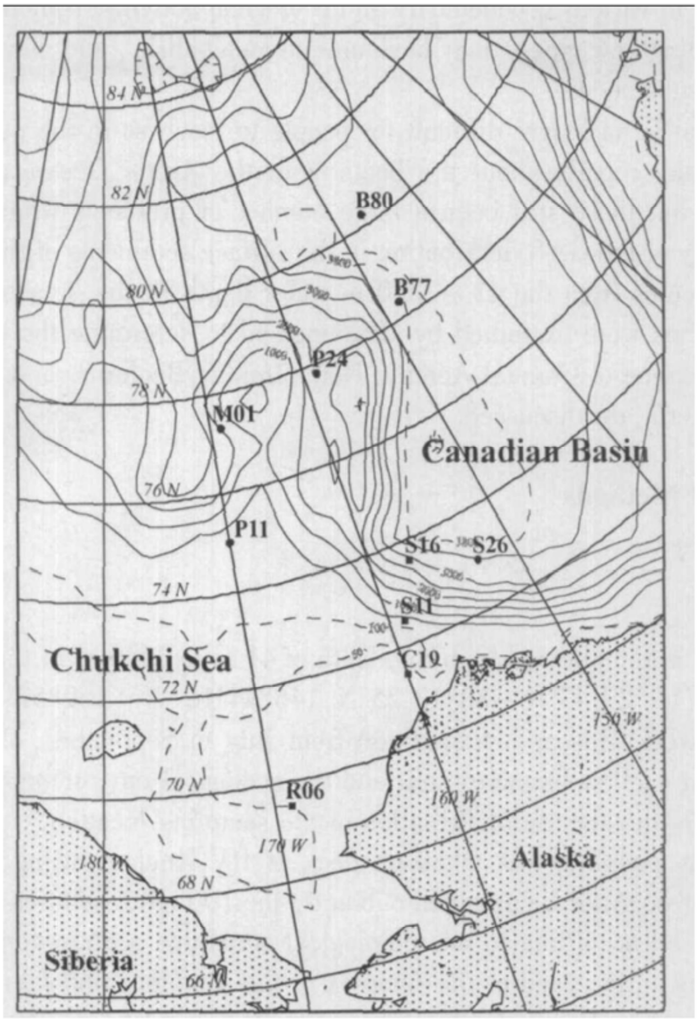

Fig. 1 Sampling stations of GAB from the Chukchi Sea and Canadian basin, the Arctic ocean

3 Result

The length range of the 10 sediment cores is 12 ~ 38 cm. The results of GAB occurrence percentage and abundance in each sediment cores or layer are listed in Tables 1 and 2.

Table 1 shows that R06 has the lowest GAB average abundance of 1.44×10^6 cell · g^{-1} while S16 has the highest abundance of 2.18×10^8 cell · g^{-1} at 4℃, the GAB average abundance ranging from 1.34×10^6 for R06 to 2.19×10^8 cell · g^{-1} for S16 at 25℃. According to table 2, overall occurrence percentage of GAB is 91.3% at 4℃. There are 11 out of 24 layers, occurrence percentage of which all is 100%; the lowest level occurred at 16-18 cm layer which is only 66.7%. The GAB abundance range from 0 to 2.40×10^9 cell · g^{-1}, average is 4.46×10^7 cell · g^{-1}. Overall GAB occurrence percentage is 96.7% at 25℃, which of 17 layers is 100%. The lowest occurrence percentage appears in the 22 ~ 24 cm layer, which is 83.3%. The GAB abundance range stay alike

Table 1. Occurrence percentages and contents of GAB in the sediment cores

core	long (cm)	4℃					25℃				
		Num.	Oc. Per.	Min.	Max.	Ave.	Num.	Oc. Per.	Min.	Max.	Ave.
R06	12	11	90.9	0	7.50×10^{6}	1.44×10^{6}	10	100.0	1.90×10^{4}	7.50×10^{6}	1.34×10^{6}
C19	22	16	100.0	9.30×10^{3}	2.40×10^{7}	7.82×10^{6}	16	100.0	1.50×10^{4}	2.40×10^{8}	5.49×10^{7}
S11	28	19	79.0	0	2.40×10^{8}	1.53×10^{7}	19	94.7	0	2.40×10^{8}	2.70×10^{7}
S16	28	19	94.7	0	2.40×10^{9}	2.18×10^{8}	19	100.0	2.10×10^{5}	2.40×10^{9}	2.19×10^{8}
S26	26	18	88.9	0	2.40×10^{8}	4.16×10^{7}	18	100.0	1.90×10^{4}	2.40×10^{8}	7.87×10^{7}
P11	38	16	81.3	0	2.40×10^{7}	3.29×10^{6}	16	87.5	0	2.40×10^{7}	6.65×10^{6}
M1	36	24	100.0	2.40×10^{3}	2.40×10^{8}	2.30×10^{7}	24	95.8	0	2.40×10^{8}	1.10×10^{7}
P24	28	19	94.7	0	2.40×10^{8}	1.61×10^{7}	19	94.7	0	2.40×10^{8}	1.68×10^{7}
B77	22	16	87.5	0	1.50×10^{7}	1.70×10^{6}	15	86.7	0	2.40×10^{7}	4.01×10^{6}
B80	38	24	79.2	0	2.40×10^{8}	2.09×10^{7}	24	100.0	3.00×10^{4}	2.90×10^{8}	5.91×10^{7}

Note: Num.: Sample number; Oc. Per.: Occurrence Percentages (%); Min.: abundance minimum; Max.: abundance maximum; Ave. abundance average; unit: cell · g^{-1} wet sediment weight; there are not data in R06-1 and B77-1 at 25℃. hereafter the same.

Table 2. Occurrence percentages and abundances of GAB in different depths in the sediment cores

layer (cm)	4℃					25℃				
	Num.	Oc. Per.	Min.	Max.	Ave.	Num.	Oc. Per.	Min.	Max.	Ave.
0-1	10	90.0	0	1.10×10^{7}	1.71×10^{6}	8	100.0	1.90×10^{4}	2.40×10^{7}	1.24×10^{7}
1-2	10	90.0	0	2.40×10^{7}	6.04×10^{6}	10	90.0	0	2.40×10^{8}	3.60×10^{7}
2-3	10	80.0	0	2.40×10^{7}	5.35×10^{6}	10	100.0	2.40×10^{5}	2.40×10^{8}	3.00×10^{7}
3-4	10	80.0	0	2.40×10^{8}	2.68×10^{7}	10	90.0	0	2.40×10^{8}	7.37×10^{7}
4-5	10	90.0	0	2.40×10^{7}	6.51×10^{6}	10	100.0	3.00×10^{4}	2.40×10^{8}	3.19×10^{7}
5-6	10	90.0	0	2.40×10^{7}	3.06×10^{6}	10	100.0	3.00×10^{4}	2.40×10^{8}	2.83×10^{7}
6-7	10	100.0	1.90×10^{4}	2.40×10^{8}	2.84×10^{7}	10	100.0	1.90×10^{4}	2.40×10^{8}	2.98×10^{7}
7-8	10	100.0	1.60×10^{4}	2.40×10^{8}	5.40×10^{7}	10	100.0	9.30×10^{4}	2.40×10^{8}	6.41×10^{7}
8-9	10	90.0	0	2.40×10^{7}	6.37×10^{6}	10	100.0	4.30×10^{4}	2.40×10^{8}	2.79×10^{7}
9-10	10	100.0	2.40×10^{3}	2.40×10^{8}	5.12×10^{7}	10	100.0	2.40×10^{3}	2.40×10^{8}	5.36×10^{7}
10-12	10	80.0	0	2.40×10^{7}	6.14×10^{6}	10	90.0	0	2.40×107	7.56×10^{6}
12-14	9	88.9	0	2.40×10^{8}	3.00×10^{7}	9	100.0	9.00×10^{2}	2.40×10^{8}	5.66×10^{7}
14-16	9	77.8	0	2.40×10^{7}	3.42×10^{6}	9	88.9	0	2.40×10^{7}	6.39×10^{6}
16-18	9	66.7	0	2.40×10^{7}	5.87×106	9	88.9	0	2.40×10^{7}	6.13×10^{6}
18-20	9	100.0	2.40×10^{5}	2.40×10^{7}	5.05×10^{6}	9	100.0	2.40×10^{5}	1.10×10^{8}	1.99×10^{7}
20-22	9	88.9	0	2.40×10^{9}	2.75×10^{8}	9	88.9	0	2.40×10^{9}	2.76×10^{8}
22-24	6	100.0	1.50×10^{6}	2.40×10^{7}	9.45×10^{6}	6	83.3	0	2.40×10^{7}	1.28×10^{7}
24-26	6	100.0	2.40×10^{6}	1.10×10^{9}	1.95×10^{8}	6	100.0	2.40×10^{5}	1.10×10^{9}	1.93×10^{8}
26-28	5	80.0	0	2.40×10^{6}	1.63×10^{6}	5	100.0	1.60×10^{6}	2.40×10^{7}	6.56×10^{6}
28-30	2	100.0	2.40×10^{6}	2.40×10^{8}	1.21×10^{8}	2	100.0	2.40×10^{6}	3.60×10^{6}	3.00×10^{6}
30-32	2	100.0	3.40×10^{6}	2.40×10^{7}	1.37×10^{7}	2	100.0	1.60×10^{4}	4.20×10^{6}	2.11×10^{6}
32-34	2	100.0	2.40×10^{7}	2.90×10^{7}	2.65×10^{7}	2	100.0	2.40×10^{6}	2.90×10^{8}	1.46×10^{8}
34-36	2	100.0	2.40×10^{6}	2.40×10^{7}	1.32×10^{7}	2	100.0	2.40×10^{6}	2.40×10^{7}	1.32×10^{7}
36-38	2	100.0	1.10×10^{8}	2.40×10^{8}	1.75×10^{8}	2	100.0	1.10×10^{8}	2.40×10^{8}	1.75×10^{8}

with that at 4℃, which is 0 ~ 2.40 × 10^9 cell · g^{-1}, with an average of 5.47 × 10^7 cell · g^{-1}. From above, the conclusion, which can be easily come to that the GAB occurrence percentage and the abundance are generally higher at 25℃ than those at 4℃.

4 Discussion

4.1 comparison of GAB in sediment cores

Table 1 shows that core C19 and M1 with 100% occurrence percentage of GAB account for 20% of all cores when cultivated at 4℃. The lowest occurrence percentage (79.0%) appears in core S11. When cultivated at 25℃, samples from core R6, C19, S16, S26 and B80 present 100% occurrence percentage of GAB, and account for 50% of all cores. The sample of the lowest occurrence percentage, which is 86.7% locates in B77. It is suggested that not only the proportion of cores with 100% occurrence percentage of GAB, but also occurrence percentages are higher at 25℃ than those at 4℃, meanwhile, which shown that GAB distributes widely in sediment cores.

When cultivated at 4℃, the layers at 6 ~ 7 cm、7 ~ 8 cm and 9 ~ 10 cm contain GAB in all cores, whereas at 25℃ the layers containing GAB in all cores increases, at 2 ~ 3 cm、4 ~ 5 cm、5 ~ 6 cm、6 ~ 7 cm、7 ~ 8 cm、8 ~ 9 cm and 9 ~ 10 cm. Table 2 also shows that the depth of the lowest occurrence percentage is deeper when cultivated at 25℃ than that at 4℃, which indicates that the occurrence percentage and abundance of GAB increase along with temperature increase.

Divided all cores into 3 segments, 0 ~ 10 cm, 10 ~ 24 cm and 24 ~ 38 cm. The occurrence percentages and average abundances of GAB of 3 segments at 4℃ are 91.0%, 86.0%, 97.1% and 1.89 × 10^8 cell · g^{-1}, 4.79 × 10^7 cell · g^{-1}, 7.81 × 10^7 cell · g^{-1}, respectively. At 25℃ the data are 98.0%, 91.4%, 100.0% and 3.88 × 10^8 cell · g^{-1}, 5.50 × 10^7 cell · g^{-1}, 7.70 × 10^7 cell · g^{-1}, respectively.

Those data show that occurrence percentage of GAB present higher within top and bottom segment, and lower in middle segment. Occurrence percentage of GAB at 25℃ are higher than that at 4℃. Occurrence percentage of GAB in short cores (< 26 cm) are higher than those in longer cores, if cultivated at the same temperature. Occurrence percentage of GAB at 4℃ and 25℃ in short cores are 90.4% and 98.8%, in longer cores are 88.9% and 93.9%, respectively.

Figure 2 shows distribution of GAB abundances of 10 cores at 4℃ and 25℃. Based on statistic of Table 2, it is known that fluctuating ranges of GAB abundance are identical at 4℃ and at 25℃. But average abundance of GAB is higher at 25℃, which is 1.2 times of that at 4℃. By statistic analysis based on Table 1 and Table 2, the variation characteristics of GAB abundance in each layers of cores can be found. Highest average abundance of GAB appear within 20 ~ 22 cm layer at both 4℃ and 25℃, and the value is 2.75 × 10^8 cell · g^{-1} and 2.76 × 10^8 cell · g^{-1}, respectively. Lowest average abundance of GAB at 4℃ appears within 26 ~ 28 cm layer, and is 1.63 × 10^6 cell · g^{-1}, which is just equal to 6‰ of highest average abundance. Lowest average abundance at 25℃ presents within 30 ~ 32 cm layer, and is 2.11 × 10^6 cell · g^{-1}, equals to 8‰ of highest average abundance. It is suggested that bacterial abundance increasing in deeper layer may be caused by raising temperature. Figure 2 also indicates that high GAB abundance does not necessarily appear at surface or near surface sediment, which is controlled by environmental factors.

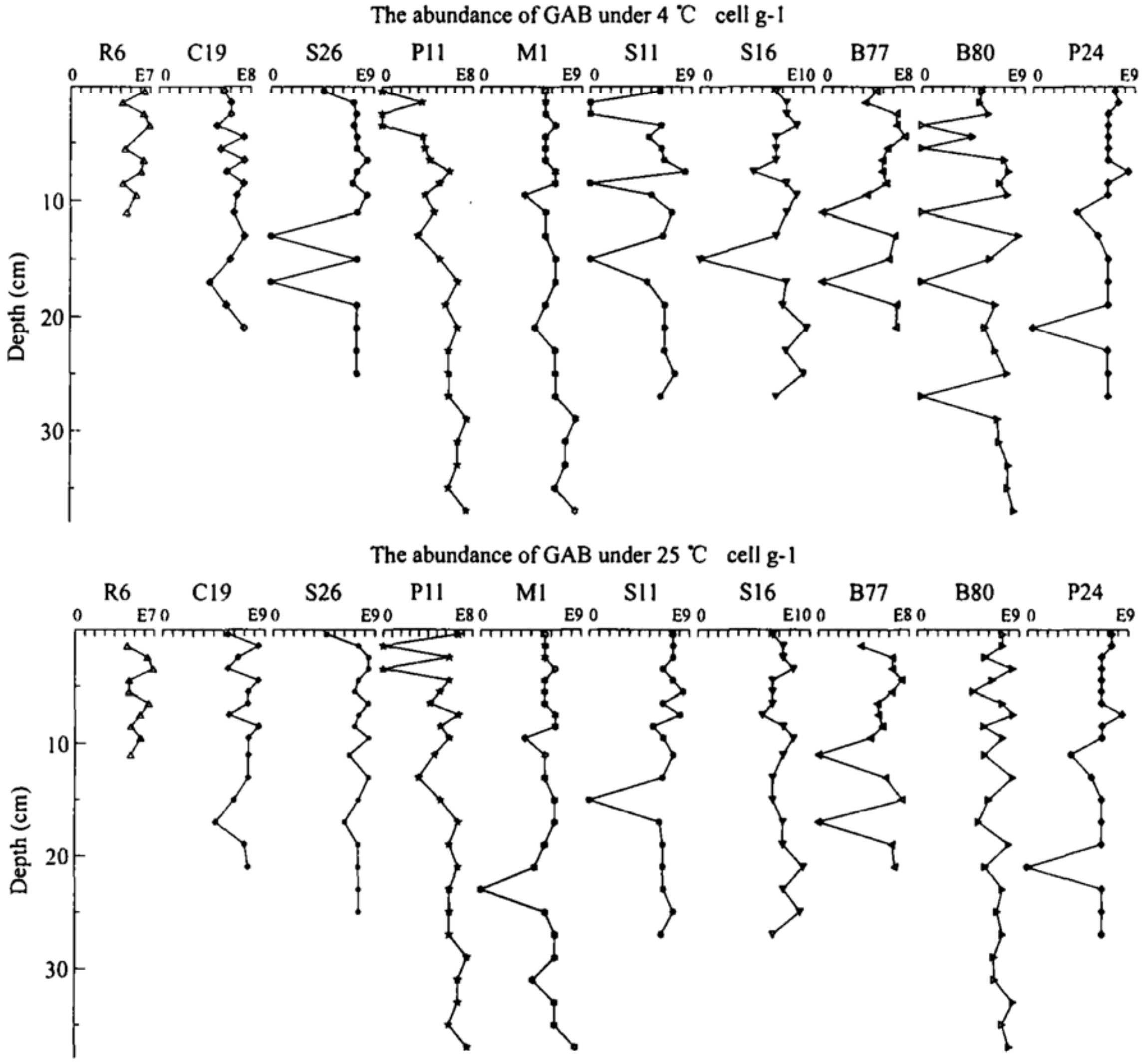

Fig. 2 Distribution of GAB among the layers of sediments in the Canada Basin and the Chukchi Sea.

Table 2 and Figure 2 shows that GAB abundance of cores do not distribute regularly. GAB abundance of cores present high value within surface and bottom layer, but the middle layer has low abundance. It may be the main reason that biogeochemical condition, ecological factors including organic matter and reducing matter, within surface sediments they are more abundant than the middle layer[11]. Then the steady condition within the bottom layer may be propitious to propagation of GAB. As for deeper layer condition, further research are required.

It can be inferred that higher temperature could change and promote enzyme production in a portion of cool or medium temperature-resistant GAB and then enlarge its survival chance and propagation[12]. The results obtained by MPN method are less than that by DAPI from Sahm *et al.* (1998)[2], but higher than the abundances of sea-ice microorganism in the adjacent sea[5].

4.2 comparison of occurrence percentages and abundances of GAB in sediment cores along latitude

The study area were divided into 4 blocks based on latitudes of 10 sampled cores:. 66° ~ 70°N, 70° ~ 74°N, 74° ~ 78°N and North of 78°N. The statistic results of calculated GAB in each block are listed in Table 3.

Table 3. Comparison of GAB in sediment cores along the latitudinal in Canada Basin and Chukchi Sea

Latitudinal (°N)	4℃					25℃				
	Num.	Oc. Per.	Min.	Max.	Ave.	Num.	Oc. Per.	Min.	Max.	Ave.
66-70	11	90.9	0	1.44×10^6	1.44×10^6	10	100.0	1.90×10^4	1.34×10^6	1.34×10^6
70-74	72	90.3	0	2.18×10^8	7.38×10^7	72	98.6	0	2.19×10^8	9.69×10^7
74-78	59	93.2	0	2.30×10^7	1.54×10^7	59	93.2	0	1.68×10^7	1.17×10^7
>78	40	82.5	0	2.09×10^7	1.32×10^7	39	94.9	0	5.91×10^7	3.79×10^7

The arrangement of occurrence percentages of GAB at 4℃ in 4 blocks is 74° ~ 78°N > 66° ~ 70°N > 70° ~ 74°N > North of 78°N, and those of the maximum or average abundance of GAB in 4 blocks is 70° ~ 74°N > 74° ~ 78°N > North of 78°N > 66-70°N, respectively.

The arrangement of occurrence percentages of GAB at 25℃ in 4 blocks is 66° ~ 70°N > 70° ~ 74°N > North of 78°N > 74° ~ 78°N, and those of the maximum or average abundances of GAB in 4 blocks is 70° ~ 74°N > North of 78°N > 74° ~ 78°N > 66° ~ 70°N, respectively.

The occurrence percentages and abundances of GAB are higher for medium latitude area than those for the southern part and northern part of study area. This may be relative with the stability of sediment environment. According to the First and Second Chinese National Arctic Research Expedition, GAB in surface sediments shows the tendency of abundances decreasing with latitude[6,7]. But the GAB abundances in sediment cores are different.

4.3 comparison of GAB in sediment cores from different water depth

GAB of the sediment cores were statistic analyzed after dividing into 4 water depth division: 41 ~ 55 m, 175 ~ 561 m, 1456 ~ 2200 m and 3000 ~ 3850 m. Comparative results are listed in Table 4.

Table 4. Comparison of GAB in sediment samples under different water depths

Depth (m)	4℃					25℃				
	Num.	Oc. Per.	Min.	Max.	Ave.	Num.	Oc. Per.	Min.	Max.	Ave.
41-55	46	89.1	0	2.40×10^8	9.37×10^6	45	97.8	0	2.40×10^8	3.12×10^7
175-561	16	81.3	0	2.40×10^7	3.29×10^6	16	87.5	0	2.40×10^7	6.65×10^6
1456-2200	43	97.7	0	2.40×10^8	2.00×10^7	43	95.4	0	2.40×10^8	1.36×10^7
3000-3850	77	87.0	0	2.40×10^9	7.05×10^7	76	97.4	0	2.40×10^9	9.29×10^7

The occurrence percentage maximum of GAB at 4℃ appears in the division 1456 ~ 2200 m, and the minimum is in division 175 ~ 561 m. The abundance maximum and minimum of GAB locates in division 3000 ~ 3850 m and 175 ~ 561 m, respectively. Moreover, average abundance of GAB generally increases with increase of water depth except for division 175-561 m, which locates in the continental slope region with especial sediment environment.

Cultivated at 25℃ the occurrence percentage maximum of GAB presents in division 41 ~ 55 m, and the minimum is in division 175 ~ 561 m like that at 4℃. Moreover, the abundance maximum and minimum of GAB at 25℃ are consistent with that at 4℃, in the division of 3000 ~ 3850m and 175 ~ 561 m, respectively. However, average abundance of GAB at 25℃ presents fluctuating variation along with water depth, with maximum in shallow water area, and minimum in the division 175 ~ 561 m. These distribution characteristics differ from that in surface sediment[7]. The occurrence percentage and abundance minimum appears in the division 175 ~ 561 m, it indicates the division may locate in transition region of geological, geographical and ecological variation. The condition like this may not suitable to the living of GAB, but the actual reason needs further study.

As for all cores, the high abundances of GAB largely exist in the middle-lower layers, and do not appear at the surface. It may be related with the possible that the decomposition of organic matter and enhancement of oxidation in middle-lower layers[11] are propitious to propagation of GAB. Medium latitude area exist higher occurrence percentage and abundance of GAB. Although northern high latitude area holds relative steady environment, but the nutritional matter required by microbes is lacking. Southern low latitude shallow water area really has abundant nutritional matter, but variation of environment is acute, such as icing and higher salinity water in winter, warm and low salinity water in summer *et al.*. Such a condition will go against propagation of some microbe. However, medium latitude area exist some advantage on source of nutritional matter and ecological environment, so higher occurrence percentage and abundance of GAB appear. Growth of GAB is affected more distinctly by water depth at 4℃ than at 25℃. Moreover the thicker the overlying water, the lower the abundance of GAB, but except for the continental slope area.

5 conclusion

GAB in 10 sediment cores from the Canada Basin and Chukchi Sea were analyzed by the methods of MPN. The occurrence percentages, range of abundance and averages of GAB cultivated at 4℃ and 25℃ are 91.3%, 0 ~ 2.40×10^9 cell · g^{-1}, 4.46×10^7 cell · g^{-1} and 96.7%, 0 ~ 2.40×10^9 cell · g^{-1}, 5.47×10^7 cell · g^{-1}, respectively. Comparisons of GAB in 10 cores indicate that GAB distribute extensively in the study area, and the occurrence percentages of GAB in shallow layer are higher than that in middle layer.

The experiments, cultivating at two temperature, show occurrence percentage and abundance of GAB increase when temperature rising. Although the cultivation temperature can't reflect the in situ environmental temperature correctly, yet the results imply that environmental temperature could be a significant factor for microbes in sediments in Arctic Ocean to some extent. Temperature change will bring the variety of physiological and biochemical state of microbes. Global climate warming is consequentially influencing the survival and evolution of microbes in sediments in Arctic Ocean, and further researches are expected to carry out.

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