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Bacterial number, heterotrophy and extracellular enzyme activity in the Bransfield Strait, Antarctica

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Abstract

To study the structure and function of bacterial populations in the Bransfield Strait, Antarctica, which is located between S 62° - 64° and W 56° - 62°, twenty nine sampling stations were chosen. Samples were collected from seawater and sediment during austral summer (1989 Dec. - 1990 Jan.) and analyzed for total bacterial number, total saprophytic bacterial number, heterotrophic activity and extracellular enzyme activities.

The number of total bacteria in seawater was between 1.0×10^4 cells/ml and 1.6×10^5 cells/ml, and total saprophytic bacteria were between 0.5×10^2 CFU/l and 8.0×10^4 CFU/l. The population density of saprophytic bacteria was significantly low, giving less than 10^{-4} of the total bacterial number, in this region. Turnover times of glucose and leucine in seawater were in the ranges of 41 to 2094 hrs, and 56 to 980 hrs, respectively. Turnover times of these organic matters were extremely variable depending on the sampling station and water depth. In the sediments, the enzyme activities of α-glucosidase, N-acetyl-β-glucosaminidase and aminopeptidase of the fast growing bacterial population were higher than those of the slow growing bacterial population, β-glucosidase activities, however, were higher in the slow growing bacterial population.

Introduction

The Antarctic is an area of outstanding scientific interest. Bacterial heterotrophy, once considered negligible in Antarctic water is now emerging as an important pathway of secondary production, and detrital material particularly that derived from krill appears to enter the food web at different trophic levels (VINCENT 1988). Bacteria in the Antarctic environment exhibit a number of interesting properties and additionally play a crucial role in the cycling of nutrients. Many scientists regard marine bacterial metabolism as a principal feature in the regeneration of primary nutrients (TANNER 1985). From this view, we carried out this study to understand the structure and function of bacterial populations in this specialized environment.

Material and methods

The locations of sampling stations are shown in Fig. 1. Sampling period was be-

tween Dec. 29, 1989 and Jan. 7, 1990, during austral summer. To estimate total bacterial cell number, epifluorescent microscopic method was used (ZIMMERMANN 1977). In addition to the basic procedures recommendations of CASSEL (1965), ZIMMERMANN and MEYER-REIL (1974), DALEY and HOBBIE (1975) and POMROY (1984) were considered. To enumerate total saprophytic bacterial number, the membrane filter method (pore size : 0.45 µm) and plate count method were used for seawater and sediment samples, respectively, using ZoBell 2216E agar medium. Plates were incubated at 8 °C for 15 days. The extracellular enzyme activities of α- and β-glucosidase, N-acetyl- β -glucosaminidase and aminopeptidase were determined by the method of KIM and HOPPE (1986). For the assessment of microbial activity, turnover time was measured by the method of WILLIAMS and ASKEW (1968) using ^{14}C-glucose and ^{14}C-leucine as substrates.

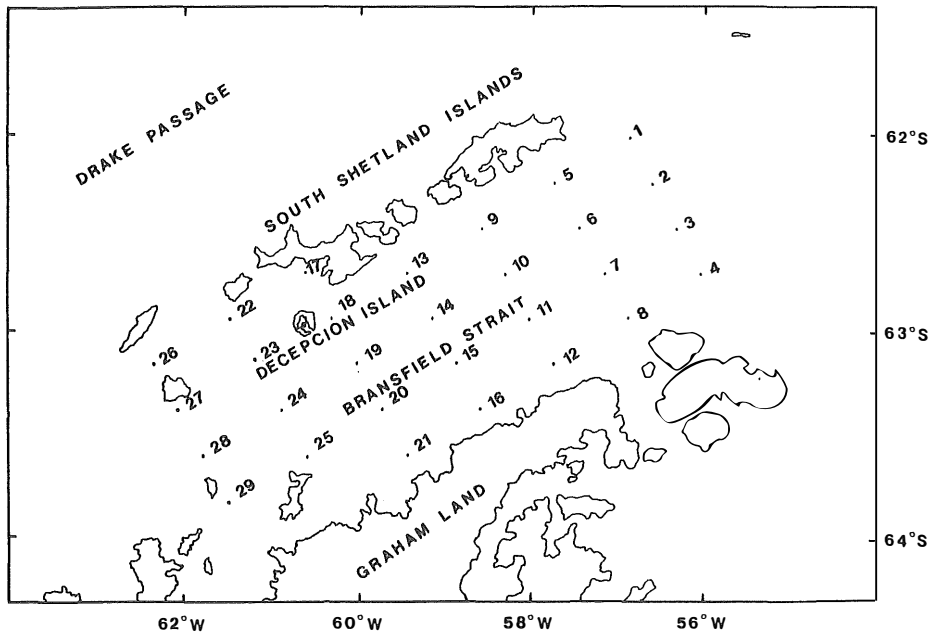

Fig. 1. Map of the Bransfield Strait with stations 1-29.

Results and discussion

Total bacteria and saprophytes

Table 1 shows the number of total bacteria and total saprophytes in each station of the Bransfield Strait at different water depths. The number of total bacterial cells varied from 1.0×10^4 cells ml^{-1} to 1.6×10^5 cells ml^{-1}. In most stations, the total cell number in the upper layer of water column was higher than that in the deeper layer. The total cell number obtained in the present investigation was found to be similar to that reported by HANSON et al. (1983) for the Drake Passage, in January, with values ranging between 1×10^4 and 2×10^5 cells ml^{-1}

Table 1. Number of total bacteria and saprophytes in each station of the Bransfield Strait.

Station	Sampling depth (m)	Total bacterial number[1]	Total number of saprophytes[2]	Station	Sampling depth	Total bacterial number[1]	Total number of saprophytes[2]
	0	13.6	31		0	4.8	17
1	30	8.3	11	16	30	3.9	16
	700	2.2	16		150	4.3	4.9
	0	7.5	24		0	12.9	-
2	30	11.3	29	17	30	6.7	-
	750	6.5	2.5		200	3.0	-
	0	6.1	15		0	8.1	-
3	30	2.3	0.5	18	30	-	-
	200	3.0	3.5		750	2.1	28
	0	8.0	24		0	5.3	-
4	30	5.8	8.5	19	30	7.3	-
	200	4.5	15		700	2.3	95
	0	5.6	29		0	8.9	-
5	30	6.6	21	20	30	4.4	10
	1500	1.3	8.0		100	3.8	31
	0	9.8	11		0	6.0	800
6	30	8.2	1.5	21	30	6.4	11
	1000	1.3	2.5		150	3.4	12
	0	7.0	30		0	11.4	-
7	30	4.9	3.5	22	30	6.9	110
	400	2.3	5.5		150	8.6	69
	0	10.5	25		0	4.0	-
8	30	8.1	30	23	20	3.7	140
	100	9.3	18		500	7.0	45
	0	4.2	62		0	8.3	-
9	30	1.9	4	24	30	5.5	-
	1000	4.7	4.5		300	2.4	19
	0	10.8	36		0	5.7	-
10	30	9.1	56	25	30	7.1	-
	750	1.6	3.5		200	4.2	-
	0	6.5	20		0	15.6	-
11	30	3.3	1	26	30	10.6	120
	500	2.3	12		150	7.9	60
	0	4.5	13		0	15.1	100
12	30	3.9	11	27	10	10.1	20
	320	2.8	8		20	7.5	23
	0	8.1	120		0	2.6	-
13	30	6.2	10	28	30	6.3	-
	1000	1.0	7.1		800	2.5	140
	0	4.4	32		0	5.4	-
14	30	6.5	39	29	30	9.2	640
	700	2.1	12.2		750	3.2	67
	0	7.9	33				
15	30	9.6	14				
	75	6.0	3.6				

1 : x 10^4 cells ml^{-1}
2 : x 10^2 CFU l^{-1}

but less than 1/10 of that in the Scotia Sea and coastal Antarctic waters (HANSON et al. 1983).

As shown in Table 1, total saprophyte number was between 5×10^1 and 8×10^4 CFU l^{-1} during the sampling period which is similar to that reported in the same sampling area in 1981 by ZDANOWSKI (1985). Compared with the total bacterial number, the saprophyte number was extremely low, giving less than 10^{-4} of the total bacterial number, in this region. The ratio of saprophytes to total bacteria in this cold environment is significantly low compared with the ratios in the temperate climatic zone. This difference might be due to the saprophyte counting method and the composition of media as pointed out by SIMIDU et al. (1986). These authors postulated that a large proportion of the bacterial population in the Antarctic waters was in the actively growing state to be almost recovered in the artificial medium.

Heterotrophic activity

The turnover times for glucose and leucine in seawater samples from the Bransfield Strait are given in Table 2. These values were in the range of 41-2094 hours and 56-980 hours, respectively and varied depending on the sampling sta-

Table 2. Turnover time of glucose and leucine in seawater samples from the Bransfield Strait.

Station	Sampling depth (m)	Glucose (h)	Leucine (h)
1	0	112.1	92.3
	30	170.1	235.5
	700	835.0	743.3
5	0	88.9	62.3
	30	664.6	499.1
	1500	1703.4	725.9
9	0	90.7	62.3
	30	212.1	192.8
	1000	1628.9	780.9
13	0	214.5	82.6
	30	167.6	110.2
	1000	1663.8	912.8
18	0	178.2	84.2
	30	-	-
	750	530.9	294.6
23	0	1291.8	816.6
	20	1168.2	979.9
	500	515.8	604.8
27	0	2093.7	687.7
	10	161.5	124.8
	20	41.3	55.6

tion and depth. The turnover times of these two compounds from 0 m, 30 m and deeper layer were increasing in this order. However, in case of the station numbers 23 and 27, the opposite pattern was observed. The turnover times of leucine were significantly shorter than those of glucose indicating that leucine could be taken up and remineralized faster than glucose in the water column of the Bransfield Strait. On the other hand, different pool sizes of these substrates in the water may also had an effect on the turnover times.

A high percentage (> 50-75 %) of the planktonic bacteria in marine environment appears to be metabolically inactive or dormant. In Antarctic water, as elsewhere, there is little or no correlation between bacterial DNA synthesis and bacterial cell concentrations (VINCENT 1988). In the present study a correlation between the turnover times of two dissolved organic compounds and the number of total bacterial cells was found, but no correlation of the turnover times with the total number of saprophytes.

Table 3. Turnover time of glucose at various locations.

Location	Tt (h)	References
Western North Pacific	6,000	SEKI et al. (1972)
Kuroshio current	2,700	SEKI et al. (1972)
Subarctic Pacific	1,300	SEKI et al. (1972)
McMurdo Sound		HODSON et al. (1981)
eastern side	116	
western side	20,454	
Bossiere Fjord, Kerguelen Island (Mussel bed)	0.5-23	DELILLE and CAHET (1985)
Kiel Fjord (Brackish water)	2.8-63.4	GOCKE (1977)
Kiel Bight (Brackish water)	5.1-523	GOCKE (1977)
Tokyo Bay	8.7	SEKI et al. (1975)
Shimoda Bay (Estuary)	31	SEKI et al. (1975)

The data summarized in Table 3 implied the great variability of heterotrophic activity in different marine areas. Compared with other results obtained from the different regions, the turnover time of glucose in the Bransfield Strait was shorter than the mean values obtained in the Subarctic Pacific (SEKI et al. 1972) and from the western side of McMurdo Sound (HODSON et al. 1981). In contrast, much shorter turnover time was reported from the samples of the eastern side of McMurdo Sound and Bossiere Fjord, Antarctica.

Water temperature is not likely to play an important role for the spatial distribution of bacterial heterotrophic potential as long as it remains unfluctuated in the Bransfield Strait. Under this circumstance, the effect of total bacterial cell number and its metabolic activity (active or dormant) together with the concentration of organic nutrients on total heterotrophic potential may become dominant.

Table 4. Percentage of positive bacterial colonies showing enzyme activities to total colonies during different incubation periods from sediment samples in the Bransfield Strait.

Station	α-glucosidase			β-glucosidase			N-acetyl-β-glucosaminidase			Aminopeptidase		
	Within 0-5 days	Within 6-15 days	Total period	Within 0-5 days	Within 6-15 days	Total period	Within 0-5 days	Within 6-15 days	Total period	Within 0-5 days	Within 6-15 days	Total period
2	59	64	62	0	47	25	17	5	11	96	79	86
3	96	50	78	0	25	7	0	0	0	100	63	90
6	86	70	82	0	33	16	5	0	4	100	87	94
9	71	63	69	0	30	8	0	0	0	100	77	93
10	96	78	91	2	5	3	0	0	0	62	64	63
11	100	0	98	0	0	0	5	0	4	75	0	67
12	21	10	17	2	7	3	68	32	56	76	50	66
14	100	63	83	0	14	5	55	17	49	63	33	56
15	24	25	24	0	25	3	100	0	85	87	33	85
16	71	8	53	0	0	0	0	0	0	75	27	52
17	54	25	46	0	18	7	15	0	8	85	56	74
18	82	23	57	0	33	11	22	0	8	93	92	92
19	92	92	92	0	76	53	50	2	13	100	94	95
21	97	40	83	0	4	2	0	0	0	84	0	59
22	85	69	80	0	39	14	38	23	35	65	25	54
23	88	100	89	0	14	3	5	0	4	88	29	69
24	88	20	69	0	9	3	6	7	7	100	75	94
25	39	21	36	0	52	15	16	0	11	96	62	86
29	31	18	26	0	20	9	26	0	15	93	56	80
Average	72.6	44.2	54.6	0.2	23.7	9.8	22.5	4.5	16.3	86.2	52.7	76.6
SD	26.8	30.2	29.4	0.9	19.9	12.3	27.9	9.2	23.3	13.3	28.7	15.3

SD : standard deviation

Bacterial extracellular enzyme activity

Table 4 shows the percentage of colonies with specified enzyme activities to total colonies during different incubation periods from sediment samples in the Bransfield Strait. The bacteria growing to colonies within 5 days were classified as the fast growing population and those growing to colonies after 6 days of incubation as the slow growing population. The enzyme activities of α-glucosidase, N-acetyl-β-glucosaminidase and aminopeptidase in the fast growing population were higher compared with those in the slow growing population. In case of β-glucosidase activities, however, the opposite result was obtained implicating that the slow growing bacterial population can play a major role for cellulose decomposition in the sediment environment of the Bransfield Strait.

The average values of each enzyme activity are increasing in the order of β-glucosidase, N-acetyl- β-glucosaminidase, α-glucosidase and aminopeptidase. Among the 4 different enzymes, aminopeptidase showed the highest activity supporting the phenomenon described by ZOBELL (1946) that most marine bacteria possess high proteolytic activity.

References

CASSELL, E.A., 1965. Rapid graphical method for estimating the precision of direct microscopic counting data. Appl. Microbiol. 13, 293-296.

DALEY, R.J. and J.E. HOBBIE, 1975. Direct counts of aquatic bacteria by a modified epifluorescent technique. Limnol. Oceanogr. 20, 875-882.

DELILLE, D. and G. CAHET, 1985. Heterotrophic processes in a Kergulen mussel-bed. In: W.R. SIEGFRIED, P.R. CONDY and R.M. LAWS (eds.), Antarctic nutrient cycles and food webs, Springer-Verlag, Berlin, 128-135.

GOCKE, K., 1977. Untersuchungen über die heterotrophe Aktivität in der zentralen Ostsee. Mar. Biol. 40, 87-94.

GOCKE, K., 1977. Heterotrophic activity. In: G. RHEINHEIMER (ed.), Microbial ecology of a brackish water environment, Springer-Verlag, Berlin, 198-222.

HANSON, R.B., H.K. LOWERY, D. SHAFER, R. SOROCCO and D.H. POPE, 1983. Microbes in Antarctic waters of the Drake Passage: Vertical patterns of substrate uptake, productivity and biomass in January 1980. Polar Biology 2, 179-188.

HODSON, R.E., F. AZAM, A.F. CARLUCCI, J.A. FUHRMAN, D.M. KARL and O. HOLM-HANSEN, 1981. Microbial uptake of dissoved organic matter in MacMurdo Sound, Antarctica. Mar. Biol. 61, 89-94.

KIM, S.-J. and H.-G. HOPPE, 1986. Microbial extracellular enzyme detection on agar plates by means of fluorogenic methylumbelliferyl-substrates. Deuxieme Colloque International de Bacteriologie marine CNRS, Brest, Actes de Colloques 3, 175-183.

POMROY, A.J., 1984. Direct counting of bacteria preserved with Lugol-iodine solution. Appl. Environ. Microbiol. 47, 1191-1192.

SEKI, H., T. NAKAI and H. OTOBE, 1972. Regional differences on turnover rate of dissolved materials in the Pacific Ocean at summer 1971. Arch. Hydrobiol. 71, 79-89.

SEKI, H., Y. YAMAGUCHI and S. ICHIMURA, 1975. Turnover rate of dissolved organic materials in a coastal region of Japan at summer stagnation period of 1974. Arch. Hydrobiol. 75, 297-305.

SIMIDU, U., K. KOGURE, K. FUKAMI and C. IMADA, 1986. Heterotrophic bacterial flora of the Antarctic ocean. Mem. Natl. Inst. Polar Res., Spec. Issue 40, 405-412.

TANNER, A.C., 1985. The role of bacteria in the cycling of nutrients within the maritime Antarctic environment. In: W.R. SIEGFRIED, P.R. CONDY and R. M. LAWS (eds.), Antarctic nutrient cycles and food webs, Springer-Verlag, Berlin, 123-127.

VINCENT, W.F., 1988. Microbial ecosystems of Antarctica, Cambridge University Press, Cambridge, 304 pp.

WILLIAMS, P.J. LeB. and C. ASKEW, 1968. A method of measuring the mineralization by microorganisms of organic compounds in seawater. Deep Sea Res. 15, 365-375.

ZDANOWSKI, M., 1985. Distribution of saprophytic bacteria. Biological investigations of marine Antarctic systems and stocks (biomass) Sp. Is. Atlas of polish oceanographic observations in Antarctic waters 1981, SCAR and SCOR Scott Polar Research Institute, Cambridge, 44-46.

ZIMMERMANN, R. and L.-A. MEYER-REIL, 1974. A new method for fluorescence staining of bacterial populations on membrane filters. Kieler Meeresforsch. 30, 24-27.

ZIMMERMANN, R., 1977. Estimation of bacterial number and biomass by epifluorescence microscopy and scanning electron microscopy. In: G. RHEINHEIMER (ed.), Microbial ecology of a brackish water environment, Springer-Verlag, Berlin, 103-120.

ZOBELL, C.E., 1946. Marine microbiology. A monograph on hydrobacteriology, Waltham, Mass., USA, Chronica Botanica Co, 240 pp.