Mem. Natl Inst. Polar Res., Spec. Issue, **40**, 405–412, 1986

HETEROTROPHIC BACTERIAL FLORA OF THE ANTARCTIC OCEAN

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Abstract: Vertical distribution of heterotrophic bacteria was surveyed at six sampling stations in the Antarctic and the Indian Oceans. Seawater samples were collected at various water depths, ranging from 0 to 2000 m. Total bacterial counts were determined by the direct microscopic method. Plate counts were carried out by the surface spreading method and the filter method using ORI agar medium. At a sampling station in the Antarctic Ocean, the enumeration of oligotrophic bacteria was also made using an MPN method.

The total bacterial counts per m*l* of seawater in the Antarctic region ranged from 10^4 to 10^5, whereas the plate counts by the filter method were 10^0 to 10^1 at the upper 500 m layers and 10^{-1} to 10^0 at the deeper water lyers. The plate counts at 2°C incubation temperature were higher than those at 20°C by 1–2 orders of magnitude for the samples at stations in the Antarctic region. In contrast, the 20°C counts for seawater from the Indian Ocean were slightly higher than 2°C counts.

The surface spreading method gave bacterial counts that were one order of magnitude higher than the filter method, and the counts with the MPN method using diluted medium were greater still, reaching about 10% of total bacterial counts. The results suggested that a fairly large proportion of the bacterial population in Antarctic seawater is in the actively growing state.

More than 87% of the bacterial strains isolated from surface water of the Antarctic Ocean were orange- and yellow-pigmented bacteria. Among the pigmented bacteria, Gram-negative, non-motile, orange-pigmented rods that appeared to constitute a single species belonging to *Flavobacterium-Cytophaga* predominated.

1. Introduction

Since the 1950's psychrophilic bacteria, whose optimum growth temperature lies below 20°C, have been isolated from the soil, lakes and sediments of Antarctica and from the seawater in the Arctic and Antarctic regions. Since the water temperature of the Antarctic Ocean is nearly 0°C throughout the year, it would be expected that the bacterial population of the seawater is composed mainly of these psychrophilic bacteria. KRISS and his colleagues (1971) and WIEBE and HENDRICKS (1974) made a survey of seawater bacteria in the Antarctic Ocean. The results of the latter authors show that the ratio of the organisms growing at 20°C to those growing at 0°C ranged from 30 to 100%, averaging 71% for samples from the Antarctic region (in the area south of 57°S).

The present report deals with the number and composition of the heterotrophic bacterial population of the Antarctic Ocean and discusses the results obtained in comparison with other oceanic seawaters.

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2. Materials and Methods

Seawater samples were collected at various water depths down to 2000m during the KH-83-4 research cruise of R. V. HAKUHO MARU from December 1983 to February 1984 at Stns. 3, 3A, 4, PI-1, 5, 6 and 7 (Fig. 1). Two different types of Niskin bacteriological samplers (chopstick and butterfly types, General Oceanics Ltd.) were used for collecting the water samples. The samples were transferred to sterile glass bottles, and plating was carried out within 4 hours after the sampling.

Total bacterial counts were determined by the direct microscopic method according to HOBBIE *et al.* (1977). Plate counts were generally carried out by the filter method using nuclepore filters of 0.2 μ pore size (General Electric Ltd.). The volume of filtered seawater samples ranged from 2–200m*l* according to the depth. For the samples of Stns. 5 and 6, the surface spreading method was also employed for the plate counts. Water samples ranging from 0.1 to 0.5m*l* were applied on agar plates. The medium used was ORI medium, the composition of which is given in Table 1. The plates were incubated at 2 and 20°C, and the colonies developed were counted for up to 30 and 14 days, respectively. After the counting, about 20 colonies from each water layer were picked up at random from the plates and purified by streaking on the agar plates. The generic composition of the isolated bacteria was determined mainly according to the scheme of SIMIDU (1985) and Bergey's Manual of Systematic Bacteriology (KRIEG and HOLT, 1984). Since flagellar staining was not carried out, Gram-negative motile rods were classified collectively under the *Pseudomonas-Alcaligenes* group.

MPN counts using a diluted medium was also made for the samples from Stn. 5. The composition of the medium (medium M) is also shown in Table 1. The concentration of the organic nutrients of the medium is so minute that the bacterial growth

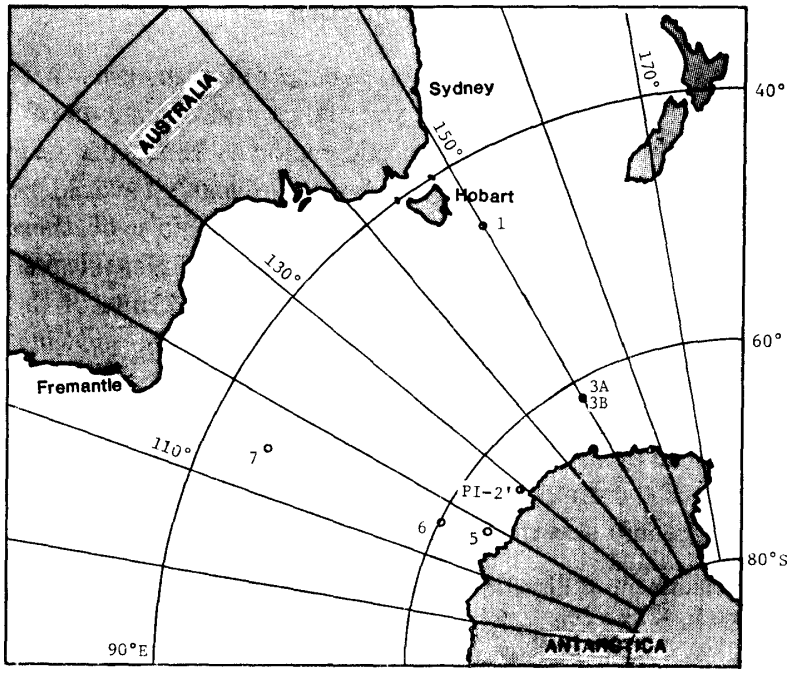

Fig. 1. Sampling locations during KH-83-4 cruise of R. V. HAKUHO MARU.

Table 1. Composition of media.

	ORI	D	M
Proteose Peptone No. 3 (Difco)	1000 mg	50 mg	4 mg
Bacto Yeast Extract (Difco)	1000	50	4
Phytone (BBL)	500	—	2
Ferric citrate	40	5	0.5
Sodium thiosulfate	200	—	2
Sodium sulfite	50	—	1
Glucose	—	2.5	0.2
Mannitol	—	2.5	0.2
Sodium acetate	—	2.5	0.2
Sodium malate	—	2.5	0.2
Agar	15.0 g	3.0 g	—
Aged seawater	900 m*l*	1000 m*l*	1000 m*l*
Distilled water	100	—	—

pH of media was adjusted to 7.8–8.0.

cannot be determined through observation of the turbidity of the medium. Hence, after incubating for 14 days at 2°C, 2m*l* of the inoculated medium were transferred into a semi-solid medium of higher organic nutrients (medium D, Table 1), and incubated for further 10 days to determine the growth.

3. Results and Discussion

Vertical distribution of the bacterial counts at various stations is shown in Table 2. The total bacterial counts per m*l*, which were determined by the direct microscopic method, varied from 10^5–10^6 at the surface to 10^4–10^5 in the deeper water layers.

The results of the viable bacterial counts depended greatly on the counting methods used. In a previous report (SIMIDU *et al.*, 1983), we pointed out the advantage of the surface spreading method over the filter method for both coastal and pelagic water samples. Again in the present results the bacterial counts by the surface spreading method were found to be higher than those determined with the filter method by more than one order of magnitude. However, the accuracy of the surface spreading method is limited since only small volumes water samples (up to 0.5 m*l*) can be applied; hence, few colonies developed on the plates for the samples of deeper water layers.

In both the water layers at Stn. 5, precise MPN counts were not obtained since all five tubes of the highest dilution—10^4 for the surface water and 10^3 for the sample from 1400m layer—gave positive bacterial growth. However, the results showed that the difference between the total bacterial counts and the MPN counts was about one order of magnitude. Our previous experiences with samples from pelagic areas of the Pacific Ocean showed that the MPN counts with the diluted medium were often 2–3 orders of magnitude lower than the total counts, which clearly contrasted with the present results. Apparently, quite a large proportion of the bacterial population in the Antarctic waters was in the actively growing state during the present sampling period, and most of them could be recovered in the artificial medium.

Most of the bacteria grown on the diluted MPN medium could grow on ORI

Table 2. Bacterial number in seawater samples.

	Depth	TC	PC			Depth	TC	PC			MPN
			2°	20°				2°	2° (spr)	20°	
Stn. 1	0 m	2.2×10^{6}	1.0×10^{0}	2.6×10^{1}	Stn. 5	0 m	3.7×10^{5}	4.3×10^{1}	1.1×10^{3}	3.4×10^{-1}	
	100	5.8×10^{5}	1.0×10^{-1}	1.7×10^{1}		20	3.6×10^{5}	3.6×10^{1}	1.8×10^{3}	1.0×10^{-1}	$2.4\times10^{4}<$
	200	3.5×10^{5}	2.4×10^{-1}	5.5×10^{0}		100	4.5×10^{5}	3.4×10^{1}	3.1×10^{2}	1.3×10^{0}	
	300	1.6×10^{5}	1.6×10^{-1}	1.5×10^{1}		150	2.7×10^{5}	3.2×10^{1}	3.3×10^{2}	1.1×10^{0}	
	500	8.0×10^{5}	3.0×10^{-2}	1.2×10^{1}		300	2.8×10^{5}	2.9×10^{1}	1.0×10^{3}	7.0×10^{-1}	
	800	2.2×10^{5}	1.9×10^{-1}	3.9×10^{0}		500	2.3×10^{5}	2.7×10^{1}	2.1×10^{2}	2.6×10^{-1}	
	1400	1.3×10^{5}	1.9×10^{-1}	3.6×10^{0}		1400	8.6×10^{4}	5.6×10^{0}	2.1×10^{1}	1.1×10^{0}	$2.4\times10^{3}<$
	2000	4.1×10^{5}	1.6×10^{-1}	1.5×10^{0}		2000	6.3×10^{4}	1.3×10^{0}	2.6×10^{1}	2.3×10^{0}	
Stn. 3A	0 m	9.5×10^{4}	2.1×10^{1}	6.7×10^{-1}	Stn. 6	0 m	1.9×10^{5}	6.5×10^{0}	2.8×10^{2}	2.6×10^{0}	
	20	1.3×10^{5}	1.8×10^{1}	5.0×10^{-1}		20	1.5×10^{5}	2.5×10^{0}	2.3×10^{2}	4.8×10^{-1}	
	100	9.3×10^{4}	2.4×10^{1}	6.4×10^{-1}		50	2.8×10^{5}	2.1×10^{0}	1.4×10^{2}		
	200	7.4×10^{4}	4.5×10^{0}	1.7×10^{0}		100	1.8×10^{5}		2.0×10^{1}	0.4×10^{-2}	
	300	7.1×10^{4}	2.8×10^{0}	5.6×10^{0}		200	1.3×10^{5}	7.6×10^{-1}	5.0×10^{0}	4.4×10^{-1}	
	500	5.4×10^{4}	2.5×10^{0}	7.8×10^{-1}		300	1.2×10^{5}		1.5×10^{1}	2.0×10^{-2}	
	800	1.3×10^{5}	1.4×10^{1}	1.3×10^{-1}		500	1.2×10^{5}	5.5×10^{-1}	1.3×10^{1}	7.0×10^{-2}	
	1200	7.9×10^{4}	5.2×10^{0}	4.2×10^{-1}		800	1.4×10^{5}	9.0×10^{-1}	4.0×10^{1}	6.3×10^{-1}	
	1500	4.0×10^{4}	6.8×10^{-1}	1.0×10^{-2}		1400	8.6×10^{4}	5.9×10^{-1}		9.9×10^{-1}	
Stn. 3B	0 m	1.1×10^{5}	3.0×10^{0}	3.7×10^{0}	Stn. 7	0 m	1.4×10^{6}	1.7×10^{0}		1.7×10^{1}	
	20	1.6×10^{5}	5.8×10^{0}	4.4×10^{0}		20	1.4×10^{6}	3.0×10^{0}		5.0×10^{0}	
	50	1.0×10^{5}	4.6×10^{0}	2.1×10^{1}		50	1.6×10^{6}	7.5×10^{0}		5.0×10^{0}	
	75	1.1×10^{5}	1.1×10^{0}	2.0×10^{0}		100	6.6×10^{5}	3.4×10^{0}		5.9×10^{0}	
	100	9.9×10^{4}	4.0×10^{0}	1.4×10^{1}		150	4.5×10^{5}	7.2×10^{0}		6.9×10^{0}	
	125	1.1×10^{5}	1.1×10^{1}	2.0×10^{0}		200	2.3×10^{5}	2.6×10^{0}		4.6×10^{0}	
	150	9.8×10^{4}	2.9×10^{1}	1.0×10^{2}		300	2.5×10^{5}	1.6×10^{0}		3.0×10^{0}	
	200	6.2×10^{4}	1.2×10^{0}	1.5×10^{1}		500	3.0×10^{5}	9.2×10^{-1}		2.0×10^{0}	
	500	6.3×10^{4}	4.5×10^{-1}	4.7×10^{0}		800	1.1×10^{5}	2.0×10^{-1}		1.0×10^{0}	
						1400	9.3×10^{4}	2.2×10^{0}		4.1×10^{0}	
						2000	7.1×10^{4}	2.9×10^{-1}		3.5×10^{-1}	

TC: total counts by direct microscopic method. PC: plate counts on ORI medium. MPN: most probable number using medium M, Table 1.

medium after the first isolation. The concentration of nutrients in ORI medium is about a half of the media such as ZoBell's 2216E and Taga's PPES-II, which are used as standard media in marine microbiology works. These results show that most of the heterotrophic bacteria in the seawater have adapted themselves to the low nutrient condition of the *in situ* environment, and oligotrophic media have definite advantages for the first isolation of bacteria in the oligotrophic state. However, after first growing in an oligotrophic medium, most of them become culturable in media of high organic nutrient concentration.

The clear contrast among the growing temperatures of bacteria from different habitats was also seen in the results given in Table 2 and Fig. 2. Psychrotrophic bacteria, which do not grow at 20°C, predominated in the samples from the Antarctic water, whereas the number of bacteria grown at 20°C were mostly larger than those grown at 2°C in the samples from the Indian Ocean.

The outstanding feature of the heterotrophic bacterial population in the Antarctic Ocean is the predominancy of orange- and yellow-pigmented bacteria. As is shown in Table 3, the proportion of orange-pigmented strains was 83–100% for the isolates from the upper 100–150m at Stns. 5 and 6. In the deeper waters the ratio of the orange-pigmented strains decreased and non-pigmented strains predominated instead.

In our experience with both the coastal and pelagic seawater, the predominancy of a single genus of over 50% occurring on such a wide scale as was seen in the present study is rarely encountered. The orange-pigmented bacteria seemed to be composed of one, or at least only a few, closely related species. They are Gram-negative, asporogenous, pleomorphic rods, often showing elongated cells. They showed no flagellar movement, did not ferment glucose, showed a positive oxidase reaction, and did not hydrolyze gelatin though most strains hydrolyzed DNA.

The composition of the bacterial population obtained by the MPN method differed from that of the agar plate method, containing a larger proportion of yellow-pigmented strains, although the latter still represent the majority in the surface water sample. Since far more bacteria were recovered by the MPN method, the results may reflect more precisely the composition of actual bacterial populations *in situ*.

The Antarctic Ocean is generally defined as the inner region of the Antarctic Convergence which, in the present cruise, lay approximately 55°S along 115°E and 56.5°S along 150°E. Around Antarctica the steady eastward circumfluent current preventing the intervening of outer environments prevails. The results of the determination of chlorophyll *a*, as well as the amount of inorganic nitrogen and phosphates, indicated that most of the phytoplankton production occurs in the layers from the surface down to 150–175m of water, the chlorophyll maximum being at about 75m. Hydrological data also showed the presence of a surface water body of low temperature (down to −1.7°C) and low salinity at the upper 100 to 200m depths. The estimation of bacterial production using radioactive thymine also showed that the active production occurs only in the upper 150–200m layers (KOGURE *et al.*, 1986).

These results show that the biological activities in the Antarctic waters are developed mostly in the surface water layers of 200m and up.

The reason why the orange-pigmented bacteria predominated in the Antarctic surface waters at the time of the cruise is uncertain. The predominancy of pigmented

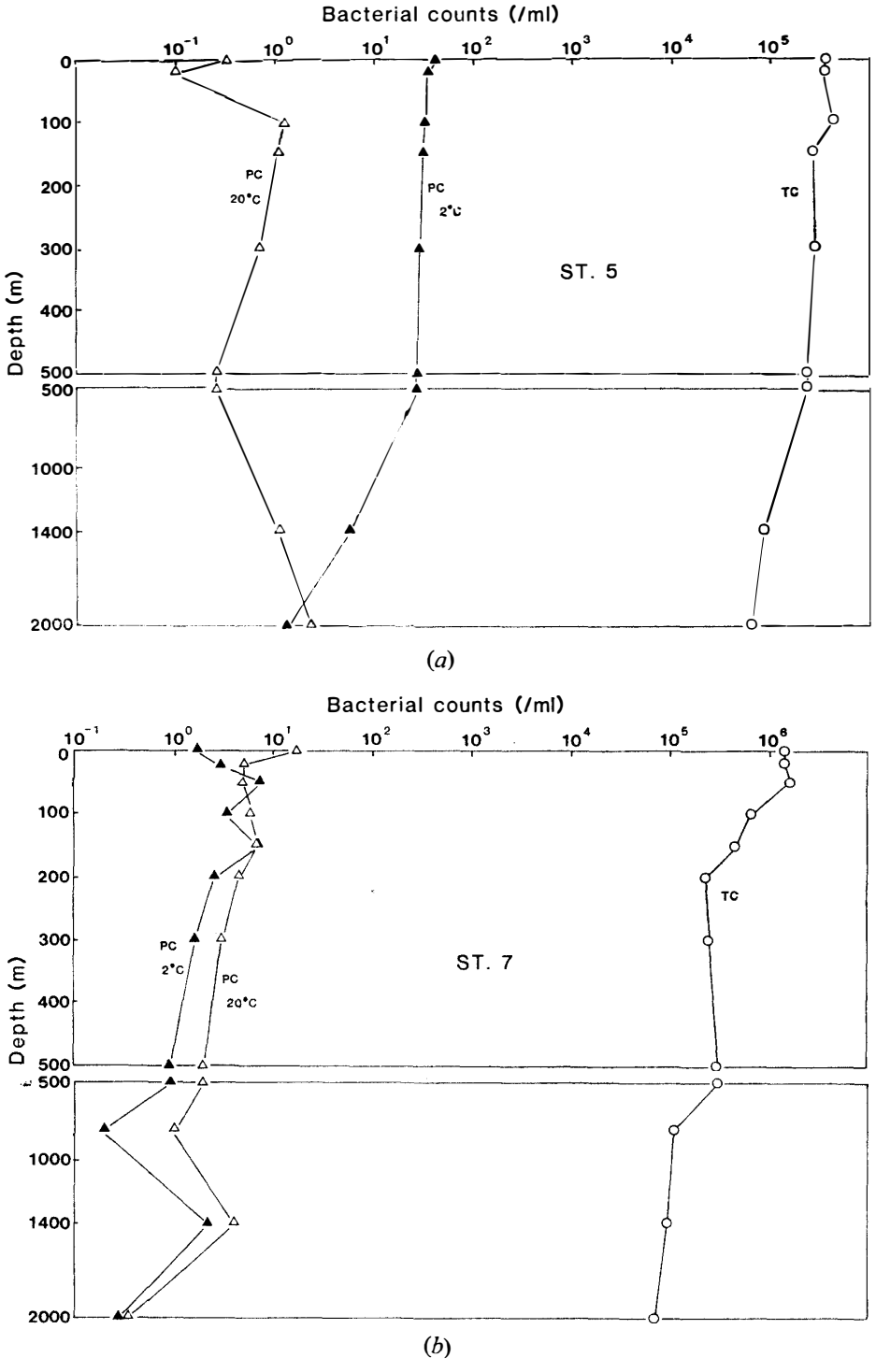

Fig. 2. Vertical profiles of bacterial counts at Stn. 5 (Fig. 2a) and Stn. 7 (Fig. 2b). TC: total counts, PC: plate counts.

bacteria in the eutrophic water has been reported by a number of authors for either marine or fresh water. In Tokyo Bay, 21–36% of the heterotrophic bacterial population of seawater samples taken in December and March was composed of *Flavobacterium-Cytophaga* (SIMIDU *et al.*, 1977). *Flavobacterium-Cytophaga* often constitute a greater

Table 3. Percentage of pigmented colonies on ORI agar plates for counting.

	Depth (m)	Orange (%)	Yellow (%)	Gray-white (%)
Stn. 5	0	82.6 (63.6)*	8.7 (36.4)*	8.7 (0.0)*
	20	87.0	0.0	13.0
	100	88.0	0.0	12.0
	150	91.3	4.3	4.3
	500	40.0	0.0	60.0
	1400	26.7 (10.0)*	0.0 (70.0)*	73.3 (20.0)*
	2000	23.8	9.5	66.7
Stn. 6	0	94.4	5.6	0.0
	20	100.0	0.0	0.0
	50	98.0	1.0	1.0
	100	93.8	0.0	6.2
	150	13.3	46.7	40.0
	200	17.6	47.1	35.3
	300	0.0	11.1	88.9
	500	25.0	12.5	62.5
	800	57.1	0.0	42.9
	1400	0.0	30.0	70.0
Stn. 7 (2°C incubation)	0	36.2	79.2	14.6
	20	16.7	66.6	16.7
	50	0.0	100.0	0.0
	100	0.0	82.4	17.6
	150	0.0	83.3	16.7
	200	0.0	0.0	100.0
	300	6.3	16.7	75.0
	500	0.0	69.6	30.4
	800	0.0	0.0	100.0
	1400	0.0	7.4	92.6
	2000	6.9	10.3	82.8
Stn. 7 (20°C incubation)	0	0.0	25.9	74.1
	20	0.0	85.2	14.8
	50	0.0	88.6	11.4
	100	0.0	86.3	13.7
	150	0.0	46.5	53.5
	200	0.0	71.7	28.3
	300	0.0	38.3	61.7
	500	0.0	64.0	36.0
	800	0.0	10.5	89.5
	1400	0.0	1.0	99.0
	2000	0.0	53.3	46.7

* Results for the isolates from MPN tubes.

part of the bacterial population that is attached to phytoplankton. The assumption that *Flavobacterium-Cytophaga* group has a close association with marine algae was also proposed (SHIBA and TAGA, 1980). During the period of the present research cruise, the amount of phytoplankton was fairly large, with layers having maximal chlorophyll *a* content reaching 0.15–0.28 $\mu g/l$, although massive blooming of the phytoplankton had been declining at the time of the sampling at Stns. 5 and 6. Accordingly, one of the explanations for the massive growth of the orange-pigmented bacteria will be the associated growth of the bacteria with phytoplankton.

The generic composition of the isolated bacteria is shown in Table 4. In the table the results for 2°C isolates at Stn. 5 and 20°C isolates at Stn. 7 are shown since psychrophilic and mesophilic bacteria predominated at Stns. 5 and 7, respectively. The results show the clear difference in the bacterial population between the upper and the deeper water layers, and also between the isolates from the Antarctic region and those from north of the Subantarctic Front, although the ratio of pigmented bacteria differed from the results of direct counting of pigmented and non-pigmented colonies before the isolation of bacteria. In the deeper water layers *Acinetobacter-Moraxella* organisms and Gram-negative, motile bacteria predominated. Most of the latter bacteria degraded DNA, hence there is a possibility that most of them are members of *Alteromonas*.

The absence of Vibrionaceae is another outstanding feature of bacterial population

Table 4. Composition of bacterial population in seawater south of the Polar Front (Stn. 5) and north of the Subantarctic Front (Stn. 7).

	Depth	Flavob.-cytoph[1] orange	Flavob.-cytoph. yellow	Acinetob.-Morax.[2]	Pseudom.-Alcal.[3]	Vibrion.[4]	Not ident.	No. of strains
Stn. 5	0 m	80.0%	8.0%	12.0%	0.0%	0.0%	0.0%	25
	20	84.0	8.0	0.0	0.0	0.0	8.0	25
	100	92.0	0.0	0.0	8.0	0.0	0.0	25
	150	91.4	4.3	0.0	0.0	0.0	4.3	23
	500	40.0	0.0	20.0	20.0	0.0	20.0	5
	1400	13.6	0.0	36.4	22.7	0.0	27.3	22
	2000	16.7	8.3	33.3	29.2	0.0	12.5	24
Stn. 7	0 m	0.0	13.3	0.0	73.4	0.0	13.3	15
	20	0.0	53.8	7.7	7.7	7.7	23.1	13
	100	0.0	43.7	37.5	0.0	0.0	18.8	16
	200	0.0	62.4	31.3	0.0	0.0	6.3	16
	300	0.0	33.3	22.2	38.9	5.6	0.0	18
	800	11.8	0.0	35.3	35.3	5.8	11.8	17
	1400	0.0	0.0	47.8	52.2	0.0	0.0	23
	2000	0.0	0.0	25.0	12.5	0.0	62.5	16

1) *Flavobacterium-Cytophaga.* 2) *Acinetobacter-Moraxella.*
3) *Pseudomonas-Alteromonas-Alcaligenes.* 4) Vibrionaceae.

of the Antarctic Ocean. Here again, as in highly eutrophic Tokyo Bay (SIMIDU *et al.*, 1977), a massive growth of phytoplankton may have prevented the growth of Vibrionaceae.

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(*Received July 24, 1985; Revised manuscript received August 30, 1985*)