TAN

Kieler Meeresforsch., Sonderh. 8, 219-224. Kiel 1991

Biomass and nutritional requirements of psychrotrophic bacterial communities in Fram Strait and Western Greenland Sea

T. L. Tan and H.-J. Rüger

Alfred-Wegener-Institut für Polar- und Meeresforschung
Am Handelshafen 12, D-2850 Bremerhaven, Germany

Abstract

During the "Polarstern" expedition ARK IV/2 in June 1987, water samples from 8 stations were taken to study biomass and substrate utilization of cold adapted bacteria. Bacterial biomasses determined from acridine orange direct counts (AODC) were between 0.4 and 31.4 µg C/l, and ATP concentrations amounted from <0.1 to 40 ng/l. Colony counts on seawater agar reached only 0.1 % of AODC, but with the MPN-method 1 to 10 % of AODC were recorded. With ^{14}C-glutamic acid or ^{14}C-glucose as tracer substrate in oligotrophic broth containing 0.5 mg trypticase and 0.05 mg yeast extract per liter of seawater, obligately oligotrophic bacteria could be detected in one water sample. Although incubation was at 2 °C, only psychrotrophic bacteria showing growth temperatures between 1 and 30 °C were obtained. Organic substrate utilizations by 106 isolates were tested at 4 and 20 °C. Most carbohydrates, organic acids, alcohols, and alanine were assimilated at both temperatures, but arginine, aspartate and ornithine were utilized only at 20 °C by almost all strains.

Introduction

For understanding the microbial processes in polar and other cold deep-sea regions, a better knowledge of the structure, activity and biomass of bacterial communities in relation to the extreme environmental conditions, like low temperature and low nutrient concentrations, is needed.

Oligotrophic bacteria were recorded in the Pacific Ocean and South China Sea (CARLUCCI et al. 1986, ISHIDA et al. 1986), but to our knowledge no investigations exist on the distribution and role of low-temperature adapted oligotrophic bacteria in the arctic ecosystem. The results of our investigations on microbial biomasses, distribution of oligotrophs, and substrate utilizations of the copiotrophic isolates at low temperatures are presented here.

Material and methods

Water samples were obtained with 20 l-Niskin samplers from 25, 200 and 1000 m depth at 8 stations in Fram Strait and the Western Greenland Sea (Fig. 1).

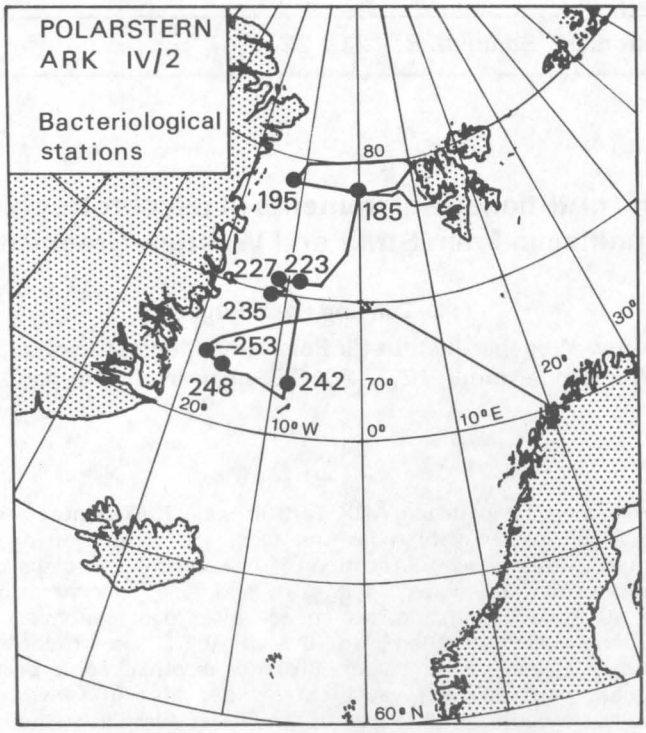

Fig. 1. Bacteriological stations in Fram Strait and the Western Greenland Sea.

The bacteria were collected on cellulose acetate filters (0.2 µm pore width, 47 mm diameter) and incubated at 2 °C on seawater agar according to ZoBell. Viable counts were estimated after 3 months, and the same plates taken for isolation of strains. Substrate utilizations by the isolates were determined as already described (RÜGER 1988).

For enumeration of bacteria with the ^{14}C-MPN method (3 flasks per dilution), the copiotrophic and oligotrophic media according to ISHIDA et al. (1986) were used; L-[U-^{14}C] glutamic acid (specific activity: 148.7 µCi/mg) or D-[U-^{14}C] glucose (29.6 µCi/mg) served as tracer substrate in the oligotrophic medium. Turbidity and liquid scintillation measurements of $^{14}CO_2$ were done after 4 months of incubation at 2 °C.

Bacterial biomasses in 50 and 100 ml of seawater were collected on polycarbonate filters (0.1 µm pore width, 47 mm diameter) and the cells preserved with glutaraldehyde vapours in petri dishes. After staining with acridine orange the bacteria were morphologically differentiated into five groups: small coccoid cells, Ø about 0.28 µm, volume 11.5 x 10^{-3} µm³; large coccoid cells, presumably cyanobacteria, Ø about 1 µm, volume 523.8 x 10^{-3} µm³; short rods, 0.28 by 0.55 µm, volume 33.9 x 10^{-3} µm; long rods, 0.55 by 2.2 µm, volume 522.9 x 10^{-3} µm³; filamentous cells, 0.55 by 5.5 µm, volume 1307.2 x 10^{-3} µm³. Biovolumes were converted into biomasses using the factor 5.6 x 10^{-13} gC per µm³. For ATP determinations, the microorganisms from 500 ml seawater were filtered, the cells

on the filters extracted in 5 ml of boiling Hepes buffer (0.1 M), and the extracts stored at -25 °C (TAN and RÜGER 1989).

Results and discussion

Bacterial biomasses from AODC were between 0.4 and 31.4 µg C/l and ATP concentrations amounted from <0.1 to 40 ng/l. Direct counts were between 1.23 and 24.0 x 10^4 cells/ml. Cyanobacteria were found in almost all water samples; the highest densities were recorded at station no. 227 in 200 m (13.8 x 10^3) and at station no. 235 (15.1 and 8.1 x 10^3), as expressed in the higher biovolumes (Table 1). The AODC results were in the same range as reported for Antarctic Ocean

Table 1. Direct counts, biovolumes and biomasses of bacteria in Fram Strait and the Western Greenland Sea.

Station No.	Depth to bottom, m	Sampling depth, m	AODC/ml x 10^4	Biovol./ml x 10^3 µm³	Biomass/ml ng C	ATP/ml pg
185	2560	25	9.45	6.92	3.9	10
		200	8.92	6.92	3.9	1
		1000	3.43	6.80	3.8	< 0.1
195	149	25	10.60	9.18	5.1	2
		135	4.50	5.64	3.2	0.3
223	2629	25	2.27	3.88	2.2	29
		200	1.33	0.75	0.4	0.4
227	747	25	1.23	5.31	3.0	10
		200	11.30	31.72	17.8	0.5
235	403	25	24.00	56.11	31.4	7
		200	19.70	39.47	22.1	0.2
242	2466	25	9.37	12.17	6.8	40
		200	5.91	11.62	6.5	1
		1000	1.97	2.31	1.3	< 0.1
248	1450	25	13.14	18.56	10.4	18
		200	1.92	1.58	0.9	1
		1000	9.68	19.88	11.1	< 0.1
253	315	25	4.52	8.96	5.0	5
		200	5.10	3.76	2.1	8

AODC: acridine orange direct counts; ATP: adenosine triphosphate

water samples by SIMIDU et al. (1986). The cyanobacteria in the East Greenland Current came presumably from the warmer Atlantic Intermediate Water mixed

with Polar Water (GRADINGER and LENZ 1989). ATP concentrations in Antarctic waters around Elephant Island and west of the Antarctic Peninsula in Austral winter (VOSJAN et al. 1987) were somewhat higher than found in Fram Strait and Western Greenland Sea in summer time.

Table 2. Densities of cultivable copiotrophic and oligotrophic bacteria in Fram Strait and the Western Greenland Sea.

Station No.	Sampling depth, m	CFU/ml copiotrophs	MPN/ml copiotrophs	MPN/ml oligotrophs, facultative	MPN/ml oligotrophs, obligate
185	25	2.50×10^2	348×10^2	9.1×10^2	0
	200	1.60×10^2	33×10^2	23.0×10^2	0
	1000	2.30×10^2	17×10^2	15.0×10^2	0
195	25	0.35×10^2	46×10^2	9.1×10^2	0
	135	0.14×10^2	5×10^2	0	0
223	25	0.48×10^2	348×10^2	3.6×10^2	5.5×10^2
	200	0.47×10^2	5×10^2	3.6×10^2	0
	1000	0.71×10^2	n.d.	n.d.	n.d.
227	25	0.93×10^2	49×10^2	0	0
	200	0.32×10^2	7×10^2	9.1×10^2	0
235	25	0.93×10^2	84×10^2	3.6×10^2	0
	200	1.60×10^2	23×10^2	15.0×10^2	0
242	25	1.10×10^2	17×10^2	3.6×10^2	0
	200	1.90×10^2	8×10^2	7.3×10^2	0
	1000	1.50×10^2	n.d.	n.d.	n.d.
248	25	1.10×10^2	542×10^2	3.6×10^2	0
	200	1.10×10^2	8×10^2	9.1×10^2	0
	1000	6.20×10^2	13×10^2	n.d.	n.d.
253	25	0.46×10^2	70×10^2	7.2×10^2	0
	200	0.33×10^2	70×10^2	n.d.	n.d.

CFU: colony forming units; MPN: most probable number; n.d.: not determined

Colony counts on seawater agar were between 0.14 and 6.20 x 10^2/ml, but with the MPN-method cell densities were 1-2 orders of magnitude higher (Table 2). This relation between the two methods is in agreement with SIMIDU et al. (1986). Colony counts from the Gulf of Alaska were lower than those reported here, but the plates were already evaluated after 3 weeks incubation at 5° C (HAUXHURST et al. 1980). In contrast to ISHIDA et al. (1986), who reported obligately oligotrophic bacteria to be the dominant populations in the South China Sea and West Pacific Ocean, obligately oligotrophic bacteria were found only in one water sample from the Western Greenland Sea.

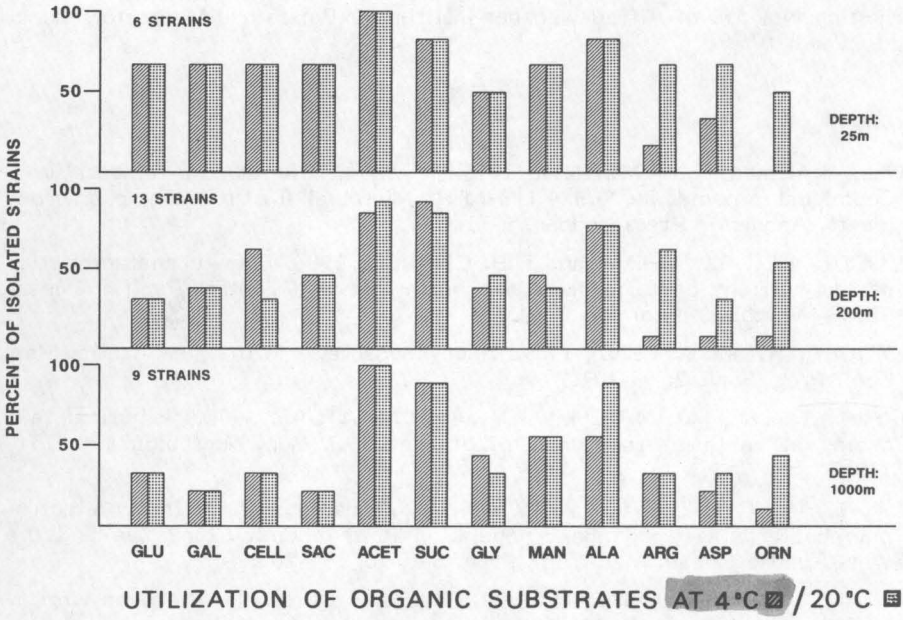

Fig. 2. Substrate utilizations of 28 psychrotrophic bacteria from station no. 248. GLU (Glucose), GAL (Galactose), CELL (Cellobiose), SAC (Saccharose), ACET (Acetate), SUC (Succinate), GLY (Glycerol), MAN (Mannitol), ALA (Alanine), ARG (Arginine), ASP (Aspartate), ORN (Ornithine).

Although incubation was at 2 °C, only psychrotrophic bacteria showing growth temperatures between 1 and 30 °C could be isolated (s. BAROSS and MORITA 1978). Organic substrate utilizations by 106 strains were tested at 4 and 20 °C. Most carbohydrates, organic acids, alcohols, and alanine were assimilated at both temperatures, but arginine, aspartate and ornithine were used only at 20 °C by almost all strains. An example for substrate utilizations by the bacterial communities from one station is shown in Fig. 2. Similarly, most of the bacteria from the Gulf of Alaska (HAUXHURST et al. 1980) were psychrotrophs and 20 representative strains utilized organic substrates at both 5 and 20 °C. On the contrary, isolates from deep-sea sediments of the western tropical Atlantic utilized most organic substrates only at 4 °C, but not at 20 °C (RÜGER 1988). NORKRANS and STEHN (1978) also reported that a great proportion of their sediment bacteria from the Norwegian and Western Greenland Sea were psychrophilic organisms showing maximum growth temperatures of 20 °C or below.

Acknowledgements

We thank Dr. K.-G. Barthel, Institut für Meereskunde Kiel, for valuable discussions and helping to get the water samples, Annegret Mädler, Karin Springer and Christa Summa for their expert technical assistence and Marion Puls for preparing the graphs. This work was supported in part by the Deutsche Forschungsgemeinschaft (Ta 63/7).

Contribution No. 358 of Alfred-Wegener-Institut für Polar- und Meeresforschung, Bremerhaven.

References

BAROSS, J.A. and R.Y. MORITA, 1978. Microbial life at low temperatures: Ecological aspects. In: D.J. KUSHNER: Microbial life in extreme environments. Academic Press London. 9-71.

CARLUCCI, A.F., S.L. SHIMP and D.B. CRAVEN, 1986. Growth characteristics of low-nutrient bacteria from the north-east and central Pacific Ocean. FEMS Microbiol. Ecol. 38, 1-10.

GRADINGER, R. and J. LENZ, 1989. Picocyanobacteria in the high Arctic. Mar. Ecol. Prog. Ser. 52, 99-101.

HAUXHURST, J.D., M.I. KRICHEVSKY and R.M. ATLAS, 1980. Numerical taxonomy of bacteria from the Gulf of Alaska. J. Gen. Microbiol. 120, 131-148.

ISHIDA, Y., M. EGUCHI and H. KADOTA, 1986. Existence of obligately oligotrophic bacteria as a dominant population in the South China Sea and the West Pacific Ocean. Mar. Ecol. Prog. Ser. 30, 197-203.

NORKRANS, B. and B.O. STEHN, 1978. Sediment bacteria in the deep Norwegian Sea. Mar. Biol. 47, 201-209.

RÜGER, H.-J., 1988. Substrate-dependent cold adaptations in some deep-sea sediment bacteria. System. Appl. Microbiol. 11, 90-93.

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.

TAN, T.L. and H.-J. RÜGER, 1989. Benthic studies of the Northwest African upwelling region: bacteria standing stock and ETS-activity, ATP-biomass and Adenylate Energy Charge. Mar. Ecol. Prog. Ser. 51, 167-176.

VOSJAN, J.H., G. NIEUWLAND, W. ERNST and T. BLUSZCZ, 1987. Shipboard comparison of two methods of extraction and measurements of ATP applied to Antarctic water samples. Neth. J. Sea Res. 21, 107-112.