

## PSYCHROPHILIC *VERSUS* PSYCHROTOLERANT BACTERIA – OCCURRENCE AND SIGNIFICANCE IN POLAR AND TEMPERATE MARINE HABITATS

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**Abstract** - The numerical dominance and ecological role of psychrophilic bacteria in bottom sediments, sea ice, surface water and melt pools of the polar oceans were investigated using isolates, colony forming units (CFU) and metabolic activities. All sediment samples of the Southern Ocean studied showed a clear numerical dominance of cold-loving bacteria. In Arctic sediments underlying the influence of cold polar water bodies psychrophiles prevailed also but they were less dominant in sediments influenced by the warm Atlantic Water. A predominance of psychrophiles was further found in consolidated Antarctic sea ice as well as in multiyear Arctic sea ice and in melt pools on top of Arctic ice floes. A less uniform adaptation response was, however, met in polar surface waters. In the very northern part of the Fram Strait (Arctic Ocean) we found bacterial counts and activities at 1°C exceeding those at 22°C. In surface water of the Weddell Sea (Southern Ocean) psychrophiles also dominated numerically in early autumn but the dominance declined obviously with the onset of winter-water and a decrease of chlorophyll *a*. Otherwise in surface water of the Southern Ocean CFUs were higher at 22°C than at 1°C while activities were *vice versa* indicating at least a functional dominance of psychrophiles. Even in the temperate sediments of the German Bight true psychrophiles were present and a clear shift towards cold adapted communities in winter observed. Among the polar bacteria a more pronounced cold adaptation of Antarctic in comparison with Arctic isolates was obtained. The results and literature data indicate that stenothermic cold adapted bacteria play a significant role in the global marine environment. On the basis of the temperature response of our isolates from different habitats it is suggested to expand the definition of Morita in order to meet the cold adaptation strategies of the bacteria in the various cold habitats.

**Key words:** Bacteria, cold adaptation, psychrophilic, sea ice, bottom sediments, Southern Ocean, Arctic Ocean, German Bight

### INTRODUCTION

The ability of bacteria to reproduce at 0°C was described by Forster in 1887 (10). A few years later Schmidt-Nielsen (28) for the first time used the term 'psychrophilic' to define this bacterial type. As only the minimum growth temperature was considered in this early definition some confusion arose due to the lack of differentiation between 'cold-loving' and 'cold-tolerant' adaptation types. However, with the isolation of true psychrophiles in the 1960's (22,29,32) cold adapted bacteria were consequently subdivided into the stenothermic and eurythermic category and Morita ended the confusion with a new definition (21). He coined the term 'psychrophilic' for those microorganisms whose cardinal growth temperatures (minimum, optimum, and maximum) are at or below 0, 15 and 20°C, respectively, while microorganisms with a higher growth optimum and maximum were called 'psychrotrophic'. This definition is

still in common use today. Only the term 'psychrotrophic' is nowadays replaced by 'psychrotolerant'. Although Morita's definition has in principal been proven to be useful, the cardinal temperatures should be reconsidered on the basis of increased numbers of cold adapted bacteria isolated from various habitats and in the light of new ecological data.

For a long period psychrophiles were considered as exotic organisms, however, in the last few years the number of psychrophilic isolates increased considerably. The psychrophiles cover a wide range of phylogenetic diversity (1) and have representatives in important functional groups like methanotrophs (2), sulfate reducers (19), and methanogens (11). A large number of psychrophiles originate from sea ice (3,13,14,17) but surprisingly a considerable number also exists in habitats which are not permanently cold (29,30). One of the most intensive studies on the occurrence of psychrophiles and controlling environmental factors was not conducted in a

stable cold environment but in the temperate inshore waters of Narragansett Bay, Rhode Island (29,30). Furthermore, the classical competition experiments of Harder and Veldkamp (15) are based on psychrophilic and psychrotolerant strains isolated from temperate waters of the North Sea. Comparatively few studies have been conducted to assess the number and, in particular, the role and significance of psychrophiles *versus* psychrotolerants in permanently cold habitats like the polar oceans and the deep-sea. Only a few investigators report on the dominance of psychrophiles in the polar marine environments. Wiebe and Hendricks (37) determined very high percentages of psychrophiles especially in deeper waters compared with surface waters. Reichhardt (25) also found among his biopolymer degrading isolates from Antarctic shelf sediments the stenothermic cold adaptation as a predominant growth characteristic. Further, obviously due to the high numbers of psychrophilic isolates from sea ice Deming (8) concedes that ice "provides the more selective environment for cold adaptation than milder unfrozen bodies of water and sediment". However, other studies (6,7,16,33) indicate that the dominance of psychrotolerants is more the rule than the exception in the polar pelagic realm. To broaden our knowledge on the distribution and relative importance of psychrophiles in polar marine

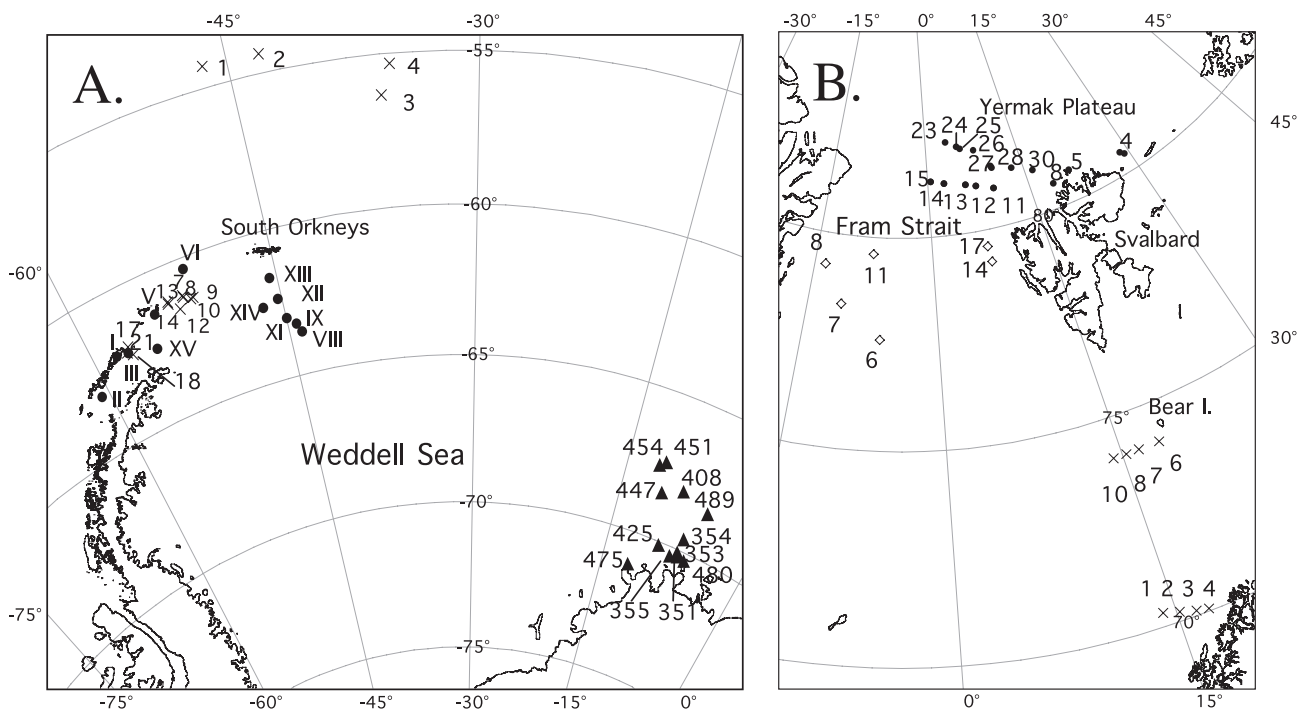
environments we have compiled results from different studies that examined temperature responses in sea ice, sea ice melt pools, surface water, deep water and bottom sediments of both polar oceans on the basis of viable counts, metabolic activities and isolates. The results were compared with those obtained for temperate sediments from the German Bight.

## MATERIAL AND METHODS

### Sampling

Samples of sea ice, melt pools, surface water, and bottom sediment were collected during the cruises ANT II/3, ANT X/3, ANT XI/4, ARK III/3 and ARK XIII/2 of RV *Polarstern*, cruise no. 56 of RV *Meteor*, cruise no. 200 of RV *Anton Dohrn*, and cruises no. 4/85 and 48/85 of RV *Victor Hensen*. Positions of all stations are shown in Fig. 1 or cited in the legends.

Sea ice was drilled with a KOVACS enterprise MARK III coring system. The ice core sections from different depths were processed as described previously by Helmke and Weyland (16). Melt pool samples on top of Arctic sea-ice floes were collected with 100 ml sterile syringes and surface water beside or beneath sea-ice floes were taken with sterile glass bottles. The sediment samples were collected with a multicorer ('*Polarstern*' cruises), box grabs or van Veen grabs. Overlying water was aseptically siphoned off and sediment subsampled up to a depth of 2 cm. Careful attention was paid to maintain sterile and cold conditions during sampling and processing.



**Fig. 1** Sampling stations in **A**) the Southern Ocean (Weddell Sea) and in **B**) the Arctic Ocean (Fram Strait) and Norwegian Sea. **A**) Crosses represent sampling stations of cruise no. 56 of R.V. *Meteor*, closed circles of cruise ANT II/3 of R.V. *Polarstern* and closed triangles of cruise ANT X/3 of R.V. *Polarstern*. **B**) Crosses represent sampling stations of cruise no. 200 of R.V. *Anton Dohrn*, closed circles of cruise ARK XIII/2 of R.V. *Polarstern* and open rhombi of cruise ARK III/3 of R.V. *Polarstern*. Maps were generated with the PanMap software (<http://www.pangea.de>).

*Viable counts*

For bacterial enumeration, 0.1 ml aliquots of the different samples or of 10 fold dilutions were spread onto the surface of precooled Chitin agar (36) containing comparably low nutrients. Duplicate sets were prepared for each dilution. One set was incubated at 1°C while the other was incubated at 22°C. Agar plates were incubated until the number of colonies on a given plate no longer increased. Representative colonies of the 1°C incubation plates were chosen and stock cultures of pure isolates were established on Marine Agar 2216 (Difco, Becton Dickinson, Sparks, USA) by standard picking and restreaking procedures.

The number of viable counts of the sediments of the German Bight was additionally estimated by the most-probable-number (MPN) technique. For MPN calculations, 3 replicates of successive 10-fold dilutions were prepared using Marine Broth 2216 (Difco). Three sets were prepared and incubated at 1°C, 20°C and 30°C (30°C results not shown). MPN growth was evaluated by means of turbidity examination.

*Temperature characteristics*

Temperature tolerance was determined as previously described by Helmke and Weyland (16). Growth of sea-ice isolates was examined at

0, 5, 10, 15, 20, 25 and 30°C (18 and 24°C were applied with the Antarctic isolates) and of sediment bacteria at 1, 7, 12, 18, 24, 30°C in Marine Broth 2216 (Difco) in Titertek plates. Turbidity was determined at 590nm with a Multiscan plus MKII (Flow Laboratories, Meckenheim, Germany).

*[<sup>3</sup>H]-thymidine uptake*

Rates of heterotrophic bacterial production were determined according to Fuhrman and Azam (12) using [methyl-<sup>3</sup>H]thymidine (spec. activity in the range of 2.6-3.2 TBq/mmol, Amersham Pharmacia Biotech, Buckinghamshire, UK) with a final concentration of 10 nmol l<sup>-1</sup>. Aliquots of the sea ice (melted), melt pool, surface water, and sediment (diluted 1:1 with overlying water) were treated with [methyl-<sup>3</sup>H] thymidine and incubated at 1°C and 22°C for 12 to 24 hr. Afterwards cells were collected on Nuclepore filters (pore size 0.2 µm) and extracted with ice cold 5% TCA. Filters of the sea ice, melt pool, and water samples were directly assayed for radioactivity with Filtercount (Perkin Elmer, Elmau, Germany) as scintillation medium in a Tricarb 2550 TR/LL (Perkin Elmer). Sediment filters were combusted in an Oxidizer OX 500 (Zinser, Frankfurt, Germany) using Oxysolve T (Zinser) as H<sub>2</sub>O absorber and scintillation medium.

**Table 1** CFUs and ratios of CFUs as well as bacterial production (thymidine incorporation) determined at 1°C and 22°C with samples of sea ice, melt pools, surface water, water from 1000 m, and water from 2000 m in the Southern Ocean and Arctic Ocean. (Locations during ANT XI/4 in the Southern Ocean are: 4 (53°23.0'S 01°W), 12 (68°35.1'S 10°43.6'E), 13 (69°48.7'S 12°26.4'E), 15 (65°59.9'S 24°59.4'E).

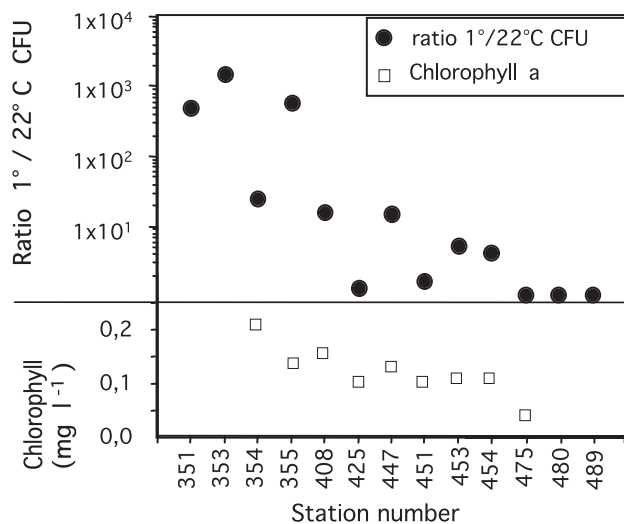
Southern Ocean (ANT XI/4)					Arctic Ocean (ARK XIII/2)				
Station Number	1°C CFU x1000 (ml <sup>-1</sup> )	22°C CFU x1000 (ml <sup>-1</sup> )	Ratio CFU 1/22°C	Ratio Activity 1/22°C	Station Number	1°C CFU x1000 (ml <sup>-1</sup> )	22°C CFU x1000 (ml <sup>-1</sup> )	Ratio CFU 1/22°C	Ratio Activity 1/22°C
<i>Sea Ice</i>					<i>Sea Ice</i>				
13	40.52	0.02	2500	3.1	4	62.9	3.83	16	
					5	7.4	0.02	370	1.9
					8	4.5	0.01	331	3.1
					11	50.2	0.78	64	
					13	36.0	9.09	4	
					23	17.1	0.61	28	
					26	9.8	0.12	82	1.2
					27	151.3	2.35	64	
					28	78.0	2.97	26	
					30	41.6	0.53	79	
					<i>Melt pools</i>				
					11	2.8	0.14	20	
					13	n.d.	n.d.	n.d.	1.8
					23a	3.0	0	3020	
					23b	8.8	0.01	771	3.0
					23c	0.5	0	505	
					26a	4.8	0.01	477	
					26b	0.2	0	81	1.2
					30a	5.3	0.01	531	
					30b	100.8	33.82	3	
					<i>Surface water</i>				
					11	1.8	0.02	91	
					13	0.3	0.05	6	
					14	2.9	0.35	9	
					24	0.4	0.01	36	
					25	26.2	0.20	134	
					27	0.5	0.01	25	1.2
					30	1.3	0.01	137	1.4
<i>Surface water</i>					<i>Surface water</i>				
4	0.04	0.07	0.57	1.8	11	1.8	0.02	91	
12	0.01	0.01	1.00	6.5	13	0.3	0.05	6	
15	0.06	0.51	0.12	2.0	14	2.9	0.35	9	
<i>Water, 1000 m</i>					<i>Water, 1000 m</i>				
4	0.06	0.08	0.75	0.25	24	0.4	0.01	36	
15	0.03	0.18	0.16	0.4	25	26.2	0.20	134	
<i>Water, 2000 m</i>					<i>Water, 2000 m</i>				
4	0.02	0.12	0.16	0.11	27	0.5	0.01	25	1.2
15	0.00	0.01	0.10	0.20	30	1.3	0.01	137	1.4

## RESULTS

The role of psychrophiles in polar environments was studied on the basis of numbers and activities. In the Arctic Ocean, in the very northern part of the Fram Strait, the 1°C colony forming units of samples from multiyear sea ice, melt pools on top of ice floes, and surface water beneath sea ice exceeded clearly the 22°C CFUs (Table 1) indicating a clear predominance of psychrophilic bacteria. Parts of these samples were additionally tested for bacterial production at low and high temperature. Although the activity-ratios (1°C/22°C) obtained were clearly less in comparison to the CFU-ratios (Table 1) the psychrophilic dominance was corroborated.

In the Southern Ocean the temperature response of bacterial sea-ice communities was studied on the basis of isolates from winter sea ice (Fig. 4 B) (16) as well as on the basis of CFUs and activities of consolidated brownish sea ice sampled during autumn. The autumnal Antarctic sea-ice sample showed clearly higher CFUs and activities at 1°C than at 22°C (Table 1) coinciding with the Arctic sea-ice samples (Table 1) and the psychrophilic response of the majority of Antarctic winter sea-ice isolates (Fig. 4B) (16).

The surface water communities investigated during the autumnal cruise ANTX/3 showed no uniform psychrophilic response. At the beginning of the cruise psychrophilic bacteria were prominent in the shelf near area of the Weddell Sea but declined during the cruise (Fig. 2) obviously in connection with the erosion of summer water (20) and a decrease of chlorophyll *a* (9). At the end of the cruise psychrotolerants prevailed indicated by higher 22°C CFUs. In a subsequent autumnal cruise (ANT XI/4)



**Fig. 2** Ratios of 1°C to 22°C CFUs and concentrations of chlorophyll *a* determined in surface water samples of the Weddell Sea taken during the autumnal cruise ANT X/3 of R.V. *Polarstern*.

surface water samples from the Lazarev Sea were studied for CFUs as well as activity. Bacterial production reached higher values at 1°C while bacterial numbers had higher values at 22°C (Table 1). As deep water communities showed higher activities as well as CFUs at 22°C (Table 1) different proportions of psychrophiles to psychrotolerants in surface and deep water has to be presumed.

In contrast to the pelagic communities the sediment communities showed a uniform and clear dominance of psychrophiles at different places in the Southern Ocean (Table 2) on the basis of CFUs. The 1°C/22°C ratios ranged from 1.7 to 11250. Sediments collected from depths below 3000m off South Orkneys formed even no colonies at 22°C. Bacterial growth was not determined with these samples but some samples were investigated for chitin decomposition. The degradation rates at 1°C were also higher than at 22°C (data not shown).

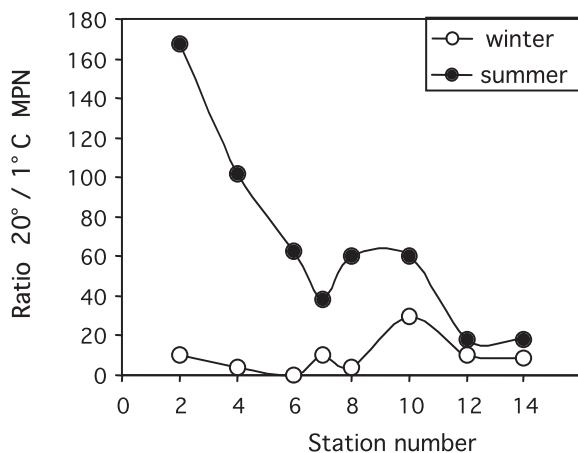
The sediment communities in the Arctic varied more in their cold adaptation than their counterparts in the south. While a clear numerical dominance of psychrophiles was found in sediments from the Greenland shelf and Yermak Plateau (Table 2) the sediments collected off Bear Island and off Tromsø showed CFU-ratios only in the range of 1. The dominance of psychrophiles in the sediments of the Yermak Plateau could be established by thymidine incorporation measurements (Table 2). Obviously the water bodies have a strong impact on the cold adaptation structure of sediment communities in the north.

Sediments in the German Bight experience seasonal temperature fluctuations from -1°C in winter (February) up to +17°C in summer (September). From summer to winter a clear increase of bacteria growing at 1°C was determined inducing a reduction of the 20°C/1°C MPN ratios (Fig. 3). Further notable is the isolation of psychrophiles from this non-permanently cold environment. About 10% of the 1°C isolates were true psychrophiles and still 68% had temperature growth maxima in the range of only 18°C to 24°C (Fig. 4).

To evaluate the degree of cold adaptation a collection of representative isolates from different habitats was investigated for its temperature tolerance. The most pronounced adaptation to low temperatures was found among the Antarctic bacteria. More than 80% of the Antarctic sediment as well as sea-ice bacteria had temperature growth maxima below 18°C (Fig. 4) and optima at about 7°C that means their cardinal temperatures are clearly below those defined by Morita (21). The Arctic sediment isolates originated from the western side (influenced by cold polar water) and eastern side (influenced by warmer Atlantic Water) of the Fram Strait. Small differences in the degree of cold adaptation became obvious between the isolates of these regions, however, even the bacteria from the western side showed a less pronounced cold adaptation than the Antarctic sediment

**Table 2** CFUs and ratios of CFUs as well as bacterial production (thymidine incorporation) determined at 1°C and 22°C with bottom sediment samples from the Southern Ocean and Arctic Ocean

Southern Ocean				Arctic Ocean				
Station Number (depth)	1°C CFUs x1000 (ml <sup>-1</sup> )	22°C CFUs x1000 (ml <sup>-1</sup> )	Ratio CFU 1/22°C	Station Number (Depth)	1°C CFUs x1000 (ml <sup>-1</sup> )	22°C CFUs x1000 (ml <sup>-1</sup> )	Ratio CFU 1/22°C	Ratio Activity 1/22°C
<i>Off South Georgia</i>				<i>Off Tromsø</i>				
4 (1150 m)	5.7	1.0	5.6	1 (2466 m)	3	17	0.2	
5 (3790 m)	1.7	0.1	20.2	2 (2342 m)	12	12	1.0	
2 (3791 m)	0.8	0.1	4.5	3 (1764 m)	52	15	3.5	
1 (4102 m)	0.5	0.3	1.7	4 (142 m)	185	200	0.9	
<i>Off South Orkneys</i>				<i>Off Bear I.</i>				
XIII (470 m)	778.1	20.6	37.7	6 (100 m)	5250	4700	1.1	
XIV (486 m)	1000	3.8	266.7	7 (284 m)	1550	2630	0.6	
XII (3059 m)	11.3	0	11250.0	8 (1453 m)	95	85	1.1	
XI (3491 m)	7.1	0	7188.0	10 (1768 m)	77	22	3.5	
IX (3789 m)	10.5	0	10552.0	<i>Greenland shelf</i>				
VIII (4419 m)	0.7	0	667.0	8 (85 m)	16125	350.0	46.1	
<i>Off Elephant I.</i>				7 (175 m)	1250	187	6.7	
7 (536 m)	7.5	0.1	60.0	11 (203 m)	23000	1443	15.9	
13 (628 m)	345	2.8	123.2	6 (312 m)	4312	912	4.7	
8 (675 m)	10.9	0.1	87.2	<i>Off Svalbard</i>				
14 (910 m)	90.0	1.3	69.2	17 (544 m)	1968	242	8.1	
9 (2395 m)	7.6	0.1	60.8	14 (698 m)	5750	732	7.8	
12 (2270 m)	27	0.1	216.0	<i>Yermak Plateau</i>				
V (2297 m)	1812	32.1	56.5	11 (1029 m)	92	7	12.8	1.7
10 (2618 m)	31.8	0.1	254.4	12 (891 m)	168	7	22.8	1.4
VI (3332 m)	35.3	0	1139.0	13 (647 m)	273	11	23.3	2.7
<i>Bransfield Strait</i>				14 (992 m)	244	14	17.3	1.6
17 (100 m)	2237	120.0	18.6	15 (2313 m)	100	5	18.4	6.1
XV (112 m)	3525	17.4	202.9					
III (300 m)	1500	18.1	82.8					
21 (923 m)	200.0	0.8	266.7					
II (1374 m)	1031	3.3	308.4					
I (1940 m)	76.6	0.7	116.5					
18 (1950 m)	4.0	0.2	21.1					

**Fig. 3** Ratios of 20°C to 1°C MPNs of sediment samples in the German Bight. Sampling positions are: stat no. 2 (53°39.05'N 08°24.3' E), no. 4 (53°45.2'N 08°11.5' E), no. 6 (53°51.3'N 08°09.0' E), no. 7 (53°54'N 08°02.2' E), no. 8 (53°57.1'N 08°00.7' E), no. 10 (54°01.6'N 08°E), no. 12 (54°09'N 07°53.3' E) and no. 14 (54°16.8'N 07° 43' E).

bacteria. About 40% of the Arctic sediment strains had temperature growth maxima between 18°C and 24°C that means they are quite close or already above the 20°C growth maximum defined by Morita for psychrophiles (Fig. 4). Their temperature growth optima, however, were in the range of 15°C or below.

The Antarctic sea-ice isolates showed a similar narrow cold adaptation as the Antarctic sediment bacteria. Only few sea-ice isolates, preponderantly those originating from young sea-ice formations, did not meet the definition of Morita. Coinciding with the Arctic sediment bacteria the Arctic sea-ice isolates showed a less pronounced cold adaptation than the Antarctic strains. More than half of the Arctic sea ice bacteria touch or surpass the 20°C temperature growth maximum. A quite similar percentage of about 10 % of sea-ice isolates from north and south were psychrotolerant (Fig. 4).

## DISCUSSION

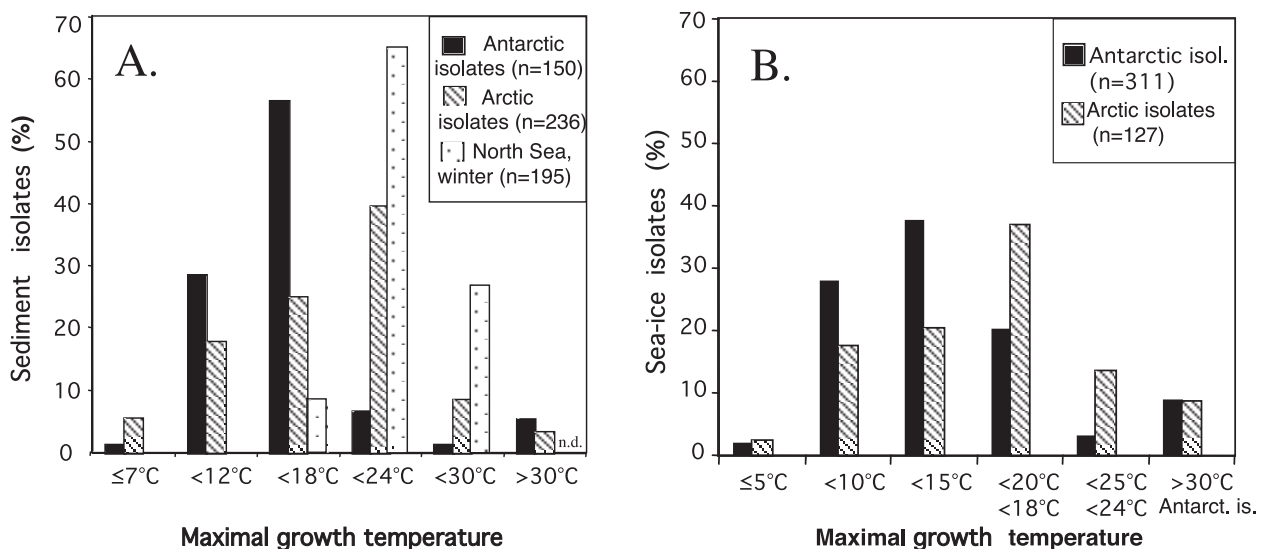
To record the numerical presence of cold adapted bacteria and to distinguish between the two temperature groups traditional cultural methods like CFUs, MPNs, or isolates need to be applied. The application of culture-independent molecular biological techniques is not appropriated as cold adaptation is not limited to specific taxonomical bacterial groups and criteria detectable with these methods and suitable for the differentiation of psychrophiles are still unknown. Cultural methods in ecological studies, however, have the disadvantage that they mostly record a very low percentage of total bacteria and that the results depend strongly on the incubation conditions used. Further, it has to be kept in mind that a numerical dominance does not inevitably mean a functional dominance. To confine the cultural shortcomings but also to evaluate the function and role of psychrophiles versus psychrotolerants we determined viable counts as well as bacterial activities at minimum and maximum growth temperatures and assessed temperature tolerances using isolates.

### *Sea ice and melt pools*

Sea ice is an excellent environment to obtain meaningful results with cultural methods since the ratios of viable to total counts are generally very high (16,18). Furthermore, sea ice is a predestined habitat for psychrophilic bacteria since temperatures are constantly below 0°C, nutrients are supplied by photosynthetic processes, and a stable matrix provides a close association of bacteria and nutrient sources. A clear dominance of psychrophiles was expected and determined in Antarctic

consolidated sea ice as well as in Arctic multiyear ice by means of isolates, CFU-ratios (1°C to 22°C), and growth activities. A closer look at the temperature response of the sea ice isolates from north and south, however, revealed differences in the degree of cold adaptation between Antarctic and Arctic sea-ice bacteria. On average the Arctic isolates had clearly broader temperature ranges as their counterparts in the Southern Ocean. Although a previous study detected almost the same bacterial phylotypes in sea ice at both poles their quantitative contribution was found to be different (5). A selective enrichment of moderate psychrophilic strains in the north may be caused by the fundamental differences in development, thickness, maturity, and ice crystal structure of Arctic versus Antarctic sea ice. Furthermore, the different community structure may be due to a different initial stock of bacteria. Sea-ice communities are recruited from seawater and the initial stock of organisms in sea ice depends on the composition of the microbial assemblages within the water column at the time and location of ice formation. In contrast to the Southern Ocean the water masses in the north are influenced by warm water as well as by terrestrial input and therefore may carry along higher concentrations of moderate psychrophilic types.

In the Southern Ocean the development of sea-ice communities during new ice formation was investigated in winter. At that time psychrotolerants dominated the seawater community. However, in the course of sea-ice formation and consolidation at the ice edge the psychrotolerants were gradually outcompeted by psychrophiles (16). This successional dynamics in the early Antarctic sea-ice communities indicate that psychrophiles rapidly can gain dominance over psychrotolerants once the



**Fig 4** A) Maximal growth temperatures of bacteria isolated from sediment of the Southern Ocean, Arctic Ocean, and German Bight. B) Maximal growth temperatures of bacteria isolated from sea ice of the Southern Ocean and Arctic Ocean.

conditions have stabilised and sufficient nutrients are available for reproduction. In the Arctic Ocean the initial stock of bacteria and/or the selection process obviously differs to a certain extent from that in the Southern Ocean. In summer shallow melt pools are a common occurrence on Arctic sea ice floes providing habitats for surface assemblages. Melt pools are freshwater systems with very extreme seasonal temperature fluctuations in the cold temperature range including frequent freezing and thawing. In contrast to consolidated sea ice the nutrient conditions here are poor (4). Under these harsh and fluctuating conditions psychrotolerant communities were expected. However, CFUs and bacterial production [also determined with tritiated leucine (4)] indicated that psychrophiles dominate numerically and functionally. The bacterial melt pool communities were revealed to be very specific communities with key organisms belonging to limnic phylotypes (4). They are obviously selected by their extraordinary survivability.

In view of the psychrophilic dominance in sea-ice communities and of the vast extent of sea ice in the polar oceans it has to be assumed that psychrophiles contribute substantially to the carbon cycles of the upper pelagic polar oceans.

#### *Bottom sediment*

The bottom sediments in the Southern Ocean offer very stable conditions for microorganisms. A clear dominance of psychrophiles in Antarctic sediment samples was therefore expected and obtained at all sites studied. A moderate dominance of psychrophiles was also reported by Reichhard (25) and Tanner and Herbert (34) among their isolates from Antarctic coastal sediments. In contrast to the Southern Ocean the sediment communities at the sites studied in the Arctic Ocean showed a less uniform and lower dominance of psychrophilic bacteria. The varying temperature structure of the bacterial sediment communities is obviously due to the more heterogeneous hydrographic situation in the north. Warm Atlantic Water and transformations of this water body strongly influence the Nordic Sea, Barent Sea and the eastern side (Svalbard) of the Fram Strait while the western side (Greenland) is subjected to cold polar waters outflowing from the central Arctic Ocean. The water mass distribution above the bottom is obviously reflected in the temperature response of the sediment communities. Considering the temperature growth maxima of Arctic sediment isolates it becomes obvious that a relatively high percentage of strains still has a stenothermic growth response but temperature growth maxima at about 24°C, that means above the limit of Morita's definition (21). CFU-ratios (5°C to 20°C) in the range of 1 to 10 were also determined by Norkrans and Stehn (23) with sediments from the deep Norwegian Sea. They also investigated the temperature range of isolates

and found one third with a maximum growth temperature of 25°C while another third of their strains isolated at 5°C were truly psychrophilic.

Rüger (26) also found a considerable number of bacteria with temperature growth maxima between 18 to 24°C in the tropical upwelling region of North Africa at depths of 800 to 1500 m where sediment temperatures were at about 8°C. Rüger (26) used the term 'psychrophilic' for this temperature group, while he called the smaller group of isolates with temperature growth maxima between 12 and 18°C 'extreme psychrophilic'. CFUs from samples collected from greater depths in that upwelling region were too low so that confident ratios could not be calculated (27). Our own Antarctic deep-sea sediment samples and the Arctic deep-sea samples studied by Norkrans and Stehn (23) also showed very low numbers. The decreasing numbers in all these samples are mainly due to the fact that methods conducted at atmospheric pressure are not adequate for deep-sea samples. Bacterial communities of deep-sea sediments were shown to be closely adapted to the pressure conditions of their site of occurrence (35). Their sensitivity to atmospheric pressure increases with their depth of origin. Strict piezophiles (barophiles) will not grow at atmospheric pressure and will therefore not be recorded with the methods employed here (35). However, piezophiles from deep-sea sediments are in principal psychrophilic (exceptions are found in the Mediterranean and the Sulu Sea) that means these organisms have also to be taken into account if the significance of psychrophiles in cold deep-sea sediments is considered. Bottom sediments from depths greater than 1000 m generally have temperatures below 10°C and these environments constitute about 60% of the earth's surface.

Psychrophiles are not only confined to permanently cold environments but also live and develop in temperate habitats. During seasonal studies in the German Bight, the enrichment of cold adapted bacteria during winter could be determined although temperatures during summer regularly reach +17°C. About 8% of the winter isolates responded truly psychrophilic and a portion of nearly 70% responded moderately psychrophilic with temperature maxima of only 24°C. Sieburth (29,30) described a similar situation in the Narraganset Bay, Rhode Island, although the summer temperatures there even attain +23°C. An enrichment of cold adapted bacteria from February to March was also determined in surface waters of the German Bight by Poremba *et al.* (24) by means of activity measurements. These results underline that if sufficient nutrients are available for psychrophiles they can attain high growth rates and outcompete psychrotolerants already in relative short periods of stable cold conditions.

#### *Surface water and deep water*

In spite of stable cold temperatures in polar surface

waters and in the deep water column most studies report psychrotolerant dominated bacterial communities for these pelagic habitats. Comparatively few studies show a prevalence of psychrophilic bacteria. Simon *et al.* (31) found a reduction in the temperature optima of bacterial growth (determined with leucine and thymidine) from  $>18^{\circ}\text{C}$  to  $4^{\circ}\text{C}$  or in other words a transition of psychrotolerant to psychrophilic dominated communities along a transect from the Polar Front to the marginal ice zone. We also found a change from a psychrophilic to a psychrotolerant community on the basis of CFUs during the replacement of summer water and the decrease in nutrients and chlorophyll *a* in the Weddell Sea (20). Most Antarctic surface water samples analysed had higher CFUs at  $22^{\circ}\text{C}$  on the one hand and higher bacterial growth activities at  $1^{\circ}\text{C}$  on the other hand, which could be interpreted as a functional dominance in spite of minor numbers of psychrophiles.

There is one report (33) of a numerical dominance of psychrotolerants in the southern Fram Strait (Arctic Ocean). In the cold Polar Surface Water of the northern Fram Strait, however, we found a clear numerical as well as functional dominance of psychrophiles. These varying results with surface waters indicate that under specific hydrographic conditions offering sufficient organic nutrients psychrophiles can outcompete psychrotolerants numerically. Such conditions, however, are limited locally and temporally in open polar oceans.

The deep-water column ( $>1000\text{ m}$ ) is stably cold but generally oligotrophic. Nutrient rich spots may occur in connection with vertical transport processes and in such niches psychrophiles may numerically dominate for a certain period. Psychrophilic/piezophilic bacteria were, however, also revealed in deep oligotrophic water where they were numerically in the minority but functionally dominant (35). Therefore, psychrophiles are assumed to play a significant functional role also in the oligotrophic cold water column which constitutes about 75% of the world's ocean by volume.

#### Definition

Based on a great number of isolates from different environments it could be shown that on average bacteria from the Southern Ocean have the smallest growth temperature ranges and meet best Morita's definition. In Arctic sediments and sea ice a considerable number of bacteria are found that have maximum growth temperatures exceeding the  $20^{\circ}\text{C}$  level of Morita's definition (21). Their maximal growth temperatures are at about  $25^{\circ}\text{C}$ , similar to the cardinal temperatures of strains isolated from cold upwelling areas as well as from temperate habitats. In order to apply a definition to all these strains, which are in principle stenothermic cold adapted, we recommend to introduce beside the 'psychrophiles' and

'psychrotolerants' according to Morita's definition (21), a further group of 'moderate psychrophiles' with a minimum growth temperature of  $\leq 0^{\circ}\text{C}$  and a maximum growth temperature of  $\leq 25^{\circ}\text{C}$ .

## CONCLUSIONS

Psychrophiles are numerically and functionally dominant in permanently cold environments with adequate nutrient supply. In non-permanently cold habitats psychrophiles may also become numerically dominant if in short cold periods sufficient nutrients are available for rapid reproduction. In the oligotrophic deep-sea psychrophilic/piezophilic bacteria are numerically in the minority but obviously functionally prevalent. Therefore, psychrophiles do not only play a significant role in the biogeochemical cycles of the polar oceans but also in other cold and even non permanently cold marine environments. Considering the considerable number of bacteria which live in the different cold environments and which surpass the  $20^{\circ}\text{C}$  limit by only few degrees we recommend to introduce an additional group of 'moderate psychrophiles' with a minimum growth temperature of  $\leq 0^{\circ}\text{C}$  and a maximum growth temperature of  $\leq 25^{\circ}\text{C}$ .

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## REFERENCES

1. Bowman, J.P., Methods for psychrophilic bacteria. In: *Marine Microbiology*, Paul, J. (ed.), Acad. Press, San Diego, 2001, pp. 591-613.
2. Bowman, J.P., McCammon, S.A. and Skerratt, J.H., *Methylospira hansonii* gen. nov., sp. nov., a psychrophilic, group I methanotroph from Antarctic marine salinity, meromictic lakes. *Microbiology* 1997, **143**: 1451-1459.
3. Bowman, J.P., McCammon, S.A., Brown, M.V., Nichols, D.S. and McMeekin, T.A., Diversity and association of psychrophilic bacteria in Antarctic sea ice. *Appl. Environ. Microbiol.* 1997, **63**: 3068-3078.
4. Brinkmeyer, R., Glöckner, F.-O., Helmke, E. and Amann, R., Predominance of  $\beta$ -proteobacteria in summer melt pools on Arctic pack ice. *Limnol. Oceanogr.* 2004, **49**: 1013-1021.
5. Brinkmeyer, R., Knittel, K., Jürgens, J., Weyland, H., Amann, R. and Helmke, E., Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. *Appl. Environ. Microbiol.* 2003, **69**: 6610-6619.
6. Delille, D., Marine bacterioplankton at the Weddell Sea ice edge,



- distribution of psychrophilic and psychrotrophic populations. *Polar Biol.* 1992, **11**: 449-456.
7. Delille, D. and Perret, E., Influence of temperature on the growth potential of southern polar marine bacteria. *Microb. Ecol.* 1989, **18**: 117-123.
  8. Deming, J.W., Psychrophiles and polar regions. *Curr. Opin. Microbiol.* 2002, **5**: 301-309.
  9. Dower, K.M., Lucas, M.I., Philips, R., Dieckmann, G. and Robinson, D.H., Phytoplankton biomass, P-I relationships and primary production in the Weddell Sea, Antarctica, during the austral autumn. *Polar Biol.* 1996, **16**: 41-52.
  10. Forster, J., Ueber einige Eigenschaften leuchtender Bakterien. *Zentralbl. Bakteriol. Parasitenkd.* 1887, **2**: 337-340.
  11. Franzmann, P., Liu, Y., Balkwill, D., Aldrich, H., Conway de Macario, E. and Boone, D., *Methanogenium frigidum* sp. nov., a psychrophilic, H<sub>2</sub>-using methanogen from Ace Lake, Antarctica. *Int. J. Syst. Bacteriol.* 1997, **47**: 1068-1072.
  12. Fuhrmann, J.A. and Azam, F., Thymidine incorporation as a measure of heterotrophic production in marine surface waters: evaluation and field results. *Mar. Biol.* 1982, **66**: 109-120.
  13. Gosink, J.J., Herwig, R.P. and Staley, J.T., *Octadecabacter arcticus* gen. nov., sp. nov. and *O. antarcticus*, sp. nov., nonpigmented, psychrophilic gas vacuolate bacteria from polar sea ice and water. *Syst. Appl. Microbiol.* 1997, **20**: 356-365.
  14. Hagen, P., Kushner, D. and Gibbons, N., Temperature induced death and lysis in a psychrophilic bacterium. *Can. J. Microbiol.* 1964, **10**: 813-823.
  15. Harder, W. and Veldkamp, H., Competition of marine psychrophilic bacteria at low temperatures. *Antonie van Leeuwenhoek* 1971, **37**: 51-63.
  16. Helmke, E. and Weyland, H., Bacteria in sea ice and underlying water of the eastern Weddell Sea in midwinter. *Mar. Ecol. Prog. Ser.* 1995, **117**: 269-287.
  17. Junge, K., Gosink, J.J., Hoppe, H.G. and Staley, J.T., Arthrobacter, Brachybacterium and Planococcus isolates identified from antarctic sea ice brine. Description of *Planococcus mcmeekinii*, sp. nov. *Syst. Appl. Microbiol.* 1998, **21**: 306-314.
  18. Junge, K., Imhoff, F., Staley, J.T. and Deming, J.W., Phylogenetic diversity of numerically important Arctic sea-ice bacteria cultured at subzero temperature. *Microb. Ecol.* 2002, **43**: 315-328.
  19. Knoblauch, C., Sahn, K. and Joergensen, B., Psychrophilic sulfate reducing bacteria isolated from permanently cold Arctic marine sediments: description of *Desulfofrigus oceanense* gen. nov., sp. nov., *Desulfofrigus fragile* sp. nov., *Desulfofaba gelida* gen. nov., sp. nov., *Desulfotalea psychrophila* gen. nov., sp. nov. and *Desulfotalea arctica* sp. nov. *Int. J. Syst. Bacteriol.* 1999, **49**: 1631-1643.
  20. Krause, G., Maul, K., Ohm, K., Plugge, R., Lütjeharms, J., Philips, R., Rigg, G. and Valentine, H., The Antarctic slope front in autumn. *Rep. Polar Res.* 1993, **121**: 9-10.
  21. Morita, R.Y., Psychrophilic bacteria. *Bacteriol. Rev.* 1975, **39**: 146-167.
  22. Morita, R.Y. and Haight, R., Temperatur effects on the growth of an obligate psychrophilic marine bacterium. *Limnol. Oceanogr.* 1964, **9**: 103-106.
  23. Norkrans, B. and Stehn, B., Sediment bacteria in the deep norwegian sea. *Mar. Biol.* 1978, **47**: 201-209.
  24. Poremba, K., Dürselen, C.-D. and Stoeck, T., Succession of bacterial abundance, activity and temperature adaptation during winter 1996 in parts of the German Wadden Sea and adjacent coastal waters. *J. Sea Res.* 1999, **42**: 1-10.
  25. Reichhardt, W., Impact of the Antarctic benthic fauna on the enrichment of biopolymer degrading psychrophilic bacteria. *Microb. Ecol.* 1988, **15**: 311-321.
  26. Rieger, H.-J., Benthic studies of the northwest African upwelling region: psychrophilic and psychrotrophic bacterial communities from areas with different upwelling intensities. *Mar. Ecol. Prog. Ser.* 1989, **57**: 45-52.
  27. Rieger, H.-J. and Tan, T., Community structures of cold and low-nutrient adapted heterotrophic sediment bacteria from the deep eastern tropical Atlantic. *Mar. Ecol. Prog. Ser.* 1992, **84**: 83-93.
  28. Schmidt-Nielsen, S., Ueber einige psychrophile Mikroorganismen und ihr Vorkommen. *Zentr. Bakteriol. Parasitenkd. Infektionsk. Hyg. Abt. II.* 1902, **9**: 145-147.
  29. Sieburth, J.M., Seasonal selection of estuarine bacteria by water temperature. *J. Exp. Mar. Biol. Ecol.* 1967, **1**: 98-121.
  30. Sieburth, J.M., Observtions on bacteria planktonic in Narragansett Bay. Rhode island; a resume. *Bull. Misaki Mar. Biol. Inst. Kyoto Univ.* 1968, **12**: 49-64.
  31. Simon, M., Glöckner, F.-O. and Amann, R., Different community structure and temperature optima of heterotrophic picoplankton in various regions of the Southern Ocean. *Aquat. Microb. Ecol.* 1999, **18**: 275-284.
  32. Stanley, S. and Rose, A., Bacteria and yeasts from lakes on Deception Island. *Roy. Soc. Phil. Trans. B.* 1967, **252**: 199-207.
  33. Tan, T. and Rieger, H.-J., Biomass and nutritional requirements of psychrotrophic bacterial communities in Fram Strait and Western Greenland Sea. *Kieler Meeresforsch.* 1991, **8**(Suppl.): 219-224.
  34. Tanner, A. and Herbert, R., A numerical study of gram-negative bacteria isolated from the Antarctic marine environment. *Publ. CNEXO (Actes Colloq)* 1982, **13**: 31-38.
  35. Weyland, H. and Helmke, E., Barophilic and psychrophilic bacteria in the Antarctic Ocean. In: *Recent Advances in Microbial Ecology*, Hattori, T., Ishid, Y., Maruyama, Y., Morita, R.Y. and Uchida, A. (eds.) Japan Science Societies Press, Tokyo, 1989, pp. 43-47.
  36. Weyland, H., Rieger, H.-J. and Schwarz, H., Zur Isolierung und Identifizierung mariner Bakterien. Ein Beitrag zur Standardisierung und Entwicklung adäquater Methoden. *Veröff. Inst. Meeresforsch. Bremerh.* 1970, **12**: 269-296.
  37. Wiebe, W. and Hendricks, C., Simple reliable cold tray for the recovery and examination of thermosensitive organisms. *Appl. Microbiol.* 1971, **22**: 734-735.