Numerical Taxonomy of Bacteria from the Beaufort Sea

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Taxonomic and ecological studies were done on 553 bacterial strains isolated in late summer 1975 from the Arctic Beaufort Sea. Numerical taxonomic analyses were employed using 300 features, assessing similarity by the Jaccard coefficient (S_J) and clustering the strains using single linkage. Bacteria isolated at 4 °C (14 clusters) and 20 °C (13 clusters) were compared with previously described genera. The dominant bacteria in the Beaufort Sea appear to be different from those found in temperate marine environments. Orange-pigmented bacteria, which appear to be *Flavobacterium* species, were dominant in surface water. Several taxonomic clusters of presumed *Vibrio* species were found. Many of the Gram-negative rods were highly pleomorphic and could not be identified. Some isolates resembled morphologically the genus *Microcyclops*. Feature analyses showed evidence of adaptation to the environment. All bacterial isolates were psychrophilic or psychrotrophic. Nutritionally, most of the organisms required organic growth factors such as vitamins.

INTRODUCTION

Microbiological studies in the Arctic Ocean are rare. As part of the U.S. Outer Continental Shelf Environmental Assessment Program, we began intensive studies on the ecology of micro-organisms in the Beaufort Sea. A major purpose of this undertaking is to characterize the microbiological communities, quantitatively and qualitatively, before mineral resources of the Alaskan Continental Shelf are extensively developed. Extensive data were gathered to characterize the predominant bacterial populations both taxonomically and ecologically. We believe that this is the first such study on bacteria in the Arctic Ocean.

This paper reports the results of sampling during summer 1975. During a normal summer, ice recedes about 80 km from the shore but shorefast ice remained throughout 1975. This restricted sampling to nearshore areas. Thus, our study was of the bacterial community during a summer with severe ice conditions. Different results might have been obtained during a more typical summer.

METHODS

Sample collection and bacterial isolation. Samples were collected in the Beaufort Sea (Fig. 1) during late summer 1975 (Aug. 20 to Sept. 25). Water samples were collected with a sterile Niskin sampler (General Oceanics, Miami, Fla., U.S.A.) and sediment samples with a mud grabber (Kahl Scientific, San Diego, Calif., U.S.A.). Samples were transferred to sterile containers, stored with ice and processed within a few hours. Temperature and salinity were measured (Table 1) with a salinity meter (Yellow Springs Instrument Co., Yellow Springs, Ohio, U.S.A.).

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Fig. 1. Chart of sampling area. Numbers indicate sampling stations. Bar marker represents 20 nautical miles.

Table 1. *Abiotic parameters and viable counts*

Station (see Fig. 1)	Depth (m)	Temp. (°C)	Salinity (‰)	Viable count* after incubation at:	
				4 °C	20 °C
<i>Water samples</i>					
1	1.0	−0.5	22.0	3.5×10^3	3.9×10^3
2	1.0	−0.5	22.2	1.4×10^3	5.7×10^2
10	1.0	−0.5	23.8	4.8×10^3	4.4×10^2
30	1.0	1.9	12.1	1.0×10^4	8.5×10^3
55	1.0	1.5	16.0	2.1×10^4	1.9×10^4
70	1.0	1.5	18.5	9.2×10^3	6.9×10^3
71	1.0	0.3	20.2	5.9×10^3	3.8×10^3
<i>Sediment samples</i>					
1	2.3	−0.5	—	1.0×10^5	1.1×10^5
2	2.3	−0.5	—	1.1×10^5	1.1×10^5
10	9.3	0.8	—	5.3×10^4	1.3×10^4
30	2.0	1.8	—	8.7×10^4	2.5×10^5
55	3.3	1.5	20.0	2.3×10^5	3.6×10^5
70	6.7	0.3	21.5	6.8×10^4	1.8×10^5
71	6.3	0.1	21.3	9.7×10^4	1.6×10^5

* Expressed as numbers ml^{-1} for water samples and numbers $(\text{g dry wt sediment})^{-1}$ for sediment samples.

Serial dilutions of samples were made in Rila marine salts solution (Rila Products, Teaneck, N.J., U.S.A.) and plated on marine agar 2216 (Difco). All materials were cooled to 5 °C before plating. For each sample, one set of replicate plates was incubated at 4 °C for 3 weeks and another at 20 °C for 2 weeks. Colonies were counted with the aid of a stereomicroscope (30 $\times$). The mean count from triplicate plates was recorded for each dilution and temperature (Table 1).

All colonies were numbered sequentially. Using random number tables, about 60 bacterial colonies from each sample were selected for isolation. After subculturing twice on marine agar to ensure purity and viability, about 25 strains from each sample were randomly selected for numerical taxonomic testing. They were maintained on marine agar slants at 4 °C and subcultured monthly. Some strains lost viability during testing (less than 8% from any source) and were discarded. A total of 276 isolates from incubation at 4 °C (designated 4 °C isolates) and 277 from incubation at 20 °C (designated 20 °C isolates) (Table 2) were used for the cluster analyses, together with 11 reference strains: *Vibrio fischeri* ATCC 15381, *Pseudomonas coenobios* ATCC 14402, *Flavobacterium halmephilum* ATCC 19717, *Flavobacterium marinotypicum* ATCC 19260, *Alcaligenes pacificus* ATCC 27122, *Pseudomonas doudoroffii* ATCC 27123, *Pseudomonas marina* ATCC 27129, *Pseudomonas nautica* ATCC 27132, *Arthrobacter citreus* ATCC 11624, *Benckea campbellii* ATCC 25920 and *Vibrio alginolyticus* ATCC 17749.

Table 2. Numbers and sources of isolates used in cluster analyses

Station (see Fig. 1)	4 °C isolates		20 °C isolates	
	Water	Sediment	Water	Sediment
1	4	5	—	—
2	4	5	—	—
2	24	24	24	24
10	14	24	24	23
30	2	24	24	24
55	23	25	19	25
70	25	25	20	25
71	25	23	21	24

Characterization of isolates. Approximately 300 phenotypic characteristics were determined for each strain (see Table 4). Unless otherwise indicated, tests on the 4 °C and 20 °C isolates were incubated at 4 °C and 20 °C, respectively.

Morphology. Cultures (1 to 4 d depending on growth rate) from marine agar slants overlaid with 1 ml Rila marine salts solution were examined for: cell shape, size and motility (wet mounts); spores, refractile granules of poly- β -hydroxybutyrate (Stanier *et al.*, 1966) and capsules (India ink stain) (phase contrast microscopy); Gram reaction (Hucker modification), acid fastness (Ziehl-Neelsen method) and fat droplets (Burdon method) (Society of American Bacteriologists, 1957). Cultures (10 d) on marine agar were examined for colony morphology and size, and for production of diffusible and non-diffusible pigments. Fluorescent pigment formation on marine agar containing 0.15% (w/v) glycerol was assessed daily with ultraviolet light (λ 260 nm). Following 10 to 15 min adaptation of the observer to the dark, bioluminescence was tested in the dark daily for 10 d [cultures grown on: Bacto-tryptone, 0.5%; Bacto-yeast extract, 0.3%; Na_2HPO_4 , 0.35%; NH_4NO_3 , 0.15%; glycerol, 3.0%; agar, 1.5% (all w/v); in $\frac{3}{4}$ -strength Rila marine salts solution, pH 7.6].

Physiological and biochemical tests. Tests were read after 14 d incubation unless stated otherwise. Growth on replicate marine agar plates was tested at 5, 10, 15, 20, 25, 37 and 43 °C, and at initial pH 3, 4, 5, 6, 7, 8, 9 and 10 (adjusted with HCl or NaOH). Salt tolerance and requirement were tested in the following medium without NaCl and with 0.5, 3, 5, 7.5, 10 and 15% (w/v) NaCl added: Bacto-tryptone, 0.5%; Bacto-yeast extract, 0.1%; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.01%; NH_4NO_3 , 0.00016%; Na_2HPO_4 , 0.0008%; Bacto-agar, 1.5% (all w/v); pH 8.0. Oxygen relations were determined from stab cultures in marine agar butts.

Distribution of growth, indole production (Kovacs method; Society of American Bacteriologists, 1957) and ammonia production (Nessler's reagent) were determined from 10 d cultures in a medium containing: Bacto-tryptone, 0.3%; Bacto-yeast extract, 0.05%; Tris, 0.6%; KH_2PO_4 , 0.01%; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.0005% (all w/v); thiamin, sodium pantothenate, riboflavin, nicotinic acid, choline, pyridoxamine and cyanocobalamin, all $1 \mu\text{g l}^{-1}$; folic acid, sodium *p*-aminobenzoate and biotin, all $0.05 \mu\text{g l}^{-1}$.

Cultures (10 d) on marine agar were tested for catalase (with 3% H_2O_2) and cytochrome oxidase production (Gaby & Hadley method, allowing 1 min for the blue colour to develop; Skerman, 1969). Methyl red and Voges-Proskauer tests (Society of American Bacteriologists, 1957) were done in MR-VP broth (Difco) prepared with full-strength Rila marine salts solution. Alkaline phosphatase was detected (Barber & Kuper, 1951) in cultures (10 d) grown in a medium containing: Bacto-tryptone, 0.5%; Bacto-yeast extract, 0.1%; NH_4NO_3 , 0.00016%; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.0005%; phenolphthalein diphosphate, 0.001% (all w/v); in $\frac{3}{4}$ -strength Rila marine salts solution, pH 7.2. Arginine, ornithine and lysine decarboxylases were detected by the Falkow method (Skerman, 1969), modified by replacing distilled water with Rila marine salts solution. These tests measure alkaline end-products and do not distinguish between arginine decarboxylase and arginine dihydrolase.

Nitrate and nitrite reduction were tested in nitrate broth (Difco) with full-strength Rila marine salts solution. Nitrite was detected with naphthylamine-sulphanilic acid reagent and residual nitrate with zinc dust (Skerman, 1969).

Acid production from D-ribose, D-fructose, cellobiose, lactose, sucrose or D-mannitol (all 1%, w/v) was detected in MOF medium (Difco). Oxidation/fermentation tests were done in MOF medium containing 1% (w/v) D-glucose (Leifson, 1963). Gas production from glucose was detected with inverted Durham tubes in the liquid medium used for determining growth distribution, supplemented with 1% (w/v) D-glucose. Substrates were filter-sterilized.

Agar hydrolysis was tested on marine agar; sunken colonies and depressions around colonies were scored as positive. Lipase activity was tested in marine agar containing 0.01% (w/v) CaCl_2 and 1% (w/v) Tween 20

or Tween 80 (Sierra, 1957). Starch hydrolysis was tested by flooding plate cultures (7 d) on marine agar containing 0.5% (w/v) potato starch with Lugol's iodine. Gelatin hydrolysis was tested by flooding cultures (7 to 10 d) on marine agar containing 10% (w/v) gelatin with acid HgCl_2 (Skerman, 1969). Casein hydrolysis was tested on marine agar overlaid with a double layer of 10% (w/v) skim milk agar. For the last three tests, clear zones around colonies were recorded as positive.

Antibiotic sensitivity tests. Antibiotic sensitivity was tested by spreading bacterial suspensions on marine agar plates and applying BBL antibiotic discs (ampicillin, 2 μg ; colistin, 10 μg ; erythromycin, 15 μg ; kanamycin, 30 μg ; neomycin, 30 μg ; nitrofurantoin, 300 μg ; novobiocin, 5 μg ; oxytetracycline, 5 μg ; penicillin G, 2 units; polymyxin B, 300 units; streptomycin, 2 μg ; tetracycline, 5 μg). Zones of inhibition were measured and sensitivity was determined against standard inhibition zones (BBL).

Nutritional tests. Basal medium B used in testing for substrate utilization was prepared as follows. Portion 1: KH_2PO_4 , 0.1 g; Tris, 6.0 g; NH_4NO_3 , 1.0 g; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.005 g; Rila marine salts solution, 500 ml; pH adjusted with HCl to 8.0. Portion 2: purified agar (Difco), 10 g; distilled water, 500 ml. Portion 3: thiamin, sodium pantothenate, riboflavin, nicotinic acid, choline, pyridoxamine and cyanocobalamin, all 1 μg ; folic acid, sodium *p*-aminobenzoate and biotin, all 0.05 μg ; distilled water, 2 ml. Portions 1 and 2 were autoclaved separately. Portion 3 was filter-sterilized. The three portions were mixed at 55 °C. Substrates were sterilized by autoclaving or filtration (Stanier *et al.*, 1966), except for hydrocarbons which were sterilized ultrasonically. Substrates were mixed with the basal medium just before pouring to give final concentrations of 0.1% (w/v), except for carbohydrates (0.15%, w/v) and phenol (0.0125%, w/v). A total of 100 substrates was tested (most are listed in Table 4). To determine growth factor requirements, two additional basal media were used. Basal medium A was basal medium B without vitamins (portion 3). Basal medium E was basal medium B supplemented with 50 mg Bacto-yeast extract, 50 mg Casamino acids and 10 mg L-tryptophan. Twelve substrates (D-ribose, D-fructose, D-glucose, acetate, succinate, fumarate, DL- β -hydroxybutyrate, DL-lactate, pyruvate, 2-ketoglutarate, D-gluconate, glycerol) were used to test the ability of strains to grow on the basal media. Four classes of growth factor requirements were tested: type 1, bacteria able to grow on all basal media (do not require growth factors); type 2, bacteria able to grow on basal media B and E but not A (require vitamins as growth factors); type 3, bacteria able to grow on basal medium E but not A or B (require complex growth factors such as amino acids); type 4, bacteria unable to grow on any basal medium (require complex unknown growth factors).

Plates were inoculated with a multiple syringe inoculator (Kaneko *et al.*, 1977a). Growth with any substrate was considered positive if within 14 d it exceeded (visually) that on the same basal medium alone.

Data processing and analysis. Data were coded in binary form according to RKC format (Rogosa *et al.*, 1971), punched on cards, and verified and proof-read by two people. Errors were also checked by computer with the CREATE program (Krichevsky, 1977). Test reproducibility was checked by periodically retesting selected strains. The estimated total error rate was less than 3%, which would not significantly affect the cluster analyses. The QUERY computer program (Krichevsky, 1977) was used to arrange the data for input to the numerical taxonomy program GTP2 (supplied by R. R. Colwell). Editing removed strains lacking more than 35% data, and non-differentiating features with more than 99% positive, 99% negative or 90% missing results (MTRXED program; Walczak & Krichevsky, 1977). Similarities were estimated with the Jaccard coefficient (S_j) and cluster analyses were done by single linkage sorting (Sokal & Sneath, 1963). Clusters of strains with similarities greater than 75% were designated as taxonomic groupings (Liston *et al.*, 1963). The input data were sorted into the same order as strains in the cluster analysis triangle. The feature frequencies of all characteristics were determined with the feature analysis program FREAK (Walczak *et al.*, 1978). Probabilistic identifications were attempted using the program IDDNEW and three identification matrices currently being developed at the American Type Culture Collection (R. Johnson, unpublished).

RESULTS

General characteristics

Table 3 summarizes the occurrence of selected features in the bacterial populations. Gram-negative rods predominated in water and sediment. The majority of 4 °C isolates from water were orange. Of the 20 °C isolates, more from sediment than from water were pigmented. The 20 °C isolates grew at higher temperatures and salinities than did the 4 °C isolates. More sediment than water isolates tolerated low pH. Most isolates grew at 5 to 15 °C, pH 6 to 8 and salinities of 3% (w/v) NaCl.

More sediment than water isolates hydrolysed starch and gelatin. The 20 °C isolates from water and sediment were equally lipolytic, but more 4 °C sediment isolates than water isolates were lipolytic. Nitrate reduction was common in 4 °C sediment isolates but rare in 4 °C water isolates. Almost equal numbers of 20 °C isolates reduced nitrate. Few strains

Table 3. Occurrence of selected features in water and sediment bacterial populations isolated at 4 and 20 °C (expressed as a percentage of the total scored in each group for the given test)

Feature	4 °C isolates		20 °C isolates	
	Water	Sediment	Water	Sediment
Rod-shaped	100	100	98	99
Curved axis	29	56	13	34
Gram-negative	99	99	94	97
Motile	4	48	5	5
Capsule	19	26	8	18
Length				
1.1–2.0 µm	29	10	33	19
2.1–3.0 µm	40	30	26	27
3.1–4.0 µm	18	40	17	27
Non-pigmented	23	56	65	32
Orange	67	23	11	30
Yellow	5	10	21	33
Growth at:				
5 °C	100	100	98	97
10 °C	100	99	98	99
15 °C	98	99	99	99
20 °C	82	56	98	99
25 °C	18	20	89	77
37 °C	1	0	7	4
pH 5	52	82	53	74
pH 6	90	96	98	100
pH 7	98	97	98	99
pH 8	89	96	98	99
pH 9	73	84	87	46
0 % (w/v) NaCl	8	16	37	32
0.5 % (w/v) NaCl	8	52	94	82
3 % (w/v) NaCl	92	93	97	99
5 % (w/v) NaCl	72	69	92	63
7.5 % (w/v) NaCl	27	20	63	48
10 % (w/v) NaCl	17	17	53	46
Starch hydrolysis	9	36	17	55
Gelatin hydrolysis	41	50	18	43
Tween 20 hydrolysis	14	62	51	48
Tween 80 hydrolysis	20	62	79	74
Phosphatase	88	86	69	79
Catalase	90	72	70	77
Oxidase	0	16	4	8
Arginine decarboxylase	63	42	16	16
Lysine decarboxylase	18	19	5	6
Ornithine decarboxylase	5	10	9	19
Ammonia from peptone	9	37	36	69
Nitrate to nitrite	3	64	27	22
Acid from:				
Glucose (oxid.)	24	62	13	38
Glucose (ferm.)	30	60	49	50
Lactose	5	5	8	12
Sucrose	44	18	43	36
Utilization of:				
Carbohydrates	55	67	90	44
Glucose	58	50	85	31
Organic acids	37	69	88	50
Acetate	20	17	78	27
Pyruvate	30	50	76	38
Alcohols	29	56	79	31
Glycerol	28	35	66	25
Amino acids	32	40	83	37
Hydrocarbons	0	0	6	6
Growth factor:				
Type 1	4	24	17	14
Type 2	66	53	73	38
Type 3	25	18	6	37
Type 4	5	5	4	10

Fig. 2. Distribution of phenotypic clusters of 4 °C isolates. The positions of the sampling stations are indicated in Fig. 1.

were oxidase-positive. Isolates utilizing at least one substrate were counted for each class of substrates. The frequencies of utilization were in the order: carbohydrates > carboxylic acids > amino acids > alcohols $\gg$ hydrocarbons. The 4 °C sediment isolates utilized more substrates than did the 4 °C water isolates; the reverse was true for 20 °C isolates. Since substrate utilization was determined with only vitamins as growth factors, fastidious organisms (growth factor requirement type 3 or 4) were not tested. Thus, only 70% of 4 °C water isolates, 77% of 4 °C sediment isolates, 90% of 20 °C water isolates and 52% of 20 °C sediment isolates could be tested. Obviously, growth factors are extremely important nutritional requirements for the Beaufort Sea bacteria.

Cluster analyses

Of the 4 °C isolates (L series), 62% fell into 14 clusters at the 75% similarity level. Four of these clusters had only two members. The remaining 38% of the 4 °C isolates did not cluster at this similarity level. The largest cluster (L4), tentatively identified as *Flavobac-*

Fig. 3. Distribution of phenotypic clusters of 20 °C isolates. The positions of the sampling stations are indicated in Fig. 1.

terium, had 60 strains (accounting for 20% of the total 4 °C isolates) of which 67% were isolated from water (Fig. 2). The second largest cluster (L6), also tentatively identified as *Flavobacterium*, contained 28 strains, 80% of which were isolated from water.

The 20 °C isolates (H series) fell into 13 clusters, which contained 60% of the isolates. Six clusters had only two members. The overall populations were very diverse (Kaneko *et al.*, 1977b). In the largest cluster (H7), which has not been identified, 85% of the strains were isolated from water and accounted for 52% of all 20 °C water isolates (Fig. 3). The second largest cluster (H4), tentatively identified as *Flavobacterium*, contained 13% of all 20 °C isolates.

Table 4. *Feature frequencies* of selected characteristics of major clusters of bacterial populations isolated at 4 and 20 °C*

Cluster	L1	L2	L4	L5	L6	L7	L9	L10	L11	L12	H3	H4	H5	H6	H7	H8	H10
No. of strains	21	9	60	4	28	7	4	10	6	13	7	36	9	7	80	3	12
Cell morphology																	
Rod-shaped	100	100	100	100	100	100	100	100	100	100	100	100	88	100	100	100	100
Curved axis	28	44	11	100	32	71	25	70	100	84	0	88	33	57	6	0	25
Coccobacillary	0	0	3	0	0	0	0	0	0	0	16	0	0	0	2	0	0
Pear-shaped	14	88	15	25	21	0	0	20	25	15	0	2	0	28	38	33	0
Pleomorphic	95	77	96	100	96	85	100	88	100	92	33	85	55	42	38	66	83
Tapered end	81	22	8	0	3	0	0	10	0	0	0	0	0	14	17	100	0
Rounded end	100	100	100	100	100	100	100	100	100	100	100	100	100	100	97	100	91
Cell length																	
0.5–1.0 µm	0	0	1	0	3	0	0	0	0	0	66	0	0	14	7	0	0
1.1–2.0 µm	0	33	42	0	29	16	0	20	0	0	0	2	55	28	42	0	8
2.1–3.0 µm	4	44	44	25	42	50	0	50	25	15	0	26	33	14	26	0	0
3.1–4.0 µm	4	22	10	50	17	33	25	30	75	69	16	45	11	0	10	100	50
4.1–5.0 µm	28	0	0	0	3	20	75	0	0	16	16	25	0	0	6	0	25
5.1–10.0 µm	65	0	1	25	0	0	0	0	0	0	0	0	0	42	6	0	16
Cell width																	
< 0.5 µm	0	0	10	50	39	0	0	0	0	0	66	8	44	14	6	0	0
0.5–1.0 µm	81	100	89	50	60	100	100	100	100	84	33	91	66	85	93	100	100
1.1–2.0 µm	19	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0
Cell arrangement																	
Presence of single cells	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Chain formation	5	33	63	0	67	0	25	50	0	0	0	0	33	14	8	33	41
Irregular aggregates	0	0	3	0	3	0	25	0	0	0	0	2	11	0	21	0	41
Miscellaneous cell features																	
PHB inclusions	81	55	0	0	0	0	0	0	0	0	0	0	0	14	12	100	8
Capsule	23	0	22	0	32	28	0	60	25	0	0	8	11	0	7	0	8
Acid fast	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sudan black	95	77	3	0	3	0	0	0	0	0	0	2	0	28	30	100	0
Gram-negative	100	100	98	100	100	100	100	100	100	100	100	100	100	100	100	100	91
Motile	0	0	0	0	0	100	75	0	100	100	0	0	0	0	0	0	0
Colony pigment																	
Non-diffusible yellow	0	0	3	66	3	0	0	0	25	0	83	11	88	0	0	0	100
Non-diffusible orange	4	22	95	33	92	0	0	10	0	0	16	68	11	0	0	0	0
White	0	0	0	0	0	0	0	0	0	7	0	0	0	14	3	0	0
Grey	95	77	0	0	3	100	100	80	75	92	0	22	0	85	97	100	0
Colony size																	
< 1 mm	100	88	100	100	85	0	25	100	0	15	83	45	100	85	43	66	100
1–2 mm	0	0	0	0	0	14	75	0	25	61	16	54	0	14	48	33	0
2–6 mm	0	0	0	0	0	85	0	0	75	23	0	0	0	0	7	0	0
Colony morphology																	
Translucent	66	77	96	66	89	0	100	90	25	7	33	74	100	85	47	0	16
Transparent	23	22	3	33	10	14	0	10	0	0	0	22	0	14	2	33	0
Opaque	9	0	0	0	0	85	0	0	75	92	66	5	0	0	50	100	83
Entire	95	100	100	100	89	100	100	100	100	100	100	100	100	100	98	66	100
Convex	100	88	96	100	100	100	50	100	75	92	100	85	100	85	79	100	100
Mucoid	0	0	0	0	0	42	25	20	0	0	16	2	0	28	1	0	0
Glistening	100	100	100	100	100	100	100	100	100	100	83	100	100	100	86	33	75
Smooth	100	100	100	100	100	100	100	100	100	100	100	100	100	100	86	100	100
Growth in liquid media																	
No growth in liquid	4	33	5	33	7	0	0	0	0	0	0	0	0	0	0	0	0
Floccular	10	0	0	0	4	16	100	0	50	0	75	5	33	0	19	33	27
Ring	0	0	3	0	0	0	0	0	25	30	0	37	77	42	0	0	0
Pellicle	5	0	1	0	0	83	100	0	50	0	0	2	0	14	3	0	0
Even	47	16	35	0	28	33	100	100	75	100	25	94	88	100	27	66	36
Slight or no turbidity	68	100	75	100	92	33	0	20	0	0	75	31	0	42	73	100	90
Moderate turbidity	26	0	24	0	12	50	100	80	100	100	25	68	66	57	26	0	9
Heavy turbidity	5	0	0	0	0	16	0	0	0	0	0	0	33	0	0	0	0

Table 4. (cont.)

Cluster	L1	L2	L4	L5	L6	L7	L9	L10	L11	L12	H3	H4	H5	H6	H7	H8	H10
No. of strains	21	9	60	4	28	7	4	10	6	13	7	36	9	7	80	3	12
Acid from																	
D-Ribose	0	0	2	0	0	0	0	0	100	81	0	9	0	14	35	0	0
D-Fructose	0	16	32	0	13	0	0	0	100	84	0	9	0	14	27	0	0
Cellobiose	0	0	0	0	0	14	0	0	0	7	0	2	0	14	2	0	8
Lactose	0	0	0	0	0	0	0	0	0	0	0	5	0	0	3	0	0
Sucrose	0	14	57	0	50	0	0	11	0	0	0	68	0	0	48	0	8
D-Mannitol	0	0	0	0	0	0	0	0	25	0	0	20	0	14	6	0	0
D-Glucose (aerobic)	26	0	15	0	60	50	100	0	100	100	0	52	11	16	9	0	9
D-Glucose (ferm.)	26	12	20	0	27	75	100	0	100	100	16	69	33	0	59	33	0
Miscellaneous features																	
Indole produced	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0
Ammonia produced	6	0	3	0	3	14	100	0	75	100	0	85	88	85	12	33	0
Nitrate reduced	0	0	0	0	0	100	100	100	100	100	83	11	0	85	3	33	100
Nitrite reduced	0	0	0	0	0	42	0	37	0	0	0	5	0	0	1	0	0
Arginine decarboxylase	56	71	70	66	65	14	0	40	50	23	50	29	22	33	15	0	0
Lysine decarboxylase	28	0	16	0	16	0	0	44	0	53	0	0	12	0	3	50	0
Ornithine decarboxylase	12	0	2	50	0	28	50	12	0	0	0	21	25	14	9	50	0
Gelatin hydrolysis	14	22	55	0	42	85	100	20	50	38	0	64	0	14	3	0	0
Starch hydrolysis	0	0	5	0	0	85	0	0	75	100	100	83	11	0	0	0	100
Tween 20 hydrolysis	12	33	1	0	21	100	100	100	100	100	28	18	100	100	45	66	50
Tween 80 hydrolysis	40	75	11	25	11	100	100	50	0	100	0	91	88	42	82	100	90
Catalase	93	66	94	66	92	28	100	100	100	100	75	75	50	100	54	66	100
Oxidase	0	0	0	0	0	28	0	20	0	9	0	0	50	0	0	0	0
Alkaline phosphatase	100	100	100	100	100	100	100	55	100	76	83	85	66	71	93	66	16
Urease	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
pH: growth at																	
pH 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	83
pH 5	18	11	94	25	14	80	100	90	100	100	28	86	100	85	25	100	100
pH 6	56	100	100	100	71	100	100	100	100	100	100	100	100	100	100	100	100
pH 7	100	100	100	100	82	100	100	100	100	100	85	100	100	100	100	98	100
pH 8	100	100	100	100	78	100	100	100	100	100	100	100	100	100	100	100	100
pH 9	57	100	100	100	17	85	100	100	100	100	0	2	77	100	97	100	100
pH 10	4	0	0	0	0	71	0	0	100	100	0	0	0	0	1	0	0
Temperature: growth at																	
5 °C	100	100	100	100	100	100	100	100	100	100	100	97	100	100	100	100	100
10 °C	100	100	100	100	96	100	100	100	100	100	100	100	100	100	100	100	100
15 °C	100	100	100	100	96	71	100	100	100	100	100	100	100	100	100	100	100
20 °C	95	100	96	0	89	0	0	100	0	0	100	100	100	100	100	100	100
25 °C	57	88	1	0	3	0	0	100	0	0	28	88	100	100	96	66	100
37 °C	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	25
43 °C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
NaCl: growth at																	
0% (w/v)	5	29	6	0	4	0	0	89	0	0	0	9	89	86	20	34	100
0.5% (w/v)	71	100	98	0	92	0	0	100	0	0	16	82	100	100	97	66	100
3% (w/v)	95	100	100	100	42	100	100	90	100	100	100	100	100	100	98	66	100
5% (w/v)	66	100	100	100	17	85	100	10	100	46	28	77	100	0	98	100	100
7.5% (w/v)	23	33	53	0	3	14	0	0	25	7	0	25	77	0	73	66	50
10% (w/v)	23	14	18	0	12	0	0	0	0	0	0	42	100	14	62	33	8
15% (w/v)	0	0	6	0	4	0	0	0	0	0	0	0	0	0	0	0	0
Carbohydrates utilized																	
L-Arabinose	4	0	8	0	0	0	0	0	0	15	0	0	0	0	5	0	83
D-Ribose	9	0	1	0	0	14	0	0	0	100	84	0	0	0	3	0	50
D-Xylose	33	88	8	0	7	0	0	0	0	0	0	0	0	0	80	0	75
D-Fructose	4	66	45	0	0	0	0	0	100	100	0	0	0	0	58	33	100
D-Galactose	4	66	78	0	3	0	0	0	0	100	0	0	0	0	59	0	33
D-Glucose	81	88	68	25	14	0	50	0	100	100	0	2	55	0	94	66	100
D-Mannose	0	0	58	0	3	0	0	0	50	100	0	0	0	0	1	0	66
L-Sorbose	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0
Salicin	0	0	3	0	0	0	0	11	25	100	0	2	0	0	1	0	0
Cellobiose	14	100	0	0	0	0	0	0	0	7	0	11	22	0	84	33	100
Lactose	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	33	0
Maltose	23	0	76	0	21	0	0	0	100	100	0	0	11	0	2	0	100
Sucrose	9	11	45	0	3	0	0	0	0	0	0	0	0	0	0	0	100
Trehalose	0	0	0	0	0	0	0	0	75	0	0	2	0	0	1	0	100
Raffinose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	91

Table 4. (cont.)

Cluster No. of strains	L1 21	L2 9	L4 60	L5 4	L6 28	L7 7	L9 4	L10 10	L11 6	L12 13	H3 7	H4 36	H5 9	H6 7	H7 80	H8 3	H10 12
Alcohols utilized																	
1-Butanol	0	0	0	0	0	0	0	11	0	0	0	0	77	0	0	0	33
Ethanol	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1-Propanol	4	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	25
D(-)-1,2-Propanediol	0	0	0	0	0	0	0	0	0	0	0	0	33	0	2	66	83
Glycerol	95	100	5	0	0	0	25	0	100	100	0	8	11	0	88	33	16
D-Arabitol	66	44	0	0	0	0	0	0	0	0	0	0	0	0	37	0	0
D-Mannitol	66	66	0	0	0	0	0	0	25	7	0	0	0	0	68	100	0
D-Sorbitol	76	22	0	0	0	0	0	0	0	0	0	0	0	0	28	0	0
meso-Inositol	4	77	0	0	0	0	0	0	0	0	0	0	0	0	67	0	0
Carboxylic acids utilized																	
Acetic acid	85	88	0	0	0	0	0	11	25	0	0	0	11	42	100	100	100
Butyric acid	76	77	0	0	0	0	0	0	0	0	14	0	100	28	64	100	83
Caproic acid	76	44	0	0	0	0	0	0	0	0	0	0	100	28	80	100	83
Caprylic acid	100	100	0	0	0	0	0	88	0	0	0	0	100	100	100	100	100
Lauric acid	14	22	0	0	0	0	0	0	0	0	0	0	33	14	22	0	41
Propionic acid	81	33	1	0	0	0	0	11	0	0	0	0	44	28	67	100	100
Valeric acid	0	33	0	0	0	0	0	11	0	0	0	0	100	14	36	33	91
Glutaric acid	95	100	13	0	0	0	0	33	100	100	0	0	44	42	100	100	33
Malonic acid	71	100	0	0	0	0	0	0	0	0	0	0	11	0	77	33	41
Succinic acid	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	33	33
Fumaric acid	95	55	16	0	3	28	25	77	100	100	0	0	55	85	98	100	58
Itaconic acid	9	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	33
DL-Glyceric acid	28	88	0	0	0	0	0	0	100	100	0	0	11	0	84	0	66
β -Hydroxybutyric acid	95	88	0	0	0	0	0	0	0	0	0	0	100	42	100	100	83
DL-Lactic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	33	0
L(+)-Tartaric acid	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	16
Citric acid	85	44	2	0	0	0	0	0	50	100	0	0	0	0	42	66	16
2-Ketogluconic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16
Pyruvic acid	100	100	6	0	0	28	100	100	100	15	0	0	88	100	84	100	100
2-Ketoglutaric acid	100	100	0	0	0	0	0	62	25	0	0	0	44	85	96	100	83
m-Hydroxybenzoic acid	76	0	0	0	0	0	0	0	0	0	0	0	0	0	15	100	100
p-Hydroxybenzoic acid	90	100	0	0	0	0	0	11	0	0	0	2	11	0	100	100	100
Galacturonic acid	4	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	8
D-Gluconic acid	0	0	0	0	0	0	0	0	0	69	0	2	11	0	2	0	16
Amino acids utilized																	
L-Alanine	85	66	0	0	0	0	0	100	25	0	0	0	0	100	92	100	0
γ -Aminobutyric acid	81	100	0	0	0	0	0	90	0	0	0	0	0	100	95	50	8
L-Arginine	28	55	31	0	0	0	0	0	0	0	0	0	0	0	50	0	33
L-Asparagine	28	0	0	0	0	0	0	40	0	0	0	0	0	14	12	50	50
L-Aspartic acid	33	88	30	0	17	0	0	90	25	92	0	0	0	100	39	100	50
L-Cysteine	42	44	0	0	0	0	0	10	0	0	0	0	22	0	45	100	16
L-Cystine	28	55	0	0	0	0	0	0	0	0	0	0	11	0	6	0	33
Glycine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
L-Leucine	0	0	0	0	0	0	0	0	0	0	0	0	22	0	1	0	50
L-Isoleucine	0	0	0	0	0	0	0	0	0	0	0	0	55	0	1	0	66
L-Methionine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0
L-Ornithine	57	55	0	0	3	0	0	0	0	0	0	0	0	28	74	50	58
L-Phenylalanine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0
L-Proline	42	55	0	0	0	0	0	10	50	0	0	0	22	28	70	0	16
L-Serine	93	22	0	0	0	0	0	50	0	0	0	0	0	50	30	0	91
L-Threonine	81	0	0	0	0	0	0	0	0	0	0	0	0	0	10	100	8
L-Tryptophan	71	0	0	0	0	0	0	0	0	0	0	0	0	0	12	100	0
L-Tyrosine	66	0	0	0	3	0	0	0	0	0	0	0	11	0	10	100	0
L-Valine	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0	0
Amines utilized																	
Histamine	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0
N-Acetylglucosamine	42	0	1	0	0	0	0	11	100	100	0	0	14	0	1	0	0

The following substrates were not utilized by any cluster: L-rhamnose, 2-propanol, dulcitol, phenol, 2-phenylethanol, benzoic acid, ascorbic acid, *o*-hydroxybenzoic acid, stearic acid, L-lysine, α -amylamine, ethanolamine, putrescine, guanine, thymine, *n*-hexadecane, *n*-pentadecane, *n*-phenyldecane, pristane, pentadecylcyclohexane.

All isolates were facultative anaerobes.

* The 'percentage positive' calculations do not include missing data, so for some tests the product of the feature frequency and the total number of organisms in the cluster is not an integral number.

From the 27 original clusters, 47 representative strains were chosen (3 from each of the 10 largest clusters and 1 from each of the others). Cluster analyses failed to show similarity (75%) between the 4 °C and 20 °C clusters. Thus, the populations isolated at 4 °C appear to be distinct from those isolated at 20 °C. Tests on the 47 representative strains were repeated at 5 °C since even identical organisms can show different test results at 5 °C and 20 °C. However, less than 3% of the repeated test results differed from the original results for both the 4 °C and 20 °C isolates.

Distribution of clusters

The distribution of the clusters is shown in Figs 2 and 3 for the 4 °C and 20 °C isolates, respectively. Some of the clusters (L1, L4, L6, H4, H7) were widely distributed geographically in water and sediment, but most clusters showed restricted distribution, e.g. cluster L12 contained only strains from sediment near Point Barrow. The distribution pattern of clusters was similar in Prudhoe Bay water samples (stations 55, 70, 71).

Classification of clusters

The features of the major clusters are shown in Table 4. The growth factor requirements for all clusters are shown in Table 5. None of the isolates clustered with the reference strains and none was identified by the probabalistic identification matrices. The Beaufort Sea isolates are not identical with the common Gram-negative rods (some of which are from marine sources) contained in the matrices.

Of the 27 phenotypic clusters, 22 could be placed into seven categories:

1. *Gram-negative rods producing yellow or orange pigments* (clusters L3, L4, L6, H1, H2, H3, H4, H5, H13). According to the 8th edition of *Bergey's Manual* (Buchanan & Gibbons, 1974), Gram-negative, facultatively anaerobic rods, motile or non-motile, producing yellow, orange, red or brown pigments are classified in the genus *Flavobacterium*. This definition is vague and encompasses a heterogeneous collection of bacteria, e.g. it could include yellow-pigmented members of the Enterobacteriaceae. There were morphological, physiological, biochemical and nutritional differences between the *Flavobacterium* type strains in *Bergey's Manual* and our isolates. Many of our strains showed pleomorphism characteristic of *Cytophaga* species: however, no swarming was observed. The distinction between *Flavobacterium* and *Cytophaga* may be difficult (Hayes, 1963; Weeks, 1969; Hendrie *et al.*, 1968). Several studies have attempted to clarify the taxonomy of yellow-pigmented Gram-negative bacteria (Goodfellow *et al.*, 1976; Floodgate & Hayes, 1963). Clearly, this group of organisms is quite heterogeneous.

2. *Gram-negative bacteria forming full or partial rings* (clusters L5, H6, H9). These organisms are morphologically similar to members of the genus *Microcycylus*, which is heterogeneous (Claus *et al.*, 1968; Raj, 1977) and of uncertain affiliation (Buchanan & Gibbons, 1974). Our strains have different physiological and nutritional characteristics from previously described species. Unlike *Microcycylus marinus* and several non-marine *Microcycylus* species (Raj, 1977), our strains grew at 5 °C and did not produce acids from carbohydrates.

3. *Gram-negative, non-pigmented, fermentative, facultatively anaerobic, curved or straight rods* (clusters L7, L8). The morphological and biochemical characteristics of these organisms closely resembled those of *Vibrio* species (Davis & Park, 1962) or *Beneckeia* species (Baumann *et al.*, 1971). All of the clustered organisms in this category were obligate psychrophiles. They had more complex growth requirements than the psychrophilic *Vibrio marinus* (Colwell & Morita, 1964) isolated from the North Pacific Ocean (Morita & Haight, 1964).

4. *Gram-negative, non-pigmented, weakly fermentative, facultatively anaerobic, highly pleomorphic rods* (clusters L1, L2, H7, H8). This category of clusters has not been identified

Table 5. *Growth factor requirements of dominant clusters of bacterial populations isolated at 4 and 20 °C*

4 °C isolates: L series													
Cluster	1	2	3	4	5	6	7	8	9	10	11	12	13
No. of strains	21	9	2	60	4	28	7	2	4	10	6	13	2
Growth factor:													
Type 1	—	—	—	—	—	—	—	—	—	+	+	—	—
Type 2	+	+	+	+	—	—	—	+	+	—	—	+	+
Type 3	—	—	—	—	±	±	+	—	—	—	—	—	—
Type 4	—	—	—	—	w	w	—	—	—	—	—	—	—
20 °C isolates: H series													
Cluster	1	2	3	4	5	6	7	8	9	10	11	12	13
No. of strains	2	2	7	36	9	7	80	3	2	12	2	2	2
Growth factor:													
Type 1	—	—	—	—	—	—	—	—	—	—	—	+	+
Type 2	—	—	—	—	+	+	+	+	+	+	+	—	—
Type 3	—	+	—	+	—	—	—	—	—	—	—	—	—
Type 4	+	—	+	—	—	—	—	—	—	—	—	—	—

+, > 80 % positive; ±, 51–79 % positive; w, 21–50 % positive; —, < 20 % positive.

with any genus described in *Bergey's Manual*. These non-motile organisms could be associated with several different genera.

5. *Gram-negative, non-pigmented, non-fermentative, aerobic rods* (cluster L10). This category has not been identified. These organisms reduce nitrate and are probably capable of anaerobic respiration. Phenotypically, they could belong to the genera *Pseudomonas*, *Alcaligenes* or *Alteromonas* (Baumann *et al.*, 1972). With no observed motility and without flagella determination, the distinction is not clear.

6. *Non-acid fast, coryneform bacteria showing rudimentary branching and snapping division* (cluster H10). The coryneform bacteria may be *Arthrobacter* species even though they appeared to be Gram-negative. Indeed, many *Arthrobacter* species fail to appear Gram-positive (Mulder, 1964). Coccoid forms of these organisms and morphogenesis typical of *Arthrobacter* were observed.

7. *Gram-negative coccobacilli* (clusters H11, H12). By the criteria of Baumann *et al.* (1968), the strains in cluster H12 appear to be *Acinetobacter* species. Cluster H11 is not closely related phenotypically and remains unidentified. Organisms in cluster H12 were oxidase-negative, did not require growth factors and utilized many substrates including hydrocarbons. Hydrocarbon utilization by *Acinetobacter* has been reported by Finnerty *et al.* (1973).

DISCUSSION

The Arctic Beaufort Sea, like other polar seas, is characterized by low temperatures, extensive ice cover, constant sunlight during summer and constant darkness during winter. Bacteria in the Beaufort Sea are adapted to these extreme environmental conditions. Our isolates clearly showed adaptation to growth at low temperatures. Of the 4 °C isolates, 25% were obligate psychrophiles unable to grow above 15 °C. The remainder of the 4 °C isolates and all of the 20 °C isolates were psychrotrophs; 95% of these could not grow at 37 °C. In comparison to our findings, McDonald *et al.* (1963) found 31% of their isolates from Canadian Arctic sediments could grow at 0 °C but not at 25 °C. In studies in the Antarctic Ocean, Wiebe & Hendricks (1974) found 37% of their 4 °C isolates could not grow above 15 °C and Morita (1975) reported that 35% of his isolates could not grow at 20 °C. Thus, the Arctic and Antarctic seas are dominated by psychrotrophs and have a high incidence of obligate psychrophiles.

Nearshore areas of the Beaufort Sea show large seasonal fluctuations in salinity. Summer

freshwater input and ice melt result in low salinities. Freezing out of salts during winter ice formation results in hypersaline layers which move to the bottom. Most of our isolates could not grow without added NaCl. The optimum salt concentration was 3% (w/v) NaCl. Most of the 20 °C isolates tolerated a wide range of salt concentrations, but many of the 4 °C water isolates were restricted to near 3% (w/v) NaCl for growth.

We are not certain why so many strains were oxidase-negative. It is possible that test conditions, e.g. age of cultures, affected the results. Using identical methods in a number of other studies, we have observed much higher proportions of oxidase-positive organisms.

All isolates were facultative anaerobes by the agar-butt stab method. The oxidative/fermentative metabolism tests on MOF medium were used to distinguish aerobes from facultative anaerobes.

Pigmentation can protect bacteria against the lethal effects of intense solar radiation (Mathews & Sistrom, 1959). A very high proportion of the bacterial isolates obtained from surface waters at 4 °C were pigmented; it is possible that the orange or yellow pigmentation is an adaptive protective mechanism for Beaufort Sea bacteria exposed to intense sunlight during the Arctic summer.

An unexpected finding was that most bacteria required vitamins or more complex growth factors. The probable source of nutrients for Beaufort Sea bacteria is primary producing phytoplankton. The annual spring bloom of under-ice algae is probably the prime source of nutrients for bacterial growth (Horner & Alexander, 1972). Phytoplankton are known producers of vitamins and amino acids which can supply bacterial growth factor requirements (Carlucci & Bowes, 1970; MacLeod *et al.*, 1954; Burkholder & Burkholder, 1956). Some flavobacteria have complex requirements for vitamins and amino acids (Prince *et al.*, 1954; Prince & Cleverdon, 1955; Weeks & Beck, 1960).

The cluster analyses and comparison with previously described taxa indicate that the dominant bacteria of the Beaufort Sea are quite different from those found in temperate marine environments. Most of the phenotypic clusters could be tentatively classified as members of genera which are listed as of uncertain affiliation in *Bergey's Manual* (Buchanan & Gibbons, 1974). *Pseudomonas* species, which are often found to be a dominant genus in marine environments (Simidu *et al.*, 1977; Pfister & Burkholder, 1965; Murchelano & Brown, 1970), did not comprise a large proportion of the Beaufort Sea bacteria. We did find several clusters of presumed *Vibrio* species; such species have been found in high proportions in temperate marine environments (Kaneko & Colwell, 1973, 1974; Lovelace *et al.*, 1967; Cook & Goldman, 1976). Flavobacteria are usually not the dominant taxa in temperate marine ecosystems. In Chesapeake Bay, Lovelace *et al.* (1967) found 56% *Vibrio*, 18% *Pseudomonas* and 6% *Flavobacterium* species. In Antarctic marine waters, Pfister & Burkholder (1965) found *Pseudomonas* species to be dominant and pigmented bacteria to comprise a low proportion of the bacterial population. The latter study, in another polar marine environment, contrasts with our findings.

With respect to the genera represented in our cluster analyses, the species were psychrophilic or psychrotrophic. In many cases these may represent new species. The environmental conditions of the Beaufort Sea have apparently resulted in selection of heterogeneous bacterial taxa that are uniquely adapted as members of the bacterial community capable of survival and proliferation in this ecosystem.

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REFERENCES

- BARBER, M. & KUPER, S. W. A. (1951). Identification of *Staphylococcus pyogenes* by the phosphatase reaction. *Journal of Pathology and Bacteriology* **63**, 65–68.
- BAUMANN, P., DOUDOROFF, M. & STANIER, R. Y. (1968). A study of the *Moraxella* group. II. Oxidase-negative species (genus *Acinetobacter*). *Journal of Bacteriology* **95**, 1520–1541.
- BAUMANN, P., BAUMANN, L. & MANDEL, M. (1971). Taxonomy of marine bacteria: the genus *Beneckea*. *Journal of Bacteriology* **107**, 268–294.
- BAUMANN, L., BAUMANN, P., MANDEL, M. & ALLEN, R. D. (1972). Taxonomy of aerobic eubacteria. *Journal of Bacteriology* **110**, 402–429.
- BUCHANAN, R. E. & GIBBONS, N. E. (editors) (1974). *Bergey's Manual of Determinative Bacteriology*, 8th edn. Baltimore: Williams & Wilkins.
- BURKHOLDER, P. R. & BURKHOLDER, L. M. (1956). Vitamin B₁₂ in suspended solids and marsh muds collected along the coast of Georgia. *Limnology and Oceanography* **1**, 202–208.
- CARLUCCI, A. F. & BOWES, P. M. (1970). Vitamin production and utilization by phytoplankton in mixed culture. *Journal of Phycology* **6**, 393–400.
- CLAUS, D., BERGENDAHL, J. E. & MANDEL, M. (1968). DNA base composition of *Microcyclops* species and organisms of similar morphology. *Archiv für Mikrobiologie* **63**, 26–68.
- COLWELL, R. R. & MORITA, R. Y. (1964). Reisolation and emendation of description of *Vibrio marinus* (Russell) Ford. *Journal of Bacteriology* **88**, 831–837.
- COOK, T. M. & GOLDMAN, C. K. (1976). Bacteriology of Chesapeake Bay surface waters. *Chesapeake Science* **17**, 40–49.
- DAVIS, H. G. & PARK, R. W. A. (1962). A taxonomic study of certain bacteria currently classified as *Vibrio* species. *Journal of General Microbiology* **27**, 101–119.
- FINNERTY, W. R., KENNEDY, R. S., SPURLOCK, B. O. & YOUNG, R. A. (1973). Microbes and petroleum: perspectives and implications. In *The Microbial Degradation of Oil Pollutants*, pp. 105–125. Edited by D. G. Ahearn & S. P. Meyers. Baton Rouge: Center for Wetland Resources. Louisiana State University Publication No. LSU-SG-73-01.
- FLOODGATE, G. D. & HAYES, P. R. (1963). The Adansonian taxonomy of some yellow pigmented marine bacteria. *Journal of General Microbiology* **30**, 237–244.
- GOODFELLOW, M., AUSTIN, B. & DICKINSON, C. H. (1976). Numerical taxonomy of some yellow-pigmented bacteria isolated from plants. *Journal of General Microbiology* **97**, 219–233.
- HAYES, P. R. (1963). Studies on marine flavobacteria. *Journal of General Microbiology* **30**, 1–19.
- HENDRIE, M. S., MITCHELL, T. G. & SHEWAN, J. M. (1968). The identification of yellow-pigmented rods. In *Identification Methods for Microbiologists*, Part B, pp. 67–78. Edited by B. M. Gibbs & D. A. Shapton. London: Academic Press.
- HORNER, R. & ALEXANDER, V. (1972). Algal populations in Arctic sea ice: an investigation of heterotrophy. *Limnology and Oceanography* **17**, 454–458.
- KANEKO, T. & COLWELL, R. R. (1973). Ecology of *Vibrio parahaemolyticus* in Chesapeake Bay. *Journal of Bacteriology* **113**, 24–32.
- KANEKO, T. & COLWELL, R. R. (1974). Distribution of *Vibrio parahaemolyticus* and related organisms in the Atlantic Ocean off South Carolina and Georgia. *Applied Microbiology* **28**, 1009–1017.
- KANEKO, T., HOLDER-FRANKLIN, M. & FRANKLIN, M. (1977a). Multiple syringe inoculator for agar plates. *Applied and Environmental Microbiology* **33**, 982–985.
- KANEKO, T., ATLAS, R. & KRICHEVSKY, M. (1977b). Bacterial diversity in the Beaufort Sea. *Nature, London* **270**, 596–599.
- KRICHEVSKY, M. (1977). Coding and management of microbiological data. *Developments in Industrial Microbiology* **18**, 309–318.
- LEIFSON, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *Journal of Bacteriology* **85**, 1183–1184.
- LISTON, J., WIEBE, W. & COLWELL, R. R. (1963). Quantitative approach to the study of bacterial species. *Journal of Bacteriology* **85**, 1061–1070.
- LOVELACE, T. E., TUBIASH, H. & COLWELL, R. R. (1967). Quantitative and qualitative commensal bacterial flora of *Crassostrea virginica* in Chesapeake Bay. *Proceedings of the National Shellfisheries Association* **58**, 82–87.
- MACLEOD, R. A., ONOFREY, E. & NORRIS, M. E. (1954). Nutrition and metabolism of marine bacteria. I. Survey of nutritional requirements. *Journal of Bacteriology* **68**, 680–686.
- MATHEWS, M. M. & SISTROM, W. R. (1959). Function of carotenoid pigments in non-photosynthetic bacteria. *Nature, London* **184**, 1892–1893.
- MCDONALD, I. J., QUADLING, C. & CHAMBERS, A. K. (1963). Proteolytic activity of some cold-tolerant bacteria from Arctic sediments. *Canadian Journal of Microbiology* **9**, 303–315.
- MORITA, R. Y. (1975). Psychrophilic bacteria. *Bacteriological Reviews* **39**, 144–167.
- MORITA, R. Y. & HAIGHT, R. D. (1964). Temperature effects on the growth of an obligate psychrophilic marine bacterium. *Limnology and Oceanography* **9**, 103–106.
- MULDER, E. G. (1964). *Arthrobacter*. In *Principle and Application in Aquatic Microbiology*, Proceedings of Rudolfs Research Conference, pp. 254–276. Edited by H. Heukelekian & N. C. Dondero. New York: John Wiley.
- MURCHELANO, R. A. & BROWN, C. (1970). Heterotrophic bacteria in Long Island Sound. *Marine Biology* **7**, 1–6.
- PFISTER, R. M. & BURKHOLDER, P. R. (1965). Numerical taxonomy of some bacteria isolated from antarctic and tropical seawaters. *Journal of Bacteriology* **90**, 863–872.
- PRINCE, H. N. & CLEVERDON, R. C. (1955). The flavobacteria. II. Utilization of nitrogen compounds. *Journal of Bacteriology* **69**, 307–309.
- PRINCE, H. N., BECK, E. S., CLEVERDON, R. C. & KULP, W. L. (1954). The flavobacteria. I. Nutritional requirements. *Journal of Bacteriology* **68**, 326–328.

- RAJ, H. D. (1977). *Microcyclops* and related ring-forming bacteria. *CRC Critical Reviews in Microbiology* **5**, 243–269.
- ROGOSA, M., KRICHEVSKY, M. I. & COLWELL, R. R. (1971). Method for coding data on microbial strains for computer manipulation. *International Journal of Systematic Bacteriology, Supplement* **21**, 1A–184A.
- SIERRA, G. (1957). A simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of contact between cells and fatty substrates. *Antonie van Leeuwenhoek* **23**, 15–22.
- SIMIDU, U., KANEKO, E. & TAGA, N. (1977). Microbiological studies of Tokyo Bay. *Microbial Ecology* **3**, 173–191.
- SKERMAN, V. B. D. (1969). *Abstracts of Microbiological Methods*. New York: Wiley-Interscience.
- SOCIETY OF AMERICAN BACTERIOLOGISTS (1957). *Manual of Microbiological Methods*. New York: McGraw-Hill.
- SOKAL, R. R. & SNEATH, P. H. A. (1963). *Principles of Numerical Taxonomy*. San Francisco: W. H. Freeman.
- STANIER, R. Y., PALLERONI, N. J. & DOUDOROFF, M. (1966). The aerobic pseudomonads: a taxonomic study. *Journal of General Microbiology* **43**, 159–271.
- WALCZAK, C. A. & KRICHEVSKY, M. I. (1977). Interactive computer editing of phenetic data for numerical taxonomy. *American Society for Microbiology Annual Meeting*, abstract no. 138.
- WALCZAK, C. A., JOHNSON, R. & KRICHEVSKY, M. I. (1978). A flexible approach to phenotypic feature frequency calculations. *American Society for Microbiology Annual Meeting*, abstract no. 156.
- WEEKS, O. B. (1969). Problems concerning the relationships of cytophaga and flavobacteria. *Journal of Applied Bacteriology* **32**, 13–18.
- WEEKS, O. B. & BECK, S. M. (1960). Nutrition of *Flavobacterium aquatile* strain Taylor and a microbiological assay for thiamine. *Journal of General Microbiology* **23**, 217–229.
- WIEBE, W. J. & HENDRICKS, C. W. (1974). Distribution of heterotrophic bacteria in a transit of the Antarctic Ocean. In *Effect of the Ocean Environment on Microbial Activities*, pp. 524–535. Edited by R. R. Colwell & R. Y. Morita. Baltimore: University Park Press.