Numerical Taxonomy of Bacteria from the Gulf of Alaska

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Numerical taxonomic analysis was performed on 247 bacterial strains isolated from the Northwest Gulf of Alaska in October 1975 and on 1010 bacterial strains isolated from the Northeast Gulf of Alaska in March 1976. Using the Jaccard similarity coefficient (S_J) and single linkage clustering, 24 clusters (containing three or more strains) from the Northeast Gulf isolates and 12 clusters from the Northwest Gulf isolates were found at the 70 % similarity level. The dominant organisms in the Northeast Gulf of Alaska were identified as being in the *Moraxella*–*Acinetobacter* group; these organisms were not found in the Northwest Gulf. The physiological and biochemical characteristics of the isolates in the clusters identified as belonging to the *Moraxella*–*Acinetobacter* group did not permit a clear separation of the two genera. Several clusters were tentatively identified as belonging to the *Beneckeia*–*Vibrio* group from samples collected from both the Northeast and Northwest Gulf regions. A high proportion of the isolates from both regions were pigmented; several clusters of *Flavobacterium* occurred. Strains belonging to the genus *Microcyclops* were isolated from the Northwest Gulf but not from the Northeast Gulf of Alaska. Most clustered strains from the Northeast Gulf were eurytolerant, did not require growth factors and utilized a wide variety of substrates. Most isolates from the Northwest Gulf of Alaska were psychrotrophic and restricted to growth at salinities approximating that of seawater; many isolates from the Northwest Gulf demonstrated complex nutritional requirements. Many strains of Gram-negative pleomorphic rods isolated from both regions could not be identified.

INTRODUCTION

The ecology of micro-organisms in marine Alaskan continental shelf regions was studied as part of the U.S. Outer Continental Shelf Environmental Assessment Program. A major purpose of this undertaking has been to characterize the microbiological communities before mineral resources of the continental shelf are extensively developed. This study is concerned with the characterization of the predominant bacterial populations found in the Gulf of Alaska. Areas within the region studied have been considered as potential sites for offshore oil development and for development of oil transport facilities. If changes in biological communities are to be monitored, it is necessary to establish a baseline inventory of biomass, activities and species distribution through reconnaissance of the region prior to developmental activities. In order to establish the needed data base on the microbiological community, numerical taxonomic analyses were performed on bacterial isolates from surface

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Fig. 1. Chart of sampling locations.

water and sediment collected from sites in the Northwest and Northeast regions of the Gulf of Alaska. A further aim was to determine whether bacterial populations are similar to those from other regions of the Pacific Ocean (ZoBell, 1946, 1961, 1963; ZoBell & Upham, 1944; Simidu *et al.*, 1977) or whether these populations from northern marine ecosystems possess adaptive features which distinguish them from populations isolated from temperate regions.

METHODS

Sample collection and bacterial isolation. Samples were collected in the Northwest Gulf of Alaska, between Unimak Pass and the mouth of Cook Inlet, during October 1975, and in the Northeast Gulf of Alaska, from Kodiak Island to Yakutat Bay, during March 1976 (Fig. 1). Surface water samples were collected with a sterile Niskin butterfly sampler (General Oceanics, Miami, Fla, U.S.A.) and sediment samples with a Van-Veen grab sampler (by courtesy of I. Kaplan). Samples were transferred to sterile containers stored at 5 °C. Enumeration (plating) procedures were initiated within 1 h of collection. Samples for bottom temperature and salinity determinations were collected with Nansen bottles (Wildco Instruments) with attached reversing thermometers. Intertidal sediment samples were collected directly with a sterile wide-mouth container.

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 approximately 5 °C before plating. For each sample, one set of replicate plates was incubated at 5 °C for 3 weeks and another at 20 °C for 2 weeks. Colonies were counted with the aid of a Quebec colony counter; the mean count, from triplicate plates for sediment samples and sextuplicate plates for water samples, was recorded for each dilution and temperature.

All colonies were numbered sequentially; they were observed using a stereo microscope (30×) so that even relatively slowly growing bacteria were included in the pool of strains available for selection for isolation. Using random number tables, approximately 25 (from the Northwest Gulf cruise) to 60 (from the Northeast Gulf cruise) bacterial colonies from each sample were selected for isolation. After subculturing

Table 1. Numbers and sources of isolates used in cluster analyses

Station (see Fig. 1)	5 °C isolates		20 °C isolates	
	Water	Sediment	Water	Sediment
<i>Northwest Gulf of Alaska</i>				
101	8	18	—	10
106	7	—	—	—
119	6	—	5	—
121	—	16	—	9
124	12	—	10	—
133	9	—	10	—
134	—	19	—	7
137	10	17	7	16
145	11	—	5	—
148	5	—	1	—
156	12	—	6	—
159	8	—	3	—
<i>Northeast Gulf of Alaska</i>				
1	30	22	35	35
4	19	29	18	20
7	—	—	27	—
29	—	—	4	—
30	17	31	8	31
32	4	27	8	21
37	—	25	—	—
52	27	29	26	25
53	24	22	7	27
57	14	28	4	23
59	—	—	10	—
A	22	17	14	36
D	31	29	23	23
E	26	—	28	—
F	28	3	20	33

twice on marine agar plates to ensure purity and viability, about 25 strains from each sample were randomly selected for inclusion in the numerical phenetic analyses. Cultures were maintained on marine agar slants at 4 °C and subcultured monthly. Some strains lost viability during testing and were discarded; more problems were found in maintaining the viability of isolates from the Northwest Gulf of Alaska than for those from the Northeast Gulf.

The following strains were successfully tested: 504 isolates from the 5 °C Northeast Gulf enumeration plates, 506 isolates from the 20 °C Northeast Gulf enumeration plates, 158 isolates from the 5 °C Northwest Gulf enumeration plates and 89 isolates from the 20 °C Northwest Gulf enumeration plates. These isolates are designated '5 °C isolates' and '20 °C isolates' depending on the plate incubation temperature. Their sources are shown in Table 1. Eleven ATCC reference strains, chosen to represent taxa associated with marine ecosystems, were included in the analyses: *Alcaligenes pacificus* 27122, *Arthrobacter marinus* 25374, *Beneckeia campbellii* 25920, *Flavobacterium halmephilum* 19717, *F. marinotypicum* 19260, *Pseudomonas coenobios* 14402, *P. doudoroffii* 27123, *P. marina* 27129, *P. nautica* 27132, *Vibrio alginolyticus* 17749 and *V. fischeri* 15381.

Characterization of isolates. Approximately 300 phenotypic characteristics were determined for each strain. Details of the test procedures have been described (see Kaneko *et al.*, 1979). Phenotypic characteristics examined included morphological examination of both cells and colonies, physiological growth ranges including tolerance to temperature, salt and pH, biochemical tests including determination of a variety of enzymic activities, antibiotic sensitivity tests, and nutritional characteristics including the abilities to utilize a large number of biochemically diverse substrates (see Tables 4 and 6).

The test error rate, measured for repetitive testing of 5 °C isolates included in this study, was approximately 3 % at both 5 and 20 °C. Substrate utilization tests on 20 representative strains from clusters composed only of strains originally isolated at 20 °C were repeated at 5 °C since identical organisms can show different test results at different incubation temperatures. Less than 3 % of the repeated test results differed from the original results, which is within the range of error normally found for repetitive testing at a single temperature.

Data processing and analysis. Data were coded and processed as previously described (Kaneko *et al.*, 1979). Clustering was performed using the Jaccard coefficient (S_J) (Sneath, 1957) and single linkage clustering (GTP2 program, by courtesy of R. R. Colwell). To minimize the formation of false clusters, an inherent problem with single linkage clustering, the data were checked prior to cluster analysis to ensure that no strains were included for which there were extensive missing data (i.e. > 30 %). Data could be missing due to loss of strain viability, contamination or other observable problems during testing. The S_J coefficient was chosen because of the wide variety and large number of features determined, many of which gave numerous negative results for the diverse strains examined. At the time these analyses were performed, only programs with single linkage clustering algorithms were available. Since then, a portion of the data has been re-analysed using both weighted and unweighted average linkage with essentially identical results. Since all the data had been already analysed, it was decided to avoid the added expense of further analysis when the average linkage clustering algorithms became available.

Separate cluster analyses were performed for isolates from the Northeast and Northwest Gulf of Alaska. Because of the large number of strains included in the Northeast Gulf study, the initial cluster analyses of strains from this region were done using four independent subsets consisting of 242 strains isolated from water at 5 °C, 232 strains isolated from water at 20 °C, 262 strains isolated from sediment at 5 °C and 274 strains isolated from sediment at 20 °C, respectively. These initial analyses yielded 85 clusters of two or more strains at about 70 % similarity level; these clusters contained 346 strains. The data on strains occurring within these clusters were then pooled into a single combined set and re-run as another cluster analysis. A limitation of the combined cluster technique is that isolates which may be part of these clusters, but which occurred as single-membered clusters in the original four subsets, will have been left out of the analysis. There could be undetected clusters of four or less strains resulting from combination of single-membered clusters from the various subsets. Feature frequencies of all characteristics were determined with an updated version of the feature analysis program FREAK (Walczak, 1979).

Identification of defined phena. In addition to the inclusion of reference strains in the phenetic analyses, probabilistic identifications were attempted using the program IDDNEW and three identification matrices developed at the American Type Culture Collection (Johnson, 1979). Further attempts to identify phena to the genus level were performed using the taxonomic keys and the full text descriptions in *Bergey's Manual of Determinative Bacteriology* (Buchanan & Gibbons, 1974) and the keys of Skerman (1967).

RESULTS

Characteristics of samples

The abiotic parameters – salinity, temperature and depth – and the viable bacterial counts found for samples collected from the Northwest and Northeast regions of the Gulf of Alaska are shown in Table 2. Temperatures at the time of sampling were higher in October when the Northwest region was sampled than in March when the Northeast region was sampled. Using viable plating enumeration procedures with incubation at both 5 and 20 °C, it was found that neither seasonal nor spatial variations were associated with corresponding fluctuations in viable counts.

Characteristics and identification of Northeast Gulf of Alaska isolates

Twenty-four clusters containing three or more strains were found at the 70 % similarity level in the combined cluster analysis (performed after elimination of organisms that formed single-membered clusters in the four individual subset analyses) of isolates from the Northeast Gulf of Alaska. Seventeen clusters containing four or more isolates were considered to be major clusters. The largest cluster (E21) contained nearly equal numbers of isolates from water and sediment, all of which were isolated at 20 °C. Nine of the clusters contained strains from multiple sampling sites (Table 3), but generally the clusters did not contain strains isolated at different temperatures nor from both water and sediment samples. Indeed, only 12 % of the clusters contained strains isolated both from water and from sediment. Clusters E4, E5, E6, E7, E8, E10, E11, E15, E16, E18, E19, E20, E22, E23 and E24 only contained isolates from plates incubated at 5 °C, and clusters E1, E3, E9, E12, E14, E17 and E21 only contained isolates from plates incubated at 20 °C.

The feature frequencies of the 17 major clusters are shown in Table 4. Most of the

Table 2. *Abiotic parameters and viable counts*

Station	Depth (m)	Temp. (°C)	Salinity (‰)	Viable count at 5 °C (ml ⁻¹ or g ⁻¹)	Viable count at 20 °C (ml ⁻¹ or g ⁻¹)
<i>Northwest Gulf of Alaska</i>					
Water samples					
101	0-2	9.1	30.8	5.5×10^1	—
106	0-2	7.7	—	6.5×10^1	—
119	0-2	8.6	29.6	3.5×10^1	4.3×10^1
124	0-2	8.3	31.8	9.7×10^1	8.0×10^1
133	0-2	8.8	30.8	9.2×10^1	1.1×10^2
137	0-2	8.6	31.3	1.2×10^2	—
145	0-2	8.0	31.4	1.3×10^2	3.1×10^2
148	0-2	7.8	31.9	1.9×10^2	5.7×10^2
156	0-2	6.4	31.7	2.5×10^2	2.0×10^3
159	0-2	7.5	31.9	2.2×10^2	5.6×10^2
Sediment samples					
101	91	9.3	30.8	1.0×10^4	—
121	230	4.7	33.5	1.1×10^6	8.4×10^5
134	154	—	—	1.4×10^6	1.2×10^6
137	99	7.7	31.9	6.2×10^5	3.5×10^5
<i>Northeast Gulf of Alaska</i>					
Water samples					
1	0-2	3.0	31.7	3.3×10^1	3.9×10^1
4	0-2	3.5	32.2	2.1×10^1	2.0×10^1
7	0-2	3.5	32.3	3.1×10^1	2.2×10^2
29	0-2	4.2	32.0	1.5×10^1	1.8×10^1
30	0-2	3.8	31.7	3.3×10^1	3.2×10^1
32	0-2	4.5	32.2	1.2×10^1	5.8×10^1
52	0-2	3.2	31.8	7.2×10^1	1.0×10^2
53	0-2	3.0	31.8	5.9×10^1	2.6×10^1
57	0-2	4.0	32.1	1.0×10^1	2.7×10^1
59	0-2	4.0	32.2	1.4×10^1	1.7×10^1
A	0	5.0	31.1	7.7×10^2	2.9×10^3
D	0	2.0	31.4	2.2×10^4	2.0×10^4
E	0	3.0	31.1	1.1×10^3	9.9×10^2
F	0	3.0	30.8	3.8×10^2	3.8×10^2
Sediment samples					
1	190	4.3	32.4	5.2×10^5	2.1×10^5
4	183	4.6	32.8	1.1×10^5	5.5×10^4
30	40	4.2	31.9	5.8×10^3	1.2×10^5
32	175	5.8	33.2	1.1×10^5	6.0×10^4
37	2282	2.1	34.6	3.7×10^3	3.3×10^3
52	46	3.7	31.9	1.7×10^5	4.7×10^5
53	303	4.9	32.5	3.0×10^6	6.1×10^6
57	71	4.3	32.3	4.2×10^5	3.1×10^5
A	0	5.0	31.1	4.0×10^6	1.7×10^7
D	0	2.0	31.4	1.8×10^5	2.4×10^6
F	0	3.0	30.8	1.5×10^3	4.4×10^3

organisms grew within the range of temperatures and salt concentrations normally found in the sampling region. Strains in clusters E8, E9, E10, E11, E12, E13, E14, E15, E16, E17, E18, E19, E20, E21, E22 and E23 did not require growth factors; those in E6, E7 and E24 required vitamins; those recovered in E3, E4 and E5 required yeast extract plus Casamino acids; while those in E1 and E2 grew only on complex media.

None of the isolates formed defined clusters with the reference strains nor were any identified using the probabilistic identification matrices.

Table 4. Feature frequencies of selected characteristics of major clusters of bacterial populations isolated from the Northeast Gulf of Alaska*

Cluster	E2	E3	E6	E7	E9	E10	E13	E14	E15	E17	E18	E19	E20	E21	E22	E23	E24
No. of strains	18	9	11	4	10	29	37	11	6	5	4	6	5	91	6	9	4
Cell morphology																	
Spherical	0	77	0	0	0	0	0	0	0	0	0	33	0	0	33	22	0
Rod-shaped	100	100	100	100	100	100	97	100	100	100	0	0	0	0	0	0	0
Curved axis	0	0	0	0	0	0	8	0	100	0	0	0	0	0	0	0	0
Coccobacillary	0	0	0	0	0	0	2	0	0	0	100	66	100	100	66	77	100
Pleomorphic	0	77	0	0	0	0	51	0	0	0	0	0	0	0	0	0	0
Cell length																	
< 2.0 μm	99	0	100	100	0	100	82	100	100	100	100	100	100	100	100	100	100
> 2.0 μm	0	100	0	0	100	0	18	0	0	0	0	0	0	0	0	0	0
Cell width																	
0.5–1.0 μm	100	100	100	100	100	100	89	100	100	100	0	16	0	5	16	33	0
1.1–2.0 μm	0	0	0	0	0	0	10	0	0	0	100	83	100	94	83	66	100
Cell arrangement																	
Single cells	100	100	100	100	100	100	100	100	100	100	0	0	0	0	33	11	0
Pairs	0	0	0	0	0	0	0	0	0	0	100	100	100	100	66	88	100
Miscellaneous cell features																	
Endospores produced	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
PHB ⁺ inclusions	0	0	0	0	0	100	2	18	0	0	0	0	0	0	0	0	0
Capsule	94	0	—	—	0	—	0	0	—	—	—	—	—	91	—	—	—
Sudan black	5	0	0	0	10	13	27	0	0	0	0	0	0	0	0	0	0
Gram-positive	0	100	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
Gram-negative	100	0	100	100	0	100	100	100	100	100	100	100	100	100	100	100	100
Motile	0	100	0	0	100	100	100	100	100	0	0	0	0	0	0	0	0
Colony pigmentation																	
Non-diffusible pink	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Non-diffusible brown	0	0	0	0	0	0	5	0	0	20	0	0	0	0	0	0	0
Non-diffusible yellow	94	0	0	100	0	0	0	0	0	20	0	0	0	0	0	0	0
Non-diffusible orange	5	0	0	0	0	0	0	0	0	60	0	0	0	0	0	0	0
White	0	0	0	0	0	0	0	0	100	0	0	100	40	0	100	100	100
Grey (no pigment)	0	0	100	0	100	100	91	100	0	0	100	0	60	100	0	0	0
Colony size																	
< 1 mm	11	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
1–2 mm	83	22	0	25	20	6	8	0	16	80	50	16	60	58	16	0	0
2–6 mm	5	77	100	75	80	93	91	100	83	20	50	83	40	40	83	100	100
Colony morphology																	
Translucent	0	0	27	25	0	58	67	54	0	20	0	0	0	1	0	0	25
Transparent	0	0	0	0	10	41	18	45	0	0	0	0	0	0	0	0	0
Opaque	100	100	72	75	90	0	13	0	100	80	100	100	100	98	100	100	75
Flat	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0
Raised	22	100	100	100	100	100	91	100	100	80	100	100	100	100	100	100	100
Umbonate	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Convex	72	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0
Mucoid	88	0	0	0	0	0	0	0	0	40	0	0	0	1	0	0	0
Acid from																	
D-Ribose	17	100	18	25	100	0	79	40	100	20	50	100	0	73	100	100	100
D-Fructose	6	50	0	50	90	0	94	63	83	0	50	0	0	39	80	100	100
Cellobiose	5	66	0	50	80	83	72	80	16	0	100	50	20	67	100	100	100
Lactose	7	44	0	25	60	76	33	100	16	0	75	100	0	51	100	88	100
Sucrose	6	0	0	50	70	41	72	37	33	0	50	0	40	12	33	100	0
D-Mannitol	0	0	9	0	50	84	85	100	83	0	50	16	20	35	66	100	100
D-Glucose (aerobic)	0	44	0	100	100	96	100	100	66	0	50	100	0	45	50	88	100
D-Glucose (fermentative)	0	100	0	50	100	41	100	100	100	80	0	33	0	73	33	22	100
Miscellaneous features																	
Indole	0	0	0	0	30	0	69	9	33	0	0	0	0	24	40	55	0
Ammonia produced	0	25	0	0	100	47	95	63	25	20	25	0	0	68	—	—	0

Table 4. (cont.)

Cluster No. of strains	E2 18	E3 9	E6 11	E7 4	E9 10	E10 29	E13 37	E14 11	E15 6	E17 5	E18 4	E19 6	E20 5	E21 91	E22 6	E23 9	E24 4
Nitrate reduced	62	56	0	0	90	8	100	0	33	20	100	100	100	99	100	100	25
Nitrite reduced	14	55	0	0	60	6	2	0	0	0	0	0	0	13	0	0	75
Gelatin hydrolysis	44	75	0	100	100	100	86	100	100	0	0	0	20	9	0	0	0
Starch hydrolysis	66	0	0	25	100	3	100	9	0	0	0	16	0	2	0	0	25
Tween 20 hydrolysis	29	11	100	100	60	82	100	100	100	100	0	100	100	97	100	100	100
Tween 80 hydrolysis	33	0	100	25	0	79	97	81	33	80	0	100	100	56	83	88	100
β -Haemolysis sheep blood	0	100	—	—	100	—	0	72	—	0	—	—	—	0	—	—	—
L-Arginine decarboxylase	62	100	27	50	90	8	69	0	100	80	50	—	100	52	100	33	0
L-Lysine decarboxylase	20	33	27	0	66	0	13	0	0	20	25	0	0	8	0	0	0
L-Ornithine decarboxylase	17	22	0	0	40	0	5	0	0	50	25	16	0	9	0	0	0
Alkaline phosphatase	92	88	100	100	100	82	97	100	100	—	0	100	100	100	100	100	100
Urease	0	0	0	0	0	0	0	0	0	0	16	0	0	33	22	0	0
Catalase	83	100	0	100	100	100	97	90	100	100	100	100	100	100	100	100	100
Oxidase	44	100	100	0	40	100	100	100	100	60	100	83	100	98	66	0	75
pH: growth at																	
pH 5	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0
pH 6	0	100	0	0	100	48	64	100	100	40	75	80	0	97	100	100	100
pH 8	88	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
pH 9	33	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
pH 10	0	0	36	50	70	100	83	36	100	100	100	100	100	65	100	100	100
Temperature: growth at																	
5 °C	94	100	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100
20 °C	83	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
25 °C	61	100	0	100	100	100	100	100	100	100	100	100	100	100	100	100	100
37 °C	0	100	0	0	100	3	0	0	0	60	100	100	100	100	100	100	100
NaCl: growth at																	
0.5 % (w/v)	0	100	0	100	100	100	48	100	100	100	100	100	100	100	100	100	100
5.0 % (w/v)	0	100	54	100	100	100	89	100	100	100	100	100	100	100	100	100	100
7.5 % (w/v)	0	100	0	100	100	100	5	100	100	100	100	100	100	100	100	100	100
10.0 % (w/v)	0	88	0	0	100	100	0	100	0	80	100	100	100	100	100	100	100
15.0 % (w/v)	0	0	0	0	100	0	0	0	0	0	0	0	0	46	0	0	0
Carbohydrates utilized																	
L-Arabinose	0	0	0	0	0	6	0	9	100	100	0	0	0	24	0	0	0
D-Ribose	0	0	0	0	100	3	97	27	33	100	50	0	0	24	100	0	0
D-Xylose	0	0	0	100	100	31	16	72	100	100	0	0	0	14	100	0	0
L-Rhamnose	0	0	0	0	0	0	0	0	0	100	0	0	0	15	0	0	0
D-Fructose	0	0	0	50	100	96	100	63	100	100	100	66	0	19	100	0	0
D-Galactose	0	0	45	50	0	79	100	100	100	100	50	16	0	29	50	0	0
D-Glucose	5	0	72	100	100	48	97	100	100	100	100	100	0	19	33	100	0
D-Mannose	0	0	0	100	100	0	100	72	100	100	0	0	0	23	33	0	0
L-Sorbose	0	0	0	0	0	100	2	36	0	40	100	100	60	14	83	0	0
Salicin	0	0	0	0	0	10	45	18	0	100	0	16	0	7	83	0	25
Cellobiose	0	0	0	0	0	41	62	90	16	100	0	0	0	17	50	0	0
Lactose	0	0	18	50	0	10	5	100	66	100	0	0	0	14	0	0	0
Maltose	0	—	100	100	100	44	90	0	50	100	0	0	0	5	16	0	0
Sucrose	0	0	0	50	100	13	75	18	16	100	0	0	0	23	16	66	0
Trehalose	0	0	0	0	100	0	88	9	100	—	0	0	0	15	16	0	0
Raffinose	0	0	0	0	0	0	2	36	0	100	0	0	0	19	0	0	0
Alcohols utilized																	
1-Butanol	0	0	0	0	30	0	0	0	0	100	0	0	0	27	0	0	0
Ethanol	0	0	0	0	0	0	5	63	0	80	0	0	0	14	0	0	0
1-Propanol	0	0	0	0	0	0	16	81	16	60	0	0	0	33	0	0	0
2-Propanol	0	0	0	0	0	0	2	36	0	100	0	0	0	1	0	0	0
D(-)-1,2-Propanediol	0	0	0	0	0	0	0	9	0	100	0	0	0	18	0	0	0
Glycerol	0	0	0	100	100	0	100	0	100	100	0	0	0	8	33	0	0
D-Arabitol	0	0	0	0	0	0	8	9	100	100	0	0	0	3	0	0	0
Dulcitol	0	0	0	0	0	0	5	0	0	100	0	0	0	0	0	55	0
D-Mannitol	5	0	0	100	100	96	97	100	100	100	0	0	0	5	33	0	0

Table 4. (cont.)

Cluster No. of strains	E2 18	E3 9	E6 11	E7 4	E9 10	E10 29	E13 37	E14 11	E15 6	E17 5	E18 4	E19 6	E20 5	E21 91	E22 6	E23 9	E24 4
D-Sorbitol	0	0	0	100	10	0	18	0	0	100	0	0	0	3	0	0	0
meso-Inositol	0	0	0	0	0	0	78	0	0	100	0	0	0	2	33	0	0
Phenol	0	0	0	0	0	0	0	9	0	100	0	0	0	2	0	0	0
2-Phenyl ethanol	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0
Carboxylic acids utilized																	
Acetic acid	0	0	0	0	100	0	54	100	66	100	0	16	40	81	0	100	0
Butyric acid	0	0	0	0	100	0	21	45	100	100	0	50	0	97	50	77	0
Caproic acid	0	0	0	0	100	13	86	72	100	100	0	0	0	100	0	11	0
Caprylic acid	0	0	0	0	100	0	73	100	100	100	0	0	0	92	0	0	0
Lauric acid	0	0	0	0	0	0	13	0	0	100	0	0	0	7	16	66	0
Propionic acid	0	—	0	0	—	17	0	—	100	—	0	0	0	—	0	0	0
Valeric acid	0	33	0	0	100	0	64	72	100	100	0	0	100	64	66	55	0
Glutaric acid	0	0	0	0	0	0	18	0	0	100	0	0	0	83	0	0	0
Malonic acid	0	0	0	0	0	0	0	63	0	100	0	0	0	4	0	0	0
Succinic acid	0	—	0	0	—	51	85	—	100	—	0	0	0	—	83	0	0
Oleic acid	0	0	0	0	0	0	0	18	0	100	0	0	0	0	0	0	0
Fumaric acid	0	0	9	0	100	10	97	100	100	100	0	66	40	64	33	77	0
Itaconic acid	0	0	0	0	0	0	5	0	0	100	0	0	0	3	0	0	0
DL-Glyceric acid	0	0	—	—	100	—	100	36	—	100	—	—	—	18	—	—	—
DL-Hydroxybutyric acid	0	0	0	0	0	0	13	27	100	100	0	0	0	60	0	0	0
DL-Lactic acid	0	0	0	0	0	0	100	27	100	100	75	83	100	82	100	55	0
L(+)-Tartaric acid	0	0	0	0	0	34	0	9	0	100	0	33	0	34	50	77	100
Citric acid	0	0	0	0	100	13	91	18	100	100	50	100	0	35	50	0	0
2-Ketogluconic acid	0	0	0	0	100	82	5	0	100	100	0	100	0	24	33	0	0
Pyruvic acid	0	0	9	0	100	100	83	100	100	100	50	83	60	100	100	44	0
2-Ketoglutaric acid	0	0	0	0	100	75	56	72	100	100	100	100	60	80	33	100	0
Benzoic acid	0	0	0	0	0	0	13	0	0	0	0	0	0	65	33	100	0
m-Hydroxybenzoic acid	0	0	0	0	0	0	0	0	0	100	0	0	0	3	0	0	0
p-Hydroxybenzoic acid	0	0	0	0	0	0	2	0	83	100	0	0	0	4	50	0	0
Ascorbic acid	0	0	0	0	0	58	16	36	0	0	0	0	0	6	0	11	0
Galacturonic acid	0	0	0	50	0	0	5	0	100	100	0	0	0	9	0	0	0
D-Gluconic acid	0	0	0	0	100	41	2	18	100	40	50	66	0	25	16	0	0
o-Hydroxybenzoic acid	0	0	0	0	10	0	2	0	0	100	100	33	60	22	66	22	0
Stearic acid	0	0	0	0	0	0	0	0	0	100	0	0	0	3	0	0	0
Amino acids utilized																	
L-Alanine	0	0	0	0	0	24	67	100	100	100	0	0	0	78	0	0	0
γ-Aminobutyric acid	0	0	0	0	0	0	2	18	100	100	0	83	0	82	0	77	0
L-Arginine	0	0	0	0	70	0	64	36	100	60	0	0	60	53	33	0	0
L-Asparagine	0	0	0	0	0	3	64	90	100	40	100	100	60	72	100	100	0
L-Aspartic acid	0	11	0	100	100	0	83	100	100	100	0	0	0	35	50	11	0
L-Cysteine	0	0	—	—	0	—	37	81	—	100	—	—	—	68	—	—	—
L-Cystine	0	0	0	0	0	0	8	0	0	100	100	83	60	22	50	100	0
L-Glutamic acid	0	0	0	0	60	58	97	63	100	100	0	0	0	42	66	22	25
Glycine	0	0	0	0	0	0	72	63	0	0	0	0	0	6	33	0	0
L-Leucine	0	0	0	0	0	3	8	0	100	100	0	0	0	76	83	0	0
L-Isoleucine	0	0	27	0	0	0	2	54	100	80	0	66	60	14	50	22	0
L-Lysine	0	0	0	0	0	0	12	36	33	0	0	66	60	80	0	0	0
L-Methionine	0	0	0	0	0	0	5	27	0	20	0	0	0	27	0	0	0
L-Ornithine	0	0	0	0	100	0	78	9	66	100	0	100	40	53	33	0	0
L-Phenylalanine	0	0	0	0	0	0	10	72	16	60	0	0	0	82	50	33	0
L-Proline	0	0	0	0	100	0	100	81	100	100	0	0	40	93	83	11	0
L-Serine	0	0	0	0	20	10	83	81	50	100	0	0	0	60	0	0	0
L-Threonine	0	0	0	0	0	0	94	72	0	100	0	0	0	5	0	0	0
L-Tryptophan	0	0	0	0	0	0	2	54	0	40	0	0	0	30	0	11	0
L-Tyrosine	0	0	0	0	0	0	14	63	0	80	0	0	0	100	0	100	0
L-Valine	0	0	0	0	0	0	0	27	0	60	0	0	0	9	0	0	0
Amines utilized																	
Histamine	0	0	0	0	0	0	0	63	0	100	0	0	0	14	0	0	0
Putrescine	0	0	0	0	0	0	0	0	100	0	0	16	0	0	16	0	0

Table 4. (cont.)

Cluster	E2	E3	E6	E7	E9	E10	E13	E14	E15	E17	E18	E19	E20	E21	E22	E23	E24
No. of strains	18	9	11	4	10	29	37	11	6	5	4	6	5	91	6	9	4
<i>N</i> -Acetylglucosamine	0	0	54	0	100	0	94	36	100	80	0	0	0	17	50	0	25
Guanine	0	0	0	0	0	0	0	36	0	100	0	0	0	4	0	0	0
Thymine	0	0	0	0	0	0	2	18	0	100	0	0	0	8	0	0	0
Hydrocarbons utilized																	
<i>n</i> -Hexadecane	0	0	0	0	0	0	5	0	0	100	0	0	0	9	0	0	0
2-Methylnaphthalene	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0
ω -Phenyldecane	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0
Pristane	0	0	0	0	0	0	0	0	0	100	0	0	0	2	0	6	0
Pentadecylcyclohexane	0	0	0	0	0	0	2	9	0	100	0	0	0	4	0	0	0

* The following features were 100% positive for all strains: rounded ends, entire colonies, glistening colonies, smooth colonies, facultative anaerobe, growth at pH 7, growth at 10 °C, growth at 15 °C and growth at 3.0% NaCl.

† PHB, Poly- β -hydroxybutyric acid.

Based on the features shown in Table 4, six categories of the major clusters can be described:

1. Gram-negative, non-motile rods—coccobacilli often occurring as pairs (clusters E18 to E24). The morphological and metabolic features of the organisms closely resembled those of the *Acinetobacter*–*Moraxella* group as described in *Bergey's Manual of Determinative Bacteriology* (Lautrop, 1974). The genera *Acinetobacter* and *Moraxella* are distinguished by the oxidase test, strains of the former being negative while those of the latter are positive (Baumann *et al.*, 1968; Lautrop, 1974). Additionally, strains of the genus *Acinetobacter* utilize carbohydrates, may form acid from sugar and are resistant to penicillin; strains of the genus *Moraxella* do not utilize carbohydrates and are sensitive to penicillin (Lautrop, 1974; Thornley, 1967; Shewan, 1971). In the present study, strains in cluster E23 meet all the above characteristics of the genus *Acinetobacter* and strains in cluster E20 all those of *Moraxella*. The other clusters (E18, E19, E21, E22 and E24) represent intermediate cases which could be classified as either *Acinetobacter* or *Moraxella* depending on which of the above criteria were used.

2. Gram-negative, motile, oxidase-positive, fermentative, curved or straight rods (clusters E10, E13, E14 and E15). Members of these clusters resembled strains classified in the genera *Aeromonas*, *Benckeia* and *Vibrio*; the distinction between these taxa is not clear (Baumann *et al.*, 1971).

3. Gram-negative, non-motile rods producing yellow, orange or brown pigments (clusters E2, E7 and E17). According to the taxonomic keys in *Bergey's Manual of Determinative Bacteriology* (Weeks, 1974), these strains are included in the genus *Flavobacterium*, a heterogeneous genus defined primarily on pigment production and failure to show 'unusual' characteristics such as ring formation or plant pathogenicity which would place the organisms in genera such as *Microcycus* and *Xanthomonas*.

4. Gram-negative, non-motile, non-fermentative, oxidase-positive, catalase-negative rods which are not actively proteolytic in gelatin media (E6). These strains resembled non-motile strains of *Alcaligenes* except for the catalase test results (Holding & Shewan, 1974).

5. Gram-positive, motile rods producing pink colonies and spherical bodies in older cultures (E3). The morphogenesis of these strains is representative of coryneform bacteria (e.g. *Arthrobacter*) which also exhibit a morphological cycle (Bousfield, 1978; Mulder, 1964; Keddie, 1974, 1978).

6. Gram-positive–Gram-variable, motile, large rods forming endospores and growing aerobically (E9). The strains in this cluster clearly belong in the genus *Bacillus* (Gordon *et al.*, 1973). The endospores were oval, terminal or subterminal and did not distend the sporangium. According to the keys of Gordon *et al.* (1973), strains occurring in this cluster are

Table 5. Sources of isolates in each cluster shown as the percentage isolated from each of the 12 sampling stations in the Northwest Gulf of Alaska

Cluster No. of strains	W1 4	W2 3	W3 20	W4 3	W5 3	W6 3	W7 5	W8 7	W9 9	W10 4	W11 3	W12 3
Station												
101	—	33	30	—	—	—	—	14	—	—	—	—
106	—	—	5	—	—	—	—	—	—	—	—	—
119	—	—	5	—	33	—	—	—	—	—	—	—
121	—	—	—	—	—	—	20	14	—	—	—	—
124	25	33	—	33	—	—	—	14	—	—	—	—
133	25	33	25	33	—	—	—	—	11	—	—	—
134	—	—	—	—	—	33	80	29	—	—	—	—
137	25	—	30	33	67	—	—	—	89	100	—	—
145	25	—	—	—	—	—	—	14	—	—	33	—
148	—	—	5	—	—	33	—	—	—	—	—	—
156	—	—	—	—	—	33	—	—	—	—	—	—
159	—	—	—	—	—	—	—	14	—	—	67	100

assigned to the Group I *Bacillus* species. The strains exhibited denitrifying activities and showed eurytolerance to physiological growth conditions, growing over a wide range of pH values, salt concentrations and temperatures.

In addition to the organisms that formed defined major clusters, several minor clusters showed characteristics of the genera *Flavobacterium* (strains in E8 were Gram-negative, motile rods producing yellow pigments), *Pseudomonas* (strains in E5 were Gram-negative, motile rods that grew only oxidatively) and *Vibrio* (strains in E11, E12 and E16 were Gram-negative rods generally with a curved axis). Other clusters (E1 and E4) could not be associated with defined genera.

Characteristics and identification of Northwest Gulf of Alaska isolates

The bacterial populations in this region were quite diverse; only 12 clusters containing three or more members were found at the 70 % similarity level and of these only four contained five or more strains. The larger clusters generally accommodated isolates from several locations (Table 5); the two largest clusters contained a mixture of isolates from water and sediment and from 5 and 20 °C isolation temperatures. Only two of the clusters were restricted to isolates from a single location.

The feature frequencies of the 12 clusters are shown in Table 6. Most isolates were psychrotrophs capable of growth at 5 and 20 °C, the normal temperature range for the area sampled; all grew well at 3 % NaCl concentrations and several would not grow in the absence of added NaCl. Four of the clusters (W6, W8, W9 and W10) contained strains which exhibited complex nutritional growth factor requirements; those of one cluster (W7) required vitamins; but those in the remainder showed no growth factor requirements (W1, W2, W3, W4, W5, W11 and W12).

None of the reference strains were recovered within the 12 clusters and none of the isolates were identified using the computer comparison with ATCC strains. Based on the features shown in Table 6, seven categories of clusters can be described, five of which show major characteristics of defined generic groups:

1. Gram-negative, oxidase-positive, non-pigmented, fermentative, motile rods (clusters W2 and W3). These organisms were similar to *Beneckea* species (Baumann *et al.*, 1971).
2. Gram-negative, oxidase-positive, non-pigmented, variably fermentative, motile, curved or straight rods (clusters W5 and W7). The morphological and biochemical characteristics of these organisms closely resembled those of *Vibrio* species (Davis & Park, 1962) or *Beneckea* species (Baumann *et al.*, 1971). Members of these clusters were stenohaline growing only at 3 % NaCl.
3. Gram-negative, facultatively anaerobic, straight rods producing non-diffusible yellow

Table 6. *Feature frequencies of selected characteristics of major clusters of bacterial populations isolated from the Northwest Gulf of Alaska**

Cluster No. of strains	W1 4	W2 3	W3 20	W4 3	W5 3	W6 3	W7 5	W8 7	W9 9	W10 4	W11 3	W12 3
Cell morphology												
Curved axis	50	0	0	0	33	0	60	57	0	0	66	100
Pleomorphic	0	0	0	0	0	0	20	14	0	0	0	0
Rounded ends	100	100	94	100	100	100	100	71	100	100	100	100
Square ends	0	0	5	0	0	0	0	28	0	0	0	0
Cell length												
0.5-1.0 μm	0	0	10	0	0	0	20	0	100	0	0	100
1.1-2.0 μm	75	33	78	100	66	0	60	85	0	100	66	0
2.1-3.0 μm	25	66	10	0	33	100	20	14	0	0	33	0
Cell width												
<0.5 μm	0	0	5	0	0	0	0	0	0	0	0	100
0.5-1.0 μm	100	100	89	100	100	100	100	100	100	100	100	0
1.1-2.0 μm	0	0	5	0	0	0	0	0	0	0	0	0
Miscellaneous cell features												
PHB $\dagger$ inclusions	0	0	5	0	0	66	0	42	0	0	0	0
Capsule	25	33	15	33	33	33	40	57	100	100	0	0
Sudan black	0	0	26	0	0	33	0	14	0	0	0	0
Gram-negative	100	100	100	100	100	100	100	85	100	100	100	100
Motile	100	66	78	100	100	66	20	42	0	0	0	0
Colony pigmentation												
Diffusible yellow	0	0	0	0	0	0	0	14	0	0	0	0
Non-diffusible violet	0	0	0	66	0	0	0	0	0	0	0	0
Non-diffusible yellow	0	0	10	0	0	0	0	0	100	100	100	100
Grey (no pigment)	100	100	89	33	100	100	100	100	0	0	0	0
Colony size												
<1 mm	0	0	0	0	0	0	0	0	22	100	0	0
1-2 mm	0	0	5	0	0	66	80	85	77	0	66	100
2-6 mm	100	100	94	100	100	33	20	14	0	0	33	0
Colony morphology												
Translucent	50	100	89	0	66	100	100	71	0	0	100	100
Transparent	50	0	0	0	0	0	0	0	0	0	0	0
Opaque	0	0	10	100	33	0	0	28	100	100	0	0
Entire	75	0	89	100	100	100	100	100	100	100	100	100
Undulate	25	100	10	0	0	0	0	0	0	0	0	0
Raised	100	100	63	33	33	66	100	100	0	0	100	100
Umbonate	0	0	31	66	66	0	0	0	0	0	0	0
Convex	0	0	5	0	0	33	0	0	100	100	0	0
Mucoid	0	0	5	0	0	33	0	0	100	100	0	0
Acid from												
D-Ribose	0	—	92	50	50	—	50	0	0	0	0	0
D-Fructose	—	100	94	50	50	—	0	0	0	0	—	—
Cellobiose	0	0	22	0	0	—	60	0	0	0	—	—
Lactose	0	0	10	0	0	—	66	—	0	0	—	—
Sucrose	0	0	0	50	33	—	0	0	0	0	—	—
D-Mannitol	0	100	94	100	66	—	0	0	0	0	—	100
D-Glucose (aerobic)	0	100	94	50	33	—	100	0	0	0	0	0
D-Glucose (fermentative)	50	100	88	100	66	0	100	0	0	25	66	0
Miscellaneous features												
Obligate aerobe	100	0	5	0	66	50	0	0	0	0	0	33
Facultative anaerobe	0	100	95	100	33	50	100	100	100	100	100	66
Indole	0	33	94	0	33	—	0	0	0	0	0	—
Ammonia produced	0	100	94	100	66	—	0	0	16	0	0	—
Nitrate reduced	0	0	95	100	66	0	100	66	75	50	0	0
Nitrite reduced	0	0	0	50	0	0	0	16	0	0	0	0
Starch hydrolysis	0	100	90	100	0	66	0	0	75	75	—	100

Table 6. (cont.)

Cluster	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12
No. of strains	4	3	20	3	3	3	5	7	9	4	3	3
Tween 20 hydrolysis	75	66	95	100	0	100	0	50	0	0	0	0
Tween 80 hydrolysis	100	66	95	100	—	100	0	0	0	0	—	0
L-Arginine decarboxylase	0	33	65	0	0	0	100	83	25	50	50	—
L-Lysine decarboxylase	0	0	0	0	0	0	66	33	0	0	0	—
L-Ornithine decarboxylase	0	0	0	0	0	0	66	25	0	25	0	—
Alkaline phosphatase	100	100	90	50	100	100	100	100	71	—	100	100
Catalase	0	100	100	50	100	0	80	85	33	75	100	100
Oxidase	50	100	100	100	100	100	100	100	11	0	0	33
pH: growth at												
pH 6	0	0	78	0	0	0	0	0	11	0	0	0
pH 8	100	100	85	100	100	100	100	100	66	100	66	100
pH 9	100	100	90	100	100	100	100	71	11	25	0	0
pH 10	100	100	95	100	0	0	0	28	0	0	0	0
Temperature: growth at												
5 °C	100	100	100	100	—	100	100	100	77	100	100	100
20 °C	100	100	100	50	100	100	80	42	100	75	100	100
25 °C	0	100	100	0	100	0	40	0	0	0	0	0
37 °C	0	0	10	0	0	0	20	0	0	—	—	—
NaCl: growth at												
0.5 % (w/v)	0	33	100	50	0	0	0	0	0	0	0	0
5.0 % (w/v)	100	100	75	50	100	0	100	42	11	0	0	0
7.5 % (w/v)	0	0	85	0	0	0	0	0	0	0	0	0
Carbohydrates utilized												
D-Ribose	0	0	100	0	0	0	0	0	0	0	0	0
D-Fructose	0	100	100	0	0	0	0	0	0	0	0	0
D-Galactose	0	100	42	0	0	0	0	0	0	0	0	0
D-Glucose	100	100	100	0	0	0	0	0	0	0	0	0
D-Mannose	0	100	95	0	0	0	0	0	0	0	0	0
L-Sorbose	0	66	0	0	0	0	0	0	0	0	0	0
Salicin	0	66	10	50	0	0	0	0	0	0	0	0
Cellobiose	25	33	20	0	33	0	0	0	0	0	0	0
Lactose	25	0	0	50	33	0	0	0	0	0	0	0
Maltose	0	0	5	0	0	0	0	0	0	25	0	0
Sucrose	0	66	5	100	33	0	0	0	0	0	0	0
Trehalose	100	100	100	0	0	0	0	0	0	0	—	0
Alcohols utilized												
1-Propanol	0	100	0	50	0	0	0	0	0	0	0	0
Glycerol	25	100	100	50	0	0	0	0	0	0	0	0
D-Mannitol	0	100	100	0	66	0	0	0	0	0	0	0
D-Sorbitol	0	100	0	0	0	0	0	0	0	0	0	0
meso-Inositol	0	33	5	0	0	0	0	0	0	0	0	0
2-Phenyl ethanol	0	66	0	50	0	0	0	0	0	0	0	0
Carboxylic acids utilized												
Acetic acid	0	100	95	50	100	0	0	0	0	0	0	100
Caproic acid	0	0	15	0	0	0	0	0	0	0	0	0
Lauric acid	100	100	10	100	0	0	0	0	22	0	0	0
Propionic acid	0	0	20	0	0	0	0	0	0	0	0	0
Glutaric acid	0	100	40	50	0	0	0	0	0	0	0	0
Malonic acid	25	100	95	0	0	0	0	0	0	0	0	0
Succinic acid	25	100	0	50	0	66	0	0	0	0	0	0
Oleic acid	0	0	0	50	0	0	0	0	0	0	0	0
Itaconic acid	0	0	5	0	0	0	20	14	0	0	0	0
DL-Glyceric acid	25	0	65	0	0	0	0	0	0	0	0	0
β -Hydroxybutyric acid	0	0	0	50	66	0	0	0	0	0	0	0
DL-Lactic acid	0	100	35	0	100	0	0	0	0	0	0	100
L(+)-Tartaric acid	0	0	0	0	0	0	0	0	0	0	100	33
Citric acid	0	100	30	50	66	0	0	0	0	0	0	0

Table 6. (cont.)

Cluster No. of strains	W1 4	W2 3	W3 20	W4 3	W5 3	W6 3	W7 5	W8 7	W9 9	W10 4	W11 3	W12 3
Pyruvic acid	75	100	95	50	100	0	0	0	0	0	100	33
2-Ketoglutaric acid	0	100	90	50	100	0	40	0	0	0	66	100
Ascorbic acid	0	0	65	0	0	0	0	0	0	0	0	0
Galacturonic acid	0	0	0	50	0	0	0	0	0	0	0	0
D-Gluconic acid	0	100	15	0	0	0	0	0	0	0	0	0
Stearic acid	0	0	0	0	0	0	0	0	25	0	—	0
Amino acids utilized												
L-Alanine	75	66	100	50	100	0	0	0	0	0	0	0
L-Arginine	100	66	40	50	100	0	100	33	0	0	—	0
L-Asparagine	25	100	100	50	100	0	80	0	0	0	0	0
L-Aspartic acid	0	100	100	0	0	0	100	14	0	0	100	100
L-Cysteine	0	100	40	50	0	0	20	0	0	0	0	0
L-Cystine	0	100	15	100	0	0	0	0	0	0	0	0
L-Glutamic acid	100	100	100	100	100	0	80	14	0	0	33	66
Glycine	75	100	100	0	0	0	0	0	0	0	0	0
L-Leucine	100	0	68	50	0	0	0	0	0	0	—	0
L-Isoleucine	0	0	15	50	66	0	0	0	0	0	—	0
L-Ornithine	0	66	15	50	100	0	0	0	0	0	0	0
L-Phenylalanine	0	0	10	50	100	0	0	0	0	0	—	0
L-Proline	100	100	100	100	100	0	100	14	0	0	0	0
L-Serine	100	100	100	0	100	0	0	0	0	0	0	0
L-Threonine	0	100	95	0	0	0	20	0	0	0	0	0
L-Tryptophan	0	0	5	0	0	0	0	0	0	0	0	0
L-Tyrosine	100	33	90	100	100	0	0	0	0	0	0	0
L-Valine	0	—	25	100	0	—	0	0	0	0	0	0
Amines utilized												
N-Acetylglucosamine	100	100	100	0	100	0	40	0	0	0	0	0
Guanine	0	33	0	50	0	0	0	0	0	0	0	33
Thymine	0	100	0	50	0	0	0	0	0	0	0	0
Hydrocarbons utilized												
n-Pentadecane	0	0	25	0	0	0	0	0	0	0	0	0
1-Methylnaphthalene	0	0	25	0	0	0	0	0	0	0	0	0
ω-Phenyldecane	0	0	10	0	0	0	0	0	0	0	0	0

* The following substrates were not used by strains in any of the clusters: L-arabinose, D-xylose, raffinose, L-rhamnose, 1-butanol, ethanol, 2-propanol, D-arabitol, dulcitol, phenol, caprylic acid, fumaric acid, benzoic acid, α-aminobutyric acid, L-lysine, L-methionine, ethanolamine, histamine, putrescine, 2-methylnaphthalene. The following features were 100% positive for all strains: rod-shaped, predominance of single cells, glistening colonies, smooth colonies, growth at pH 7, growth at 10 °C, growth at 15 °C and growth at 3.0% NaCl.

† PHB, Poly-β-hydroxybutyric acid.

pigments (clusters W9 and W10). According to taxonomic keys in *Bergey's Manual of Determinative Bacteriology* (Weeks, 1974), strains in these clusters are classified in the genus *Flavobacterium*. Members of these clusters were fastidious; they were restricted to growth at a salt concentration of 3% NaCl and required complex media for growth.

4. Gram-negative, yellow-pigmented, non-motile rods forming partial rings (clusters W11 and W12). These organisms were morphologically similar to members of the genus *Microcycylus* (Claus *et al.*, 1968; Raj, 1977; Staley, 1974). The strains were oxidase-negative and grew on a very limited number of substrates.

5. Gram-negative, oxidase-positive rods producing violet pigments (cluster W4). Isolates within this cluster produced violet pigments which were soluble in ethanol, but not water, and gave an absorption maximum at 579 nm characteristic of the pigment violacein produced by strains of the genus *Chromobacterium* (Sneath, 1956, 1974). The strains were motile and lost their pigment-producing ability on repeated subculturing.

6. Gram-negative, straight or curved rods that are catalase-negative (clusters W1 and

W6). These strains have not been identified. Members of cluster W1 were non-fermentative; those of cluster W6 failed to grow in fermentation tests and thus could not be scored for these features.

7. Gram-negative, non-pigmented, pleomorphic rods exhibiting bipolar inclusions (cluster W8). These bacteria have not been identified. They showed a high degree of morphological variability and were nutritionally fastidious.

In addition to the organisms that were recovered in defined clusters, several individual organisms showed characteristics of coryneform bacteria (large rods forming spherical bodies in older culture) and several others were Gram-positive cocci which exhibited characteristics of the genus *Micrococcus* (Gram-positive cocci producing pigments and occurring singly or in pairs). Numerous other organisms were observed which were not readily associated with previously described taxa.

DISCUSSION

The dominant *Moraxella*-*Acinetobacter* populations found in the Northeast Gulf of Alaska were absent from the Northwest Gulf of Alaska. Indeed, while *Acinetobacter* and *Moraxella* strains are readily isolated from marine habitats, they have not previously been found as dominant marine bacterial populations. Some caution must be used in comparing and drawing firm conclusions about regional differences in species compositions in Alaskan Outer Continental Shelf areas since samples were collected at different times in each area; caution should also be used when comparing the sizes of populations in these different studies since different numbers of isolates were used in each study.

As in the Beaufort Sea (Kaneko *et al.*, 1979), there were several clusters that were equated with *Vibrio*-*Beneckea* species and there was a notable lack of *Pseudomonas* strains in both the Northeast and Northwest Gulf regions. *Vibrio*-*Beneckea* species, which are commonly found in marine ecosystems (Kaneko & Colwell, 1973, 1974), were frequently identified in this study. The repeated failure to find dominant populations of *Pseudomonas* is not likely to be an artefact of isolation procedures since, using the same basic medium marine agar 2216, ZoBell and colleagues (ZoBell & Upham, 1944; ZoBell, 1946) isolated numerous *Pseudomonas* species in the Pacific between Hawaii and California. Simidu *et al.* (1977) also isolated *Pseudomonas* species from the Pacific Ocean.

A general feature of the isolates from the Northeast Gulf of Alaska is their eurytolerance to temperature, salt and pH; most isolates grew at 5 to 37 °C, 0.5 to 10 % NaCl and pH values of 6 to 10. There was a high incidence of diverse pigmented bacteria (44 % of total isolates) in this region, many of which formed single-membered clusters; this finding is similar to our observations in other Alaskan Continental Shelf regions. Some are undoubtedly flavobacteria, but some of these pigmented bacteria may be coryneforms (Keddie, 1978); difficulties in identifying marine members of the coryneform group have been discussed by Bousfield (1978). There appears to be a spatial discontinuity in the distribution of *Microcycylus* species; this genus is common to the contiguous region of the Northwest Gulf of Alaska, Bering Sea and Arctic Ocean, but not the Northeast Gulf of Alaska.

A surprisingly high proportion of the isolates from the Northwest Gulf of Alaska appear to be 'true marine bacteria' as shown by their salt requirements. Most of the organisms isolated from this region possess the features of autochthonous marine micro-organisms. Most isolates are psychrotrophs capable of growth over the full range of temperatures that occur in surface and bottom water layers of these habitats. No obligate psychrophiles were found. There is no universal definition for a true marine bacterium. ZoBell (1961, 1963) considers a marine bacterium to be one which, on initial isolation, grows in full-strength seawater (3 % NaCl), but not at one-quarter to one-third the osmotic pressure of seawater. Bacteria which, on initial isolation, are restricted to growth at or near 3 % NaCl and which grow in the range of 0 to 20 °C were considered to meet the requirements of true marine

bacteria. Other eurytolerant bacteria exist quite well in marine ecosystems as evidenced by the large number of such organisms isolated, but it is virtually impossible to classify such bacteria as truly marine since they could also exist in terrestrial and freshwater ecosystems. It is best to consider such organisms, which are able to grow in seawater under ambient conditions and which are isolated from marine ecosystems, as indigenous marine bacteria; they are functionally marine bacteria if not indeed true marine bacteria.

There was a lack of a dominant population in the Northwest Gulf of Alaska. Rather, the populations in this region were very diverse and most organisms formed single-membered clusters. There was a relatively high mortality rate among these original isolates as evidenced by the reduction from the original 25 isolates from each station to those used in the analyses which are shown in Table 1. We attribute this loss of viability in part to the complex nutritional requirements shown by many of the isolates which may not have been met during subculturing. The more stringent nutritional requirements of the Northwest Gulf isolates, compared with the Northeast Gulf isolates, are interesting to note; they probably reflect regional differences in nutrient availability.

Many of the pigmented isolates, which comprised 37 % of the strains included in the cluster analysis, formed single-membered clusters. The incidence of pigmented bacteria in the Northwest Gulf of Alaska was somewhat lower than in the Northeast Gulf and higher than has been reported for some temperate marine waters (Lovelace *et al.*, 1967). *Microcyclops* species were tentatively identified, based on morphology, near the Aleutian Islands; they have also been found in the Beaufort Sea (Kaneko *et al.*, 1979) and Bering Sea (unpublished data) but not in the Northeast Gulf of Alaska.

The strains examined in this study show a heterogeneity of features that does not permit a clear separation of genera based on the use of individual 'key' phenotypic features; this is evidenced by the spectrum of features found for isolates that appear to be *Moraxella*-*Acinetobacter* species. Problems with relying on a single feature for determining taxonomic status are illustrated in this study, e.g. the reliance on yellow-orange pigmentation for classification of *Flavobacterium* species and on a negative oxidase test for classification of *Acinetobacter*.

The results of the catalase tests present a particular problem in classifying several phenetic groups in this study. The only Gram-negative, catalase-negative strains described in *Bergey's Manual of Determinative Bacteriology* are included in the genus *Derxia* (Becking, 1974). While the relationship of strains isolated from the Gulf of Alaska to the genus *Derxia* cannot be positively excluded, the features of the catalase-negative strains from the Gulf of Alaska do not resemble those in the description of *Derxia gummosa*. The catalase-negative isolates could represent a new group of generic rank, but it is not possible to make this conclusion based largely on this phenotypic feature which may be of dubious taxonomic value.

This paper highlights the difficulties in classifying bacteria from diverse habitats, which have been poorly studied, even when a large number of phenotypic features are considered. Many of the isolates examined in this study could not be identified.

The failure to recover reference strains within phenetic clusters makes identification of the Gulf of Alaska isolates difficult. The reference strains were selected because they represent genera normally reported for marine ecosystems. Most of the ATCC strains selected as reference strains were originally isolated from marine ecosystems, many from the Pacific Ocean near the Gulf of Alaska regions sampled in this study. In retrospect, it is not surprising that ATCC reference strains from temperate regions of the Pacific Ocean would not be included in phenetic clusters containing strains isolated from sub-Arctic Gulf of Alaska ecosystems. Most of the ATCC strains included in constructing the probabilistic identification matrices are not marine isolates and would not be expected to show a high similarity with marine isolates from the Gulf of Alaska. Species distribution appears to be quite different between the Northeast and Northwest Gulf of Alaska regions as well as between

these regions and elsewhere in the Pacific Ocean and in other Alaskan Continental Shelf regions. Many new 'species' or genera probably exist within the Gulf of Alaska and other northern marine ecosystems.

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