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A Numerical Taxonomic Study of Pelagic and Benthic Surface-layer Bacteria in Seasonally-cold Coastal Waters

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Summary

Heterotrophic bacteria from the pelagic zone and the benthic surface-layer of the continental shelf, near Newfoundland, were isolated. Using numerical taxonomy, clusters of related strains were identified and the characteristics of the clusters determined. The majority of the pelagic strains were facultatively anaerobic and were identified as *Vibrio*, although a few strains were not identified and others were assigned to the family *Vibrionaceae*. The few strictly aerobic strains from the pelagic zone were *Alteromonas*. The benthic bacterial population was more diverse and bacteria with strictly respiratory metabolism predominated. Among these were strains of *Pseudomonas, Flavobacterium,* and *Alteromonas*. The remaining strains were *Vibrio,* and some *Vibrionaceae*. Although some pelagic and benthic strains were fastidious, most utilized a broad range of substrates. This was most evident among, although not restricted to, the marine pseudomonads and the flavobacteria of the benthos. In seasonally-cold oceans the benthic surface-layer is an important zone for detrital breakdown and nutrient recycling. The nutritional versatility of *Pseudomonas* and *Flavobacterium* may, in part, account for their abundance in the substrate enriched benthic surface-layer.

Key words: Taxonomy – Marine bacteria – Pelagic bacteria – Benthic bacteria – Sediment-water interface – Coastal water

Introduction

The microbial ecology of coastal waters of the Newfoundland continental shelf has been studied by *Gow* and *Mills* (1984), *Hollohan* et al. (1986), *Smith* et al. (1986), *Pomeroy* and *Deibel* (1986), and *Powell* et al. (1987). *Gow* and *Mills* (1984) showed that the bacterial population was predominately psychrophilic and psychrotrophic and *Powell* et al. (1987) showed that, for much of the year, the region supports a bacterial population that is characteristic of cold oceans. Depending upon temperature, biodegradation of organic matter can be slow in this region (*Hollohan* et al., 1986; *Pomeroy* and *Deibel,* 1986). As a result, a significant proportion of detritus sinks to the sediment-water interface before it decomposes (*Pomeroy* and *Deibel,* 1986). *Novitsky* (1983a; 1983b) has shown that the microbial population at the sediment-water interface is able to respond quickly and repeatedly to relatively large nutrient additions. It is his opinion that, on a weight or volume basis, the sediment-water interface is the most active microbial habitat in the pelagic and benthic ecosystem.

The heterotrophic bacterial flora of cold-ocean water and sediments has been investigated in several numerical taxonomic studies. Two of the most extensive studies are those of *Kaneko* et al. (1979) and *Hauxhurst* et al. (1980). *Kaneko* et al. (1979) showed that the dominant bacterial flora in water and sediments of the Beaufort Sea was different from the flora of temperate waters. *Hauxhurst* et al. (1980) compared strains from the Northeastern and Northwestern gulfs of Alaska. Their study showed that most strains tended to cluster with strains of similar regional origin and they attributed this to regional differences in nutrient availability in the water column. From these

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observations it is apparent that the bacterial population of one region cannot necessarily be predicted from the results of other regions.

This study was conducted to determine characteristics of the bacterial flora of the sediment-water interface and that of the planktonic zone above the interface. The extent to which the bacterial populations in the two zones may differ is not known, although differences may be expected because of differences in nutrient availability.

Materials and Methods

Study site and sampling procedures. The study site was located at latitude 47°33′N, longitude 53°01′W, in Conception Bay, Newfoundland. The pelagic samples were collected at a depth of 40 m and the benthic-surface sample was from sediment at a depth of 60 m. A J–Z water sampler (*ZoBell,* 1941) was used to collect pelagic samples. The core sample of sediment was collected using an A.K.B. design core sampler (*Wildco* Instruments, Michigan) fitted with a sterile plastic core tube. After collection, the core tube was sealed in a sterile plastic bag. Samples were stored in seawater and ice until they were processed in the laboratory. The time that elapsed between collection and complete processing of the samples was less than 6 h.

Growth and selection of bacterial strains. Details of the methods used to cultivate the bacteria have been described by *Gow* and *Mills* (1984) and *Hollohan* et al. (1986). Benthic bacteria were obtained from surface-sediment contained in the sterilized plastic cylinder of the core sampler. The flocculent surface-layer of sediment was removed aseptically and 1 g wet wt. was added to a 99-ml dilution blank. This sample is called the benthic sample. This was serially diluted using dilution blanks containing 75% natural seawater and 25% deionized water. Plates of YEPN medium were inoculated by the spin-plating technique (*Gow* and *Mills,* 1984). To determine the dry wt. of sediment, subsamples were dried to constant weight at 105 °C. Pelagic samples were plated directly, by surface-spreading, using the spin-plating technique.

Plates inoculated with pelagic and benthic samples were incubated at 15 °C for 5 weeks. Colonies with diameters 1-mm or greater were numbered and representative strains selected using a table of random numbers. The colonies were subcultured onto a medium that was a modification of the Lib X medium described by *Griffiths* et al. (1974). The modified Lib X medium (MLX) contained: 1% (w/v) BBL Trypticase, 1% (w/v) yeast-extract, 0.03% (w/v) sodium citrate, 0.03% (w/v) $NaNO_3$, and 0.001% (w/v) $Fe(NH_4)_2(SO_4)_2.6H_2O$. These ingredients were added to aged 75% (w/v) natural seawater buffered with 50 mM tris (hydroxymethyl) methylamine (Tris)-hydrochloride (pH 7.5). Solidified medium was prepared with 1.2% Oxoid technical agar No. 3.

Colonies were subcultured by streak-plating until purity was assured. Stock cultures were maintained on slants of MLX medium. For increased storage life, slant cultures were overlayed with sterile mineral oil and stored at 4 °C.

The following type cultures, from the American Type Culture Collection (ATCC), Rockville, Maryland, were used in the study for comparative purposes. The ATCC number follows the strain name: *Alteromonas undina,* 29660; *Alteromonas espejiana,* 29659; *Alteromonas macleodii,* 27126; *Alcaligenes aquamarinus,* 14400; *Deleya cupida,* 27124; *Deleya venusta,* 27125; *Deleya marina,* 25374; *Oceanospirillum commune,* 27118; *Oceanospirillum vagum,* 27119; *Photobacterium phosphoreum,* 11040; *Photobacterium angustum,* 25915; *Pseudomonas doudoroffii,* 27123; *Pseudomonas fluorescens,* E13525; *Pseudomonas nautica,* 27132; *Vibrio vulnificus,* 27562; and *Vibrio splendidus,* 25914.

Characterization tests. Most characterization tests were performed by procedures described by *Hollohan* et al. (1986). In addition to determination by Gram staining, the Gram reaction was tested by the non-staining KOH technique (*Buck,* 1982). The tests performed are shown in Table 1.

Numerical taxonomy. The cluster analysis programme used was part of the Clustan package of programs (*Wishart,* 1978). The Euclidian distance coefficient was used to estimate pairwise dissimilarities between OTU, followed by cluster analysis using Ward's clustering method.

Results

Strain selection

Bianchi and *Bianchi* (1982) have shown that as few as 20 to 30 bacterial strains are sufficient to show diversity. Initially, samples were collected during mid-November and 30 and 40 strains were isolated from the pelagic zone and the sediment surface, respectively. As characterization tests proceeded, it became apparent that the benthic sample contained a diverse bacterial population and that the pelagic sample was relatively uniform. To ensure a representative sample from the pelagic zone, this zone was sampled a second time during the following June. This is a period of increasing microbial activity in the pelagic zone of the region studied (*Powell* et al., 1987). This time 28 strains were selected. The final set of OTU that were characterized consisted of type cultures and strains from three isolations. These included 40 OTU from the benthic zone sampled during November, 30 OTU from the pelagic zone sampled at the same time, and 28 OTU from the pelagic zone sampled during June. The first pelagic sample was designated no. 1, and the second no. 2. Among the 115 OTU were the 16 type cultures listed in *Materials and Methods.* One of the type cultures, *Pseudomonas fluorescens,* was duplicated to serve as an internal control.

Clustering of benthic and pelagic OTU

A data matrix of 115 OTU and most of the characters shown in Table 1 was made. Characters that were omitted were those that were either positive or negative for all OTU, or were possessed by only a few type cultures. All OTU were Gram-negative, catalase positive, unable to utilize γ-aminovalerate, and Voges-Proskauer negative. With the exception of some type cultures and those that were nonmotile, all had polar flagella. Only some type cultures produced arginine dihydrolase or accumulated PHB as an intracellular storage product. These tests were not included in the numerical analysis.

Using Euclidean distance, with Ward's clustering method, a dendrogram revealed 3 major clusters at a phenon line of dissimilarity level 3.0 (Fig. 1). This dendrogram showed that benthic OTU made up at least one-third of the two clusters A and B, and were present in cluster C but to a lesser extent. The OTU from the first pelagic sample were absent from cluster A, predominated in cluster B, and were present in cluster C. OTU from the second pelagic

Table 1. Characterization tests used in the study

Morphological: Gram reaction, cell shape, motility, number and position of flagella.
Physiological: Growth at 5, 35 and 40 °C; Leifson MOF reactions, Na^+-requirement for growth, denitrification, luminescence.
Other Tests: Oxidase, catalase, arginine dihydrolase, Voges-Proskauer.
Exoenzymes: Amylase, gelatinase, lipase, cellulase, alginase, laminarinase, chitinase.
Storage product: PHB accumulation.

Categories of substrates and substrates used as sole sources of carbon and energy:

1. Carbohydrates; D-ribose, D-xylose, L-arabinose, sucrose, trehalose, maltose, cellobiose, mellibiose, lactose, L-rhamnose, D-mannose, D-fructose, D-galacturonate, salicin, D-gluconate, D-glucuronate, N-acetylglucosamine, D-fucose, inulin, D-glucose, D-saccharose, D-galactose.
2. Alcohols; ethanol, *n*-butanol, *n*-propanol, isopropanol.
3. Polyalcohols; D-sorbitol, *meso*-inositol, erythritol, D-mannitol, glycerol, adonitol.
4. Aliphatic amino acids; glycine, L-α-alanine, D-α-alanine, β-alanine, L-serine, L-leucine, L-isoleucine, DL-norleucine, L-valine, L-aspartate, L-lysine, L-citrulline, γ-aminobutyrate, L-arginine, L-ornithine, γ-aminovalerate, L-threonine, α-aminovalerate, γ-aminovalerate, L-glutamate, pelargonate.
5. Aromatic amino acids; L-proline, L-histidine, L-phenylalanine, L-tyrosine, L-tryptophan, *p*-aminobenzoate.
6. N-substituted amino acids; betaine, hippurate, sarcosate.
7. Other N-containing substances; allantoin, putrescine, glycholate, kynurenic acid, ethanolamine, benzylamine, creatine, niacinamide, adenine.
8. Aliphatic carboxylic, and hydroxycarboxylic acids; acetate, heptanoate, isobutyrate, isovalerate, propionate, valerate, butyrate, DL-glycerate, DL-β-hydroxybutyrate, DL-lactate, formate, glycolate.
9. Aliphatic dicarboxylic, hydroxy- and keto-dicarboxylic acids; adipate, azelate, fumarate, maleate, malonate, sebacate, suberate, succinate, citrate, DL-malate, α-ketoglutarate, pyruvate, mucate, oxyalate, pimelate, L-malate, tartrate, itaconate.
10. Aliphatic tricarboxylic acid; aconitate.
11. Aromatic carboxylic and substituted carboxylic acids; benzoate, *p*-hydroxybenzoate, *m*-hydroxybenzoate, phenylacetate, quinate, mandelate.

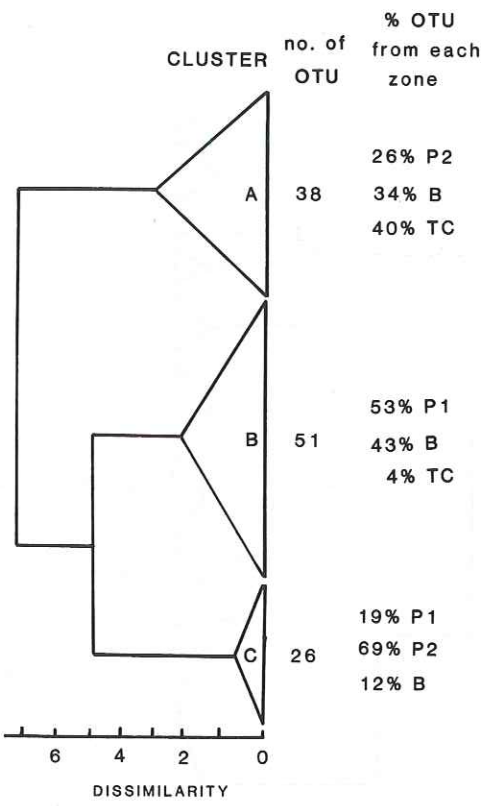

Fig. 1. Dendrogram showing the distribution of pelagic and benthic OTU in clusters at a phenon line of dissimilarity 3.0: OTU from benthos are shown as B; OTU from pelagic samples 1 and 2 are shown as P1 and P2, respectively; Type cultures are shown as TC.

sample were absent from cluster B, but predominated in cluster C and also made up a significant proportion of cluster A. OTU from the benthic zone made up significant proportions of clusters A and B and were present in cluster C. These results showed that most OTU from the two pelagic samples were different from each other, although both shared properties in common with OTU from the benthic zone. Of the type cultures, all but two, *A. undina* and *P phosphoreum,* were found in cluster A.

Identification of OTU in the clusters

A second dendrogram, using a phenon line of dissimilarity level of 0.60, resulted in 10 clusters (Fig. 2). The figure shows the identification of the predominant genera in the clusters and the sources of the OTU. The sources are from data presented in Table 2 and identifications are based on criteria presented in Tables 3 and 4. In most instances (Table 2) either facultatively anaerobic OTU or OTU with a strictly respiratory metabolism predominated a cluster and it was only in clusters i and j that there was a relatively uniform distribution of the two groups. However, if these latter clusters were further subdivided into i1, i2, j1, and j2 (Table 2) then either facultatively anaerobic OTU or OTU with strictly respiratory metabolism predominated in each subcluster.

Using the determinative criteria shown in Table 3 it was established that OTU in clusters d, f, h, and subcluster i1 were *Vibrio*. In subcluster j1, approximately one-half of the OTU did not require Na^+ for growth and none produced lipase. The Na+-independent OTU could not be described as either *Vibrio* or *Aeromonas*. They could have been *Plesiomonas,* but further studies showed that they did not utilize maltose, trehalose, inositol, and glycerol. Because strains of *Plesiomonas* should utilize these compounds (*Schubert,* 1984) it was decided that the eight strains that did not require Na^+ for growth should be assigned, at the family level, to the *Vibrionaceae*. The remaining strains of facultatively anaerobic OTU in cluster j1 were identified as *Vibrio* because they required Na^+ for growth. The organisms in cluster a were the most difficult

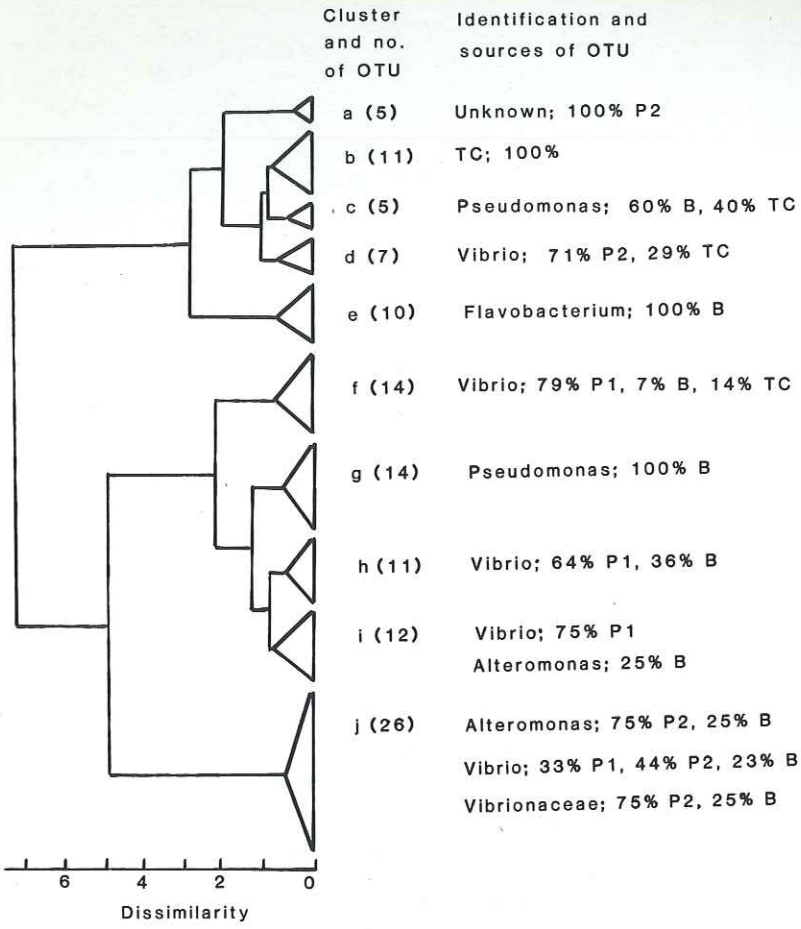

Fig. 2. Dendrogram showing the identification and distribution of OTU in clusters at a phenon line of dissimilarity 0.60: OTU from benthos are shown as B; OTU from pelagic samples 1 and 2 are shown as P1 and P2, respectively; Type cultures are shown as TC. Cluster j contained 9 *Alteromonas*, 7 *Vibrio*, and 10 *Vibrionaceae*.

to identify. These were Gram-negative, facultatively anaerobic, Na^+-independent bacteria that had orange-yellow colony pigments. Based on morphology, and some aspects of their physiology, they resembled *Flavobacterium* but were excluded from this genus because of their fermentative metabolism (*Holmes* et al., 1984). These organisms are not named in this study.

The OTU with strictly respiratory metabolism were assigned to several genera (Table 4). Those in clusters c and g were *Pseudomonas*. OTU in cluster e were *Flavobacterium* and OTU in subclusters i2 and j2 were *Alteromonas*.

Some generalizations could be made about the distribution of the bacteria in the samples. The majority of the OTU at the sediment-surface interface were *Pseudomonas* and *Flavobacterium* although *Alteromonas* and *Vibrio* were present. Pelagic sample no. 1 contained mostly *Vibrio*. Pelagic sample no. 2 had a more diverse population than pelagic sample no. 1 although facultatively anaerobic bacteria still predominated. The latter contained *Vibrio*, Vibrionaceae, and the 5 pigmented, facultatively anaerobic strains in cluster a that were not identified. Seven strains from pelagic sample no. 2, comprising 25% of this sample, were strict aerobes and identified as *Alteromonas*.

Properties of the OTU in the clusters

The clusters were examined for other characteristics. This was done by listing characters possessed by 85% or more OTU within a cluster (Table 5). An examination of Table 5 shows that the organisms in most clusters were

Table 2. Relationship between the zone of isolation and strictly respiratory or facultatively anaerobic metabolism of OTU in the 10 clusters shown in Fig. 2

Zone of isolation, or type cultures, and metabolism	Cluster											
	a	b	c	d	e	f	g	h	i		j	
									i1	i2	j1	j2
Zone												
benthic			3		10	1	14	4		3	4	1
pelagic no. 1						11		7	9		3	
pelagic no. 2	5			5							11	7
type cultures		11	2	2		2						
Metabolism												
strictly respiratory		9	5	2	10	1	14	2	2	3	1	8
facultatively anaerobic	5	2		5		13		9	7		17	

Table 3. Differential characteristics of *Vibrio* and similar facultatively anaerobic Gram-negative bacteria which inhabit aquatic environments

Characteristics	Genera[a]				Cluster or subcluster from Fig. 2				
	Vibrio	*Photo-bacterium*	*Aero-monas*	*Plesio-monas*	d	f	h	i1	j1
Motile	+	+	D	+	+	+	+	+	+
Polar flagella if motile	+	+	+	+	+	+	+	+	+
Na^+ required for growth or growth stimulation	+	+	−	−	+	+	+	+	d
Accumulation of poly-β-hydroxy-butyrate coupled with the inability to utilize β-hydroxybutyrate	−	+	−	−	−	−	−	−	−
Production of lipase	[+][b]	D	+	−	−	+	d	−	−
Utilization of D-mannitol	[+]	−	[+]	−	+	+	+	d	d

Note: Symbols for strains tested in this study; +, 85% or more of the strains are positive; −, 85% or more of the strains are negative; d, 16–84% of strains are positive; for symbols pertaining to established genera see the appropriate reference[a].

[a] From criteria given *Baumann* and *Schubert* (1984).

[b] [+] The majority, but not all strains, possess this character.

Table 4. Differential characteristics of aerobic Gram-negative bacteria which inhabit aquatic environments

Characteristics	Genera[a]						Cluster or subcluster				
	Altero-monas	*Pseudo-monas*	*Deleya*	*Alcali-genes*	*Oceano-spirillum*	*Flavo-bacterium*	c	e	g	i2	j2
Motile	+	+	+	D	+	−	+	−	+	+	+
Motile by polar flagella	+	+	D		+		+	−	+	+	+
Motile by peritrichous flagella			D	+			−	−	−	−	−
Na^+ required for growth	+	D	+	−	+	−	+	−	+	+	+
Accumulation of poly-β-hydroxybutyrate	−	D	+	+	+	−	−	−	−	−	−
Produces:											
gelatinase	D	−	−	−	D	D	D	−	−	−	−
Utilizes:											
DL-malate	D	+	+	+	D		+	−	+	−	−
D-sorbitol and *m*-hydroxybenzoate	D	−	D	D			−	−	−	−	−
Can have:											
orange-yellow pigmented colonies	D	D	−	−	−	+	−	+	−	−	−

Note: Symbols for strains tested in this study; +, 85% or more of the strains are positive; −, 85% or more of the strains are negative; for symbols pertaining to established genera see the appropriate references[a].

[a] From criteria given in *Baumann* et al. (1983), *Palleroni* (1984), *Holmes* et al. (1984), *Kersters* and *Deley* (1984), *Krieg* (1984), and *Baumann* et al. (1984).

able to utilize a variety of types of compounds as sole sources of carbon and energy. The OTU in cluster j were an exception to this. Although each organism in this cluster was able to metabolize one or another compound as a sole source of carbon energy, the only characteristic that members of the group had in common was the ability to utilize glucose. Other distinguishing characteristics of individual clusters, or groups of clusters, were denitrification shown by the benthic marine pseudomonads in cluster c, and the absence of growth at 35 and 40 °C shown by OTU in clusters f, g, h, and i. The latter organisms had all been isolated in November and made up cluster B in Fig. 1. A characteristic of OTU in all clusters, except j, was the production of the exoenzyme amylase. This indicates that polysaccharides were important substrates for most of the bacteria.

Table 5. Properties possessed by 85% or more of the OTU in the clusters shown in Fig. 2[a]

Cluster and (no. of OTU)	Identification and source of majority of OTU in the Cluster	Properties	
		Category	Name
a (5)	Unknown pelagic no. 2	Substrates from all categories were utilized	All substrates were utilized except α-aminovalerate, hippurate, sarcosate, sebacate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, quinate
		Other characters	Production of cellulase and amylase
c (5)	*Pseudomonas* benthic	Carbohydrates	D-Glucose, maltose, D-glucuronate, D-gluconate, D-galacturonate
		Alcohols	Ethanol, *n*-butanol, *n*-propanol
		Polyalcohols	D-Mannitol, glycerol
		Aliphatic amino acids	γ-Aminobutyrate, γ-aminovalerate, L-alanine, D-alanine, glycine, L-glutamate, L-leucine, L-isoleucine, pelergonate
		Aromatic amino acids	L-Histidine, L-proline
		Aliphatic carboxylic, and hydroxycarboxylic acids	Glycolate, DL-glycerate, L-lactate, DL-β-hydroxybutyrate
		Aliphatic dicarboxylic, hydroxy- and keto-dicarboxylic acids	Malonate, pimelate, suberate, succinate, adipate, fumarate, DL-malate, citrate, pyruvate, α-ketogluterate
		Aliphatic tricarboxylic acid	Aconitate
		Other characters	Production of amylase, denitrification
d (7)	*Vibrio* pelagic no. 2	Carbohydrates	D-Glucose, D-fructose
		Alcohols	Ethanol
		Polyalcohols	Erythritol, *meso*-inositol, D-mannitol, D-sorbitol
		Aliphatic amino acids	L-Glutamate, L-leucine
		Aromatic amino acids	L-Proline, L-tyrosine
		Aliphatic carboxylic, and hydroxycarboxylic acids	DL-Lactate
		Aliphatic dicarboxylic, hydroxy- and keto-dicarboxylic acids	Citrate, fumarate, α-ketoglutarate, DL-malate, succinate, suberate, pyruvate
		Other characters	Production of amylase
e (10)	*Flavobacterium* benthic	Carbohydrates	D-Glucose, lactose, D-cellobiose, D-ribose, D-trehalose, salicin
		Alcohols	Ethanol, *n*-propanol
		Polyalcohols	Adonitol, *meso*-inositol, erythritol, D-sorbitol
		Aliphatic amino acids	D-Alanine, DL-aspartate, γ-aminobutyrate
		Aromatic amino acids	L-Histidine, L-phenylalanine, L-proline, L-tyrosine
		N-substituted amino acids	Sarcosate
		Other N-containing substances	Adenine, creatine
		Aliphatic carboxylic, and hydroxy- and keto-dicarboxylic acids	DL-Glycerate, malonate, pimelate, succinate
		Aromatic carboxylic and substituted carboxylic acids	D-Mandelate
		Other characters	Production of amylase
f (14)	*Vibrio* pelagic no. 1	Carbohydrates	D-Glucose, N-acetylglucosamine, D-ribose, D-cellobiose, D-fructose, D-mannose, maltose
		Polyalcohols	D-Mannitol
		Aromatic amino acids	L-Proline
		Aliphatic dicarboxylic hydroxy- and keto-dicarboxylic acids	Pyruvate, succinate
		Other characters	Production of gelatinase, cellulase, lipase, growth at 5 °C only
g (14)	*Pseudomonas* benthic	Carbohydrates	Glucose
		Alcohols	Ethanol
		Aliphatic amino acids	L-Alanine, D-Alanine, L-arginine, γ-aminobutyrate, γ-aminovalerate, L-glutamate, valerate
		Aromatic amino acids	L-Histidine, L-proline
		Aliphatic carboxylic and hydroxycarboxylic acids	DL-Lactate, acetate, propionate, valerate
		Aliphatic dicarboxylic, hydroxy- and keto-dicarboxylic acids	Citrate, α-ketoglutarate, fumarate, DL-malate, pyruvate, succinate, suberate
		Aliphatic tricarboxylic acid	Aconitate
		Other characters	Production of amylase, growth at 5 °C only

Table 5. Continued

Cluster and (no. of OTU)	Identification and source of majority of OTU in the Cluster	Properties	
		Category	Name
h (11)	*Vibrio* pelagic no. 1 and benthic	Carbohydrates	D-Glucose, D-cellobiose, D-fructose, D-galactose, maltose, D-ribose, D-trehalose
		Alcohols	Ethanol
		Polyalcohols	D-Mannitol
		Aliphatic amino acids	L-Alanine, D-alanine, DL-aspartate, L-arginine, γ-aminovalerate, γ-aminobutyrate, L-leucine, L-glutamate
		Aromatic amino acids	L-Histidine, L-proline
		Aliphatic carboxylic, and hydroxycarboxylic acids	Acetate, glycolate, isobutyrate, isovalerate, propionate, valerate
		Aliphatic dicarboxylic, hydroxy- and keto-dicarboxylic acids	Citrate, fumarate, α-ketoglutarate, malonate, DL-malate, pyruvate, suberate, succinate
		Alipathic tricarboxylic acid	Aconitate
		Other characters	Production of amylase, growth at 5 °C only
i (12)	*Vibrio* pelagic no. 1	Carbohydrates	D-Glucose, D-fructose, D-ribose
		Alcohols	Ethanol
Alteromonas	benthic	Aliphatic amino acids	D-Alanine, L-arginine, γ-aminovalerate, γ-aminobutyrate, DL-aspartate, L-glutamate, L-leucine
		Aromatic amino acids	L-Histidine, L-proline
		Aliphatic carboxylic and hydroxycarboxylic acids	Glycolate, heptanoate, DL-β-hydroxybutyrate, DL-lactate, proprionate, valerate
		Aliphatic dicarboxylic, hydroxy- and keto-dicarboxylic acids	Fumarate, α-ketoglutarate, suberate, succinate, pyruvate
		Aliphatic tricarboxylic acid	Aconitate
		Other characters	Production of amylase, growth at 5 °C only
j (26)	*Alteromonas* pelagic no. 2 and benthic *Vibrio* pelagic no. 1 pelagic no. 2 and benthic *Vibrionaceae* pelagic no. 2 and benthic	Carbohydrates	Glucose

[a] Characteristics of cluster b, which contained 73% of the type cultures, are not included.

Discussion

In a study of the bacterial flora of a cold-ocean environment *Kaneko* et al. (1979) showed that, for the Beaufort Sea, orange-pigmented *Flavobacterium,* or *Cytophaga,* predominated the surface waters, although strains of *Microcyclus, Vibrio, Arthrobacter,* and *Acinetobacter* were present. *Pseudomonas* was isolated, but only in small numbers. The study did not emphasize differences between open-water and sediment bacteria based on different genera found in each zone, although some differences were noted, such as the predominance of *Flavobacterium* in the open-water. In 1979 the genus *Flavobacterium* included facultatively-anaerobic pigmented bacteria in addition to strictly aerobic bacteria (*Weeks,* 1974). This would include a wider range of bacteria than allowed by the current description which includes only strictly aerobic strains (*Holmes* et al., 1984). The *Flavobacterium* strains described by *Kaneko* et al. (1979) were facultatively anaerobic and most closely resembled strains in cluster a that were isolated, also from the pelagic zone, in our study.

Hauxhurst et al. (1980) compared strains from the Northeastern Gulf and the Northwestern Gulf of Alaska. Using numerical taxonomy they identified strains of *Vibrio, Flavobacterium, Bacillus, Moraxella-Acinetobacter,* and a small number of *Pseudomonas* from the Northeastern Gulf. *Vibrio, Flavobacterium, Microcyclus* and *Chromobacterium* were identified from the Northwestern Gulf. They did not discuss, in detail, taxonomic differences between pelagic and sediment bacteria, but they did observe that cold-ocean bacterial communities were nutritionally diverse. In a later study, *Hauxhurst* et al. (1981) suggested that it would be of adaptive advantage if a diverse, nutritionally versatile population was maintained.

These bacteria could have physiological tolerances whose ranges exceed those required to survive within their natural environment. The authors noted that it is possible to have a community with high nutritional or physiological diversity and also have low taxonomic diversity. Our study of bacteria in the pelagic zone and sediment-surface of coastal Newfoundland has shown that the population at the sediment-surface was more diverse than that of the pelagic zone. It also showed that these seasonally-cold waters supported a nutritionally versatile bacterial population of low taxonomic diversity. Greater taxonomic diversity has been reported for some temperate estuarine waters (*Mallory* et al., 1977; *Austin* et al., 1979).

In this study, facultatively anaerobic bacteria predominated the pelagic zone. Most strains from the first isolation were *Vibrio*. Strains from the second isolation were more diverse, but *Vibrio* still predominated. These strains, for the most part, clustered separately from the pelagic strains isolated earlier. The diversity in the second sample could have been the result of the time of isolation. This was after the spring phytoplankton bloom in this region (*Pomeroy* and *Deibel,* 1986) and it is a period of enhanced bacterial activity (*Powell* et al., 1987). Some of the groups of bacteria in the benthic surface layer had properties in common with pelagic bacteria from both isolations. This was most evident among the *Vibrio,* Vibrionaceae, and *Alteromonas* and shows that the populations in the two zones were not independent of each other.

Pseudomonas and *Flavobacterium* predominated in the benthic surface-layer. Of these, the flavobacteria are strict aerobes. The pseudomonads had the capacity for anaerobic respiration. In a study of bacterial succession during seaweed decomposition *Hollohan* et al. (1986) showed that pseudomonads were more prominent during the later stages of decomposition. If, in cold waters, the surface layer of the sediment is an important zone for detrital breakdown, then *Pseudomonas* and *Flavobacterium* may be growing on biodegradation products that are more readily available in the benthic surface-layer. Bacteria belonging to these groups were among the most nutritionally versatile isolated in this study.

Not all of the bacteria characterized in this study required Na^+ for growth. The most notable exceptions were the pigmented bacteria and the Vibrionaceae from the pelagic zone, and the benthic *Flavobacterium*. The significance of the Na^+ requirement of marine bacteria has been reviewed by *Baumann* and *Baumann* (1981). They have suggested that all, or most, Gram-negative marine bacteria have a specific requirement for Na^+. Generally, Na^+ requirement is not a determinative characteristic of many pigmented bacteria, including *Flavobacterium*, although these organisms are common to marine and other salt-water ecosystems. By using oligonucleotide cataloguing, it has been shown that *Flavobacterium* belongs to an ancient phylogenetic unit (*Paster* et al., 1985; *Woese,* 1987). The evolution of Na^+-dependency in other groups of marine bacteria, such as *Vibrio* and *Pseudomonas,* may have occurred while flavobacteria were established residents of the marine environment. It has been shown that a bacterium, that did not have Na^+-dependency for D-alanine transport, had the V_{max} for D-alanine enhanced after incorporation of a hybrid plasmid containing genes for Na^+-dependant transport (*MacLeod* and *MacLeod,* 1986). *MacLeod* (1986) suggested that Na^+-dependency of the transport process permits faster growth and gives organisms possessing it a competitive advantage in a saline environment. It is possible that Na^+-independent bacteria in the marine environment grow best when nutrients are not limiting. This could account for the presence of *Flavobacterium* in the benthic surface-layer although it is noted that strains of marine *Flavobacterium* have not been studied specifically to determine if Na^+ stimulates substrate uptake. Substrate availability could also account for the Vibrionaceae and the small group of Na^+-independent pigmented bacteria in the pelagic zone following the annual spring phytoplankton bloom.

The benthic surface layer is a zone of enhanced bacterial activity (*Novitsky,* 1983a; 1983b). The extent to which diversity contributes to enhanced activity is not known. Taxonomic information about the bacteria that inhabit the zone is necessary if the contribution of different groups, to activity, is to be investigated. This study has shown that aerobic and facultatively anaerobic bacteria with and without Na^+-dependency, are part of this ecosystem.

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