INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY, Jan. 1987, p. 27–34
0020-7713/87/01027-08$02.00/0

Vol. 37, No. 1

Halomonas subglaciescola, a New Species of Halotolerant Bacteria Isolated from Antarctica

P. D. FRANZMANN,[1]* H. R. BURTON,[2] AND T. A. McMEEKIN[1]

Department of Agricultural Science, University of Tasmania, Hobart, Tasmania, 7001, Australia[1] and Antarctic Division, Department of Science, Kingston, Tasmania, 7150, Australia[2]

***Halomonas subglaciescola* sp. nov. is proposed, based on the characteristics of 29 strains of halotolerant, nonpigmented bacteria isolated from an Antarctic, hypersaline, meromictic lake. These strains and three reference strains of halotolerant bacteria were tested for 92 attributes. The data were analyzed by numerical taxonomic procedures. The new isolates did not cluster with the three reference strains, which included the type strain of *Halomonas elongata*. However, some of the isolates did share the following attributes which are characteristic of members of the genus *Halomonas*: a guanine-plus-cytosine content of 60.9 ± 1.0 to 62.9 ± 0.7 mol%, halotolerance, largely oxidative mode of metabolism, motility, and peritrichous flagellation. The following are distinguishing features of the new species: cytochrome oxidase positive, no growth at 37°C, and glucose and other sugars are not utilized for growth. The type strain is strain ACAM 12 (= UQM 2926). The species has two biovars; biovar I contains motile strains and is represented by the type strain, and biovar II contains nonmotile strains and is represented by strain ACAM 21 (= UQM 2927).**

Organic Lake (68° 27.2′ S, 78° 12.3′ E) is a meromictic, hypersaline lake situated in the Vestfold Hills, Antarctica. This lake has been the subject of a recent biological and limnological investigation due to the high concentrations of dimethyl sulfide in it (P. D. Franzmann, P. P. Deprez, H. R. Burton, and J. van den Hoff, Aust. J. Mar. Freshwater Res., in press). As part of this ecological study, a group of halotolerant, nonpigmented bacterial strains were isolated. Such strains have not been documented previously from aquatic environments in Antarctica.

Halotolerant bacteria grow in media containing a wide range of salt concentrations (ca. 0.1 to 32.5% [wt/vol] NaCl), unlike the extremely halophilic bacteria, which survive only in solutions which contain more than 15% (wt/vol) NaCl (17). Attempts to identify the Antarctic strains as members of previously described species were unsuccessful. A taxonomic study of these strains is reported here.

MATERIALS AND METHODS

Bacterial strains. The sources of the original isolates, their strain numbers, the lake depths from which they were obtained, and some environmental parameters associated with each depth are given in Table 1. Although salinity in the lake increased with depth, the ratios of the concentrations of the major ions were essentially the ratios found in seawater (Franzmann et al., in press). The environmental parameters were measured with the methods cited by Franzmann et al. (in press). The following taxonomic and test reference strains were used in this study: *Halomonas elongata* ATCC 33173^T (T = type strain), *Paracoccus halodenitrificans* NCB 700^T, and *Vibrio costicola* NCMB 701^T. *Escherichia coli* UQM 1803^T (originally obtained as ATCC 11775^T) was used as a reference strain for determinations of deoxyribonucleic acid (DNA) base composition. The reference cultures were obtained from the following culture collections: American Type Culture Collection (ATCC), Rockville, Md.; University of Queensland Department of Microbiology (UQM), St. Lucia, Queensland, Australia; and National Collection of Industrial and Marine Bacteria (NCMB), Torry Research Station, Aberdeen, Scotland, United Kingdom.

Media. All media were sterilized at 121°C for 15 min unless otherwise stated. For Organic Lake water agar (OLWA), water collected at a depth of 2 m from Organic Lake was filtered through a 2.0-μm-pore Sartorious membrane filter and solidified with 1.5% agar. The pH was not adjusted (pH 7.0). For cystine-Organic Lake water agar, 0.5 g of L-cystine was added to 1 liter of OLWA before sterilization.

Artificial Organic Lake medium (AOL medium) consisted of 80.0 g of NaCl, 9.5 g of $MgSO_4 \cdot 7H_2O$, 5.0 g of KCl, 0.2 g of $CaCl_2 \cdot 2H_2O$, 0.1 g of $(NH_4)_2SO_4$, 0.1 g of KNO_3, and 1.0 g of yeast extract in 960 ml of distilled water. The pH was adjusted to 7.0, and the medium was sterilized. The medium was cooled to 60°C, and 20 ml of sterile Hutner salts solution (14) was added. Then 1 ml of a filter-sterilized vitamin solution, which consisted of 10.0 mg of cyanocobalamine, 2.0 mg of biotin, 10.0 mg of thiamine, 5.0 mg of calcium pantothenate, 2.0 mg of folic acid, 5.0 mg of nicotinamide, and 10.0 mg of pyridoxine hydrochloride in 100.0 ml of distilled water, was added. The medium was completed by adding 20.0 ml of sterile phosphate supplement, which consisted of 50.0 mg of K_2HPO_4 and 50.0 mg of KH_2PO_4 in 20.0 ml of distilled water. If solid medium was required (AOL agar [AOLA]), 15.0 g of agar was added prior to sterilization. For AOL-peptone medium, 5.0 g of peptone was included in each 1 liter of AOL medium. For AOL-peptone agar, AOL-peptone medium was solidified with 1.5% agar.

Enriched AOL medium contained the ingredients required for 1 liter of AOL medium but was prepared to a final volume of 900 ml. For glucose oxidation-fermentation medium, 0.5 g of peptone, 0.3 g of K_2HPO_4, 3.0 ml of 1.0% aqueous bromothymol blue, 3.0 g of agar, and 100 ml of a filter-sterilized aqueous solution of D-glucose were added to 900 ml of enriched AOL medium. For each carbon source utilization test medium, 100 ml of a 1.0% filter-sterilized aqueous carbon source solution was added to 900 ml of enriched AOL medium in which the yeast extract concentration was reduced to 0.1 g/liter. Amino acids which were not sufficiently soluble in distilled water to sterilize as 1.0%

* Corresponding author.

TABLE 1. Original isolates from Organic Lake used in this study, dates of sample collection, and salinity, oxygen concentration, and temperature of the lake at the depth from which the isolates were obtained

Strains	Date	Depth (m)	Environmental parameters			
			Salinity (g/liter)	Oxygen concn (mg/liter)	Temp (°C)	E_h (mV)
ACAM 3, ACAM 4	24 October 1984	7	210	0.0	−6.3	156
ACAM 5, ACAM 6, ACAM 7, ACAM 8, ACAM 9	24 October 1984	3	168	0.4	−6.8	382
ACAM 10, ACAM 11, ACAM 12	24 October 1984	2	167	2.9	−7.3	384
ACAM 13, ACAM 14, ACAM 15	24 October 1984	5	201	0.0	−5.3	131
ACAM 16, ACAM 17, ACAM 18	26 January 1985	3	165	1.4	−0.5	413
ACAM 19, ACAM 20, ACAM 21, ACAM 22	26 January 1985	4	218	0.0	−5.0	210
ACAM 23, ACAM 24, ACAM 25, ACAM 26	26 January 1985	5	202	0.0	−6.1	134
ACAM 27, ACAM 28, ACAM 29, ACAM 30, ACAM 31	26 January 1985	6	228	0.0	−6.5	142

solutions by filtration were sterilized as suspensions at 121°C for 15 min.

Isolation procedure. The original isolates were obtained from water samples collected from Organic Lake (68° 27′ 10″ S, 78° 12′ 15″ E) (Franzmann et al., in press). Single drops of water samples were allowed to flow across the surface of either OLWA or cystine-Organic Lake water agar plates. These plates were incubated aerobically or anaerobically (GasPak; BBL Microbiology Systems) at 10°C. After 14 days, the plates were examined with a Leitz Phaco ×32 phase-contrast objective. However, there was no evidence of bacterial growth on the plates incubated anaerobically. Anaerobes or facultative anaerobes must be present in the lake as oxygen was absent from the lake hypolimnion (Table 1). Cells from microcolonies on plates incubated aerobically were isolated by micromanipulation, as described by Skerman (13). All strains isolated on OLWA and cystine-Organic Lake water agar plates were later transferred to AOLA.

Cellular morphology and staining reactions. Cells were grown in microcolonies on OLWA and were photographed as previously described (4). Cells grown on AOLA for 7 days at 10°C were tested for their response to Gram stain (12). Cell size was determined from cell photographs and cells stained by the Gram method. After incubation in AOL medium at 10°C for 7 days, evidence of motility was sought in a hanging drop of the broth. Cells grown for the motility test were stained with 1.0% uranyl acetate and examined with a JEOL JEM-1200EX transmission electron microscope.

Biochemical and physiological tests. The oxidative or fermentative utilization of glucose was tested by the method of Hugh and Leifson (7) at 25°C for 10 days. The ability to grow anaerobically was tested by inoculating strains onto AOLA supplemented with 1.0% peptone and 0.2% KNO_3 and onto AOLA supplemented with 1.0% peptone (KNO_3 omitted) and incubating these preparations in an anaerobic atmosphere (GasPak; BBL) for 10 days at 25°C. Growth characteristics were tested in thioglycolate medium supplemented with 8.0% NaCl and incubated at 10°C for 14 days.

Tests for the presence of catalase, Kovac oxidase (12), and cytochrome oxidase (Oxoid Ltd.) were performed on colonies grown on AOLA (with potassium nitrate omitted) for 10 days at 20°C. Reduction of nitrate to nitrite, phenylalanine deaminase, lysine decarboxylase, production of H_2S from cysteine, production of indole from tryptophan, the Voges-Proskauer test, urease production, esculin hydrolysis, malonate utilization, ornithine and lysine decarboxylases, and production of beta-galactosidase were tested by using the methods of Vreeland et al. (17). Gas production from nitrate was determined by observing the accumulation of gas in an inverted tube in peptone-nitrate broth (12) supplemented with 8.0% NaCl and incubated at 25°C for 14 days under a layer of sterile paraffin. Hydrolysis of starch was tested by the method of Skerman (12), with AOLA as the basal medium and incubation at 10°C for 14 days. Gelatin liquefaction was tested in AOL broth with gelatin-charcoal disks (Oxoid) and incubation at 25°C for 1 month. The methyl red test was performed in glucose-phosphate-peptone water (12) supplemented with 8.0% NaCl by the method of Skerman (12) after growth of each strain for 7 days at 20°C. Phosphatase production was tested in a basal medium (AOL-peptone agar) by using the methods of Holding and Collee (6), with incubation at 20°C for 10 days.

The pH range for growth of each strain was tested on AOL-peptone agar (without phosphate supplement), with the pH of separate batches of media adjusted to 5.0, 6.0, 7.0, 8.0, and 9.0. The test media were inoculated and incubated at 10°C for 14 days. The temperature range for growth was tested in AOL-peptone broth that was incubated for 2 months at −5, 0, 4, 10, 25, 30, and 37°C.

Susceptibility to antibiotics. Disks impregnated with antibiotics (Oxoid) were laid on AOL-peptone agar plates which had been surface inoculated with the test strain. The following disks were used: penicillin G (5 IU), streptomycin (10 μg), chloramphenicol (10 μg), tetracycline (10 μg), neomycin (10 μg), polymyxin B (300 IU), gentamicin (10 μg), and kanamycin (30 μg). Susceptibility to vibriostatic agent 0/129 (10 μg; Oxoid) was also tested by using disks. Susceptibility to $HgCl_2$ was tested by the method of Vreeland et al. (17).

Determination of DNA base composition. DNA was isolated by the method of Marmur (11), with the addition of two pronase treatments, one after the sodium lauryl sulfate treatment and the other after the ribonuclease treatment (2). The guanine-plus-cytosine (G+C) contents of the DNAs were determined from melting curves (2).

Numerical analysis. The results from tests were coded in a binary format. The information statistic (18) was used as the measure of similarity between strains, and the fusion strategy between strains or groups involved centroid sorting (18). The MACINF program, which was originally named MULTBET (10), was used to calculate this statistic and to perform polythetic agglomerative fusions between strains or groups of strains. The fusions in this hierarchical classification occur when the information gain (ΔI) resulting from the fusion is the minimum possible compared with the ΔI of each possible fusion between ungrouped strains, groups, or ungrouped strains and groups. This method of clustering has been used in bacteriology by Thomson and Skerman (15) and by Blackall et al. (2). The position of each strain in a hypothetical three-dimensional space was determined by

TABLE 2. Frequency of occurrence of attributes for groups formed with the MACINF program

Attribute	Group A+B (18)[a]	Group C (11)	*H. elongata* ATCC 33173[T]	*P. halodenitrificans* NCMB 700[T]	*V. costicola* NCMB 701[T]
Cell shape					
Rods	+[b]	+	+	−	+
Cocci	1	−	−	+	−
Filaments (10 μm)	−	9	+	−	−
Swollen cells	6	1	−	−	−
Motile cells	−	+	+	−	+
Peritrichous flagella	−	+	+	−	−
Polar flagellum	−	−	−	−	+
Gram negative	+	+	+	+	+
Anaerobic growth	−	−	−	−	+
Anaerobic growth with KNO_3	3	1	−	+	+
Surface growth in thioglycolate broth	17	1	+	+	+
Growth on CAS medium	15	8	+	+	+
Oxidative or fermentative growth on glucose[c]	O	O	O	O	F
Oxidase (cytochrome)	+	+	−	+	+
Oxidase (Kovac)	+	+	+	+	+
Catalase	+	+	+	+	+
Voges-Proskauer test	−	−	−	−	+
Methyl red test	−	−	−	−	+
Acid produced from:					
Arabinose	−	−	+	−	−
Adonitol	−	−	+	−	−
Inositol	−	−	−	−	−
Sorbitol	−	−	−	−	−
Nitrate reduced to nitrite	12	8	+	+	−
Gas produced from nitrate	2	−	−	+	−
Phenylalanine deaminase	−	−	−	−	−
H_2S produced	−	−	−	−	−
Indole	−	−	−	−	−
Ornithine decarboxylase	4	4	+	+	+
Lysine decarboxylase	−	−	+	−	−
Malonate	−	−	−	−	−
Urease	−	−	+	−	−
Esculin	−	−	+	−	−
Beta-galactosidase	−	−	+	−	−
Phosphatase	−	−	+	+	+
Gelatin hydrolysis	−	−	+	−	−
Starch hydrolysis	−	−	−	−	−
Carbon source utilization					
Glucose	−	−	+	+	+
Sucrose	−	−	+	+	+
Cellobiose	−	−	+	−	−
Lactose	−	−	+	−	−
Glycerol	−	−	+	+	+
Citrate	−	−	+	+	−
Acetate	−	−	−	−	−
Succinate	+	+	+	+	+
Lactate	+	+	+	+	+

Continued

principal coordinate analysis with the PCOA program. The programs which we used were available in the Cyber model 840 computer and compose part of the Taxon Library Programs of the Commonwealth Scientific and Industrial Research Organisation Division of Computing Research, Canberra, Australia.

RESULTS

The numerical analysis formed six groups (groups A through F) at a ΔI level of 25.9. All of the original isolates (29 strains) were in groups A through C, whereas each of the three reference type strains was in a single-strain group (group D, E, or F). Table 2 shows the frequency at which each attribute was present in each group as well as in a group composed of strains from groups A and B (group A + B).

After growth on AOLA at 10°C for 21 days, colonies of the original isolates were less than 2.0 mm in diameter and raised with entire edges. On most media, colonies were white. However, on media enriched with alanine and in some cases hydroxy-L-proline (carbon source utilization media), the isolates formed yellow colonies. All isolates were rod shaped (Fig. 1A and B), and 9 of 11 group C strains also produced flexible filaments more than 10 μm long, as well as the shorter rods, throughout their growth cycle (Fig. 1A), unlike *H. elongata*, which produces filaments in stationary phase (17). Some strains from groups A, B, and C (Table 2)

TABLE 2—*Continued*

Attribute	Group A+B (18)[a]	Group C (11)	*H. elongata* ATCC 33173^T	*P. halodenitrificans* NCMB 700^T	*V. costicola* NCMB 701^T
Gluconate	−	−	+	+	+
Leucine	−	−	−	−	−
Valine	−	−	+	+	−
Glycine	17	+	+	+	+
Alanine	+	+	+	+	+
Isoleucine	−	−	−	+	−
Arginine	+	10	+	−	−
Lysine	+	+	+	+	−
Aspartic acid	−	−	−	−	−
Glutamic acid	−	−	−	−	−
Asparagine	1	−	−	+	−
Glutamine	5	8	+	−	+
Methionine	−	−	−	−	−
Cystine	−	−	−	−	−
Cysteine	−	−	−	−	−
Histidine	−	−	−	−	−
Threonine	+	+	−	+	+
Tryptophan	1	−	−	−	−
Tyrosine	−	−	+	−	−
Serine	−	−	+	+	+
Phenylalanine	−	−	−	−	−
Proline	+	+	+	+	+
Hydroxy-L-proline	9	−	+	−	−
Susceptibility to:					
$HgCl_2$	+	+	+	+	+
Penicillin G	−	6	−	−	−
Streptomycin	−	−	−	−	−
Chloramphenicol	8	2	−	−	+
Tetracycline	−	−	−	−	−
Neomycin	−	−	−	−	−
Polymyxin B	13	10	−	+	+
Gentamicin	−	−	−	−	−
Kanamycin	−	−	−	−	−
Vibriostatic agent 0/129	6	3	−	−	−
Growth at:					
37°C	−	−	+	+	+
25°C	+	+	+	+	+
0°C	+	+	+	+	+
−5°C	13	2	−	−	−
Growth on:					
0.0% NaCl	−	−	−	−	−
0.5% NaCl	6	3	+	+	+
1.0% NaCl	15	6	+	+	+
2.5% NaCl	+	+	+	+	+
17.5% NaCl	+	+	+	+	+
20.0% NaCl	7	6	+	−	+
25.0% NaCl	−	−	+	−	−
30.0% NaCl	−	−	−	−	−

[a] The numbers in parentheses are the numbers of strains in the groups.
[b] +, All strains positive; −, all strains negative; numbers indicate the numbers of strains positive.
[c] O, Oxidative; F, fermentative.

produced large, swollen forms on OLWA (Fig. 1C). When motile, the isolates possessed peritrichous flagella and thicker appendages, which perhaps represented mixed flagellation (Fig. 2). The elongated cells moved slowly, and motility coincided with cell flexing.

All strains were moderately halophilic and grew in AOLA containing as little as 2.5% NaCl and as much as 17.5% NaCl. Although all of the isolates produced a weak acid reaction from glucose in the oxidation-fermentation test medium, growth was not stimulated by glucose or other sugars in the carbon source utilization test media. Similarly, the other sugars tested did not stimulate growth of the isolates in the carbon source utilization test media.

Only four of the strains, strains ACAM 13, ACAM 15, ACAM 26, and *P. halodenitrificans* NCMB 700^T, showed stimulated growth in an anaerobic jar in media which contained 0.2% KNO_3. All of these strains except ACAM 15 produced gas from nitrate. In our test system, *H. elongata* ATCC 33173^T did not grow anaerobically with nitrate supplied as a terminal electron acceptor, nor did it produce acid from glucose in the freshly boiled oxidation-fermentation medium when sealed under paraffin. Vreeland (16) stated that *H. elongata* possesses a mainly respiratory type of metabolism, although growth on glucose could occur under anaerobic conditions without nitrate. The following additional results showed variance with results previously re-

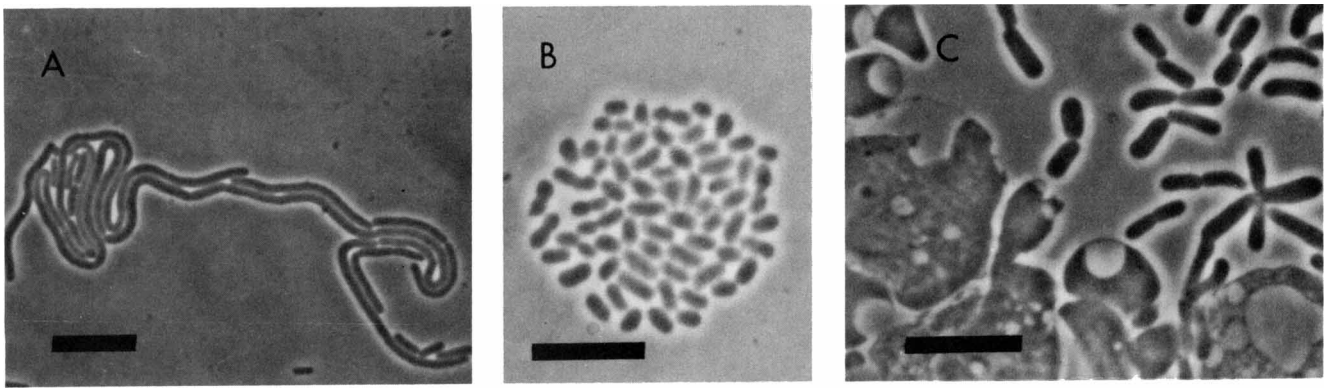

FIG. 1. *H. subglaciescola* grown on OLWA at 10°C for 24 h. Bars = 10 μm. (A) Strain ACAM 12^T. (B) Strain ACAM 21 (biovar II). (C) Swollen cells of strain ACAM 11.

ported for *H. elongata* ATCC 33173^T (17): malonate not utilized and beta-galactosidase produced.

Most strains (25 of 32) grew on CAS medium, which was the primary basal medium used for the characterization of *H. elongata* (17). Although all strains were catalase positive, the catalase reactions for the Organic Lake isolates were considerably more vigorous than the catalase reaction for *H. elongata* ATCC 33173^T.

Eleven strains were analyzed for the G+C contents of their DNAs and were easily lysed with the lysozyme and sodium dodecyl sulfate treatments used. The results of these analyses are shown in Table 3.

A diagrammatic representation of how the strains clustered in the numerical analysis is shown as a dendrogram in Fig. 3. The distribution of strains in a hypothetical space as analyzed by the PCOA program is shown in Fig. 4.

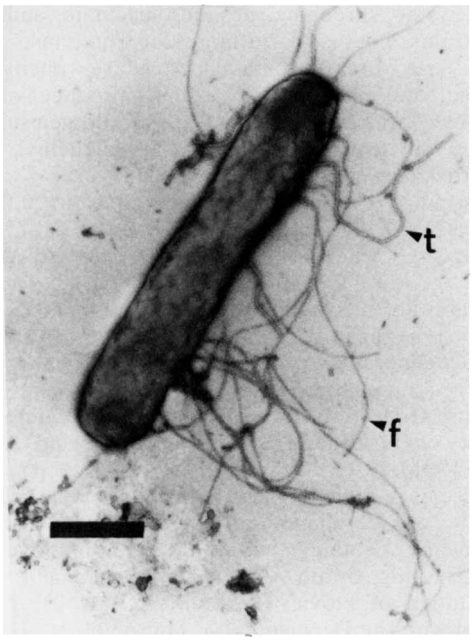

FIG. 2. Electron micrograph of an *H. subglaciescola* ACAM 12^T cell which was negatively stained after growth at 20°C for 48 h. The micrograph shows the presence of both flagella (f) and thick appendages (t). Bar = 1.0 μm.

DISCUSSION

At a ΔI of 25.9, six groups (groups A through F) were formed. A postori diagnosis of the attributes present in each group (Table 2) showed that there was no single attribute which was present for 100% of the strains in group A which was absent in 100% of strains in group B and vice versa. Groups A and B were separated polythetically at a ΔI of 33.61. The following characteristics were the most significant attributes and accounted for 55% of the total ΔI in the fusion of groups A and B: utilization of asparagine (group A, all 7 strains; group B, 1 of 11 strains), utilization of hydroxy-L-proline (group A, all 7 strains; group B, 1 of 11 strains), and growth on 0.5% NaCl (group A, 5 of 7 strains; group B, 1 of 11 strains).

As groups A and B are inseparable on monothetic grounds and large data banks are not readily available for polythetic identification of strains, it is useful to lump groups A and B into a single group, group A+B, for taxonomic purposes.

Group C was distinguished from group A+B on the basis of a number of attributes, most notably motility, flagellation, filament formation, susceptibility to penicillin G, utilization of hydroxy-L-proline, utilization of asparagine, and surface growth in thioglycolate broth. The only attributes which were wholly present in one group (group C) and wholly

TABLE 3. G+C contents of the DNAs of strains isolated from an Antarctic saline lake and of three reference strains

Strain or organism	G+C content (mol%)
ACAM 5	62.6 ± 0.6
ACAM 10	61.7 ± 0.3
ACAM 11	62.9 ± 0.7
ACAM 12^T	61.7 ± 0.2
ACAM 15	62.9 ± 0.7
ACAM 18	61.1 ± 0.6
ACAM 21	61.0 ± 0.7
ACAM 22	60.9 ± 1.0
ACAM 25	61.1 ± 0.1
ACAM 28	62.8 ± 0.7
ACAM 30	61.5 ± 0.1
H. elongata ATCC 33173^T	60.5 ± 0.5[a]
V. costicola ATCC 33508^T	50.0[b]
Paracoccus denitrificans	64 to 66[c]

[a] Data from reference 17.
[b] Data from reference 1.
[c] Data from reference 8.

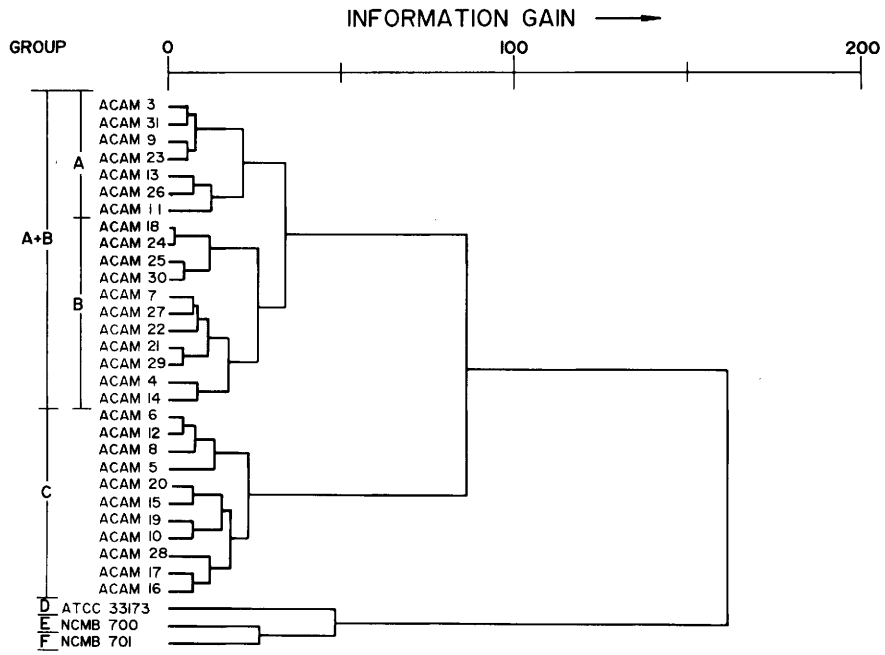

FIG. 3. Diagrammatic representation of the fusions formed with the MACINF program within a set of isolates of *H. subglaciescola* and three reference strains of halotolerant bacteria. This program uses ΔI resulting from each fusion as the similarity index.

absent from the other (group A+B), were motility and peritrichous flagellation. Thus, the groups are probably best separated at the biovar level.

The strains from Organic Lake did not cluster with any of the reference strains included in this study. In studies of the microflora of previously unexamined environments, reference strains were rarely recovered within phenetic clusters (3, 5, 8).

Although the isolates appeared to be taxonomically distant from the type strain of *H. elongata* on the basis of the tests used (Fig. 4), these organisms, particularly the group C strains, shared the following attributes classically regarded as of importance when assigning strains to taxa at the generic level: G+C content of the DNA (Table 3), morphology, halotolerance, largely oxidative mode of metabolism, motility, and flagellation. We believe that the Organic Lake isolates should be assigned to the genus *Halomonas*. Nucleic acid hybridization studies would be a useful next step to examine the genetic relatedness between these isolates and *H. elongata*.

The Organic Lake isolates could be distinguished from the only species in the genus *Halomonas*, *H. elongata*, by (i) their inability to utilize a number of carbon sources (glucose, sucrose, cellobiose, lactose, glycerol, citrate, valine, tyrosine, serine, and malonate), (ii) their inability to grow at 37°C, and (iii) their production of cytochrome oxidase. Therefore, we propose a new species of *Halomonas*, *Halomonas subglaciescola*.

***Halomonas subglaciescola* sp. nov.** *Halomonas subglaciescola* (sub. gla' ci. es. co. la. L. prefix *sub* below; L. n. *glacies* ice; L. suffix *cola* to dwell; *subglaciescola* a species which dwells below ice) cells are rod shaped and occur as single cells, in pairs, or in short chains. Some strains produce long and flexuous cells up to 50 μm long. Cell widths range from 0.5 to 1.1 μm. Strains have peritrichous flagellation when motile. Cells are gram negative. Colonies are white to cream on solid media, although yellow colonies can form on media enriched with alanine and, in some cases, hydroxy-L-proline.

Members of the species are aerobic, although some strains can utilize nitrate as a terminal electron acceptor. Nitrate is reduced to nitrite by most strains, and a few strains produce gas from nitrate. The species is catalase positive, Kovac oxidase positive and cytochrome oxidase positive. Sugars do not stimulate growth on complex media, although a number of amino acids and organic acids do so, including alanine, arginine, lysine, threonine, proline, succinate, and lactate. Some strains possess ornithine decarboxylase. Lysine decarboxylase, urease, beta-galactosidase, phenylalanine deaminase, and phosphatase are not produced. Gelatin, esculin, and starch are not hydrolyzed. Hydrogen sulfide and indole are not produced. The species is methyl red and Voges-Proskauer negative.

Most strains grow on media containing 0.5 to 20.0% NaCl but not on 25.0% NaCl at 10°C. Growth occurs at 25 and 0°C but not at 37°C. Many strains grow at −5°C. Growth occurs from pH 5 to 9.

All strains are susceptible to $HgCl_2$, and most are susceptible to polymyxin B. All strains are resistant to streptomycin, tetracycline, neomycin, gentamicin, and kanamycin.

The type strain, strain ACAM 12, has been deposited with the Culture Collection of the Department of Microbiology, University of Queensland, St. Lucia, Queensland, Australia, as strain UQM 2926.

The species has two biovars. Biovar I contains cells which are motile, can be filamentous, and are more than 10 μm long. Biovar II contains cells which are nonmotile and less than 10 μm long. Strain ACAM 12^T is a biovar I strain. A representative of biovar II, strain ACAM 21, has been deposited with the Department of Microbiology, University of Queensland, as strain UQM 2927. Strain ACAM 25 has also been deposited with the Department of Microbiology, University of Queensland, as strain UQM 2925. As some attributes are variable within the species, the results for

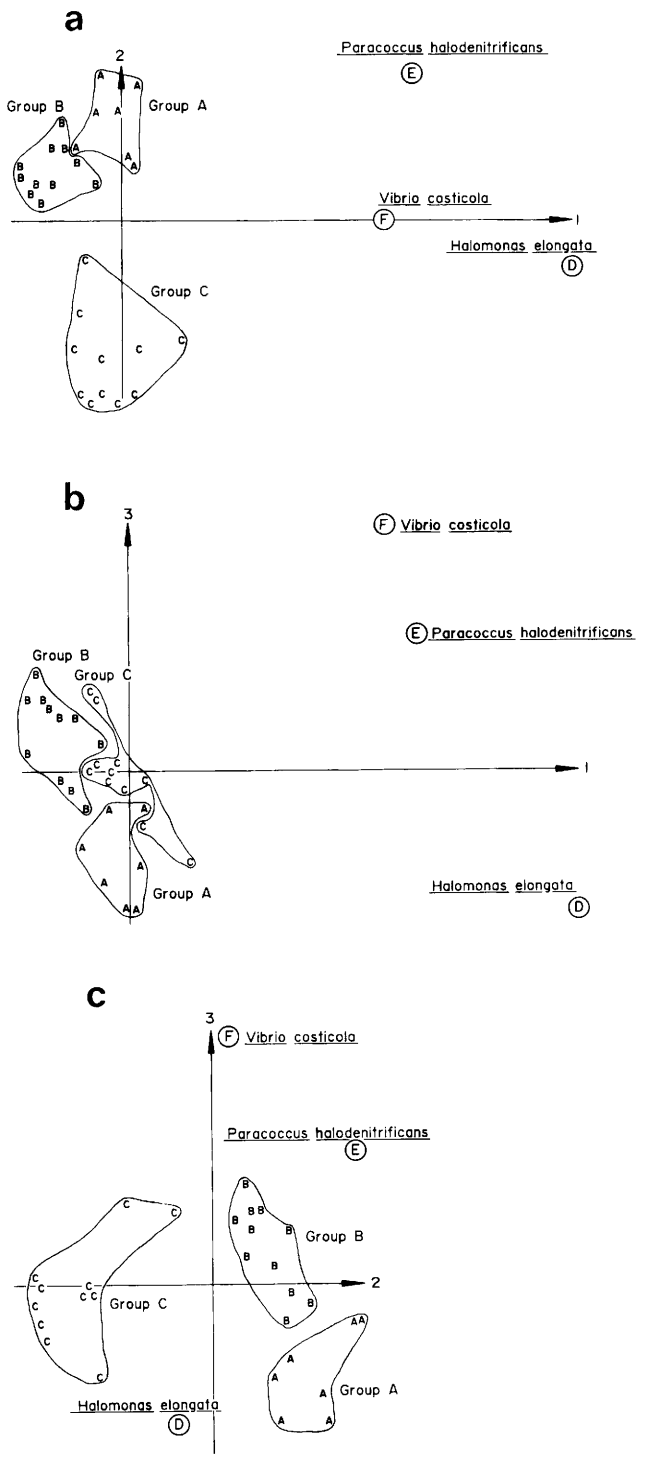

FIG. 4. Distribution of strains from three phena (groups A, B, and C) of *H. subglaciescola* and three reference strains of halotolerant bacteria determined by principal coordinate analysis with the PCOA program. The three panels represent the strains in a hypothetical three-dimensional space. (a) Plane 1, 2. (b) Plane 1, 3. (c) Plane 2, 3.

these tests, as determined for type strain ACAM 12 and biovar II reference strain ACAM 21, are shown in Table 4.

The G+C contents of the DNAs range from 60.9% ± 1.0 to 62.9% ± 0.7 mol%.

TABLE 4. Characteristics of *H. subglaciescola* type strain ACAM 12 and biovar II reference strain ACAM 21 for attributes which are variable for strains from each of the biovars

Attribute	Strain ACAM 12^T	Strain ACAM 21
Cell dimensions (μm)	0.5-1.1 by 1.1-56.5	0.5 by 0.5-3.9
Swollen cells formed	−	−
Motile cells	+	−
Peritrichous flagella	+	−
Anaerobic growth with NO_3	−	−
Surface growth in thioglycolate broth	−	+
Growth on CAS medium	+	+
Nitrate reduced to nitrite	+	−
Gas produced from nitrate	−	−
Ornithine decarboxylase	−	−
Carbon source utilization		
Glycine	+	+
Arginine	+	+
Asparagine	−	−
Glutamine	+	+
Tryptophan	−	−
Hydroxy-L-proline	−	−
Susceptibility to:		
Penicillin G	+	−
Chloramphenicol	−	−
Polymyxin B	+	+
Vibriostatic agent 0/129	−	−
Growth at −5°C	−	+
Growth on 20% NaCl	−	+
Growth on 0.5% NaCl	−	+
Growth on 1.0% NaCl	−	+
G+C content (mol%)	61.7 ± 0.2	61.0 ± 0.7

Ecology. Unlike the type species of *Halomonas*, *H. elongata*, which was isolated from a solar salt facility, *H. subglaciescola* was isolated from an Antarctic saline lake. Vreeland (16) speculated that members of this genus inhabit intertidal areas and saline lakes. Although the strains were aerobic, many were isolated from the hypoliminion of the lake, from which oxygen was absent (Table 1). Their presence in the hypolimnion was presumably due to sedimentation of the cells. As far as we know, there was only one eucaryote in the lake which was predacious on bacteria or phytoplankton, a choanoflagellate, *Acanthoecopsis unguiculata*. The lake receives an input of penguin molt feathers each summer when it is not ice covered, and this may account for the preference of these strains for amino acids as sources of carbon and energy, although surprisingly, cysteine and cystine were not utilized.

The salinity of the surface waters of the lake in January 1985 was 8.31‰ or 0.8% after relatively fresh water (present as ice up to 1.2 m thick throughout most of the year) appeared. The free water below the ice plug in October had a salinity of 158.0‰ or 15.8%. Organisms which inhabit such an environment would be expected to have tolerance to a wide range of salt concentrations.

Temperatures in the lake reached as high as 9.5°C in summer and fell to as low as −14.0°C in winter.

ACKNOWLEDGMENTS

We thank C. Nunn for assistance with medium preparation and the Department of Microbiology, University of Queensland, for the use of their Gilford model 2600 temperature-controlled spectrophotometer and model 2527 thermoprogrammer.

The Australian Collection of Antarctic Microorganisms is funded by the Australian Research Grants Scheme, the Antarctic Division,

and the University of Tasmania. This project was funded in part by the Australian Research Grants Scheme.

LITERATURE CITED

1. **Baumann, P., A. L. Furniss, and J. V. Lee.** 1984. Genus 1. *Vibrio*, p. 518–538. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
2. **Blackall, L. L., A. C. Hayard, and L. I. Sly.** 1985. Cellulolytic and dextranolytic Gram-negative bacteria: revival of the genus *Cellvibrio*. J. Appl. Bacteriol. **59**:81–97.
3. **Colwell, R. R.** 1973. The use of numerical taxonomy in estuarine microbiology, p. 91–114. *In* L. H. Stevenson and R. R. Colwell (ed.), Estuarine microbial ecology. University of South Carolina Press, Columbia.
4. **Franzmann, P. D., and V. B. D. Skerman.** 1981. *Agitococcus lubricus* gen. nov., sp. nov., a lipolytic, twitching coccus from freshwater. Int. J. Syst. Bacteriol. **31**:177–183.
5. **Hauxhurst, J. D., M. I. Krichevesky, and R. M. Atlas.** 1980. Numerical taxonomy of bacteria from the Gulf of Alaska. J. Gen. Microbiol. **120**:131–148.
6. **Holding, A. J., and J. G. Collee.** 1971. Routine biochemical tests, p. 1–33. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 6A. Academic Press, Inc., New York.
7. **Hugh, R., and E. Leifson.** 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram-negative bacteria. J. Bacteriol. **66**:24–26.
8. **Kaneko, T., M. I. Krichevesky, and R. M. Atlas.** 1979. Numerical taxonomy of bacteria from the Beaufort Sea. J. Gen. Microbiol. **110**:111–125.
9. **Kocur, M.** 1984. Genus *Paracoccus*, p. 399–402. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
10. **Lance, G. N., and W. T. Williams.** 1967. Mixed data classificatory programs. I. Agglomerative systems. Aust. Comp. J. **1**:15–20.
11. **Marmur, J.** 1961. A procedure for the isolation of DNA from microorganisms. J. Mol. Biol. **3**:208–218.
12. **Skerman, V. B. D.** 1967. A guide to the identification of the genera of bacteria, 2nd ed. The Williams & Wilkins Co., Baltimore.
13. **Skerman, V. B. D.** 1968. A new type of micromanipulator. J. Gen. Microbiol. **54**:287–298.
14. **Staley, J. T.** 1981. The genera *Prosthecomicrobium* and *Ancalomicrobium*, p. 456–460, *In* M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes. Springer-Verlag, Berlin.
15. **Thompson, J. P., and V. B. D. Skerman.** 1979. *Azotobacteriaceae*: the taxonomy and ecology of the aerobic, nitrogen-fixing bacteria. Academic Press, Inc., New York.
16. **Vreeland, R. H.** 1984. Genus *Halomonas*, p. 340–343. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
17. **Vreeland, R. H., C. D. Litchfield, E. L. Martin, and E. Elliot.** 1980. *Halomonas elongata*, a new genus and species of extremely salt-tolerant bacteria. Int. J. Syst. Bacteriol. **30**:485–495.
18. **Williams, W. T., and J. M. Lambert.** 1966. Multivariate methods in plant ecology. V. Similarity analyses and information-analysis. J. Ecol. **54**:427–445.