

***Halomonas meridiana*, a New Species of Extremely Halotolerant Bacteria Isolated from Antarctic Saline Lakes**

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Summary

Halomonas meridiana sp. nov., is proposed for seven strains of halotolerant, non-pigmented bacteria isolated from several hypersaline lakes of the Vestfold Hills, Antarctica. These strains, plus 17 new isolates of the Antarctic species *Halomonas subglaciescola* and six reference strains of halotolerant bacteria, were tested for 134 physical and biochemical attributes. The data were analysed by numerical taxonomic procedures. The new isolates clustered most closely with the reference strains *Halomonas elongata* (ATCC 33173^T) and *Halomonas halmophila* (NCMB 1971^T) and furthest from *Halomonas subglaciescola* (UQM 2926^T and UQM 2927), but were sufficiently distinct to be considered as a new species. The strains of *Halomonas meridiana* separated into two phenons but representatives of both groups had DNA with 59 ± 1 mol% G+C.

Key words: *Halomonas meridiana* – Halotolerant – Haloversatile – Antarctica – Saline lakes

Introduction

During 1987–88, a limnological survey was undertaken on several of the saline lakes of the Vestfold Hills, Antarctica (Table 1). Part of this investigation examined the distribution of *Halomonas subglaciescola*, a species first isolated from Organic Lake in 1984–85 (Franzmann et al., 1987a). Of the bacterial strains isolated, 26 randomly selected, non-pigmented strains were investigated in detail (Table 2). A parallel taxonomic investigation on pigmented strains from Organic Lake is reported elsewhere (Dobson, S. J., Bsc. (Hons.) thesis, University of Tasmania, 1989).

The saline lakes of the Vestfold Hills are relics of seawater catchments isolated by isostatic uplift in the last 6000 years (Burton, 1981). Since the isolation of these catchments, factors such as evaporation, freezing concentration, cooling and marine and meltstream influx have changed the ionic ratios of the lakes. As the water temperature of concentrated brines drops below -10°C , salts pre-

cipitate selectively depleting the lake of various ions (Thompson and Nelson, 1956). Although the lakes originated from the same marine source, their current ionic concentrations may vary greatly (Burton, 1981).

Nutrients of Antarctic saline lakes are derived largely from meltstreams, although bird and seal excreta may provide organic matter (Burton, 1981). All of the lake samples supported microalgal populations, most commonly *Dunaliella* sp. and *Chaetoceros* sp., which would also be effective nutrient sources. Terrestrial algae from the lake shores, or feathers blown onto the lake, provide organic matter in some cases. Physico-chemical conditions in Antarctic saline lakes can vary markedly with season. The summer thaw lowers lake salinity through meltstream influx and ice cover melt; microalgae bloom and wind mixing changes nutrient status. Bacteria able to colonise this harsh Antarctic environment are of interest, particularly their origin and relationships to halotolerant bacteria from other geographical locations. Their origin may be marine, as studies of the lakes of the Vestfold Hills area have yielded a variety of bacterial taxa, many of which have marine relatives (Franzmann et al., 1988). Halotolerant bacteria are also known to be active in sea ice micro-

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Table 1. Hypersaline lakes sampled during 1987–1988

	Latitude			Longitude			Species present:	
	"	'	"(S)	°	'	"(E)	<i>H. meridiana</i>	<i>H. subglaciescola</i>
Ace Lake	68	28	18	78	11	03	No	No
Burch Lake	68	27	20	78	15	41	Yes	Yes
Cemetery Lake	68	37	36	77	57	55	No	No
Ekho Lake	68	31	12	78	15	57	No	No
Fletcher Lake	68	27	10	78	15	09	No	No
"Horse" Lake	68	28	18	78	09	14	Yes	No
Johnstone Lake	68	29	40	78	24	10	No	No
Laternula Lake	68	39	03	77	58	06	Yes	Yes
"Lake Island" Lake	68	32	44	77	59	24	Yes	No
Organic Lake	68	27	24	78	11	11	Yes	Yes
Rookery Lake	68	29	51	78	04	02	No	No
unnamed Lake	68	38	29	77	54	28	No	No

"" = unofficial name

Table 2. Site data and physical parameters for test strains

Strain Designation	Collection date	Site	Depth (m)	Sal. (ppt)	Temp. (°C)
ACAM 230, 234, 256	Apr–May 1987	Organic Lake	1	70.3	-8 ^a
ACAM 233	Apr–May 1987	Organic Lake	2	152	-6 ^a
ACAM 223, 247, 251	Apr–May 1987	Organic Lake	3	181	-3 ^a
ACAM 221, 250	Apr–May 1987	Organic Lake	4	187	-2 ^a
ACAM 225, 229, 254	Apr–May 1987	Organic Lake	5	208	-3 ^a
ACAM 224, 227, 231, 255, 259	Apr–May 1987	Organic Lake	6	225	-3 ^a
ACAM 252	Apr–May 1987	Organic Lake	7	ND	ND
ACAM 253	Nov 1987	"Horse" Lake	1	44	-2.5
ACAM 239	Nov 1987	"Horse" Lake	6	158	2
ACAM 242, 246	Nov 1987	Burch Lake	6	184	-4
ACAM 235	Nov 1987	"Lake Island" Lake	1	147	-8
ACAM 222, 236	Nov 1987	Laternula Lake	0	150 ^b	ND

^a = Aug 1987; ^b = Nov 1987 (Unpubl. data, J. Gibson); ND = No Data

bial communities in McMurdo Sound, Antarctica (Kottmeier and Sullivan, 1988), and such organisms could be pre-adapted for life in cold, hypersaline lakes.

Materials and Methods

Isolation procedure. Bacteria were isolated and routinely maintained on Organic Lake Water Yeast Agar (OLWYA) which contained 0.2 µm filtered water from Organic Lake, collected from a depth of 2 m, plus yeast extract (0.1 g/l) and agar (15 g/l).

Strains were isolated with a Skerman micromanipulator (Skerman, 1968). A single drop of water from each sample was allowed to run across the surface of an agar plate which was then incubated at 10 °C in the dark. After 10 days, the plates were examined under a Leitz Laborlux 12 microscope with a × 32 long working distance objective lens. Single cells were manipulated from microcolonies to different localities on the agar surface. After incubation, isolated colonies developed (2–4 weeks) from the isolated single cell. Colonies were transferred to fresh culture media.

Media. All media were sterilized for 15 min at 121 °C unless otherwise stated. The basal media for all tests was 3% NaCl

Artificial Organic Lake Peptone Agar/Broth (3% AOLPA and 3% AOLPB). This medium was based on AOLPA (Franzmann et al., 1987b) and consisted of NaCl, 30 g; MgSO₄ · 7H₂O, 9.5 g; KCl, 5.0 g; CaCl₂ · 2H₂O, 0.2 g; (NH₄)₂SO₄, 0.1 g; KNO₃, 0.1 g; peptone, 5.0 g; yeast extract, 1.0 g made up to 960 ml with distilled water. The pH was adjusted to 7.3 with 0.1 M KOH and the medium sterilized. The medium was cooled to 60 °C then 20 ml of sterile Hutner Modified Salts Solution (HMSS) (Staley, 1981), 20 ml sterile Phosphate Supplement (PS) and 1 ml of 0.2 µm filter-sterilized vitamin solution (Vit.) was added aseptically. For solid media, 15 g of agar was added prior to sterilization. All strains were incubated at 25 °C unless otherwise stated.

The isolates and the halotolerant reference strains *Halomonas elongata* (ATCC 33173^T), *Halomonas balmophila* (NCMB 1971^T), *Halomonas subglaciescola* (UQM 2926^T, 2927), *Deleya aesta* (NCMB 1980^T) and *Deleya halophila* (CCM 3662^T) were maintained on 3% AOLPA, incubated for 10 days, then stored at 4 °C. All strains were subcultured at three-month intervals.

Results for the type strain of *Halomonas halodurans* ATCC 29686^T were not included as the culture received gave 2 colony types. These showed 25% dissimilarity across the range of tests and neither strain was consistent with the results reported by Hebert and Vreeland (1987).

For each carbon source utilization test, 1.0 g of the carbon source was added to 100 ml of distilled water then sterilized by

filtration ($0.2\text{ }\mu\text{m}$). These solutions were added to sterile modified 3% AOLPA (without peptone but with 0.1 g of yeast extract and made up to 860 ml). Carbon sources not sufficiently soluble in distilled water to sterilize by filtration were autoclaved at 121°C for 15 min before addition.

A modified Oxidative/Fermentative (O/F) medium was based on that of *Smibert* and *Krieg* (1981); 600 ml 3% AOLPA (with 1 g of peptone, 0.1 g of yeast extract and 3 g of agar), 0.5 g of $(\text{NH}_4)_2\text{SO}_4$, 0.5 g of tris buffer, 0.25 g of bromocresol purple, distilled water to volume (960 ml). Five g of glucose was added for O/F media with glucose. All O/F media was adjusted to pH 7.5.

Cellular morphology and staining reactions. Cells grown in microcolonies on 3% AOLPA were photographed as described by *Franzmann* and *Skerman* (1981). Cell size was determined from photographs. Cells in early logarithmic phase grown in 8% AOLPA (with 8% NaCl) were examined for number and position of flagella with a JEOL JEM-1200EX transmission electron microscope after adhesion to 1 mg/ml poly-L-lysine hydrochloride soaked FORMVAR coated 75 mesh copper disks and staining with 1.0% uranyl acetate for 5 sec.

Broth cultures in early logarithmic phase were examined for motility by the hanging drop method (*Skerman*, 1967). Similar cultures were examined by the Gram method (*Skerman*, 1967). Colony characteristics were examined after 5 days growth on 3% AOLPA.

Biochemical and physiological tests. All tests were incubated at 25°C for 10 days unless otherwise stated. The tests for catalase, Kovac's oxidase (*Smibert* and *Krieg*, 1981) and cytochrome oxidase (Oxidase BR64, Identification sticks, Oxoid) were done on 3% AOLPA (without KNO_3). Nitrate reduction was tested in 3% AOLPB (with 10 g/l of peptone and 2 g/l of KNO_3). Gas production was observed in inverted Durham tubes. Nitrate reduction

was tested after 4 weeks by the method of *Smibert* and *Krieg* (1981). The bathochromic shift of flexirubin pigments was tested to indicate presence of the pigment (*Reichenbach* et al., 1981) in colonies on 3% AOLPA. Anaerobic growth was determined after 4 weeks on 3% AOLPA (with 10 g/l peptone and 2 g/l KNO_3 and with KNO_3 excluded) in airtight jars under anaerobic conditions (BBL gas pack).

Phenylalanine deaminase, ornithine decarboxylase, lysine decarboxylase malonate utilization, urease, aesculin hydrolysis and β -galactosidase activity were tested using Micro ID units (Organan Teknika Pty. Ltd.) (*Vreeland* et al., 1980) incubated for 24 hours.

Agar hydrolysis was indicated by pitting or liquefaction of the agar surface of 3% AOLPA plates after 4 weeks growth. Amylase activity was tested on 3% AOLPA supplemented with 5% starch by the method of *Skerman* (1967). Casein hydrolysis was indicated by clear zone on 3% AOLPA supplemented with 10% skim milk. Chitin hydrolysis was indicated by a clear zone on media prepared as described by *Skerman* (1967) using 3% AOLPA as the basal medium. DNase activity was confirmed, for colonies grown on DNA agar (Oxoid) supplemented with 3% AOLPA salts, by a clear zone after flooding with 1 N HCl (*Jeffries* et al., 1957). Gelatin hydrolysis was tested with Oxoid "Chargels" after 4 weeks incubation. Phosphatase production was tested on 3% AOLPA with 10 ml/l of a filter sterilized solution of sodium phenolphthalein diphosphate (1%) by exposure to 25% ammonia solution (*Smibert* and *Krieg*, 1981). Hydrolysis of Tween 20 and Tween 80 was tested on 3% AOLPA supplemented with either 10 ml/l of Tween 20 or Tween 80, incubated for 4 weeks (*Skerman*, 1967). For each carbon source test, the strains were examined after 4 weeks for growth stimulation. The O/F media was examined for colour change after 4 weeks. Acid production and alkaline reaction from carbohydrates was

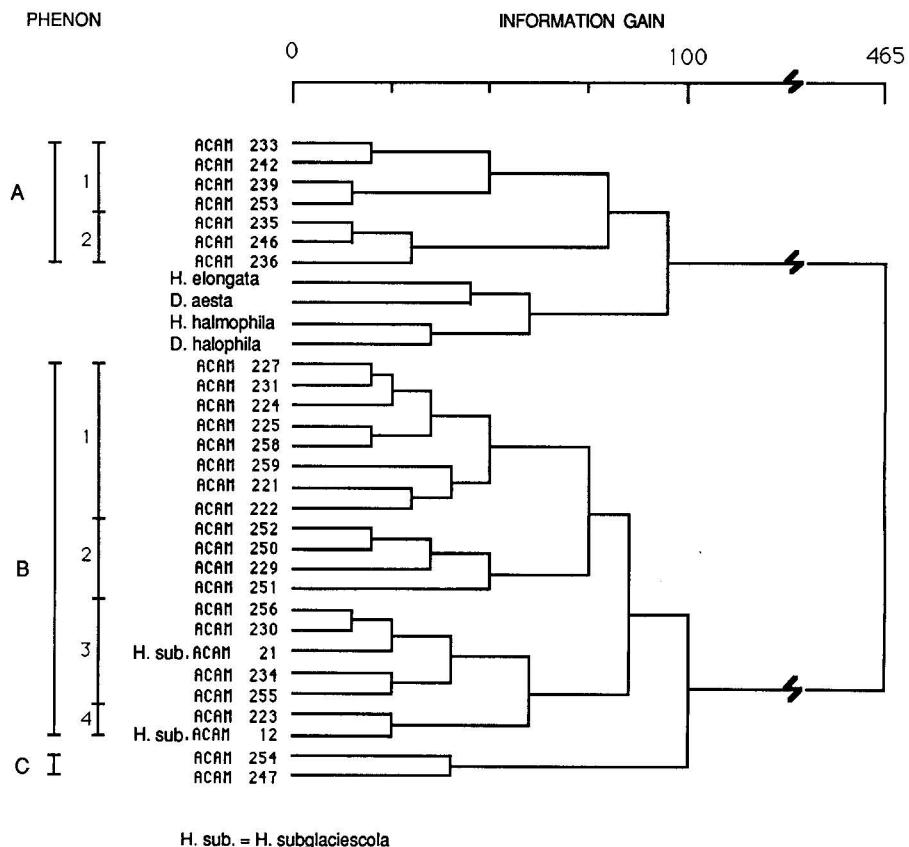


Fig. 1. Diagrammatic representation of the fusions formed with the MACINF program which uses information gain (ΔI) as the polythetic measure of phenotypic similarity between strains or clusters, and a centroid sorting fusion strategy.

tested in 3% AOLPB with the addition of 2 drops of 0.12% (w/v) aqueous solution of bromothymol blue after growth.

Strains inoculated into 3% AOLPB in screw-capped 30 ml bottles were examined for growth after 8 weeks incubation at -5° or 37°C. Strains inoculated onto 3% AOLPA with pH adjusted with 1N HCl and 1N NaOH to 4, 5, 6, 8 or 9 were examined for growth after 8 weeks. Strains inoculated into AOLPB with a final NaCl concentration of 0%, 5%, 10%, 15% or 20% (w/v) were examined for growth after 8 weeks incubation. Plates of 3% AOLPA were surface inoculated and incubated for 4 weeks with 3 different antibiotic discs (Oxoid) on each plate. Mercuric chloride discs were prepared by soaking filter paper discs in 1:5000 mercuric chloride and sterilized at 115°C 15 min. A uniform inhibition zone of at least 2 mm from the edge of the disc was required for sensitivity to be recorded.

Numerical analysis. The results of the tests were coded into binary format. Two types of analysis were conducted. The first, MACINF, used information gain ($\Delta 1$) as the measure of similarity and cluster generation was by centroid sorting. Principal coordinate analysis vectors were obtained using the PCOA program. The second, MJAC, used the Jaccard coefficient as the similarity coefficient with complete linkage (or furthest neighbor cluster generation). All programs are available in the TAXON suite of programs of the Commonwealth Scientific and Industrial Research Organisation, Division of Computing Research, Canberra, Australia.

Salt and temperature tolerance. Strains inoculated into AOLPB with a final NaCl concentration from 0% (residual 0.01%) to 24% were incubated with gentle shaking at 37°C for 7 days. Optical density was measured during incubation with a Corning Nephelometer. The slope of optical density against time (hours) was plotted against salinity for each strain and the NaCl concentration range for optimal growth was determined. The maximum NaCl concentration for growth was estimated by extrapolation. Strains inoculated into 3% AOLPB with a final NaCl concentration of 3% were incubated with gentle shaking at a range of temperatures from 13° to 44°C for 7 days. Optical density was measured during incubation using a Corning Nephelometer. The NaCl concentration range for optimal growth was determined by the method of McMeekin et al. (1988).

DNA base composition. Bacteria were grown in 3% AOLPA in 1 l batches, DNA was extracted by a modification of the method of Marmur (1961), with the addition of a pronase treatment after the sodium dodecyl sulphate step (Blackall et al., 1985).

The mol % G+C of *Halomonas meridiana* was determined by the method of Marmur (1961). Thermal denaturation was observed as a change in absorbance at 260 nm using a Pye Unicam SP8-200 series UV/Vis Spectrophotometer and a Pye Unicam SPY 876 series 2 Temperature Program Controller as described by Sly et al. (1986) using DNA extracted from *E. coli* (UQM 1803) with mol % G+C 51.7 as a control. Replicate mol % G+C for each of the unknowns were determined and the mean and standard deviation calculated.

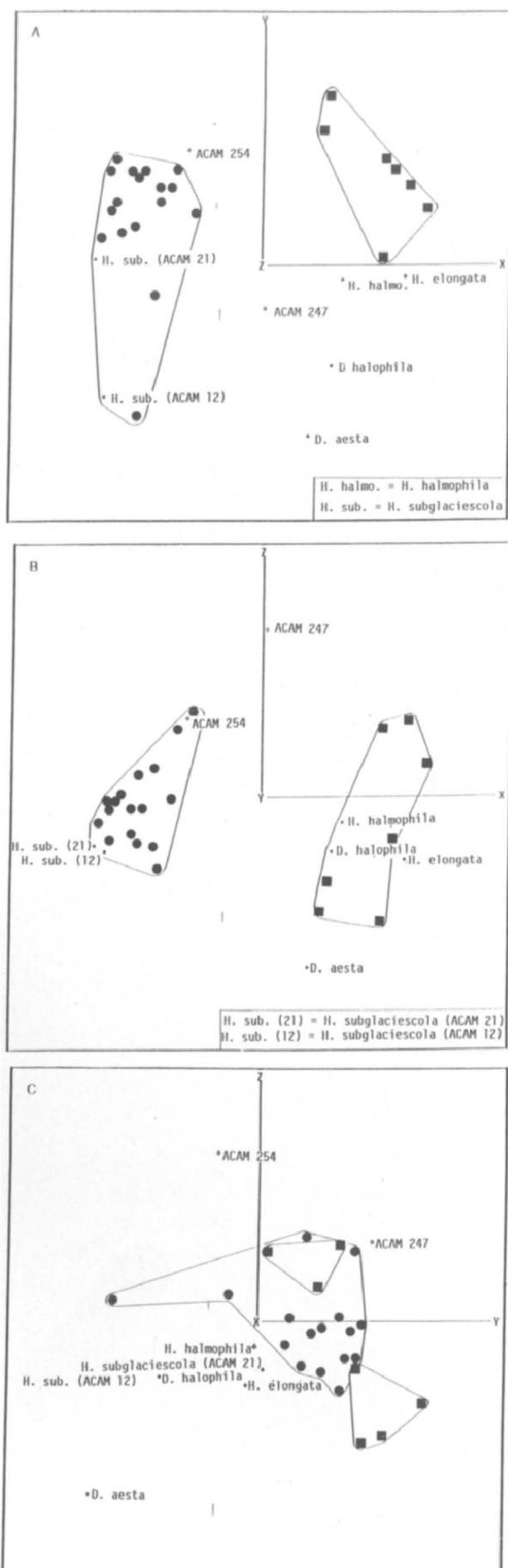


Fig. 2. Distribution of phena A (■) and B (●) defined by the cluster analysis, reference strains, and the strains ACAM 254 and ACAM 247 in a three-dimensional hypothetical space determined by principal coordinate analysis of phenotypic attributes by the program PCOA.

Results

Nine clusters were produced at the information gain (ΔI) of 50 (Fig. 1). Four of the reference strains, *Halomonas elongata/Deleya aesta* and *Halomonas halophila/Deleya halophila* clustered together. *Halomonas subglaciescola* [strain UQM 2926^T (ACAM 12^T)] clustered with phenon B4 and *Halomonas subglaciescola* [strain UQM 2927^T (ACAM 21^T)] with phenon B3.

MJAC, which excludes negative matches, produced a very similar dendrogram. At a S_J of 50%, the groupings were very similar; the only differences were that ACAM 221 and ACAM 222 clustered in phenon B3 instead of B2; ACAM 234 grouped with phenon B1 instead of B3; phenon A1 clustered with *Halomonas halophila*, and A2 with *Halomonas elongata*; *Deleya aesta* and *Deleya halophila* clustered together.

Phenon A consisted of two groups that were associated with the reference strains *Halomonas halophila*, *Halomonas elongata*, *Deleya aesta* and *Deleya halophila* (Figs. 1 and 2). The strains of this phenon were motile by peritrichous flagella and were distinguished from strains of other phenons by their 1–2 lateral flagella (Fig. 3) and

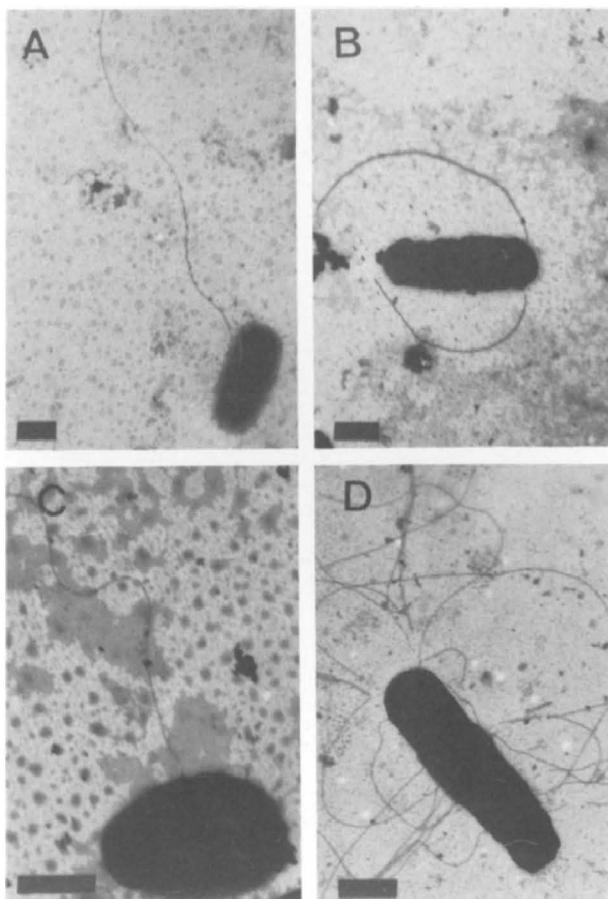


Fig. 3. Electron micrographs of *Halomonas meridiana* (A) and (B) biovar I, UQM 3352 (ACAM 233), (C) biovar II, UQM 3352^T (ACAM 246^T), showing lateral flagella and *Halomonas subglaciescola* (D) ACAM 257, showing peritrichous flagella. Bar = 500 nm.

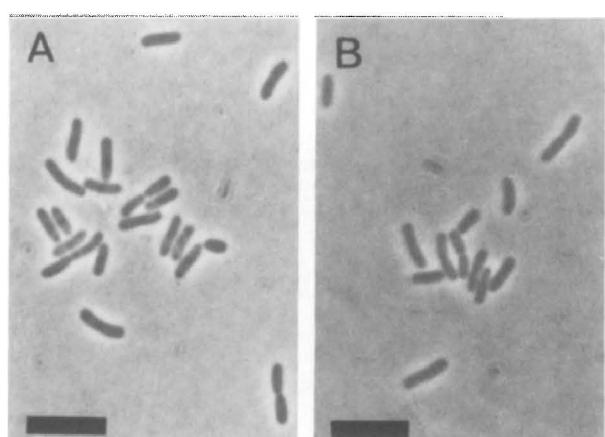


Fig. 4. *Halomonas meridiana* grown on 3% AOLPA for 24 hours at 25°C (A) biovar I, UQM 3353 (ACAM 233). (B) biovar II, UQM 3352^T (ACAM 246^T). Bar = 10 µm.

their ability to utilize a wide range of compounds as sole carbon and energy sources. Both UQM 3353 (ACAM 233) and UQM 3352^T (ACAM 246^T) have mol % G+C values of 59 ± 1 . Fig. 4 shows the cellular morphology of UQM 3353 (ACAM 233) and UQM 3352^T (ACAM 246^T).

Phenon B contained 17 strains, including the reference strains of *Halomonas subglaciescola* [UQM 2926^T (ACAM 12^T) and UQM 2927 (ACAM 21)]. These bacteria, when motile, were peritrichously flagellated and utilized comparatively few compounds as sole carbon and energy sources.

Phenon C has only two representatives (ACAM 254, ACAM 247). Both strains fell between phenons A and B on the principal coordinate analysis (PCOA) (Fig. 2). ACAM 254 appears more closely related to phenon B and ACAM 247 to phenon A. Both these strains were non-motile.

Table 3 contains the characteristics for phenons A1 and A2 (which represent biovars of the new species *Halomonas meridiana* discussed below). Detailed characteristics of the reference strains, the new isolates of *Halomonas subglaciescola* (Phenon B) and Phenon C (two isolates) are also presented in Table 3.

The type strain ACAM 246^T (UQM 3352^T) and reference strain ACAM 233 (UQM 3353) were examined for salt tolerance at 37°C and temperature tolerance in 3% NaCl. ACAM 246^T (biovar I) showed optimal growth at 1% to 3% NaCl and 28° to 40°C. The maximum salt tolerance was 20–25% NaCl and the maximum growth temperature 45°C. ACAM 233 (biovar II) demonstrated optimal growth at 0.5–3% NaCl and at 34–38°C. The maximum salt tolerance was 25–30% NaCl and maximum growth temperature 47°C.

Discussion

The physical and biochemical features of phenon A indicated that these strains are members of the family

Table 3. Characteristics of original isolates (phena A, B, C) and reference strains *Halomonas elongata* (ATCC 33173^T), *H. halmophila* (NCMB 1971^T), *Deleya aesta* (NCMB 1980^T), *D. halophila* (CCM 3662^T) and *H. subglaciescola* (UQM 2926^T, UQM 2927)

Table 3. continued

	Phenon				Reference strains				Phenon					
	A1 [4] ^a	A2 [3]	ATCC 33173	NCBM 1971	NCMB 1980	CCM 3662	UQM 2926	UQM 2927	B1 [8]	B2 [5]	B3 [4]	B4 [1]	C [2]	
<i>Organic compounds as sole carbon and energy sources:</i>														
Adonitol	2	+	+	—	+	—	—	—	—	—	—	1	—	—
Arginine	+	+	+	+	—	—	—	—	—	6	+	+	—	1
Cellbiose	1	+	—	—	—	+	—	—	—	1	—	+	—	—
Cysteine	—	+	+	+	—	—	—	—	—	1	—	—	—	1
D-Arabinose	1	+	—	—	—	—	—	—	—	—	—	—	—	1
D-Galactose	—	+	+	+	+	+	—	—	—	1	1	—	+	1
D-Gluconate	+	+	+	—	+	+	—	—	—	—	—	—	—	+
D-Glucose	+	+	+	+	+	—	—	—	—	6	+	—	—	—
D-Xylose	2	+	+	+	+	+	—	—	—	—	—	—	—	+
DL-Tryptophan	2	+	+	+	—	—	—	—	—	—	—	—	—	1
Fructose	+	2	+	+	—	—	—	—	—	3	3	1	+	—
Fumarate	+	+	+	+	+	+	—	—	+	+	+	—	+	+
Glycerol	—	+	+	+	—	—	—	—	—	—	2	—	+	1
Glycine	1	+	—	—	—	—	—	—	—	—	—	—	—	1
Histidine	—	—	+	+	—	—	—	—	—	—	—	—	—	—
Hydroxy-L-proline	—	+	+	—	—	—	—	—	—	3	1	—	+	—
Inositol	3	+	+	—	—	—	—	—	—	2	1	—	—	+
Isoleucine	+	+	+	+	+	+	—	—	—	—	—	1	—	+
L-Arabinose	2	+	+	+	—	—	—	—	—	—	—	—	—	1
L-Asparagine	+	+	+	+	—	—	—	—	—	—	+	+	—	—
L-Rhamnose	—	+	+	—	—	—	—	—	—	2	1	—	—	1
Lactose	1	2	+	—	—	—	—	—	—	—	—	—	—	+
Leucine	+	+	+	+	+	+	—	—	—	—	—	—	—	+
Lysine	+	1	+	+	+	+	+	+	+	6	1	+	+	+
Maltose	+	+	+	+	+	+	+	—	—	—	—	—	+	1
Mannitol	+	+	+	+	+	+	—	—	—	—	—	—	+	1
Mannose	+	+	+	+	+	+	—	—	—	—	—	—	—	—
Methionine	2	2	—	—	—	—	—	—	—	—	1	—	—	1
Ornithine	2	+	+	+	+	+	+	+	—	6	3	3	—	+
Proline	+	+	+	+	+	+	—	—	—	5	+	—	+	+
Propionate	+	+	+	+	—	—	—	—	—	4	—	—	—	+
Raffinose	2	2	—	—	—	—	—	—	—	—	—	—	—	1
Salicin	1	+	+	—	—	—	—	—	—	—	1	—	—	1
Serine	+	2	+	+	+	+	+	—	—	—	2	1	—	+
Succinate	+	+	+	—	—	—	—	—	—	—	+	+	+	+
Sucrose	+	+	+	+	—	—	—	—	—	—	—	—	—	+
Threonine	+	2	—	—	—	—	—	—	—	4	+	3	—	+
Trehalose	2	+	—	—	—	—	—	—	—	—	1	—	—	1
Tyrosine	—	+	+	—	—	—	—	—	—	—	—	—	—	—
Valine	+	+	+	—	—	—	—	—	—	—	—	—	—	—
β-hydroxybutyrate	+	+	+	+	—	—	—	—	—	—	—	—	—	1
<i>Susceptibility to:</i>														
Ampicillin (10 µg)*	—	—	—	—	+	—	+	+	+	+	+	2	+	+
Bactritacin (10 i.u.)	—	—	—	—	+	—	+	—	—	4	2	1	+	—
Cephalothin (30 µg)	1	—	—	+	+	—	—	+	+	+	2	+	+	+
Chloramphen. (30 µg)	+	2	+	+	+	+	+	+	+	+	3	+	+	+
Erythromycin (15 µg)	1	—	—	—	—	—	—	—	—	5	—	+	+	1
Gentamicin (10 µg)	2	+	+	+	+	+	+	+	+	+	+	+	+	+
HgCl ₂ (1:5000)	—	—	—	+	—	—	—	—	+	3	2	1	—	+
Nalidixic acid (30 µg)	1	—	—	+	+	—	—	+	—	—	—	1	—	—
Neomycin (30 µg)	2	2	+	+	+	+	+	+	+	+	+	3	+	+
Novobiocin (20 µg)	—	—	—	—	—	—	—	—	—	6	2	+	+	1
O/129 (10 µg)	—	—	—	—	—	—	—	—	—	5	—	3	—	1
Penicillin G (10 i.u.)	—	—	—	—	—	—	—	—	—	6	2	+	—	+
Streptomycin (10 µg)	—	—	+	—	—	—	—	—	—	—	+	—	—	+
Tetracycline (30 µg)	—	—	—	—	—	—	—	—	—	—	1	—	+	—

* Disc concentration

All positive: Gram-negative, cytochrome oxidase, circular and smooth colonies, growth at pH and 10% NaCl, acid from maltose, growth on alanine and pyruvate and susceptibility to polymyxin B.

All negative: Bathochromatic shift, anaerobic growth without KNO₃, nitrogen from nitrate, agar hydrolysis, chitin hydrolysis, gelatin liquefaction, malonate utilization, phenylalanine deaminase, width ≤ 0.4 µm, growth at pH 4, alkaline reaction from fructose, galactose, glucose, mannitol, sucrose and maltose, yellow or orange colour and spreading colonies.^a The numbers in parentheses are the number of strains in groups.^b +, all strains positive; —, all strains negative; numbers indicate numbers of strains positive; space indicates no data.

Halomonadaceae (Franzmann et al., 1988). The genera of this family, *Deleya* and *Halomonas*, are difficult to separate on the basis of phenotypic tests (Dobson, S. J., Bsc. (Hons.) thesis, University of Tasmania, 1989) and chemotaxonomic data (Franzmann and Tindall, in press).

The strains in phenon A had a mol % G+C range of 58–60, that is, within the range quoted for members of the genus *Deleya* (53–68) and at the lower end of the range for previously described *Halomonas* spp. (60–64). Visualization by principal coordinate analysis (Fig. 2) shows a greater similarity of phenon A to the type strains of *Halomonas elongata* and *H. halmophila* than to *Deleya aesta* or *D. halophila*. On this basis, and because of the difficulty in separating *Deleya* and *Halomonas* by phenotypic or chemotaxonomic means, the strains of phenon A were attributed to the genus *Halomonas*, which has nomenclatural priority, as *Halomonas meridiana*.

Description of *Halomonas meridiana* sp. nov. me.r.i.di.a'na L. fem., adj. *meridiana*, of the south.

Cells are rod-shaped, occurring singly, in pairs or as short chains, $0.6 \times 1.9 \mu\text{m}$ to $1.0 \times 4.5 \mu\text{m}$, and produce 1–2 lateral flagella. Cells are Gram-negative, catalase, cytochrome oxidase and Kovac's oxidase positive. Colonies are smooth, circular and white to off-white. Yellow colonies may be produced on media containing alanine or pyruvate. Aerobic, do not reduce nitrate to nitrite. Other characteristics are given in Table 3. The mol % G+C content of the DNA ranges from 58.8 ± 0.6 to 59.1 ± 0.8 .

Table 4. Differential characteristics for *Halomonas meridiana* (UQM 3352^T, UQM 3353), *H. elongata* (ATCC 33173^T), *H. halmophila* (NCMB 1971^T) and *H. subglaciiscola* (UQM 2626^T, UQM 2927)

	Strains			
	UQM 3352-3	ATCC 33173	NCBM 1971	UQM 2926-7
Kovac's oxidase	+	+	-	+
Nitrate to nitrite	-	+	-	V
Anaerobic growth with KNO	-	+	-	-
DNAase	-	+	+	-
Esculin utilization	-	+	-	-
Lysine decarboxylase	-	+	-	-
Phosphatase	-	+	-	-
β -galactosidase	-	+	-	-
Growth at 0 °C	+	-	-	-
Colony Flat	-	+	-	-
Colony Convex	+	-	+	+
<i>Organic compounds as sole carbon and energy sources:</i>				
Histidine	-	+	+	-
Succinate	+	+	-	+
Valine	+	+	-	-
<i>Susceptibility to:</i>				
HgCl ₂ (1:5000)	-	-	+	V
Streptomycin (10 µg)	-	+	-	ND
Tetracycline (30 µg)	-	-	-	+

V = Variable, ND = No Data

The species has 2 biovars. The type strain, ACAM 246^T (UQM 3352^T) is from biovar II and has been deposited with the Culture Collection of the Department of Microbiology, University of Queensland, together with a representative of biovar I, ACAM 233 (UQM 3353).

Table 5. Characteristics for representative strains of biovar I (ACAM 233) and biovar II (ACAM 246^T). Those characters shown are variable within *Halomonas meridiana*

	ACAM 233	ACAM 246
Ornithine decarboxylase	+	+
Alkaline from Lactose	-	+
<i>Cell length:</i>		
$L \leq 2 \mu\text{m}$	-	-
$2 < L \leq 4 \mu\text{m}$	+	+
$4 < L \leq 6 \mu\text{m}$	-	-
$L > 6 \mu\text{m}$	-	-
<i>Cell width:</i>		
$0.8 < W \leq 1.2 \mu\text{m}$	+	+
$W > 1.2 \mu\text{m}$	-	-
<i>Growth at:</i>		
pH 5	+	-
-5 °C	-	+
37 °C	+	+
0.01% NaCl	+	+
<i>Acid from:</i>		
Glucose	+	+
L-Arabinose	-	+
Mannitol	-	+
<i>Colony morphology:</i>		
Width < 1 mm (7 days)	-	+
Width > 1 mm (7 days)	+	-
Entire	+	+
Undulate	-	-
<i>Organic compounds as sole carbon and energy sources:</i>		
Adonitol	+	+
Cellobiose	-	+
D-Arabinose	+	+
D-Xylose	+	+
DL-Tryptophan	+	+
Fructose	+	+
Glycine	-	+
Inositol	-	+
L-Arabinose	+	+
Lactose	-	+
Lysine	+	+
Ornithine	+	+
Salicin	-	+
Serine	+	+
Threonine	+	+
Trehalose	+	+
<i>Susceptibility to:</i>		
Cephalothin (30 µg)	-	-
Chloramphenicol (30 µg)	+	+
Erythromycin (15 µg)	-	-
Gentamicin (10 µg)	-	+
Nalidixic acid (30 µg)	-	-

Characteristics to distinguish *Halomonas meridiana* from other *Halomonas* spp. are presented in Table 4 and differences between the type strain ACAM 246^T (biovar II) and reference strain ACAM 233 (biovar I) are listed in Table 5.

Most marine bacteria may be accommodated in the slight halophile category (Kushner, 1978) and less than 5% of bacteria isolated from oceanic waters are moderate or non halophiles (Ventosa et al., 1984). According to the definition of Kushner (1978), *Halomonas meridiana* sp. nov. is a slight halophile, belonging to that group of bacteria with optimal growth in media with NaCl concentrations between 1 and 3%. This definition does not convey the wide range of salt concentrations tolerated by *H. meridiana*. The terms halotolerant and extremely halotolerant have been used to describe halophilic bacteria that have a wide range of salt tolerances (Vreeland et al., 1980; Franzmann et al., 1987a; Hebert and Vreeland, 1987). Whilst both the terms halotolerant and slightly halophilic may be used to describe the novel species *Halomonas meridiana*, a new term, HALOVERSATILE, conveys more clearly the salinity growth relationships of this organism.

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