The distribution of *Methylosphaera hansonii* outside of these lakes is unknown; however, the Vestfold Hills lakes are of recent origin (8000–10000 years old), formed from a marine uplift (Franzmann, 1996) that followed the retreat of the ice sheet after the last ice age. This leads to the hypothesis that *Methylosphaera hansonii* may also be common in Antarctic coastal sediments.

Enrichment and Isolation Procedures

Low temperature marine sediments and hypolimnetic zones of polar marine-salinity lakes (and possibly coastal marine zones) are the best sites for isolation of *Methylosphaera* strains. Enrichment is performed in mineral salts media prepared in serum vials with either natural or artificial seawater. A headspace of 1:1 methane/air is created and the vials incubated at 2–4°C. After 3–4 weeks, a white turbidity forms that is accompanied by white sedimented material. *Methylosphaera hansonii* enrichments contain a high population of nonmethanotrophic, usually methylotrophic, co-contaminants. Known strains of *Methylosphaera* cannot be grown on solid agar and, consequently, the isolation procedure described for most methanotrophs (see enrichment and isolation details for the genus *Methylococcus*) is not possible. Isolation can be achieved by serially diluting enrichments to extinction in 96-well plastic microtiter trays (Bowman et al., 1997c) containing NMS seawater liquid media and incubated in containers containing 1:1 methane/air. Several strains may be purified in the same tray simultaneously. After sufficient incubation, the wells containing the highest dilutions that show growth are examined by microscopy. A number of separate transfers may be required to eventually obtain morphologically homogenous cultures.

Maintenance Procedures

Long term preservation of *Methylosphaera hansonii* is currently problematic. The best procedure is to keep strains in serum vials with NMS seawater media under a methane/air atmosphere held at about 2°C, periodically subculturing them. Viability is rapidly lost following freezing, including snap freezing in liquid nitrogen.

Differentiation of the genus *Methylosphaera* from other genera

Phenotypic, genotypic, and chemotaxonomic traits that differentiate *Methylosphaera* from other type I methanotrophs are shown in Tables BXII.γ.67 and BXII.γ.68 of the chapter describing the family *Methylococcaceae*.

Taxonomic Comments

16S rDNA based phylogenetic analysis indicates that *Methylosphaera hansonii* forms a separate branch at the periphery of the cluster of type I methanotrophs that includes the genera *Methylobacter*, *Methylomicrobium*, and *Methylomonas*. The closest relatives are methanotrophic mytilid endosymbionts (evolutionary distance 0.09–0.10).

DNA–DNA hybridization analysis indicates that strains from different lake isolation sites are closely related, showing 65–100% hybridization with ACAM 549.

List of species of the genus Methylosphaera

1. **Methylosphaera hansonii** Bowman, McCammon and Skerratt 1998d, 327[VP] (Effective publication: Bowman, McCammon and Skerratt 1997c, 1457.)
han.son' i.i. M.L. gen. n. *hansonii* of Hanson, named after American microbiologist R.S. Hanson.

Cells are coccoidal and nonmotile. Colonies do not form on solid agar media. Liquid media cultures possess no pigment. Characteristics are as described for the genus and as listed in Tables BXII.γ.67 and BXII.γ.68 of the chapter describing the family *Methylococcaceae*.

Optimum temperature for growth, ~10–15°C; range, −2–20°C. pH range for growth, 6.0–8.5; optimum, ~7.5.

Known habitat includes certain Antarctic marine-salinity meromictic lakes.

The mol% G + C of the DNA is: 43.5–45.9 (T_m).

Type strain: AM6, ACAM 549.

GenBank accession number (16S rRNA): U67929.

Order VIII. **Oceanospirillales** *ord. nov.*

George M. Garrity, Julia A. Bell and Timothy Lilburn

O.ce.an.o.spi.ril.la' les. M.L. neut. n. *Oceanospirillum* type genus of the order; *-ales* ending to denote order; M.L. fem. n. *Oceanospirillales* the *Oceanospirillum* order.

The order *Oceanospirillales* was circumscribed for this volume on the basis of phylogenetic analysis of 16S rDNA sequences; the order contains the families *Oceanospirillaceae*, *Alcanivoraceae*, *Hahellaceae*, *Halomonadaceae*, *Oleiphilaceae*, and *"Saccharospirillaceae"*.

Most genera halotolerant or halophilic. Motile except for *Alcanivorax*. Aerobic, microaerophilic, or facultatively anaerobic chemoorganotrophs.

Type genus: **Oceanospirillum** Hylemon, Wells, Krieg and Jannasch 1973, 361[AL].

Family I. **Oceanospirillaceae** *fam. nov.*

GEORGE M. GARRITY, JULIA A. BELL AND TIMOTHY LILBURN

O.ce.an.o.spi.ril.la' ce.ae. M.L. neut. n. *Oceanospirillum* type genus of the family; *-aceae* ending to denote family; M.L. fem. pl. n. *Oceanospirillaceae* the *Oceanospirillum* family.

The family *Oceanospirillaceae* was circumscribed for this volume on the basis of phylogenetic analysis of 16S rDNA sequences; the family contains the genera *Oceanospirillum* (type genus), *Balneatrix*, *Marinomonas*, *Marinospirillum*, *Neptunomonas*, *Oceanobacter*, *Oleispira*, and *Pseudospirillum*. *Oceanobacter*, *Pseudospirillum* and *Oleispira* were proposed after the cut-off date for inclusion in this volume (June 30, 2001) and are not described here (see Satomi et al. (2002) and Yakimov et al. (2003a), respectively).

Motile by polar flagella. Aerobic; strictly respiratory except for *Neptunomonas*, which gives weak fermentation reactions. Aquatic; *Balneatrix* is found in fresh water, whereas other genera are marine.

Type genus: **Oceanospirillum** Hylemon, Wells, Krieg and Jannasch 1973, 361[AL].

Genus I. **Oceanospirillum** *Hylemon, Wells, Krieg and Jannasch 1973, 361[AL]**

BRUNO POT AND MONIQUE GILLIS

O.ce.an.o.spi.ril' lum. M.L. n. *oceanus* ocean; Gr. n. *spira* a spiral; M.L. dim. neut. n. *spirillum* spirillum a small spiral; *Oceanospirillum* a small spiral (organism) from the ocean (seawater).

Rigid, helical cells 0.4–1.2 μm in diameter. Motile by bipolar tufts of flagella. A polar membrane underlies the cytoplasmic membrane at the cell poles in all species so far examined by electron microscopy. **Intracellular poly-β-hydroxybutyrate is formed. All species form thin-walled coccoid bodies that predominate in old cultures.** Gram negative. **Aerobic**, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. Nitrate respiration does not occur. Nitrate can be reduced to nitrite in all oceanospirilla. Optimum temperature for growth, 25–32°C. **Oxidase positive.** Indole and aryl sulfatase negative. Casein, starch, hippurate, and esculin are not hydrolyzed. **Seawater is required for growth. Carbohydrates are neither oxidized nor fermented.** Amino acids or the salts of organic acids serve as carbon sources. Growth factors are not usually required. Isolated from coastal seawater, decaying seaweed, and putrid infusions of marine mussels.

The mol% G + C of the DNA is: 45–50.

Type species: **Oceanospirillum linum** (Williams and Rittenberg 1957) Hylemon, Wells, Krieg and Jannasch 1973, 374 (*Spirillum linum* Williams and Rittenberg 1957, 82.)

FURTHER DESCRIPTIVE INFORMATION

All species of *Oceanospirillum* consist of helical cells; however, variants having less curvature may arise after prolonged transfer. For example, the type strain of *O. japonicum* consisted initially of long, helical cells with several turns (Watanabe, 1959), but now consists of slightly curved or S-shaped cells. The cells have a constant and characteristic type of clockwise (right-handed) helix. Only one phylogenetically unrelated species (*O. pusillum*) has a counterclockwise (left-handed) helix (Terasaki, 1972). Photographs showing the size and shape of various species of oceanospirilla and *O. minutulum* (now *Marinospirillum minutulum*) are presented in Fig. BXII.γ.104.

An unusual elaboration of the plasma membrane, the "polar membrane", occurs in all of the species so far examined (Beveridge and Murray, unpublished results). It is attached to the inside of the plasma membrane by bar-like links and is located, most commonly, in the region surrounding the polar flagella (Murray and Birch-Andersen, 1963). Such a membrane has been found mainly in genera of helical bacteria, such as *Spirillum*, *Campylobacter*, *Aquaspirillum*, *Ectothiorhodospira*, and *Rhodospirillum*.

All species have intracellular poly-β-hydroxybutyrate, but granules may not be evident in cells having a small diameter and chemical analysis may be required to demonstrate the polymer.

All species have bipolar tufts of flagella and all species show extensive formation of coccoid bodies (sometimes termed "microcysts") in old cultures. These bodies have thin walls and resemble spheroplasts; however, they are resistant to lysis in distilled water (Kelly, 1959). Whether coccoid bodies are resistant to desiccation is not known. Three main modes of formation of coccoid bodies were described by Williams and Rittenberg (1957), as follows: (a) two cells may entwine and apparently fuse. The cells become shorter and thicker and a protuberance develops at the point of fusion. This gradually enlarges and absorbs the organisms to form the coccoid body. More than one coccoid body may develop from a pair of entwined spirilla; (b) a spirillum may become shorter and thicker and a protuberance arises from the center of the cell or from each end of the cell. The protuberances enlarge and eventually merge into a single coccoid body as the helical cell is absorbed; (c) a spirillum may undergo a gradual shortening and rounding to form a coccoid body. The majority of coccoid bodies present in old cultures appears to be viable and can "germinate" when placed into a fresh medium (Williams and Rittenberg, 1956). Germination is by unipolar or bipolar growth of a helical cell from the coccoid body, with the latter being absorbed into the developing helical cell.

Seawater is required for the growth of all species. Media prepared with natural seawater or with 2.75% NaCl have been used for enrichment and isolation (Williams and Rittenberg, 1957; Terasaki, 1963, 1970, 1980). Commonly used culture media for

**Editorial Note:* The genus *Oceanospirillum* has recently undergone taxonomic reevaluation. *O. minutulum* has been transferred to the genus *Marinospirillum* as *Marinospirillum minutulum* (Satomi et al., 1998); see the Taxonomic Comments section in this chapter. *O. commune* and *O. vagum* are homotypic synonyms of *Marinomonas communis* and *Marinomonas vaga*, respectively. After the cut-off date for inclusion of taxonomic changes in this volume of the *Systematics*, Satomi et al. (2002) emended the description of *Oceanospirillum* and proposed the transfer of *O. jannaschii* to the genus *Marinobacterium* as *Marinobacterium jannaschii*, the transfer of *O. japonicum* to the new genus *Pseudospirillum* as *Pseudospirillum japonicum*, the transfer of *O. kriegii* to the new genus *Oceanobacter* as *Oceanobacter kriegii*, and the transfer of *O. pusillum* to the new genus *Terasakiella* as *Terasakiella pusilla*.

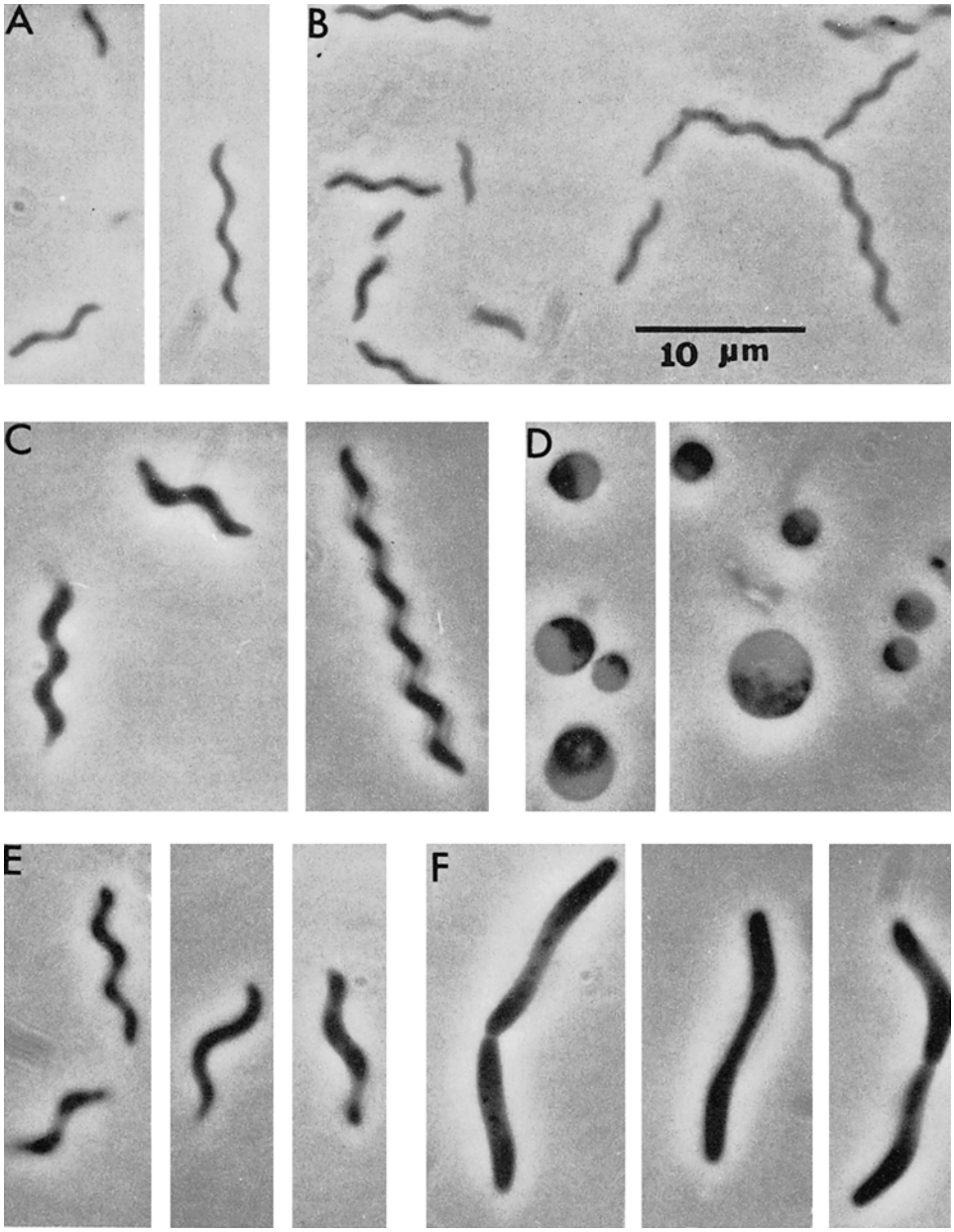

FIGURE BXII.γ.104. Phase contrast photomicrographs of several species of the genus *Oceanospirillum*. All photomicrographs were taken at the same magnification. *A*, *Marinospirillum minutulum* ATCC 19193. *B*, *O. linum* ATCC 11336. *C*, *O. maris* ATCC 27509. *D*, coccoid bodies of *O. maris* formed after 7 d of incubation. *E*, *O. beijerinckii* subsp. *beijerinckii* ATCC 12754. *F*, *O. japonicum* ATCC 19191. Reproduced with permission from N.R. Krieg, Bacteriological Reviews, *40*: 55–115, 1976, ©American Society for Microbiology.)

oceanospirilla are nutrient broth prepared with natural seawater and PSS, or MPSS broth[1] prepared with artificial seawater[2].

Oceanospirilla generally produce moderate to abundant, turbid growth in 2–3 d in PSS seawater broth (Hylemon et al., 1973). In seawater-nutrient broth, membranous masses are often formed at the surface and can be dispersed with shaking to yield turbid cultures (Terasaki, 1972).

Colonies of oceanospirilla generally develop within 2–3 d on PSS seawater agar and are usually white, circular, and convex, ranging from pinpoint to 1.5 mm in diameter (Hylemon et al., 1973). Colonies on seawater-nutrient agar are generally pinpoint in size at 48 h but become larger (up to 2.0 mm in diameter) at 7 d; they are usually convex or umbonate, glistening, opaque, pale yellow, and butyrous (Terasaki, 1972). Rough (R) colonies may arise on prolonged transfer; for example, the colonies of the type strain of *O. japonicum* are presently of the R type. Some species produce a water-soluble, yellow-green fluorescent pigment on PSS seawater agar.

Most species grow best at a temperature of 30–32°C; however, *O. maris* subsp. *hiroshimense* grows best at 25°C (Terasaki, 1972).

1. See the genus *Aquaspirillum* for recipes for these media.

2. Artificial sea water for use in PSS broth, g/l of distilled water: NaCl, 27.5; $MgCl_2$, 50; $MgSO_4$, 2.0; $CaCl_2$, 0.5; KCl, 1.0; and $FeSO_4$, 0.01.

The nutrition of oceanospirilla is generally simple. Most species grow in simple defined media with amino acids or the salts of organic acids as carbon sources and ammonium ions as the nitrogen source. However, *O. linum* is specifically stimulated by methionine in a medium containing succinate and malate as carbon sources, and *O. maris* subsp. *williamsae* has a growth factor requirement that has not yet been identified. A listing of the carbon sources for oceanospirilla is given below in Table BXII.γ.77. Some apparent contradictions occur between the results obtained from different laboratories, although the results within each laboratory are reproducible. These differences are likely attributable to differences in definitions of what constitutes a positive growth response, and in some cases to the use of different strains.

The use of antisera in agglutination tests with a limited number of strains has indicated that the species of *Oceanospirillum* can be distinguished serologically (McElroy and Krieg, 1972). The antisera were prepared against whole cells and adsorbed with heated cells, leaving only antibodies against thermolabile antigens.

Oceanospirilla have been isolated from coastal seawater (Williams and Rittenberg, 1957), decaying seaweed (Jannasch, 1963), and putrid infusions of marine mussels (Terasaki, 1963, 1970, 1980). By direct microscopic counts of the bacteria present in clear and turbid seawaters near Port Aransas, Texas, Oppenheimer and Jannasch (1962) found that spirilla comprised only 0.1–2.5% of the total bacteria present. Whether oceanospirilla occur in the open sea is not known. Based on chemostat experiments, Jannasch (1963) suggested that the growth of oceanospirilla might be restricted to environments of higher nutrient concentration than is found in ordinary seawater, such as in zones surrounding decaying particulate matter. With regard to occurrence of oceanospirilla in putrid infusions of marine mussels, the source is most likely marine mud adherent to the mussels (Terasaki, 1970).

Enrichment and Isolation Procedures

The enrichment and isolation method used by Williams and Rittenberg (1957) is as follows. A seawater sample is mixed with an equal volume of Giesberger's base medium (NH_4Cl, 0.1%; K_2HPO_4, 0.05%; $MgSO_4$, 0.05%) plus 1.0% calcium lactate. After incubation and appearance of spirilla, a portion of the initial culture is sterilized and mixed with an equal volume of sterile Giesberger's medium lacking NH_4Cl. This mixture is then inoculated from the unsterilized portion of the initial culture. One to three subcultures done in this manner are sufficient to establish the spirilla as the predominant type. For isolation, the enrichment is diluted 1:100 to 1:100,000 with sterile seawater. The dilution bottles are shaken vigorously and allowed to stand at room temperature for 20 min to allow migration of spirilla to the surface of the dilution. Isolation is then accomplished by streaking the surface water onto a suitable agar medium such as nutrient agar prepared with seawater and containing 0.3% yeast autolysate. Plates are incubated at 30°C and after 24 h examined for distinctive, granular, umbonate or pulvinate colonies with a ground-glass appearance.

The method of Terasaki (1970) has yielded excellent results for the isolation of oceanospirilla from putrid infusions. Marine mussels are smashed with a hammer and placed in a Petri dish with a teaspoon of marine mud. Sterilized seawater is poured into the dish until the mussels sink completely in the solution. The infusion is incubated at 27–28°C and examined for the development of spirilla after 1, 2, 4, and 7 d. Isolation is accomplished by streaking dilutions onto suitable agar media.

For enrichment by use of continuous cultures, see Jannasch (1967).

Maintenance Procedures

Oceanospirilla may be maintained in semisolid PSS seawater medium (containing 0.15% agar to give a jelly-like consistency) at 30°C with weekly transfer (Hylemon et al., 1973). Cultures may also be maintained as stabs in seawater-nutrient agar at room temperature with monthly transfer (Terasaki, 1972).

Preservation is most easily accomplished by suspending a dense concentration of cells in seawater-nutrient broth containing 10% (v/v) dimethylsulfoxide, with subsequent freezing in liquid nitrogen. A method for freeze-drying oceanospirilla has been reported by Terasaki (1975).

Procedures for Testing Special Characters

Characterization methods for oceanospirilla have been described in detail by Terasaki (1972, 1979) and Hylemon et al. (1973). The comments given in this *Manual* for the genus *Aquaspirillum* also apply to the genus *Oceanospirillum*, except that media containing natural or artificial seawater must be used for all characterization tests.

Differentiation of the genus *Oceanospirillum* from other genera

See the genus *Aquaspirillum*, in Volume 2 Part C in this *Manual*, for characteristics of *Oceanospirillum* that distinguish the genus from other morphologically or physiologically similar genera.

Taxonomic Comments

In the eighth edition of *Bergey's Manual of Determinative Bacteriology* (Krieg, 1974), a single genus, *Spirillum*, contained all of the various aerobic and microaerophilic spirilla, including freshwater and marine species. However, the DNA base composition for the genus ranged from 38 to 65 mol% G + C and appeared to be unusually broad for a bacterial genus. Moreover, three groups were evident within the genus: (a) the aerobic, freshwater spirilla that could not tolerate 3% NaCl (mol% G + C 50–65); (b) the aerobic, marine spirilla that required seawater for growth (mol% G + C = 42–48); and (c) the large, microaerophilic spirilla that belong to the species *S. volutans* (mol% G + C = 38). Accordingly, Hylemon et al. (1973) divided the genus into the three genera *Spirillum*, *Aquaspirillum*, and *Oceanospirillum*, with the marine organisms comprising the latter genus. This subdivision was used in the first edition of *Bergey's Manual of Systematic Bacteriology* (Krieg, 1984a). Although this scheme proved useful for practical purposes, it was only gradually that the phylogenetic aspects of the three subdivisions were revealed.

In an analysis of the 16S rRNA oligonucleotide catalogs of the species *O. minutulum* (now *Marinospirillum minutulum*) and *Oceanospirillum maris*, Woese et al. (1982) found that both organisms belonged to group III of the phototrophic bacteria as defined by Gibson et al. (1979), but they were not closely related to each other. Later, Woese et al. (1985) studied three additional species of *Oceanospirillum*—*O. japonicum*, *O. linum*, and *O. beijerinckii*. These species, together with the families *Enterobacteriaceae* and *Vibrionaceae*, constituted the core of 'subgroup 3' of the *Gammaproteobacteria* (Stackebrandt et al., 1988).

An organism known as *"Spirillum lunatum"* (Williams and Rit-

TABLE BXII.γ.77. Carbon sources used by *Oceanospirillum* species and *Marinospirillum minutulum*

Characteristic	*O. linum*		*O. beijerinckii* subsp. *beijerinckii*		*O. beijerinckii* subsp. *pelagicum*	*O. maris* subsp. *maris*	*O. maris* subsp. *hiroshimense*	*O. multiglobuliferum*	*O. jannaschii*	*O. japonicum*		*O. kriegii*	*O. pusillum*	*M. minutulum*	
Method[a,b]	A[c]	B[d]	A	B	B[d]	A[e]	B	B	A[f]	A	B	A[f]	B	A	B
Carbon source:															
Citrate	−	−	−	−	d	−	−	+	+	−	−	+	+	−	−
Aconitate	−	nd	−	nd	nd	−	nd	nd	−	−	nd	−	nd	−	nd
Isocitrate	−	nd	−	nd	nd	−	nd	nd	nd	−	nd	nd	nd	−	nd
α-Ketoglutarate	−	nd	−	nd	nd	−	nd	nd	d	−	nd	+	nd	d	nd
Succinate	−	−	−	+	+	−	+	+	+	−	+	+	+	+	+
Fumarate	−	−	−	+	+	−	+	+	+	−	+	+	+	+	+
Malate	−	−	+	−	+	d	+	+	+	+	+	+	+	+	+
Oxaloacetate	−	nd	−	nd	nd	+	nd	nd	nd	+	nd	nd	nd	+	nd
Pyruvate	−	−	+	−	+	−	+	+	+	+	+	+	+	+	+
Lactate	−	−	−	−	+	−	+	+	+	+	+	+	+	+	+
Malonate	−	−	−	−	−	−	−	−	−	−	−	d	+	−	−
Tartrate	nd	−	nd	−	+	nd	+	−	−	nd	nd	−	−	d	−
Acetate	d	−	−	−	+	−	+	+	+	+	+	+	+	−	+
Propionate	−	−	−	−	+	−	+	+	+	−	+	+	+	+	+
Butyrate	nd	−	−	−	nd	nd	nd	+	+	nd	+	d	+	nd	+
Caproate	−	nd	−	nd	nd	−	nd	nd	d	−	nd	d	nd	−	nd
β-Hydroxybutyrate	−	nd	−	nd	nd	−	nd	nd	+	−	nd	+	nd	−	nd
p-Hydroxybenzoate	−	nd	−	nd	d	−	d	nd	−	−	nd	+	nd	−	nd
Ethanol	−	−	−	−	d	−	d	−	+	−	−	+	−	−	−
n-Propanol	−	−	−	−	d	−	d	−	+	−	−	+	−	−	−
n-Butanol	−	−	−	−	−	−	−	−	d	−	−	+	−	−	−
Glycerol	−	−	−	−	nd	−	nd	−	−	−	−	−	+	−	−
L-Histidine	−	nd	−	nd	nd	−	nd	nd	−	−	nd	−	nd	−	nd
L-Tyrosine	−	nd	−	nd	nd	−	nd	nd	−	−	nd	−	nd	−	nd
L-Phenylalanine	−	nd	−	nd	nd	−	nd	nd	d	−	nd	−	nd	−	nd
L-Alanine	−	nd	−	nd	nd	−	nd	nd	+	+	nd	+	nd	−	nd
L-Glutamate	−	nd	−	nd	nd	+	nd	nd	d	+	nd	d	nd	+	nd
L-Aspartate	−	nd	−	nd	nd	−	nd	nd	d	−	nd	−	nd	−	nd
L-Glutamine	−	nd	−	nd	nd	−	nd	nd	nd	+	nd	nd	nd	d	nd
Asparagine	−	nd	−	nd	nd	−	nd	nd	nd	−	nd	nd	nd	−	nd
L-Proline	−	nd	−	nd	nd	d	nd	nd	+	−	nd	+	nd	+	nd
L-Hydroxyproline	−	nd	−	nd	nd	−	nd	nd	nd	−	nd	nd	nd	−	nd
L-Ornithine	−	nd	−	nd	nd	−	nd	nd	+	−	nd	−	nd	−	nd
L-Citruline	−	nd	−	nd	nd	−	nd	nd	+	−	nd	−	nd	−	nd
L-Arginine	−	nd	−	nd	nd	−	nd	nd	+	−	nd	d	nd	−	nd
L-Lysine	−	nd	−	nd	nd	−	nd	nd	d	−	nd	−	nd	−	nd
Putrescine	−	nd	−	nd	nd	−	nd	nd	+	−	nd	+	nd	−	nd
L-Methionine	−	nd	−	nd	nd	−	nd	nd	nd	−	nd	nd	nd	−	nd
L-Serine	−	nd	−	nd	nd	−	nd	nd	d	−	nd	−	nd	−	nd
L-Cysteine	−	nd	−	nd	nd	−	nd	nd	nd	−	nd	nd	nd	−	nd
Glycine	−	nd	−	nd	nd	−	nd	nd	d	−	nd	−	nd	−	nd
L-Leucine	−	nd	−	nd	nd	−	nd	nd	d	−	nd	−	nd	−	nd
L-Isoleucine	−	nd	−	nd	nd	−	nd	nd	d	−	nd	−	nd	−	nd
L-Valine	−	nd	−	nd	nd	−	nd	nd	d	−	nd	−	nd	−	nd
L-Tryptophan	−	nd	−	nd	nd	−	nd	nd	−	−	nd	−	nd	−	nd

[a]Method A (Hylemon et al., 1973): A turbidimetrically standardized cell suspension in synthetic seawater was inoculated into a defined, vitamin-free medium containing the carbon sources (0.1%) and ammonium sulfate as the nitrogen source. Growth responses were measured turbidimetrically after one 72-h serial transfer from the initial cultures, using a Klett colorimeter with the blue (420 nm) filter and 16-mm cuvettes. Symbols: +, 10 or more Klett units of turbidity for all strains tested; −, less than 10 Klett units of turbidity; d, differs among strains; nd, not determined.

[b]Method B (Terasaki, 1972, 1979): A cell suspension washed in basal, defined, vitamin-free medium (Williams and Rittenberg, 1957) containing natural seawater and lacking carbon sources. The cells were inoculated into similar media containing the test compounds (0.05%) and ammonium chloride as the nitrogen source. After 7 d, growth was estimated turbidimetrically. Symbols: +, a turbidity of 0.025 absorbance units or greater for all strains tested; −, a turbidity of less than 0.025; d, differs among strains; nd, not determined.

[c]Strain ATCC 12753 failed to grow with any sole carbon source, while strain ATCC 11336 grew only with acetate. Both strains grew abundantly when succinate plus malate were supplied as carbon sources and L-methionine as the nitrogen source.

[d]Strain OF3 (Terasaki, 1972, 1973) differs from the results given in the table in that it grows with a large veriety of sole carbon sources: citrate, succinate, fumarate, malate, pyruvate, lactate, acetate, propionate, and butyrate. Whether this strain should be included in the species *O. linum* is uncertain.

[e]The results are given for *O. maris* subsp. *maris*. *O. maris* subsp. *williamsae* fails to grow with any sole carbon (or sole nitrogen) source and, therefore, appears to have an auxotrophic growth requirement. This requirement has not yet been defined.

[f]As reported by Bowditch et al. (1984a).

tenberg, 1957) was included in the genus *Oceanospirillum* by Hylemon et al. (1973), but this posed taxonomic problems. The characteristics of the type strain (ATCC 11337 or NCMB 54) did not fit the original description of the species, and Linn and Krieg (1978) found that NCMB strain 54 consisted of a mixture of two dissimilar organisms. The first type was a short, vibrioid rod that possessed a single polar flagellum, grew in either the presence or absence of seawater, catabolized sugars, did not form coccoid bodies, and had a mol% G + C of 63–64. The second type was a larger, helical organism that possessed bipolar flagellar tufts, required seawater, failed to attack sugars, formed coccoid bodies, and had a mol% G + C of 45. The smaller organism did not appear to belong to either *Oceanospirillum* or *Aquaspirillum* and it remains unclassified. The larger organism had characteristics more in accord with the original description of *"S. lunatum"* but differed in certain respects; it has been classified as a new subspecies of *O. maris*: *O. maris* subsp. *williamsae*.

Bowditch et al. (1984a, b) added four species to the genus *Oceanospirillum*, mainly based on immunological relationships. These species were *Oceanospirillum commune*, for the organism previously named *Marinomonas communis* (Van Landschoot and De Ley, 1983, 1984), *Oceanospirillum vagum* for *Marinomonas vaga* (Van Landschoot and De Ley, 1983, 1984), and two species *Oceanospirillum jannaschii* and *Oceanospirillum kriegii* for two groups of unnamed marine bacteria I-1 and H-1, respectively. As a result, the genus definition of *Oceanospirillum* needed to be changed drastically, with the unfortunate loss of most of the readily determinable phenotypic features from the genus definition (Krieg, 1984a) and the extension of the upper mol% G + C limit for the genus from 51 to 57. By this extension, a considerable overlap of mol% G + C range was introduced between the genera *Aquaspirillum* (49–65 mol% G + C) and *Oceanospirillum* (42–51 mol% G + C). In this way, one of the most reliable genotypic features discriminating both genera was lost. Phylogenetic data (Pot et al. 1989, Pot, 1996; Satomi et al., 1998), however, have since shown that all four species cannot be regarded as members of the genus *Oceanospirillum*.

On the basis of a polyphasic approach including DNA–DNA and DNA–rRNA hybridizations, Pot et al. (1989) showed that only five species, including the type species, constituted a separate rRNA branch in the *Gammaproteobacteria* and redefined the genus *Oceanospirillum* to contain *O. linum*, *O. maris*, *O. beijerinckii*, *O. multiglobuliferum*, and, more distantly, *O. japonicum*. Based on DNA–DNA hybridizations (as suggested by Krieg, 1984a) and numerical comparison of whole-cell proteins, *O. maris* subsp. *hiroshimense* and *O. beijerinckii* subsp. *pelagicum* were created for the former species *O. hiroshimense* and *O. pelagicum*. *O. pusillum* was shown to belong to the *Alphaproteobacteria*, and *O. commune* and *O. vagum* were relegated to their original generic positions as *Marinomonas communis* and *Marinomonas vaga*, respectively. The two species *O. jannaschii* and *O. kriegii* were shown to be phylogenetically too remote to be considered members of the genus *Oceanospirillum*, and, together with *O. minutulum*, they constituted separate rRNA branches in the *Gammaproteobacteria*.

Subsequently, this phylogenetic heterogeneity was confirmed by studies of fatty acid, quinone, and polyamine compositions (Hamana et al., 1994; Sakane and Yokota, 1994; Bertone et al., 1996). All species, except *O. pusillum*, contained ubiquinone-8 (Q-8) as a major respiratory quinone (Table BXII.γ.78). Like other spirilla from the *Alphaproteobacteria* (see the genus *Aquaspirillum* in this book), *O. pusillum* contained over 90% Q-10. The thirteen strains of *Oceanospirillum* that have been investigated for their fatty acid composition by Sakane and Yokota were divided into three groups (Table BXII.γ.79 and BXII.γ.80). Group I included the 10 strains belonging to *O. linum*, *O. maris* subsp. *hiroshimense*, *O. maris* subsp. *williamsae*, *O. beijerinckii* subsp. *beijerinckii*, *O. beijerinckii* subsp. *pelagicum*, *O. multiglobuliferum*, and *O. japonicum*, all of which have a low mol% G + C (42.5–48.4). Group II included the two type strains of *O. jannaschii* and *O. kriegii* and had a high mol% G + C content (54.8–54.9). Group III included only *O. pusillum* and could be clearly distinguished from other marine spirilla in having $C_{14:0\ 3OH}$ as the major 3-hydroxy fatty acid, besides Q-10 (Table BXII.γ.80). Bertone et al. (1996) confirmed the separate position of *O. japonicum*, *O. jannaschii*, and *O. kriegii*.

All *Oceanospirillum* species including *O. jannaschii* and *O. kriegii* contain both putrescine and spermidine. The relative content (Table BXII.γ.81) of putrescine is very small when compared with the level found in members of the *Alphaproteobacteria*. The relative concentration of putrescine for *O. pusillum* corresponds with that of other members of the *Alphaproteobacteria*. The absence of 2-hydroxy putrescine and homospermidine is a unifying character for the *Gammaproteobacteria*. The polyamine profile of *Oceanospirillum* I and II is not different, nor are their fatty acid profiles.

Later, 16S rDNA sequence analysis of all *Oceanospirillum* spe-

TABLE BXII.γ.78. Cellular quinone systems in *Oceanospirillum* species and *Marinospirillum minutulum*[a]

Species[b]	Strain	Group	Quinone system				
			Q-6	Q-7	Q-8	Q-9	Q-10
O. linum	IFO 15448^T	Ic	3	4	91	2	
	IFO 15449	Ic	1	2	96	1	
O. beijerinckii subsp. *beijerinckii*	IFO 15445^T	Id	1	12	83	4	
O. beijerinckii subsp. *pelagicum*	IFO 13612^T	Id	2	7	91	1	
O. maris subsp. *hiroshimense*	IFO 13616^T	Ic	1	4	94	1	
O. maris subsp. *williamsae*	IFO 15468^T	Ic	12	7	80	1	
O. multiglobuliferum	IFO 13614^T	Ic	1	4	94	1	
O. jannaschii	IFO 15466^T	IIb	3	7	89	1	
O. japonicum	IFO 15446^T	Ib	1	14	84	1	
	IFO 15447	Ib		9	88	3	
O. kriegii	IFO 15467^T	IIa	2	5	89	4	
O. pusillum	IFO 13613^T	III			1	6	93
Marinospirillum minutulum	IFO 15450^T	Ia	1	1	97	1	

[a]After Sakane and Yokota (1994).

[b]*Oceanospirillum maris* subsp. *maris* has not been investigated.

TABLE BXII.γ.79. Cellular concentrations of non-polar fatty acids in *Oceanospirillum* species and *Marinospirillum minutulum*[a]

Species[b]	Strain	Non-polar fatty acid[c]										
		$C_{12:0}$	$C_{12:1}$	$C_{14:0}$	$C_{14:1}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{17:1}$	$C_{18:0}$	$C_{18:1}$	$C_{20:0}$
O. linum	IFO 15448^T	3	2	1			16	48				30	
	IFO 15449	4	4	1			16	45				29	
O. beijerinckii subsp. *beijerinckii*	IFO 15445^T	4		4		1	32	50				9	
O. beijerinckii subsp. *pelagicum*	IFO 13612^T	4		2	1		22	46				23	3
O. maris subsp. *hiroshimense*	IFO 13616^T	4	2	1			27	49			1	15	
O. maris subsp. *williamsae*	IFO 15468^T	4	4	2			31	47			1	11	
O. multiglobuliferum	IFO 13614^T	3	2	2			28	44				20	
O. jannaschii	IFO 15447	2		1			19	46			1	31	
O. japonicum	IFO 15466^T	3		1			25	57				14	
	IFO 15446^T	3		1			22	53				21	
O. kriegii	IFO 15467^T	7	4	1	1	2	16	36	2	3	1	27	
O. pusillum	IFO 13613^T	3		1	3		15	18			1	58	
Marinospirillum minutulum	IFO 15450^T	2			4		35	26				32	

[a]After Sakane and Yokota (1994).

[b]*Oceanospirillum maris* subsp. *maris* has not been investigated.

[c]The percentage of the acid relative to the total non-polar acids.

TABLE BXII.γ.80. Cellular concentrations of 2- and 3-hydroxy fatty acids in *Oceanospirillum* species and *Marinospirillum minutulum*[a]

Species[b]	Strain	3-hydroxy fatty acids[c]						2-hydroxy fatty acid[d]
		$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{14:1}$	$C_{16:0}$	$C_{18:0}$	
O. linum	IFO 15448^T	100						–
	IFO 15449	100						–
O. beijerinckii subsp. *beijerinckii*	IFO 15445^T	63		30		6		–
O. beijerinckii subsp. *pelagicum*	IFO 13612^T	60		30		9		–
O. maris subsp. *hiroshimense*	IFO 13616^T	100						–
O. maris subsp. *williamsae*	IFO 15468^T	100						–
O. multiglobuliferum	IFO 13614^T	100						–
O. jannaschii	IFO 15447	100						–
O. japonicum	IFO 15466^T	4	96					–
	IFO 15446^T	3	97					–
O. kriegii	IFO 15467^T	19	54			27		–
O. pusillum	IFO 13613^T			87		2	10	+ ($C_{18:1}$)
Marinospirillum minutulum	IFO 15450^T		61	3	36			–

[a]After Sakane and Yokota (1994).

[b]*Oceanospirillum maris* subsp. *maris* has not been investigated.

[c]The percentage of the acid relative to the total 3-hydroxy acids.

[d]–, absent; +, present

cies confirmed the above findings. Kawasaki et al. (1997), in a phylogenetic study of helically shaped bacteria in the *Alphaproteobacteria*, showed that *O. pusillum* was not related to other taxa of spirilla and constituted a separate branch in the *Alphaproteobacteria*, also confirming previous findings of Woese et al. (1985) and Pot (1996). Therefore, *O. pusillum* cannot belong to the genus *Oceanospirillum* (Kawasaki et al., 1997). Phenotypic characteristics support the removal of *O. pusillum* from the genus: a single flagellum at each pole, a counterclockwise type of helix, and a mol% G + C of 51, which is slightly higher than the range of 42–48 for the rest of the genus. As a formal transfer has not been proposed, *O. pusillum* is therefore listed below as "Species assigned but phylogenetically not belonging in *Oceanospirillum*".

Satomi et al. (1998) determined and compared 16S rDNA sequences of all the *Oceanospirillum* species. They found that *O. linum*, *O. maris*, *O. beijerinckii*, and *O. multiglobuliferum* constituted a single rRNA cluster, separate from the branches formed by *Marinobacter*, *Marinobacterium*, and *Marinomonas*. Based on their findings, they also excluded *O. japonicum* from the genus *Oceanospirillum*. Phenotypically, *O. japonicum* is different from other *Oceanospirillum* species since it does not form coccoid bodies and its flagella appear to be crescent shaped with less than one helical turn, whereas those of other species have one or more helical turns. Moreover, *O. japonicum* grows best at 35–37°C. As a formal new description has not been proposed, *O. japonicum* is therefore listed below as a "Species assigned but phylogenetically not belonging in *Oceanospirillum*".

In the same study, it was shown that *O. minutulum* clustered on a separate branch together with new isolates from kusaya gravy (Satomi et al., 1998). For this branch, a new genus *Marinospirillum* has been proposed, containing the two species *M. minutulum* and *M. megaterium* (Satomi et al., 1998).

Compared to the other *Oceanospirillum* species, *O. jannaschii* and *O. kriegii* both have a higher mol% G + C (54.8–54.9) and occupy a separate phylogenetic position (Satomi et al., 1998). Many phenotypic characteristics discriminate these species from the genus *Oceanospirillum* (Table BXII.γ.82). Although not discussed separately by the authors, *O. jannaschii* occurred on the same branch as *Marinobacterium* (González et al., 1997). Further taxonomic research, including DNA–DNA hybridizations, should be performed to substantiate the exact level of genotypic relationship between *O. jannaschii* and *Marinobacterium georgiense*. *O. kriegii* constituted a separate branch in the 16S rRNA dendrogram (Satomi et al., 1998).

TABLE BXII.γ.81. Cellular concentrations of polyamines in *Oceanospirillum* and *Marinospirillum minutulum*[a,b,c]

Species[d]	Strain	Medium[e]	Dap	H-Put	Put	Cad	Spd	HSpd
O. linum	IFO 15448^T	199SW	–	–	0.01	–	0.65	–
	IFO 15449	199SW	–	–	0.02	–	0.80	–
O. beijerinckii subsp. *beijerinckii*	IFO 15445^T	199SW	–	–	0.01	–	0.64	–
O. beijerinckii subsp. *pelagicum*	IFO 13612^T	199S	–	–	0.06	–	0.48	–
		199SW	–	–	0.01	–	0.65	–
O. maris subsp. *hiroshimense*	IFO 13616^T	199S	–	–	0.02	–	0.86	–
		199SW	–	–	0.03	–	0.90	–
O. maris subsp. *williamsae*	IFO 15468^T	199SW	–	–	0.03	–	0.90	–
O. multiglobuliferum	IFO 13614^T	199S	–	–	0.08	–	0.40	–
		199SW	–	–	0.01	–	0.45	–
O. jannaschii	IFO 15466^T	199SW	–	–	0.02	–	0.80	–
O. japonicum	IFO 15446^T	199SW	–	–	0.01	–	1.11	–
	IFO 15447	199SW	–	–	0.01	–	1.23	–
O. kriegii	IFO 15467^T	199SW	–	–	0.03	–	0.84	–
O. pusillum	IFO 13613^T	199S	–	–	0.15	–	1.40	–
		199SW	–	–	0.24	–	1.19	–
Marinospirillum minutulum	IFO 15450^T	199SW	–	–	0.10	–	0.72	–

[a]Cells were harvested at stationary growth phase.

[b]After Hamana et al. (1994).

[c]Abbreviations: Dap, diaminopropane; H-Put, 2-hydroxyputrescine; Put, putrescine; Cad, cadaverine; Spd, spermidine; HSpd, homospermidine; –, not detectable (<0.005).

[d]*Oceanospirillum maris* subsp. *maris* has not been investigated.

[e]Media:199, polyamine-free growth medium from Flow Lab., Irvine, U.K., pH 7.0.; 199S, medium 199 dissolved in 70% synthetic seawater, pH 7.0; 199SW, medium 199 dissolved in seawater, pH 7.0.

Based on 16S rRNA gene sequence analysis, the genus *Oceanospirillum* should therefore be limited to *O. linum*, *O. maris* subsp. *maris*, *O. maris* subsp. *hiroshimense*, *O. maris* subsp. *williamsae*, *O. beijerinckii* subsp. *beijerinckii*, *O. beijerinckii* subsp. *pelagicum*, and *O. multiglobuliferum*. Consequently, *O. japonicum*, *O. jannaschii*, *O. kriegii*, and *O. pusillum* should be removed from the genus. The genus definition described above has been adapted accordingly. The precise taxonomic affiliation of the last four species needs to be further determined.

Note added in proof: Satomi et al. (2002) have formally revised the taxonomic status of the genus *Oceanospirillum* and proposed the formal transfer of the species assigned to but not belonging to the genus *Oceanospirillum* to new and existing genera. (See Kimura et al., 2002 in Further Reading section below.)

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Further Reading

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Satomi, M., B. Kimura, T. Hamada, S, Harayama, and T. Fujii. 2002. Phylogenetic study of the genus *Oceanospirillum* based on 16S rRNA and *gyrB* genes: emended description of the genus *Oceanospirillum*, description of *Pseudospirillum* gen. nov., *Oceanobacter* gen. nov. and *Terasakiella* gen. nov. and transfer of *Oceanospirillum jannaschii* and *Pseudomonas stanieri* to *Marinobacterium* as *Marinobacterium jannaschii* comb. nov. and *Marinobacterium stanieri* comb. nov. Int J Syst Evol Microbiol. *52*: 739–747.

Differentiation of the species of the genus *Oceanospirillum*

Morphological and physiological characteristics of the species of *Oceanospirillum* are indicated in Tables BXII.γ.77 and BXII.γ.82. Chemotaxonomic characteristics of the species are indicated in Tables BXII.γ.78, BXII.γ.79, BXII.γ.80, and BXII.γ.81.

List of species of the genus Oceanospirillum

1. **Oceanospirillum linum** (Williams and Rittenberg 1957) Hylemon, Wells, Krieg and Jannasch 1973, 374^{AL} (*Spirillum linum* Williams and Rittenberg 1957, 82.)
li′ num. L. n. *linum* flax, thread.

The morphological characters are depicted in Fig. BXII.γ.104 and listed in Table BXII.γ.82. The physiological and chemotaxonomic characters are indicated in Tables BXII.γ.78, BXII.γ.79, BXII.γ.80, BXII.γ.81, and BXII.γ.82. Sole carbon sources are listed in Table BXII.γ.77. Growth in defined media is usually poor; however, abundant growth occurs in defined media containing malate plus succinate as carbon sources and methionine as the nitrogen source. Nitrate is not used.

Strain OF3, isolated by Terasaki (1972, 1973), differs from other strains of *O. linum* in that it can grow well in defined media with a variety of sole carbon sources and

TABLE BXII.γ.82. Morphological and physiological characteristics of the species of *Oceanospirillum* and of *Marinospirillum minutulum*[a]

Characteristics	*O. linum*	*O. beijerinckii*	*O. maris*	*O. multiglobuliferum*	*O. jannaschii*	*O. japonicum*	*O. kriegii*	*O. pusillum*	*Marinospirillum minutulum*
Type of helix[b]	C	C	C	C	SR	C	SR	CC	C
Wavelength of helix, µm	1.8–4.0	6.3–7.2	3.5–7.0	3.5–5.0	nd	7.0–20.0	nd	1.7–2.0	2.0–2.8
Helix diameter, µm	0.8–1.4	1.5–3.0	1.4–2.8	1.0–2.0	nd	2.0–5.0	nd	1.0–1.2	0.6–1.5
Length of helix, µm	4.0–30.0	2.0–15.5	2.5–40.0	2.0–10.0	nd	5.0–75.0	nd	1.2–4.0	3.0–8.0
Cell diameter, µm	0.4–0.6	0.6–1.2	0.6–1.1	0.5–0.9	nd	0.8–1.4	nd	0.3–0.5	0.3–0.4
Flagellar arrangement[c]	BT	BT	BT	BT	M1-2	BT	MS	BS	BT
Polar membrane present	nd	+d	+[d]	nd	nd	+	nd	nd	+
Intracellular poly-β-hydroxybutyrate formed	+	+	+	+	+	+	+	+	+[e]
Coccoid bodies predominant:									
After 3–4 weeks	+	+	+	+	nd	−	nd	+	+
After 24–48 h	−	−	−	+	nd	−	nd	−	−
Range of NaCl (%) for growth in peptone water after 7 d[f]	0.5–8.0	0.5–8.0	nd	0.5–4.0	nd	0.5–8.0	nd	0.5–8.0	0.5–8.0
Growth in PSS-seawater broth containing:[g]									
9.75% total NaCl	+	+	+	nd	nd	nd	nd	nd	+
12.75% total NaCl	−	−	−	nd	nd	nd	nd	nd	d
Temperature range for growth (°C)[h]	11–39	8–41	2–35	6–37		10–43		6–40	11–37
Optimum growth temperature 25°C rather than 30–32°C	−	−	d[i]	−	+	−	+	−	−
Phosphatase	+	+	d[i]	+	nd	w	nd	w	−
Nitrate reduced to nitrite	−	−	−	−	+	−	−	+	+
Oxidase	+	+	+	+	+	+	+	+	+
Catalase	+ or w	+ or w	di	+	nd	w or −	nd	w or −	−
Anaerobic growth with nitrate	−	−	−	−	nd	−	nd	−	−
Denitrification	−	−	−	−	−	−	−	−	−
Acid produced from sugars	−	−	−	−	nd	−	nd	−	−
Hydrolysis of esculin, hippurate, starch, or casein	−	−	−	−	nd	−	nd	−	−
Indole test	−	−	−	−	nd	−	nd	−	−
Sulfatase (0.1% phenolphthalein disulfate)[g]	−	−	−	nd	nd	nd	nd	nd	−
Gelatin liquefaction at 20°C after: [f,j]									
7 d	−	d	−	−	−	d	−	−	−
28 d	+	d	−	−	−	d	−	−	−
42 d	+	d	−	−	−	d	−	−	−

(*continued*)

TABLE BXII.γ.82. *(cont.)*

Characteristics	*O. linum*	*O. beijerinckii*	*O. maris*	*O. multiglobuliferum*	*O. jannaschii*	*O. japonicum*	*O. kriegii*	*O. pusillum*	*Marinospirillum minutulum*
Gelatin hydrolysis at 30°C after:									
4 d[d,g]	−	−	−	nd	−	−	−	nd	−
Reduction of 0.3% H_2SeO_3[g]	+	−	−	nd	nd	nd	nd	nd	+
Water-soluble brown pigment formed in the presence of:[g]									
0.1% tyrosine	+	+	d	nd	nd	nd	nd	nd	−
0.1% phenylalanine	−	−	−	nd	nd	nd	nd	nd	−
0.1% tryptophan	d	−	d	nd	nd	nd	nd	nd	d
Growth in the presence of:[g]									
0.1% oxgall	+	+	+	nd	nd	nd	nd	nd	+
0.1% glycine	+	−	+	nd	nd	nd	nd	nd	+
Growth on:[g]									
Eosin methylene blue agar	−	−	d	nd	nd	nd	nd	nd	−
MacConkey agar	−	−	−	nd	nd	nd	nd	nd	−
Triple-Sugar iron agar	+	−	+	nd	nd	nd	nd	nd	+
Sellers agar	−	−	−	nd	nd	nd	nd	nd	−
Methyl red-Voges–Proskauer broth	+	−	+	nd	nd	nd	nd	nd	−
Water-soluble yellow-green fluorescent pigment formed[g]	+	−	d	nd	−	nd	−	nd	−
Deoxyribonuclease[g]	−	+	−	nd	nd	nd	nd	nd	−
Ribonuclease[g]	d	+	−	nd	nd	nd	nd	nd	−
Urease[g]	−	−	−	nd	nd	nd	nd	nd	−
Auxotrophic growth requirement	+[k]	−	d[k]	−	nd	−	nd	−	−
Mol% (G + C) of DNA	48–50	47–49	45–47	46	56–57	45	54–56	51	42–44

[a]Phenotypic data are from Krieg (1984a), Baumann et al. (1972), and Bowditch et al. (1984a); +, present in all strains; −, lacking in all strains; d, depending on the strain; w, weak reaction; nd, not determined.

[b]C, clockwise helix; CC, counterclockwise helix (determined by focusing on the bottom of the cells.

[c]SR, straight rod; BT, bipolar tufts, BS, bipolar single; M1-2, 1–2 flagella at one pole; MS, monopolar single.

[d]Characteristic has not been tested in *O. maris* subsp. *hiroshimense* and *O. beijerinckii* subsp. *pelagicum*.

[e]No granules are visible microscopically in *Marinospirillum minutulum* but chemical analysis indicates presence of the polymer.

[f]Data from Terasaki (1972, 1979).

[g]Data from Hylemon et al. (1973).

[h]*Oceanospirillum kriegii* grows at 35°C but not at 40°C; *Oceanospirillum jannaschii* does not grow at either 35°C or 40°C.

[i]*Oceanospirillum maris* subsp. *hiroshimense* has an optimal growth temperature of 25°C; catalase reaction can be negative, weak, or positive; phosphatase reaction can be positive or negative. For detailed information: see Hylemon et al. (1973), Terasaki (1972, 1979), and Table BXII.γ.83.

[j]Gelatin liquefaction was not tested in *O. maris* subsp. *maris*. It was negative in *O. maris* subsp. *hiroshimense*, positive in *O. beijerinckii* subsp. *beijerinckii*, and differed depending on the strain in *O. beijerinckii* subsp. *pelagicum*.

[k]*Oceanospirillum linum* grows poorly or not at all in a defined medium with a single carbon source and ammonium ions as the nitrogen source; however, abundant growth ocurs in a defined medium containing succinate plus malate as carbon source and methionine as the nitrogen source. (See also footnote [d] in Table BXII.γ.82.) *Oceanospirillum maris* subsp. *williamsae* ATCC 2954^T does not grow in vitamin-free medium and requires an unidentified growth factor.

ammonium ions as the nitrogen source (see footnote d, Table BXII.γ.77). Other characteristics of this strain are similar to those of the type strain (Terasaki, 1973), but whether it should be included in this species is uncertain.

The species includes organisms previously assigned to the two species *Spirillum linum* and *Spirillum atlanticum* by Williams and Rittenberg (1957). The two species were combined into the single species *O. linum* by Hylemon et al. (1973), based on a high degree of similarity in phenotypic characters and in DNA base composition.

Isolated from coastal seawater.

The mol% G + C of the DNA is: 48–50 (T_m).

Type strain: ATCC 11336, DSM 6292, NCMB 56.

2. **Oceanospirillum beijerinckii** (Williams and Rittenberg 1957) Hylemon, Wells, Krieg and Jannasch 1973, 374[AL] (*Spirillum beijerinckii* Williams and Rittenberg 1957, 90.)

bei.jer.inck′ i.i. M.L. gen. n. *beijerinckii* of Beijerinck; named after Prof M.W. Beijerinck of Delft, Holland.

The morphological characters are depicted in Fig. BXII.γ.104 and listed in Table BXII.γ.82. The physiological and chemotaxonomic characters are indicated in Tables BXII.γ.78, BXII.γ.79, BXII.γ.80, BXII.γ.81, and BXII.γ.82. Sole carbon sources are listed in Table BXII.γ.77. Ammonium ions can serve as a sole nitrogen source; nitrate is not used.

Isolated from coastal seawater.

The mol% G + C of the DNA is: 47 (T_m).

Type strain: ATCC 12754, DSM 7166, NCMB 52.

a. **Oceanospirillum beijerinckii** *subsp.* **beijerinckii** (Williams and Rittenberg 1957) Hylemon, Wells, Krieg and Jannasch 1973, 375[AL] (*Spirillum beijerinckii* Williams and Rittenberg 1957, 90.)

Differs from *O. beijerinckii* subsp. *pelagicum* as indicated in Table BXII.γ.83.

The mol% G + C of the DNA is: 47 (T_m).

Type strain: ATCC 12754 , DSM 7166, NCMB 52.

b. **Oceanospirillum beijerinckii** *subsp.* **pelagicum** (Terasaki 1973) Pot, Gillis, Hoste, Van de Velde, Bekaert, Kersters and De Ley 1989, 32[VP] (*Spirillum pelagicum* Terasaki 1973, 65.)

pe.la′ gi.cum. L. neut. adj. *pelagicum* belonging to the sea.

Differs from the *O. beijerinckii* subsp. *beijerinckii* as indicated in Table BXII.γ.83.

Isolated from putrid infusions of marine mussels.

The mol% G + C of the DNA is: 49 (T_m).

Type strain: ATCC 33337, DSM 6288, IFO 13612, NCMB 2228.

GenBank accession number (16S rRNA): AB006761.

3. **Oceanospirillum maris** Hylemon, Wells, Krieg and Jannasch 1973, 376[AL]

ma′ ris. L. n. *mare* the sea; L. gen. n. *maris* of the sea.

The morphological characters are depicted in Fig. BXII.γ.104 and listed in Table BXII.γ.82. The physiological and chemotaxonomic characters are indicated in Tables BXII.γ.78, BXII.γ.79, BXII.γ.80, BXII.γ.81, and BXII.γ.82. Sole carbon sources are listed in Table BXII.γ.77.

Isolated from coastal seawater (Jannasch, 1967).

The mol% G + C of the DNA is: 45–46 (T_m).

Type strain: ATCC 27509, DSM 6286, LMG 5213.

GenBank accession number (16S rRNA): AB006771.

TABLE BXII.γ.83. Differentiating characteristics for the subspecies of *Oceanospirillum beijerinckii*[a]

Characteristic	*O. beijerinckii* subsp. *beijerinckii*	*O. beijerinckii* subsp. *pelagicum*
Temperature range for growth (°C)	14–37	8–41
Growth with:		
Lactate, citrate, malate, pyruvate, acetate, propionate, tartrate	−	+
p-Hydroxybenzoate, ethanol, *n*-propanol	−	d
Gelatin liquified at 20°C	+	d
Growth after 7 d in peptone water with:		
0.5–6.0% NaCl	+	−
0.5–8.0% NaCl	−	+
Mol% G + C of DNA	47	49

[a]Phenotypic data from Terasaki (1972, 1979); +, present in all strains; −, lacking in all strains; d, depending on the strain

c. **Oceanospirillum maris** *subsp.* **maris** Hylemon, Wells, Krieg and Jannasch 1973, 376[AL]

Differs from the *O. maris* subsp. *williamsae* and the *O. maris* subsp. *hiroshimense* as indicated in Table BXII.γ.84.

The mol% G + C of the DNA is: 46 (T_m).

Type strain: ATCC 27509, DSM 6286, LMG 5213.

GenBank accession number (16S rRNA): AB006771.

d. **Oceanospirillum maris** *subsp.* **hiroshimense** (Terasaki 1973) Pot, Gillis, Hoste, Van de Velde, Bekaert, Kersters and De Ley 1989, 33[VP] (*Spirillum hiroshimense* Terasaki 1973, 62.)

hi.ro.shi.men′ se. M.L. neut. adj. *hiroshimense* pertaining to Hiroshima. Japan.

Characters are as described for the species. Differs from the *O. maris* subsp. *maris* and the *O. maris* subsp. *williamsae* as indicated in Table BXII.γ.84.

Isolated from putrid infusions of marine mussels.

The mol% G + C of the DNA is: 47 (T_m).

Type strain: IFO 13616, DSM 9524.

e. **Oceanospirillum maris** *subsp.* **williamsae** Linn and Krieg 1984, 355[VP]

will′ iam.sae. M.L. gen. n. *williamsae* of Williams; named after Marion A. Williams, who was the first to describe species of marine spirilla.

Characters are as described for the species. Differs from the *O. maris* subsp. *maris* and the *O. maris* subsp. *hiroshimense* as indicated in Table BXII.γ.84.

Isolated from a mixture of organisms comprising NCMB strain 54 by Linn and Krieg (1978).

The mol% G + C of the DNA is: 45 (T_m).

Type strain: ATCC 29547, IFO 15468.

GenBank accession number (16S rRNA): AB006763.

4. **Oceanospirillum multiglobuliferum** (Terasaki 1973) Terasaki 1979, 143[AL] (*Spirillum multiglobuliferum* Terasaki 1973, 69.)

mul.ti.glo.bu.li′ fe.rum. L. adj. *multus* much, many; L. dim. n. *globulus* a small sphere, globule; L. v. *fero* to bear, carry; M.L. neut. adj. *multiglobuliferum* bearing many globules.

The morphological characters are listed in Table BXII.γ.82. The physiological and chemotaxonomic characters are indicated in Tables BXII.γ.78, BXII.γ.79, BXII.γ.80, BXII.γ.81, and BXII.γ.82. Sole carbon sources

TABLE BXII.γ.84. Differentiating characteristics for the subspecies of *Oceanospirillum maris*[a]

Characteristic	*O. maris* subsp. *maris*	*O. maris* subsp. *hiroshimense*	*O. maris* subsp. *williamsae*
Optimal growth temperature (°C)	30–32	25	30–32
Catalase reaction	strongly +	d	weakly +
Phosphatase activity	−	+	−
DNase activity	−		+
RNase activity	−		+
Growth with 1% glycine	+		−
Growth in vitamin-free, defined growth medium	+		−
Growth with:			
L-Glutamate, oxaloacetate	+		−
Succinate, pyruvate, lactate, tartrate, acetate, propionate	−	+	−
Mol% G + C of DNA	46	47	45

[a]Phenotypic data from Hylemon et al. (1973); +, present in all strains; −, lacking in all strains; d, depending on the strain

are listed in Table BXII.γ.77. Differs from other species by forming unusually large numbers of coccoid bodies even in 24- to 48-h-old broth cultures. Ammonium ions can serve as a sole nitrogen source; nitrate is not used.

Isolated from putrid infusions of marine mussels.

The mol% G + C of the DNA is: 46 (T_m).

Type strain: IFO 13614.

GenBank accession number (16S rRNA): AB006764.

Species assigned but phylogenetically not belonging in *Oceanospirillum*

1. **Oceanospirillum jannaschii** Bowditch, Baumann and Bauman 1984b, 503[VP] (Effective publication: Bowditch, Baumann and Bauman 1984a, 227.)
jan.nasch′i.i. M.L. gen. n. *jannaschii* of Jannasch; named after H.W. Jannasch.

Straight rods. Some morphological, physiological, and chemotaxonomic characters are listed in Tables BXII.γ.78, BXII.γ.79, BXII.γ.80, BXII.γ.81, and BXII.γ.82 (see also Baumann et al., 1972). Sole carbon sources are listed in Table BXII.γ.77. Utilize 39–46 organic compounds including fatty acids, tricarboxylic acid cycle intermediates, alcohols, amino acids, and amines. Utilizes γ-aminovalerate and histamine (Baumann et al., 1972).

Isolated from seawater after enrichment (Baumann et al., 1972).

The mol% G + C of the DNA is: 56–57 (T_m).

Type strain: ATCC 27135, DSM 6295, IFO 15466.

GenBank accession number (16S rRNA): AB006765.

2. **Oceanospirillum japonicum** (Watanabe 1959) Hylemon, Wells, Krieg and Jannasch 1973, 375[AL] (*Spirillum japonicum* Watanabe 1959, 78.)
ja.pon′i.cum. M.L. neut. adj. *japonicum* pertaining to Japan.

The morphological characters are depicted in Fig. BXII.γ.104 and listed in Table BXII.γ.82; however, the type strain presently has morphological features that differ from the original description, in that the cells are no longer helical with several waves but instead are curved, straight, or S-shaped; moreover, colonies of this strain are presently of the R (rough) type. Therefore, it is likely that the type strain has undergone alteration since its isolation in 1959. Three reference strains isolated by Terasaki (1972, 1973) have morphological features that more nearly correspond to those given in the original description (strains IF4, IF8, and UF3), and also they form colonies of the S (smooth) type.

The physiological and chemotaxonomic characters are indicated in Tables BXII.γ.78, BXII.γ.79, BXII.γ.80, BXII.γ.81, and BXII.γ.82. Sole carbon sources are listed in Table BXII.γ.77.

Isolated from putrid infusions of marine mussels.

The mol% G + C of the DNA is: 45 (T_m).

Type strain: ATCC 19191, DSM 7165.

3. **Oceanospirillum kriegii** Bowditch, Baumann and Bauman 1984b, 503[VP] (Effective publication: Bowditch, Baumann and Bauman 1984a, 227.)
krie′gi.i. M.L. gen. n. *kriegii* of Krieg; named after N.R. Krieg.

Straight rods. Some morphological and physiological characters are listed in Tables BXII.γ.78, BXII.γ.79, BXII.γ.80, BXII.γ.81, and BXII.γ.82 (see also Baumann et al., 1972). Sole carbon sources are listed in Table BXII.γ.77. Utilize 29–33 organic compounds including D-glucose and D-fructose but no other pentose, hexose, or disaccharide; also utilize tricarboxylic acid cycle intermediates, alcohols, amino acids, and amines. Produces an extracellular lipase (Baumann et al., 1972).

Isolated from see water after enrichment (Baumann et al., 1972).

The mol% G + C of the DNA is: 54–56 (T_m).

Type strain: ATCC 27133, DSM 6294, IFO 15467, NCMB 2042.

GenBank accession number (16S rRNA): AB006767.

4. **Oceanospirillum pusillum** (Terasaki 1973) Terasaki 1979, 142[AL] (*Spirillum pusillum* Terasaki 1973, 67.)
pu.sil′lum. L. dim. neut. adj. *pusillum* very small.

The morphological characters are listed in Table BXII.γ.82. The physiological and chemotaxonomic characters are indicated in Tables BXII.γ.78, BXII.γ.79, BXII.γ.80, BXII.γ.81, and BXII.γ.82. Sole carbon sources are listed in Table BXII.γ.77. Ammonium ions can serve as a sole nitrogen source; nitrate is not used.

Isolated from putrid infusions of marine mussels.

Belongs to the *Alphaproteobacteria*.

The mol% G + C of the DNA is: 51 (T_m).

Type strain: ATCC 33338, DSM 6293, IAM 14442, IFO 13613, NCMB 2229.

GenBank accession number (16S rRNA): AB006768.

Genus II. **Balneatrix** *Dauga, Gillis, Vandamme, Ageron, Grimont, Kersters, De Mahenge, Peloux and Grimont 1993b, 624[VP] (Effective publication: Dauga, Gillis, Vandamme, Ageron, Grimont, Kersters, De Mahenge, Peloux and Grimont 1993a, 42)*

CATHERINE DAUGA

Bal' ne.a.trix. L. fem. n. *balneatrix* bather.

Straight or curved rods, 0.5–0.7 × 2.8–5 μm, sometimes elongated and flexuous. Gram negative. **Motile by single polar flagellum. Aerobic**, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. Produce convex, round, smooth colonies on solid media. Optimum temperature, 30°C; range, 20–46°C. Optimum pH, 6.5–7.5. Growth factors are not required. **No growth occurs in medium containing more than 1% NaCl.** Chemoorganotrophic. Acid is produced from glucose and certain other carbohydrates. **Oxidase positive.** Weakly catalase positive. **Indole positive.** Urease negative. Reduce nitrate to nitrite. Freshwater organisms; occasionally pathogenic for humans.

The mol% G + C of the DNA is: 54.

Type species: **Balneatrix alpica** Dauga, Gillis, Vandamme, Ageron, Grimont, Kersters, De Mahenge, Peloux and Grimont 1993b, 624 (Effective publication: Dauga, Gillis, Vandamme, Ageron, Grimont, Kersters, De Mahenge, Peloux and Grimont 1993a, 43).

FURTHER DESCRIPTIVE INFORMATION

Balneatrix strains grow after a 2-d incubation at 30°C. The center of the colony is pale yellow after 2–3 d, and pale brown after 4–5 d.

Balneatrix strains are nonfermentative, as verified by oxidation–fermentation medium (Casalta et al., 1989). All strains tested are Voges–Proskauer negative by the Barrit reference method. Nutritional tests can be done using Biotype-100 strips (BioMerieux, Marcy l' Etoile, France) with M70 minimal medium (Véron, 1975) supplemented with 0.15% agar. Strains of *Balneatrix* are nutritionally diverse, and some strains can grow with citrate, D-alanine, ethanolamine, D-glucosamine, L-histidine, DL-lactate, D(+)-malate (see Table BXII.γ.85). Arginine dihydrolase, lysine and ornithine decarboxylases, acetamide, and starch hydrolysis are invariably negative. Tetrathionate is not reduced. Gelatin is weakly hydrolyzed. Citrate can serve sometimes as the sole carbon source for growth. Tween-80 hydrolysis also differs among strains. Tributyrin is hydrolyzed, and the egg yolk reaction (lecithinase) is positive. The gamma-glutamyl transferase test is positive. There is no ONPG hydrolysis and no extracellular DNase (Casalta et al., 1989).

Balneatrix strains are susceptible *in vitro* to a variety of antimicrobial agents, including β-lactam, macrolides, and aminoglycoside antibiotics, sulfamethoxazole–trimethoprim, chloramphenicol, deoxycycline, minocycline, ofloxacin, and nalidixic acid. They are resistant to clindamycin and vancomycin.

At this writing, *Balneatrix* strains have been isolated only from thermal water and clinical specimens at a spa therapy center in southern France. Thirty-five cases of pneumonia and two cases of meningitis have been caused by *Balneatrix* strains (Hubert et al., 1991). According to epidemiological data, the bacteria were present in the hot water spring spa, and favorable growing conditions were found only in vapor baths. After disinfection of water pipes by chlorination, no further cases of infection were observed.

ENRICHMENT AND ISOLATION PROCEDURES

Balneatrix strains can be isolated by traditional culture techniques and incubation under aerobic conditions at 20–41°C. Environmental and clinical strains can grow in 24 h in trypto-casein soy broth (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France). Brain–heart infusion and IsoVitaleX chocolate agar (Sanofi Diagnostics Pasteur) can be used for blood and CSF (cerebrospinal fluid) samples, respectively. Strains can be subcultured on trypto-casein soy agar or nutrient agar. Antimicrobial disks can be tested on Mueller–Hinton agar (Sanofi Diagnostics Pasteur).

TABLE BXII.γ.85. Nutritional characteristics of *Balneatrix alpica*, as determined with Biotype-100 strips[a]

Substrates	Growth response[b]
N-acetyl-glucosamine, *cis*-aconitate, L-alanine, DL-γ-amino-*n*-butyrate, DL-δ-amino-*n*-valerate, L-aspartate, betaine, D-fructose, fumarate, D-gluconate, D-glucose, L-glutamate, glutarate, glycerol, DL-β-hydroxybutyrate, *myo*-inositol, α-ketoglutarate, L-malate, maltose, maltotriose, D-mannitol, D-mannose, L-proline, propionate, putrescine, D-ribose, L-serine, D-sorbitol, succinate, L-tyrosine	+
trans-Aconitate, adonitol, D-arabinose, D-arabitol, L-arabitol, benzoate, caprate, caprylate, D-cellobiose, *m*-coumarate, dulcitol, *i*-erythritol, esculin, L-fucose, D-galactose, D-galacturonate, gentisate, β-gentiobiose, D-glucuronate, DL-glycerate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, histamine, itaconate, 2-keto-D-gluconate, 5-keto-D-gluconate, lactose, lactulose, D-lyxose, malonate, maltitol, D-melezitose, D-melibiose, 1-*O*-methyl-α-galactopyranoside, 1-*O*-methyl-β-galactopyranoside, 3-*O*-methyl-D-glucopyranose, 1-*O*-methyl-α-D-glucopyranoside, 1-*O*-methyl-β-D-glucopyranoside, mucate, palatinose, phenylacetate. 3-phenylpropionate, protocatechuate, quinate, D-raffinose, L-rhamnose, D-saccharate, saccharose, L-sorbose, D(−)-tartrate, L(+)-tartrate, *meso*-tartrate, D-tagatose, D-trehalose, tricarballylate, trigonelline, tryptamine, L-tryptophan, D-turanose, xylitol, D-xylose	−
D-Alanine	d (7)
Citrate	d (1)
Ethanolamine	d (2)
D-Glucosamine	d (2)
L-Histidine	d (6)
DL-Lactate	d (7)
D(+)-Malate	d (6)

[a]Symbols : +, all strains positive ; −, all strains negative ; d, differs among strains. Numbers in parentheses indicate the number of strains that are positive.

[b]Based on results for eight strains.

MAINTENANCE PROCEDURES

The organisms survive at 30°C in trypto-casein soy broth for several weeks. They can survive for several years when frozen at −80°C in brain–heart infusion containing 50% glycerol.

DIFFERENTIATION OF THE GENUS *BALNEATRIX* FROM OTHER GENERA

The characteristics which differentiate *Balneatrix* from other genera are listed in Table BXII.γ.86.

Because *Balneatrix* shares some properties with *Flavobacterium*, such as isolation requirements, pigmented colonies, and biochemical reactions, it was initially compared with this genus; how-

TABLE BXII.γ.86. Phenotypic characteristics differentiating the genera *Balneatrix*, *Oceanospirillum*, *Marinomonas*, *Halomonas*, and *Flavobacterium*[a]

Characteristic	*Balneatrix*	*Flavobacterium*[b]	*Halomonas*[c]	*Marinomonas*[d]	*Oceanospirillum*[e]
Cell diameter, μm	0.5–0.7	0.5–3	0.6–1.9	0.5–4	0.3–1.4
Shape:					
Curved rods	+			+	
Straight rods	+	+	+	+	
Helical					+
Flagellar arrangement:					
Number	1–2	none	1–2	single	single or tufts
Arrangement	polar		polar	bipolar	bipolar
Salt tolerance (%NaCl, w/v)	0–1	nd	0.1–32	0.7–3.5	2.75–9.75
Maximum temperature (°C)	46	42	45	40	39–43
Nitrate reduced to nitrite	+	D	+	−	D
Anaerobic growth with nitrate	−	−	+	−	−
Indole	+	D	d	nd	−
Urease	−	D	d	−	−
Esculin hydrolyzed	−	+	d	nd	−
Acid from carbohydrates	+	+	+	+	−
Mol% G + C of DNA	54	31–42	60–61	45–50	45–51

[a]Symbols: +, positive for all species; −, negative for all species; nd, not determined; D, differs among species; d, differs among strains.

[b]Data from Holmes et al., 1984.

[c]Characteristics of *Halomonas elongata*. Data from Vreeland, 1984.

[d]Characteristics of *Marinomonas vaga* and *Marinomonas communis*, which were misnamed *Oceanospirillum vagum* and *O. commune* (Pot et al., 1989). Data from Baumann et al., 1972.

[e]Data from Krieg, 1984b.

ever, the mol% G + C value of 54 for the DNA of *Balneatrix* does not correspond to that of *Flavobacterium*.

Despite its environmental habitat and oxidative ability, *Balneatrix* is easily differentiated from such genera as *Oceanospirillum*, *Marinomonas*, or *Halomonas* based on salt tolerance, shape, flagellar arrangement, and ability to catabolize several carbohydrates. *Balneatrix* can be quickly differentiated from these genera by the inability to grow with more than 1% NaCl.

Taxonomic Comments

Strains of *Balneatrix* are closely related by DNA–DNA hybridization and belong to the same species. Strain 4-87, isolated from cerebrospinal fluid, has been selected as the type strain.

rRNA–DNA hybridization and 16S rDNA sequencing have shown that the genus *Balneatrix* belongs to the class *Gammaproteobacteria*. Phylogenetically, *Balneatrix* is located within a subline including many *Oceanospirillum* species (Fig. BXII.γ.105). In this group, bacteria are heterogeneous and difficult to classify. Like *Balneatrix*, *Halomonas halmophila* was first misidentified as *Flavobacterium halmophilum* (Franzmann et al., 1988). *Halomonas aquamarina*, *Halomonas halophila*, and *Halomonas marina* were first assigned to the genus *Deleya* (Dobson et al., 1993).

Phylogenetically, *Balneatrix* branches in the vicinity of *Oceanospirillum* species, but *O. vagum*, *O. commune*,* *O. jannaschii*, and *O. kriegii* are misnamed according to DNA–rRNA hybridization (Pot et al., 1989). This, in addition to the fact that *Balneatrix alpica* is the only freshwater pathogenic bacterium in the subline, argues that *Balneatrix* is a new genus.

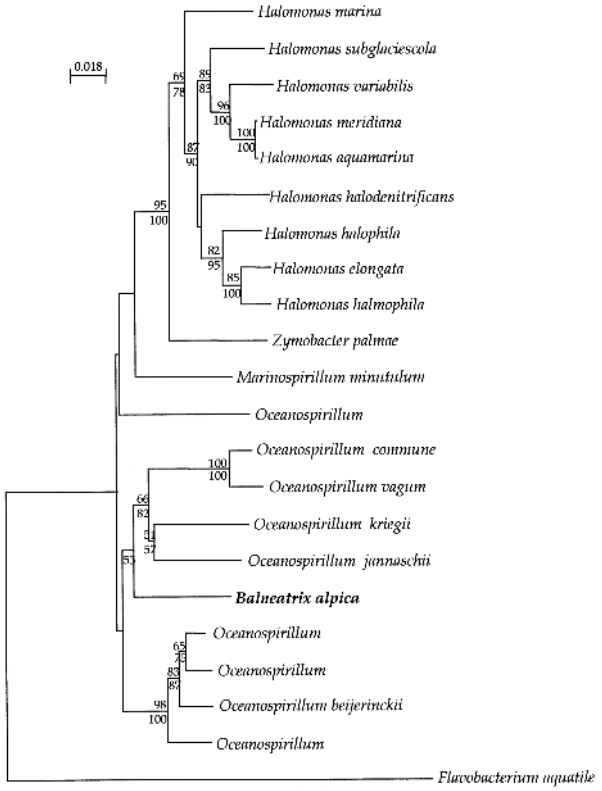

FIGURE BXII.γ.105. Relationships between the marine bacteria of the class *Gammaproteobacteria* and *Balneatrix*. *Flavobacterium aquatile*, which shares some phenotypic characteristics with *Balneatrix*, served as an outgroup. The tree shown is the result of distance analysis (NEIGHBOR routine in PHYLIP 3.5c, Kimura 2-parameters). The same tree topology was obtained with parsimony analysis (heuristic search option in PAUP 3.1.1 with 1000 replicates of the random-addition sequence). Percentages of bootstrap replicates supporting a branching pattern are given above and below the corresponding branches; the value above the branch is the parsimony analysis bootstrap percentage, and that below is from the distance analysis (100 bootstrap replicates).

*Editorial Note: *Oceanospirillum vagum* and *O. commune* are junior objective synonyms of *Marinomonas vaga* and *M. communis*.

List of species of the genus Balneatrix

1. **Balneatrix alpica** Dauga, Gillis, Vandamme, Ageron, Grimont, Kersters, De Mahenge, Peloux and Grimont 1993b, 624^{VP} (Effective publication: Dauga, Gillis, Vandamme, Ageron, Grimont, Kersters, De Mahenge, Peloux and Grimont 1993a, 43.)
al' pi.ca. L. fem. adj. *alpica* pertaining to the Alps.

The description is the same as that given for the genus. Other characteristics are listed in Table BXII.γ.85. The type strain has all the properties given for the species and hydrolyses Tween-80.

The mol% G + C of the DNA is: 54 (HPLC).
Type strain: 4-87, CIP 103589.
GenBank accession number (16S rRNA): Yl7112.

Genus III. **Marinomonas** *Van Landschoot and De Ley 1984, 91^{VP} (Effective publication: Van Landschoot and De Ley 1983, 3071)*

ANTONIO SANCHEZ-AMAT AND FRANCISCO SOLANO

Ma.ri.no.mo' nas. L. adj. *marinus* pertaining to the sea; Gr. n. *monas* a unit, monad; M.L. *Marinomonas* sea monad.

Gram-negative, straight or curved rods. Motile by means of polar flagella at one or both poles. **Aerobic**, having a strictly respiratory type of metabolism. **Oxidase positive or negative**. **Na^+ is required for growth**. **Do not accumulate poly-β-hydroxybutyrate**. Do not require organic growth factors. **Do not produce extracellular amylase**. **Utilize acetate but not butyrate or valerate**. Utilize glutamate, sorbitol, and malate. Commonly isolated from seawater.

The mol% G + C of the DNA is: 45–50.

Type species: **Marinomonas communis** (Baumann, Baumann, Mandel and Allen 1972) Van Landschoot and De Ley 1984, 91 (Effective publication: Van Landschoot and De Ley 1983, 3071) (*Alteromonas communis* Baumann, Baumann, Mandel and Allen 1972, 420.)

FURTHER DESCRIPTIVE INFORMATION

Species of *Marinomonas* are rods, either curved such as *M. communis*, or straight such as *M. vaga* and *M. mediterranea* (Fig. BXII.γ.106). They are motile; *M. communis* and *M. vaga* show a single, unsheathed, polar flagellum inserted at one or both poles of the cell. *M. mediterranea* shows only a single flagellum at one pole of the cell (Fig. BXII.γ.106).

Marinomonas strains grow in typical marine media such as Marine Agar (Zobell, 1941). In this complex medium, only *M. mediterranea* shows pigmentation; it synthesizes a brown to black melanin pigment derived from L-tyrosine as main precursor. This pigment is observed both in the colonies and diffusing into the surrounding medium (Fig. BXII.γ.107). *Marinomonas* strains also grow in basal marine agar (BMA) containing 0.2% D-glucose as carbon and energy source (Baumann et al., 1972). This basal medium is used in studies of carbon and energy source utilization. No organic growth factor is required; however, growth of *M. mediterranea* is favored in media containing organic compounds as the nitrogen source. The most suitable chemically defined medium for growing this species is MMM[1]. All culture media for *Marinomonas* must contain a marine salts base composition. *Marinomonas* species show an absolute requirement for Na^+ ion.

The physiological characteristics of the *Marinomonas* species are listed in Tables BXII.γ.87 and BXII.γ.88. Thirty-three strains of *M. communis* and 17 *M. vaga* strains isolated from the Pacific Ocean have been phenotypically characterized (Baumann et al., 1972). In contrast, only a single *M. mediterranea* strain—isolated from the Mediterranean Sea as its species name indicates—has been described (Solano et al., 1997). Unless otherwise indicated, the characterization of this strain has been also performed according to the protocols of Baumann et al. (1972), described as well by Gauthier and Breittmayer (1992).

Members of the genus *Marinomonas* are aerobic and use oxygen as a universal electron acceptor. No *Marinomonas* species is able to denitrify, although *M. mediterranea* is able to reduce nitrate to nitrite. *M. communis* is oxidase positive whereas the other two species are oxidase negative. In some cases permeabilization of *M. vaga* strains with toluene will allow a positive oxidase reaction to occur. It has been suggested that this result is due to a low level of cytochrome *c* in *M. vaga* cells (Baumann et al., 1984d); however, *M. mediterranea* always gives a negative result in cytochrome *c* oxidase tests, even when the cells are toluene-treated (Solano et al., 1997).

In assays for extracellular degradative activities, *Marinomonas* species give negative results, with the exception of *M. mediterranea*, which shows lipase and gelatinase activities. Gelatin hydrolysis was detected by comparison of the solidification of Marine Broth, supplemented with 12% gelatin and inoculated with *M. mediterranea*, with an uninoculated control. Under these conditions, a weak gelatinase activity was detected (Solano et al., 1997).

Marinomonas species are able to utilize acetate, but not butyrate or valerate, as a sole carbon and energy source. All of the *M. communis* and *M. vaga* strains metabolize *m*-hydroxybenzoate, *p*-hydroxybenzoate, and quinate by means of *meta* cleavage of the intermediate protocatechuate (Baumann et al., 1972). In contrast, *M. mediterranea* is unable to metabolize *m*-hydroxybenzoate. All *Marinomonas* species can use D-glucose. *Marinomonas communis* and *M. vaga* metabolize D-glucose and D-fructose via the Entner–Doudoroff pathway (Sawyer et al., 1977a). Aspartate kinase activity has been detected in these two species (Baumann and Baumann, 1974). All species can use glutamate as a sole carbon and energy source, but none can use glycine or L-tyrosine. Data for other carbon sources are listed in Tables BXII.γ.87 and BXII.γ.88.

1. MMM is a chemically defined medium containing (per liter): NaCl, 20 g; $MgSO_4 \cdot 7H_2O$, 7.0 g; $MgCl_2 \cdot 6H_2O$, 5.3 g; KCl, 0.7 g; $CaCl_2$, 1.25 g; $FeSO_4 \cdot 7H_2O$, 25 mg; $CuSO_4 \cdot 5H_2O$, 5 mg; K_2HPO_4, 75 mg; sodium glutamate, 2 g; and Tris base, 6.1g. The pH of this medium is adjusted to 7.4.

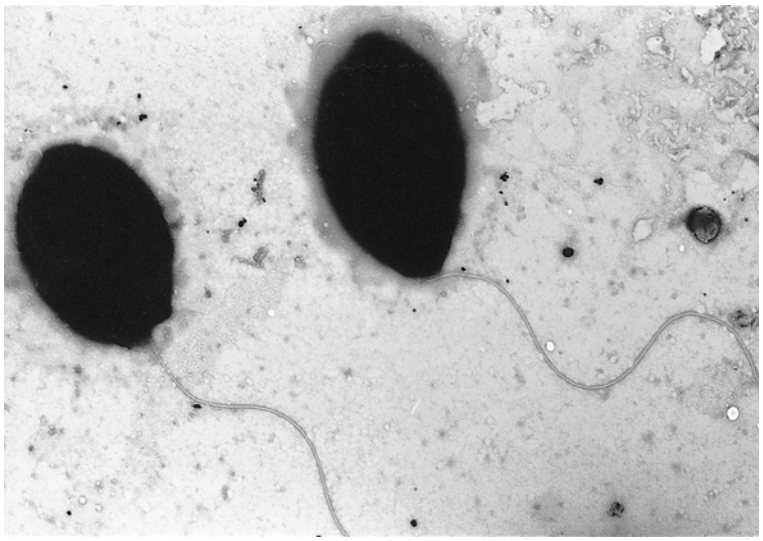

FIGURE BXII.γ.106. Electron micrograph of *Marinomonas mediterranea* MMB-1. Negatively stained with phosphotungstic acid (× 20,000).

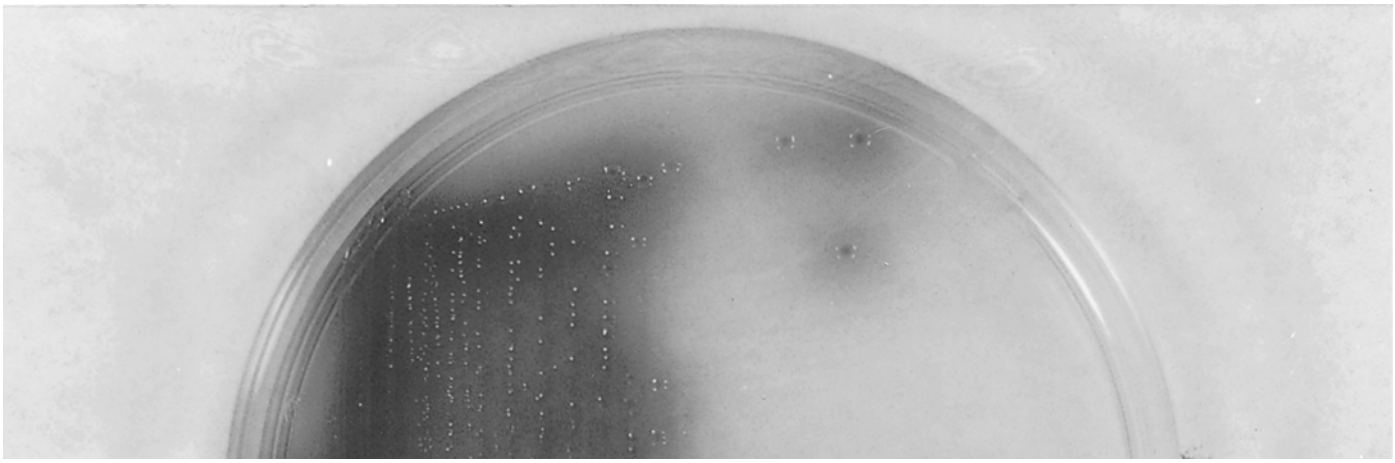

FIGURE BXII.γ.107. Colonies of *Marinomonas mediterranea* MMB-1 on Marine Agar after four days of incubation at 25°C (× 2.1).

Marinomonas communis and *M. vaga* can serve as prey for the growth of marine bdellovibrios (Taylor et al., 1974).

The mol% G + C content of the DNA of *M. communis* and *M. vaga* has been calculated using the buoyant density method. The mol% G + C of the DNA of *M. mediterranea* strain MMB-1 was determined by HPLC analysis after DNA hydrolysis and dephosphorylation (Solano et al., 1997). In this strain, the chromatogram revealed two additional peaks besides those of the four expected standard deoxynucleosides. The base modifications in the compounds giving these peaks remain unidentified, although their UV adsorption spectra suggest that they are methylated derivatives of the purine deoxynucleosides. The method used to estimate the mol% G + C content in the other two species of *Marinomonas* does not allow one to know whether this feature is shared by all the *Marinomonas* species.

Resistance of *M. mediterranea* to some antimicrobial agents was tested in order to evaluate them as genetic markers. This species was sensitive to ampicillin (50 μg/ml), chloramphenicol (10 μg/ml), gentamicin (10 μg/ml), kanamycin (40 μg/ml), streptomycin (10 μg/ml), rifampicin (50 μg/ml), and tetracycline (10 μg/ml). Resistance to the latter compound was assayed in media lacking Mg^{2+}.

TABLE BXII.γ.87. Differential characteristics of the species of the genus *Marinomonas*[a]

Characteristic	1. *M. communis*	2. *M. mediterranea*	3. *M. vaga*
Cell morphology:			
Straight rod	−	+	+
Curved rod	+	−	−
Growth at:			
5°C	−	−	−
25°C	+	+	+
35°C	+	−	+
40°C	+	−	−
NO_3^- reduction to NO_2^-	−	+	−
Cytochrome *c* oxidase	+	−	−
Pigmentation	−	+	−
Lipase	−	+	−
Gelatinase	−	+	−
Utilization of:			
D-Fructose	+	−	+
m-Hydroxybenzoate	+	−	+
α-Ketoglutarate	+	−	+

[a]Symbols: see standard definitions.

Several molecular techniques have been applied to *M. mediterranea* (Solano et al., 2000). Vectors containing the RP4 *mob* site are mobilizable by conjugation. Conjugation can be performed at 25°C on the surface of plates containing the medium LB2216[2]. Plasmids, such as pKT230, with the p15 origin of replication are stable in this species and hence can be used as cloning vectors. On the contrary, plasmids containing the *oriR6K* behave as suicidal vectors in *M. mediterranea*, which makes them useful as transposon delivery vectors. Tn*5* and Tn*10* derivatives have been assayed for this purpose. Higher transposition frequencies were obtained with Tn*10* derivatives encoding kanamycin and gentamicin resistance than with Tn*10* transposons with a different marker, or with Tn*5* transposons. Other genetic markers such as ampicillin and chloramphenicol resistance were not properly expressed in *M. mediterranea* even if they were present in plasmids. *M. mediterranea* is normally lac(−), but after transposon mutagenesis with one mini-Tn10 derivative containing the *lacZ* reporter gene, approximately 55% of the colonies showed blue coloration when grown in media containing X-Gal, thus indicating the usefulness of *lacZ* as a reporter gene in *M. mediterranea*. Mutants of *M. mediterranea* can also be obtained by nitrosoguanidine mutagenesis (Solano et al., 1997). UV radiation is not an efficient mutagen for this strain, however.

A relevant feature in *M. mediterranea* is its capacity to synthesize melanins. These pigments are made from L-tyrosine as precursor and by the involvement of the enzyme tyrosinase (EC 1.14.18.1) (Solano et al., 1997). Tyrosinase is a copper protein that belongs to the group of polyphenol oxidases (PPOs). The other important copper enzyme in this group is laccase (EC 1.10.3.2). *M. mediterranea* also shows this activity, due to a multipotent enzyme showing both tyrosinase and laccase activities (Sanchez-Amat and Solano, 1997).

Some other aerobic, Gram-negative, marine proteobacteria are able to synthesize brown to black pigments in complex media. However, aside from *M. mediterranea*, actual PPO activity has been described only in the strain 2-40 (Kelley et al., 1990; Solano and Sanchez-Amat, 1999). In some cases, melanins result from spontaneous oxidation of intermediates of L-tyrosine catabolism, such as homogentisate in *Shewanella colwelliana* (Ruzafa et al., 1994; Kotob et al., 1995). In this case, melanin-like pigments are synthesized only in aerated media with a high amount of tyrosine. These pigments, designated pyomelanins, are very similar in their chemical properties to melanins obtained by activity of the enzyme tyrosinase. To differentiate between these two possibilities, actual PPO activity should be determined spectrophotometrically or revealed by staining after PAGE (Solano et al., 1997).

A high number of *M. communis* and *M. vaga* strains have been isolated using enrichment methods with different compounds as carbon sources (Baumann et al., 1972). These isolations led to the view that *Marinomonas* is a usual component of the bacterial flora in marine waters; however, there are no data about the ecological distribution of *M. mediterranea*. The single strain of this species was detected after screening thousands of colonies of marine microorganisms for the ability to synthesize melanins. No other strains with pigmentation identical to *M. mediterranea* were detected, which suggests that it may not be very abundant. It is important to bear in mind that it is not easy to differentiate between *Marinomonas* and other aerobic marine bacteria using only phenotypic characteristics (see below). Strains for which 16S rDNA sequence analyses clearly indicate they belong to the genus *Marinomonas* have been isolated from North Sea bacterioplankton (Eilers et al., 2000). In addition, a clone showing a close relationship to the genus *Marinomonas* was detected in a 16S rDNA sequence clone library obtained from bacterial communities associated with the seagrass *Halophila stipulacea* (Weidner et al., 2000).

Enrichment and Isolation Procedures

Marinomonas species can be isolated by direct plating of seawater samples on a complex medium such as Marine Agar (Zobell, 1941). *M. communis* and *M. vaga* are able to use *m*-hydroxybenzoate, and this property can be used for an enrichment method. Seawater (500 ml) is transferred to a 2-liter Erlenmeyer flask and supplemented with 25 ml 1M Tris-HCl (pH 7.5), 0.5 g NH_4Cl, 38 mg K_2HPO_4, 14 mg $FeSO_4 \cdot 7H_2O$, and 0.5 g *m*-hydroxybenzoate. The culture is incubated at 20–25°C for up to 10 d. When growth is observed, the culture is streaked onto BMA containing 0.1% (w/v) *m*-hydroxybenzoate in order to obtain pure cultures (Baumann et al., 1984d).

Maintenance Procedures

Long-term preservation of *Marinomonas* species can be achieved by lyophilization. A suitable protocol for preparation of the cells has been described by Gauthier and Breittmayer (1992). The lyophilized cultures are reconstituted by adding 0.5 ml of Marine Broth. A few drops are streaked onto Marine Agar and the remaining is transferred to a tube containing 4 ml of Marine Broth. It is advisable to avoid high aeration of the culture during the first hours of incubation. Growth is observed after 2–3 d.

An alternative method of preservation is freezing. Glycerol is added to an overnight culture in Marine Broth to a final concentration of 20% and the culture is immediately stored at −75°C. Using this protocol, frozen *M. mediterranea* cells have remained viable for more than five years.

Strains can be maintained by serial transfer on Marine Agar for routine work in the lab. After 2–3 days of growth at 25°C, the plates can be preserved for 3–4 weeks at 15°C. It is not recommended to keep the cultures at 4°C, because viability is lost much faster than when the cultures are stored at 15°C.

2. LB2216 is obtained by mixing—after autoclaving—equal amounts of LB medium (containing 1.5% NaCl) and Marine Agar 2216.

TABLE BXII.γ.88. Other characteristics of the species of the genus *Marinomonas*[a]

Characteristics	1. *M. communis*[b]	2. *M. mediterranea*	3. *M. vaga*[b]
Motility	+	+	+
Flagellar arrangement:			
Polar	+	+	+
Peritrichous	−	−	−
Anaerobic growth	−	−	−
Na^+ requirement	+	+	+
Poly-β-hydroxybutyrate accumulation	−	−	−
Denitrification	−	−	−
Luminescence	−	−	−
Amylase	−	−	−
Utilization of:			
D-Glucose, D-mannose, D-sorbitol, citrate, acetate, succinate, malate, glycerol	+	+	+
DL-β-Hydroxybutyrate	+	+	d
β-Alanine, L-serine, L-lysine, L-arginine, spermine	+	nd	d
Ethanol, propanol	+	nd	−
D-Galactose, L-rhamnose, L-arabinose	d	nd	+
Maltose, propionate	d	−	d
DL-Glycerate, ethanolamine	d	nd	−
Cellobiose, *N*-acetylglucosamine, erythritol, heptanoate	−	nd	+
Lactose, butyrate, valerate, methanol, L-glycine, L-tyrosine	−	−	−
D-Ribose, D-arabinose, caproate, caprylate, pelargonate, ribitol, trigonelline	−	nd	d
Butanol	−	nd	−
Mol% G + C of DNA	45.9–48	46.3 ± 0.9	46.4–49.3

[a]Symbols: see standard definitions; nd, not determined.

[b]*M. communis* and *M. vaga* are able to use the following compounds as principal sources of carbon and energy: D-gluconate, fumarate, DL-lactate, aconitate, D-mannitol, γ-aminobutyrate, sarcosine, putrescine, saccharate, pyruvate, *m*-inositol, L-alanine, D-alanine, L-ornithine, L-histidine, L-proline, betaine.

The following compounds are not used as sole or principal sources of carbon and energy by *Marinomonas communis* or *M. vaga*: sucrose, melibiose, salicin, D-fucose, inulin, cellulose, mucate, L-threonine, L-leucine, L-isoleucine, L-valine, L-tryptophan, D-tryptophan, L-norleucine, DL-α-aminobutyrate, DL-α-aminovalerate, δ-aminovalerate, formate, isobutyrate, isovalerate, oxalate, malonate, maleate, glutarate, adipate, pimelate, suberate, azelate, sebacate, D-tartrate, *meso*-tartrate, glycolate, levulinate, D-mandelate, L-mandelate, citraconate, itaconate, mesaconate, ethyleneglycol, 2,3-butanediol, methanol, isopropanol, isobutanol, *n*-butanol, propyleneglycol, benzoylformate, DL-kynurenine, kynurenate, anthranilate, *m*-aminobenzoate, *p*-aminobenzoate, methylamine, benzylamine, histamine, tryptamine, butylamine, α-amylamine, 2-amylamine, pentylamine, creatine, hippurate, pantothenate, acetamide, nicotinate, nicotinamide, allantoin, adenine, guanine, cytosine, thymine, uracil, *n*-dodecane, phenylethanediol, phenol, naphthalene.

11–89% of the strains included in *M. communis* and *M. vaga* are able to use the following compounds as principal sources of carbon and energy: D-xylose, D-trehalose, D-glucuronate, D-galacturonate, caprate, L-tartrate, L-aspartate, L-phenylalanine, L-citrulline.

Differentiation of the genus *Marinomonas* from other genera

The characteristics that differentiate *Marinomonas* species from other aerobic, marine, Gram-negative bacteria are listed in Table BXII.γ.89. Further characterization of more strains might help to establish other phenotypic criteria for differentiating these genera; however, all of these genera share many physiological and biochemical features and their differentiation can be problematic. In this regard, some chemotaxonomic markers such as isoprenoid quinone and fatty acid composition have been tested (Akagawa-Matsushita et al., 1992a; Ivanova et al., 2000c). Molecular analysis, such as rRNA–DNA hybridization and particularly rDNA sequencing, have shown that *Marinomonas* species form a well-defined cluster (Van Landschoot and De Ley 1983; Solano and Sanchez-Amat, 1999). 16S rDNA sequencing of additional *Marinomonas* strains could be useful for designing nucleic acid probes for the identification and quantification in natural habitats of microorganisms belonging to this genus.

Taxonomic Comments

There are many Gram-negative, nonfermentative, heterotrophic, marine bacteria with polar flagella. Those with mol% G + C contents below 50 were temporarily assigned to the genus *Alteromonas*, and those with a mol% G + C of 57–64 were included in the genus *Pseudomonas* (Baumann et al., 1972). Further characterization of the genus *Alteromonas* revealed a great diversity

TABLE BXII.γ.89. Differential characteristics of the genus *Marinomonas* and other aerobic, Gram-negative, marine proteobacteria with polar flagellation[a]

Characteristic	*Marinomonas*	*Oceanospirillum*[b]	*Alteromonas*[c]	*Marinobacterium*[d]	*Marinobacter*[e]	*Neptunomonas*[f]	*Pseudoalteromonas*[c]	*Pseudomonas*[g]
Poly-β-hydroxybutyrate granules	−	+	−	−	−	+	−	D
Amylase	−	−	+	−	−	−	+[h]	D
Utilization of:								
Carbohydrates	+	−	+	+	−	+	+	+
Malate	+	D	−	+		−	−	+
D-Sorbitol	+		−		−	−	−	−
Mol% G + C of DNA	45–50	42–51	44–47	54.9	52.7	46	37–50	55–64

[a]For symbols see standard definitions.

[b]Data from Krieg (1984b).

[c]Data from Gauthier et al. (1995a).

[d]Data from González et al. (1997).

[e]Data from Gauthier and Brittmayer (1992).

[f]Data from Hedlund et al. (1999a).

[g]Data from Baumann et al. (1972, 1983b).

[h] *Pseudoalteromonas nigrifaciens* is negative.

(Van Landschoot and De Ley, 1983; Gauthier et al., 1995a). Two of the species originally included in the genus *Alteromonas* were *A. communis* and *A. vaga*. Immunological analysis of Fe-containing superoxide dismutase and glutamine synthetase established a relationship between these species and the genus *Oceanospirillum*, and hence their assignment to this genus under the species names *Oceanospirillum commune* and *O. vagum* was proposed (Bowditch et al., 1984a). DNA–rRNA hybridization studies applied to some Gram-negative bacteria, including those initially assigned to the genus *Alteromonas*, revealed that *A. communis* and *A. vaga* constituted a separate rRNA branch (Van Landschoot and De Ley, 1983). Taking into consideration this fact and also phenotypic traits, the genus *Marinomonas* was created to contain the two species as *M. communis* and *M. vaga*. Phylogenetic analysis based on 16S rDNA sequences of these two species and a new strain, MMB-1, revealed that they were closely related, with percentage identities greater than 95% (Fig. BXII.γ.108). These data supported creation of the genus *Marinomonas*, in which the strain MMB-1 constituted the third species, namely *M. mediterranea* (Solano and Sanchez-Amat, 1999). The data also showed that these species were more closely related to members of the genus *Oceanospirillum* than to the species remaining in the modified genus *Alteromonas* or *Pseudoalteromonas*.

Further Reading

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Gauthier, M.J. and V.A. Breittmayer. 1992. The genera *Alteromonas* and *Marinomonas*. *In* Balows, Trüper, Dworkin, Harder and Schleifer (Editors), The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, 2nd Ed., Vol. 3, Springer-Verlag, New York. 3046–3070.

Solano, F. and A. Sanchez-Amat. 1999. Studies on the phylogenetic relationships of melanogenic marine bacteria: proposal of *Marinomonas mediterranea* sp. nov. Int. J. Syst. Bacteriol. *49*: 1241–1246.

Van Landschoot, A. and J. De Ley. 1983. Intra- and intergeneric similarities of the rRNA cistrons of *Alteromonas*, *Marinomonas* (gen. nov.) and some other Gram-negative bacteria. J. Gen. Microbiol. *129*: 3057–3074.

Differentiation of the species of the genus *Marinomonas*

Table BXII.γ.87 shows the main differential characteristics of the three *Marinomonas* species described. Other characteristics are listed in Table BXII.γ.88.

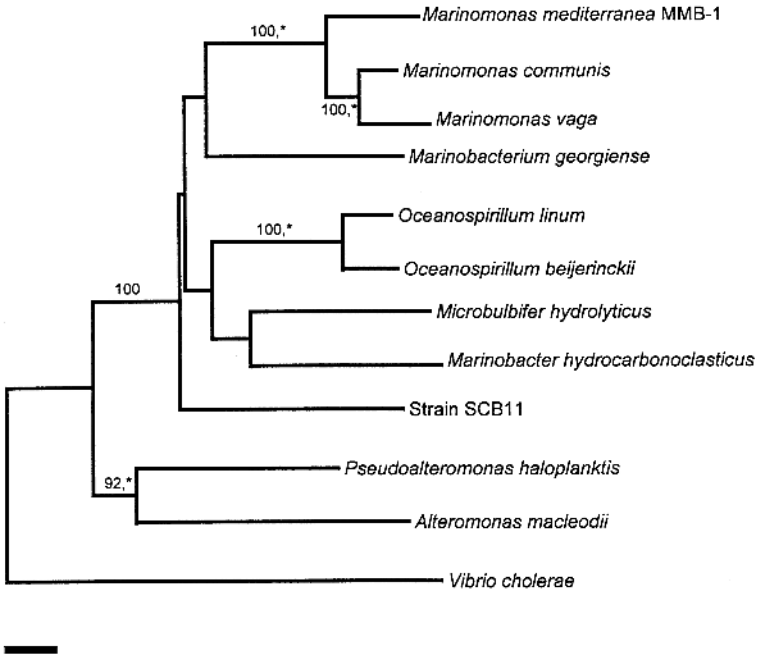

FIGURE BXII.γ.108. Phylogenetic position of *Marinomonas mediterranea* strain MMB-1 in the context of other marine bacteria. The tree was generated by the neighbor-joining method from complete 16S rDNA sequences. Numbers indicate the support for these branches by bootstrap analysis. Asterisks indicate branches also identified by maximum parsimony method. Only values higher than 90% are included (bar = 1% dissimilarity). (Reprinted with permission from F. Solano and A. Sanchez-Amat, International Journal of Systematic Bacteriology *49:* 1241–1246, 1999 ©International Union of Microbiological Societies.)

List of species of the genus Marinomonas

1. **Marinomonas communis** (Baumann, Baumann, Mandel and Allen 1972) Van Landschoot and De Ley 1984, 91[VP] (Effective publication: Van Landschoot and De Ley 1983, 3071) (*Alteromonas communis* Baumann, Baumann, Mandel and Allen 1972, 420.)
com.mu′nis. L. adj. *communis* common.

The characteristics are as described for the genus and as listed in Tables BXII.γ.87 and BXII.γ.88. Curved rods, 0.7–1.5 × 1.8–3.0 μm. Motile by means of polar flagella at one or both poles. Oxidase positive. Metabolize *m*-hydroxybenzoate, *p*-hydroxybenzoate, and quinate, but not benzoate or *o*-hydroxybenzoate, by means of *meta* cleavage of the intermediate protocatechuate.

The mol% G + C of the DNA is: 45–48 (Bd).
Type strain: ATCC 27118, DSM 5604, LMG 2864.

2. **Marinomonas mediterranea** Solano and Sanchez-Amat 1999, 1245[VP]
med.i.terr.an′e.a. L. gen. n. *mediterranea* of the Mediterranean, referring to the isolation of the type strain from the Mediterranean Sea in the region of Murcia, on the southeastern Spanish coast.

The characteristics are as described for the genus and as listed in Tables BXII.γ.87 and BXII.γ.88. Gram-negative rods, 0.5–0.9 × 1.1–1.8 μm. Motile by a single polar flagellum. Cytochrome oxidase negative. No denitrification occurs. Lipase positive; gelatinase weakly positive. Utilize D-glucose, D-mannose, D-sorbitol, citrate, β-hydroxybutyrate, succinate, glycerol, and malate. Produce melanin. Contain a pluripotent polyphenol oxidase and a tyrosinase activated by SDS.

The mol% G + C of the DNA is: 46.3 ± 0.9 (HPLC).
Type strain: MMB-1, ATCC 700492, CECT 4803.
GenBank accession number (16S rRNA): AF063027.

3. **Marinomonas vaga** (Baumann, Baumann, Mandel and Allen 1972) Van Landschoot and De Ley 1984, 91[VP] (Effective publication: Van Landschoot and De Ley 1983, 3071) (*Alteromonas vaga* Baumann, Baumann, Mandel and Allen 1972, 420.)
va′ga. L. adj. *vaga* wandering.

The characteristics are as described for the genus and as listed in Tables BXII.γ.87 and BXII.γ.88. Straight rods 0.7–1.5 × 1.8–3.0 μm. Motile by means of polar flagella at one or both poles. Oxidase negative. Metabolize *m*-hydroxybenzoate, *p*-hydroxybenzoate and quinate, but not benzoate or *o*-hydroxybenzoate, by means of *meta* cleavage of the intermediate protocatechuate.

The mol% G + C of the DNA is: 46–50 (Bd).
Type strain: ATCC 27119.
GenBank accession number (16S rRNA): X67025.

Genus IV. **Marinospirillum** *Satomi, Kimura, Hayashi, Shouzen, Okuzumi and Fujii 1998, 1346*^{VP}

MASATAKA SATOMI, BON KIMURA AND TATEO FUJII

Ma.ri.no.spi.ril' lum. L. adj. *marinus* of the sea; Gr. n. *spira* a spiral; M.L. dim. neut. n. *spirillum* a small spiral; *Marinospirillum* a small spiral from the sea.

Helical cells. Motile by bipolar tufts of flagella. Thin-walled coccoid bodies are formed in aging cultures. Endospores are not formed. Aerobic, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. **Na^+ is required for growth.** Chemoheterotrophic. **Accumulate poly-β-hydroxybutyrate. Oxidase positive. Carbohydrates are neither oxidized nor fermented.** The major isoprenoid quinone type is Q-8. Belongs to the class *Gammaproteobacteria*. One species has been isolated from putrid infusions of marine mussels; another has been isolated from kusaya gravy.

The mol% G + C of the DNA is: 42–45.

Type species: **Marinospirillum minutulum** (Watanabe 1959) Satomi, Kimura, Hayashi, Shouzen, Okuzumi and Fujii 1998, 1346 (*Oceanospirillum minutulum* (Watanabe 1959) Hylemon, Wells, Krieg and Jannasch 1973, 373; *"Spirillum minutulum"* Watanabe 1959, 83.)

FURTHER DESCRIPTIVE INFORMATION

Because *Marinospirillum minutulum*—like most members of the genus *Oceanospirillum*—was isolated from putrid infusions of marine mussels, its source is most likely marine mud adherent to the mussels (Terasaki, 1970).

Marinospirillum megaterium was isolated from kusaya gravy (Fujii et al., 1990), which is used for producing Japanese traditional dried fish, and its true ecological niche is unknown. Under microscopic observation, this species is usually observed as the dominant microbial population in the gravy, characterized by large helical cells, but is unculturable on agar surfaces. The main chemical characteristics of kusaya gravy are as follows: salinity of approximately 3% NaCl concentration, low dissolved oxygen concentration, and large amounts of volatile basic nitrogen compounds (200–500 mg/100 g) (Satomi et al., 1997).

ENRICHMENT AND ISOLATION PROCEDURES

Terasaki (1970) enriched and isolated *M. minutulum* using the following methods. Marine mussels were smashed with a hammer and placed in a Petri dish with a teaspoon of marine mud. Sterilized seawater was poured into the dish until the mussels sank completely in the solution. The infusion was incubated at 27–28°C and examined for the development of spirilla after 1, 2, and 7 d. Isolation was accomplished by streaking dilutions onto suitable agar media.

Fujii et al. (1990) enriched and isolated *M. megaterium* as follows. A loopful of kusaya gravy was used to inoculate the inlet tube of a horizontal glass tube, in which the inlet and outlet ends were bent upwards. After an appropriate time, the isolates accumulated predominantly in the outlet because of their high motility. By repeating this selective accumulation and purification process, the isolates could almost be purified (by microscopic observation). Isolation was accomplished by streaking a large amount of cells onto the suitable agar medium, because the ability of *M. megaterium* to form colonies on an agar surface was low. The ability to form colonies on the agar surface was lost after subsequent transfers of the culture.

MAINTENANCE PROCEDURES

Marinospirillum species may be maintained on stab cultures in TSSY semi-solid agar, which has the following composition (g/l): Trypticase peptone (BBL), 17.0; Phytone peptone (BBL), 3.0; Yeast nitrogen base (Difco), 1.0; NaCl, 30.0; and agar, 2.0; pH 8.0. Cultures are incubated at 25°C with biweekly transfer. Long-term preservation of *M. minutulum* by freeze-drying has been reported by Terasaki (1975).

PROCEDURES FOR TESTING SPECIAL CHARACTERS

Characterization methods for marinospirilla have been described in detail by Terasaki (1972, 1973) and Satomi et al. (1998). Media containing natural or artificial seawater or 2.5–3.0% sodium chloride must be used for all characterization tests. Media for *M. megaterium* must be supplemented with 0.1–0.3% agar because the organism requires semisolid media for growth.

DIFFERENTIATION OF THE GENUS *MARINOSPIRILLUM* FROM OTHER GENERA

The genus *Marinospirillum* cannot be differentiated phenotypically from *Oceanospirillum*, but each *Marinospirillum* species can be differentiated from *Oceanospirillum*, by positive nitrate reduction (*M. minutulum*) and a prominent microaerophilic nature with no growth on agar surfaces (*M. megaterium*).

TAXONOMIC COMMENTS

Phylogenetic analysis based on 16S rDNA gene sequences of the genus *Marinospirillum*, consisting of two species *M. minutulum* and *M. megaterium*, showed that this genus was closely related to the genera *Oceanospirillum* and *Halomonas* in the *Gammaproteobacteria* group (Fig. BXII.γ.109). Although members of the genera *Marinospirillum* and *Oceanospirillum* share helical cell morphology, there is a large phylogenetic distance between *Marinospirillum* and *Oceanospirillum*.

DIFFERENTIATION OF THE SPECIES OF THE GENUS *MARINOSPIRILLUM*

Table BXII.γ.90 lists the characteristics that differentiate *M. minutulum* from *M. megaterium*.

List of species of the genus Marinospirillum

1. **Marinospirillum minutulum** (Watanabe 1959) Satomi, Kimura, Hayashi, Shouzen, Okuzumi and Fujii 1998, 1346^{VP} (*Oceanospirillum minutulum* (Watanabe 1959) Hylemon, Wells, Krieg and Jannasch 1973, 373; *"Spirillum minutulum"* Watanabe 1959, 83.)
mi.nu' tu.lum. L. dim. neut. adj. *minutulum* very little.

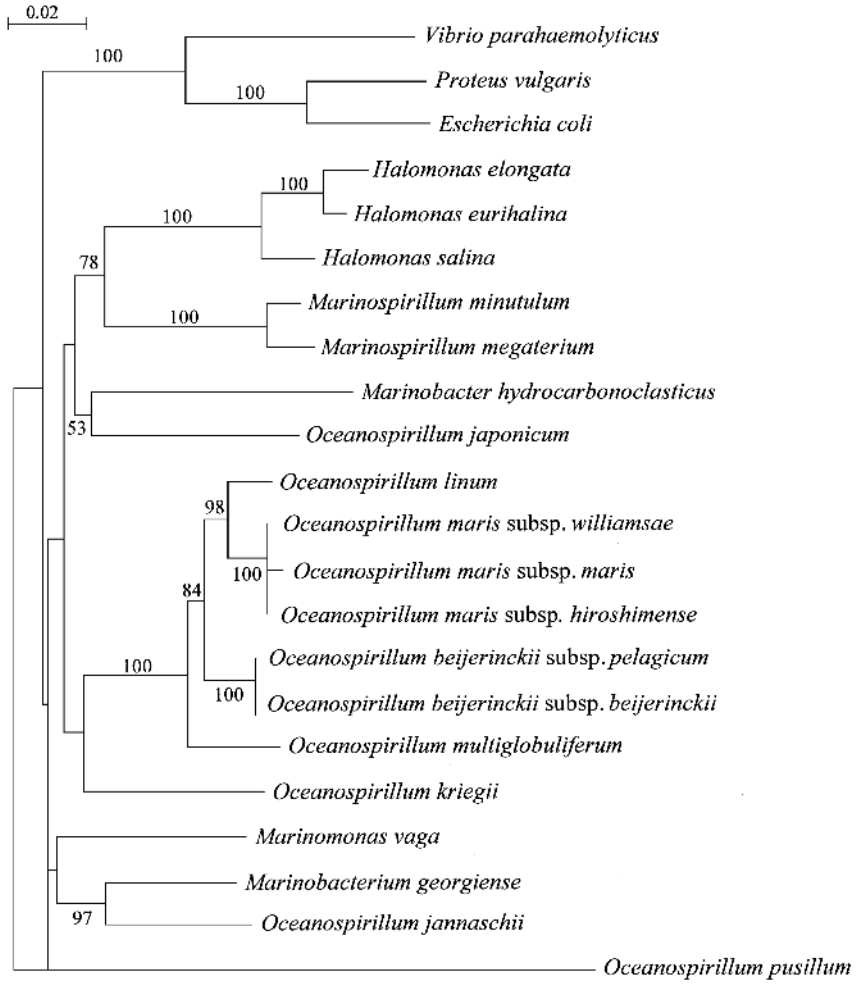

FIGURE BXII.γ.109. Phylogenetic trees of the genus *Marinospirillum* and related bacteria based on the nucleotide sequences of the 16S rRNA gene. The tree was constructed using the neighbor-joining method, and the nucleotide substitution rates (*K*nuc values) were computed by Kimura's 2-parameter model. The scale bar indicates the genetic distance of 0.02 *K*nuc. The numbers at the nodes indicate the percentages of occurrence in 1000 bootstrapped trees. Only values greater than 40% are shown. *Oceanospirillum pusillum* was used as an outgroup. Accession numbers for sequences used: *Marinospirillum minutulum* (AB006769), *M. megaterium* (AB006770), *Oceanospirillum linum* (M22365), *O. maris* subsp. *maris* (AB006771), *O. maris* subsp. *hiroshimense* (AB006762), *O. maris* subsp. *williamsae* (AB006763), *O. beijerinckii* subsp. *beijerinckii* (AB006760), *O. beijerinckii* subsp. *pelagicum* (AB006761), *O. multiglobuliferum* (AB006764), *O. japonicum* (AB006766), *O. kriegii* (AB006767), *O. jannaschii* (AB006765), *O. pusillum* (AB006768), *Halomonas elongata* (X67023), *Halomonas eurihalina* (X87218), *Halomonas salina* (X87217), *Marinomonas vaga* (X67025), *Marinobacter hydrocarbonoclasticus* (X67022), *Marinobacterium georgiense* (U58339), *Vibrio parahaemolyticus* (X56580), *Escherichia coli* (J01859), *Proteus vulgaris* (X07652).

TABLE BXII.γ.90. Characteristics differentiating the species of the genus *Marinospirillum*[a,b]

Characteristic	1. *M. minutulum*[c]	2. *M. megaterium*[d]
Cell diameter, μm	0.3–0.4	0.8–1.2
Microaerophilic	−	+
Catalase	+	− or W
Nitrate reduced to nitrite	+	−
Growth in the presence of:		
1% oxgall	+	−
1% glycine	+	−

[a]Symbols: see standard definitions.

[b]Data from Satomi et al. (1998).

[c]ATCC 19193.

[d]Strains H7 and Sp5.

Rigid, helical cells with clockwise turns (Terasaki, 1973). Cell diameter, 0.3–0.4 μm; wavelength, 2.0–2.8 μm; helix diameter, 0.6–1.5 μm; length of helix, 3.0–8.0 μm. Coccoid bodies are predominant at 3–4 weeks but not after 24–48 h growth. NaCl is required for growth: optimum concentration, 2.5–3.0%; range 0.2–10%. Optimum temperature, 25–30°C; range, 4–30°C. Optimum pH, 8.0; range, 7.0–10.5. Aerobic. Grow in liquid media under static or aerobic conditions with shaking, or on plates. Catalase positive. Do not hydrolyze gelatin, hippurate, or starch. Reduce nitrate to nitrite. Do not produce DNase, RNase, urease, or phosphatase. The type strain was isolated from putrid infusions of marine mussels.

The mol% G + C of the DNA is: 42–44 (HPLC).
Type strain: ATCC 19193, DSM 6287.
GenBank accession number (16S rRNA): AB006769.

2. **Marinospirillum megaterium** Satomi, Kimura, Hayashi, Shouzen, Okuzumi and Fujii 1998, 1346^{VP}
me.ga.te' ri.um. Gr. adj. *mega* large; Gr. n. *teras, teratis* monster, beast; M.L. n. *megaterium* big beast.

Rigid, helical cells, 0.8–1.2 × 5–15 μm, having clockwise turns; thin-walled coccoid bodies 2.0–2.5 μm in diameter. NaCl is required for growth: optimum concentration, 3%; range, 0.5–9.0%. Optimum temperature, 20–25°C; range, 4–25°C. Optimum pH, 8; range, 7.5–9.0. Microaerophilic: no growth occurs in liquid media under static or aerobic conditions with shaking, or on plates. Catalase weakly positive or negative. Do not hydrolyze gelatin, hippurate, or starch. Do not reduce nitrate. Do not produce DNase, RNase, urease, or phosphatase. The type strain was isolated from kusaya gravy.

The mol% G + C of the DNA is: 44–45 (HPLC).
Type strain: H7, JCM 10129.
GenBank accession number (16S rRNA): AB006770.

Genus V. **Neptunomonas** *Hedlund, Geiselbrecht, Bair and Staley 1999b, 1325^{VP} (Effective publication: Hedlund, Geiselbrecht, Bair and Staley 1999a, 258)*

BRIAN P. HEDLUND

Nep.tu.no.mo' nas. Rom. myth n. *Neptune* the Roman god of the sea; Gr. n. *monas* a unit, monad; M.L. n. *Neptunomonas* Neptune's monad.

Rod-shaped or slightly curved bacteria. Approximately 0.7–0.9 × 2.0–3.0 μm. (See Fig.BXII.γ.110). Cells may produce a capsule that is visible by India ink staining. Coccoid bodies may predominate in old cultures and are associated with a loss of viability. Poly-β-hydroxybutyrate is accumulated and may form small inclusions that are visible by phase-contrast microscopy. **Motile by a single polar flagellum. Aerobic; however, fermentation tests with some sugars and sugar alcohols are weakly positive. Acid is produced slowly from some carbohydrates. $Na^+$is required for growth. Oxidase and catalase positive.** Phosphatase positive. **Utilize some carbohydrates, polycyclic aromatic hydrocarbons**, amino acids, organic acids, and sugar alcohols as sole carbon sources for chemoorganotrophic growth. Temperature range for known strains, ≤4–30°C. Indigenous to coastal marine sediments. Belong to the *Oceanospirillum* group of the class *Gammaproteobacteria* based on phylogenetic analyses of 16S rDNA gene sequences.

The mol% G + C of the DNA is: 46.

Type species: **Neptunomonas naphthovorans** Hedlund, Geiselbrecht, Bair and Staley 1999b, 1325 (Effective publication: Hedlund, Geiselbrecht, Bair and Staley 1999a, 258.)

FURTHER DESCRIPTIVE INFORMATION

Only one species of *Neptunomonas* is currently known, *N. naphthovorans.*

Polycyclic aromatic hydrocarbon (PAH) catabolism by *N. naphthovorans* strains NAG-2N-113 and NAG-2N-126 has been studied in some detail (Hedlund et al., 1999a). Both strains grow on naphthalene or 2-methylnaphthalene as a sole carbon and energy source. Only strain NAG-2N-113 grows weakly on phenanthrene. Both strains degrade 1-methylnaphthalene and acenaphthene under certain conditions; however, the degradation of these molecules is not coupled to growth. Colonies produce indigo when

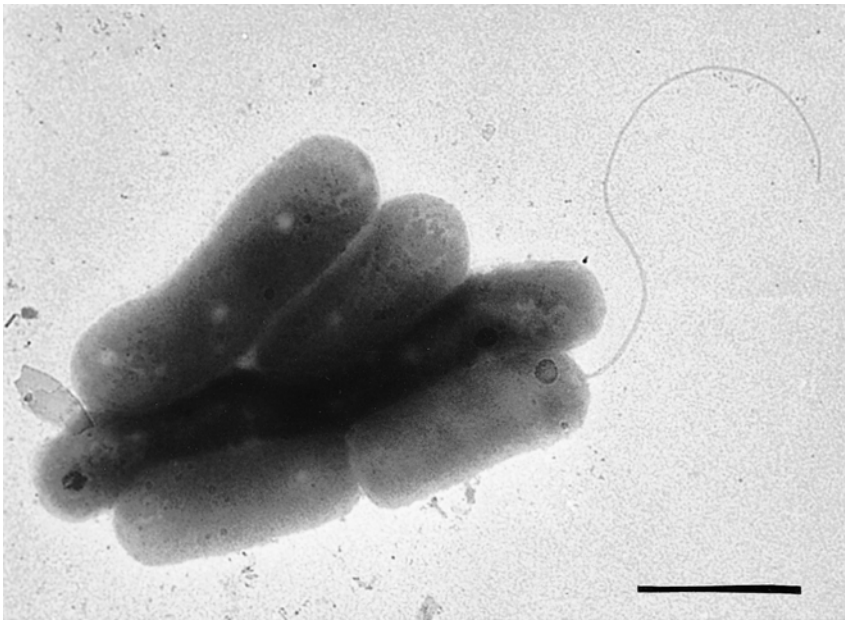

FIGURE BXII.γ.110. Electron micrograph of negatively stained *Neptunomonas naphthovorans* strain NAG-2N-126. Bar = 2 μm. (Reproduced with permission from B. Hedlund et al., Applied and Environmental Microbiology *65:* 251–259, 1999 ©American Society for Microbiology, Washington, D.C.)

naphthalene-induced cells are exposed to indole vapors, a characteristic *Neptunomonas* shares with naphthalene-degrading strains of *Pseudomonas*, *Burkholderia*, and *Marinobacter*, among others. This reaction is catalyzed by the naphthalene dioxygenase. A portion of the gene encoding the naphthalene dioxygenase large subunit has been sequenced from strains NAG-2N-113 and NAG-2N-126; they bear roughly 50–65% sequence identity to *Pseudomonas* and *Burkholderia* naphthalene dioxygenases. However, the low mol% G + C for the *Neptunomonas* dioxygenase gene indicates it is not newly acquired from organisms with a high mol% G + C by horizontal gene transfer. *Neptunomonas* does not produce colored intermediates characteristic of the *meta*-cleavage pathway when grown on naphthalene or other aromatic compounds.

A variety of carbon sources are used by *Neptunomonas* as sole carbon and energy sources, including naphthalene, 2-methylnaphthalene, *p*-hydroxybenzoate, D-fructose, D-glucose, citrate, DL-β-hydroxybutyrate, glutarate, succinate, DL-lactate, pyruvate, L-arginine, L-serine, L-glutamate, acetate, L-proline, DL-alanine, glycerol, mannitol, and D-arabitol.

All strains of this species have been isolated from a single area in Puget Sound near Seattle, WA USA.

Enrichment and Isolation Procedures

Neptunomonas strains have been isolated from coastal marine sediment contaminated with coal tar creosote, which consists mainly of polycyclic aromatic hydrocarbons. Two alternative methods have been used. The first method involves using a most probable number (MPN) approach to enumerate marine bacteria capable of growth on naphthalene and subsequently obtaining isolates from the positive MPN tubes. All manipulations have been done at 4°C with media that have been equilibrated to that temperature for several hours, but whether this is a necessary precaution is not yet known. Serial dilutions (10-fold) of the sediment are made into 5- or 10-ml portions of the artificial seawater solution ONR7a,[1] and naphthalene crystals are added as a sole carbon and energy source. The tubes are typically incubated near *in situ* temperature without shaking and monitored daily for turbidity. It should be noted that *Neptunomonas* strains do not remain viable for long periods in ONR7a with naphthalene; therefore, sampling the dilution tubes within days of the culture becoming turbid is recommended. Samples from positive tubes are diluted with ONR7a, and the dilutions are spread onto plates of ONR7a solidified with 0.8% agarose or onto a complex marine medium, such as Marine Broth 2216 solidified with 1.5% agar. Naphthalene crystals are added to the Petri dish lids, the plates are inverted, and the plates are sealed with Parafilm. If *Neptunomonas* is outnumbered by other naphthalene-degrading bacteria such as *Cycloclasticus*, *Neptunomonas* strains can be selected based on their ability to grow well in complex media such as Difco Marine Broth 2216.

A second isolation method employs a direct plating approach. Sediment is diluted into ONR7a and spread onto plates of ONR7a solidified with 0.8% agarose. Naphthalene crystals are added to the Petri dish lids, the plates are inverted, and then sealed.

Maintenance Procedures

Strains of *Neptunomonas* can be maintained for up to 1 month at 4°C on Parafilm-sealed plates of solidified Difco Marine Broth 2216. Additionally, freezer stocks containing glycerol or DMSO can be stored at −80°C for at least 2 years. Lyophilized cultures have also been revived; however, their long-term viability has not yet been evaluated.

Differentiation of the genus *Neptunomonas* from other genera

The characteristics that differentiate *Neptunomonas* from phylogenetically similar genera are listed in Table BXII.γ.91.

Several other genera of naphthalene-degrading marine bacteria exist, including *Cycloclasticus* (Dyksterhouse et al., 1995), *Marinobacter* (Gauthier et al., 1992), *Vibrio* (Geiselbrecht et al., 1996), *Pseudoalteromonas* (Hedlund et al. 1996a), and *Sphingomonas* (Zylstra, personal communication). Differential characteristics of these bacteria are shown in Table BXII.γ.92. However, it is generally recommended that 16S rRNA genes be sequenced in order to confidently identify new isolates of marine PAH-degraders.

Taxonomic Comments

Based on phylogenetic analyses using nearly complete 16S rRNA gene sequences, *Neptunomonas* is a member of the *Oceanospirillum* group of the class *Gammaproteobacteria*. However, the exact phylogenetic relationship among *Neptunomonas*, *Oceanospirillum*, *Marinomonas*, and *Marinobacterium* is uncertain, as evidenced by the low bootstrap value for the node connecting *Neptunomonas* to the tree (Fig. BXII.γ.111).

The phylogeny and taxonomy of the *Oceanospirillum* group is further complicated by nomenclatural problems associated with some members of the group. Four species, *O. linum*, *O. maris*, *O. beijerinckii*, and *O. multiglobuliferum* form a monophyletic cluster based on rRNA hybridization (Pot et al., 1989), multilocus enzyme electrophoresis (Pot et al., 1989), quinone analysis (Sakane and Yokota, 1994), fatty acid analysis (Sakane and Yokota, 1994), and 16S rRNA gene phylogenetic analysis (Hedlund et al., 1999a). Other taxa, such as *O. kriegii*, and *O. jannaschii*, are not as closely related by any measurement, and it is likely each will be assigned to a new genus. *O. japonicum* is phylogenetically distinct from the true members of the genus *Oceanospirillum*; however, it does share many phenotypic similarities with them. *O. pusillum* obviously should not be included in the genus *Oceanospirillum* since it belongs to the class *Alphaproteobacteria* (Satomi et al., 1998). Therefore, the taxonomy of the *Oceanospirillum* group will likely change dramatically in the near future.

Further Reading

Hedlund, B.P., A.D. Geiselbrecht, T.J. Bair and J.T. Staley. 1999. Polycyclic aromatic hydrocarbon degradation by a new marine bacterium, *Neptunomonas naphthovorans* gen. nov., sp. nov. Appl. Environ. Microbiol. *65*: 251–259.

1. ONR7a consists of several solutions that are prepared separately and mixed when cool. First, a 10× seawater salts solution is prepared, consisting of (g/l distilled water): NaCl, 228; Na_2SO_4, 40; KCl, 7.2; NaBr, 0.83, $NaHCO_3$, 0.31; H_3BO_3, 0.27; and NaF, 0.026. This 10× solution is diluted to 1×, and the following are added (g/l 1× solution): $Na_2HPO_4 \cdot 7H_2O$, 0.089; NH_4Cl, 0.27; and TAPSO buffer (3-[N-tris(hydroxymethyl)methylamino]-2-hydroxypropanesulfonic acid), 1.3. After the solution has been autoclaved and cooled to at least 50°C, 20.0 ml of a 50× divalent cation solution and 5.0 ml of a 200× Fe(II) solution are added per liter. The 50× divalent cations solution, which is prepared and autoclaved separately, contains (g/l): $MgCl_2 \cdot 6H_2O$, 559.1; $CaCl_2 \cdot 2H_2O$, 72.8; and $SrCl_2 \cdot 6H_2O$, 1.21. The 200× solution of Fe(II) is prepared by dissolving 0.04 g of $FeCl_2 \cdot 4H_2O$ with a few drops of concentrated HCl; water is then added to 100 ml, and the solution is sterilized by filtration. Long-term storage of the complete ONR7a solution is not recommended.

List of species of the genus Neptunomonas

1. **Neptunomonas naphthovorans** Hedlund, Geiselbrecht, Bair and Staley 1999b, 1325[VP] (Effective publication: Hedlund, Geiselbrecht, Bair and Staley 1999a, 258.)
naph.tho.vo′rans. Chem. n. *naphtho-* combining form of *naphthalene*, a white, crystalline hydrocarbon; L. v. *voro* to devour; M.L. part. adj. *naphthovorans* naphthalene-devouring.

The characteristics are as described for the genus and as listed in Table BXII.γ.91. The type strain was isolated from Eagle Harbor, a creosote-contaminated EPA Superfund site in Puget Sound, Washington, USA.

The mol% G + C of the DNA is: 46.3 (T_m).

Type strain: NAG-2N-126, ATCC 700637.

GenBank accession number (16S rRNA): AF053734.

TABLE BXII.γ.91. Characteristics differentiating the genus *Neptunomonas* from related genera[a]

Characteristic	*Neptunomonas*	*Marinobacterium*	*Oceanospirillum*[b]	*O. japonicum*	*O. kriegii*
Cell shape:					
Curved rods	+				+
Straight rods	+	+			+
Spirilla			+	+	
Flagellar arrangement	single polar	single polar	bipolar tufts	bipolar or single tufts	single polar
Cell diameter, μm	0.7–0.9	0.5–0.7	0.3–1.2	0.8–1.4	0.8–1.2
Poly-β-hydroxybutyrate accumulation	+	−	+	+	+
NO_3^- reduced to NO_2^-	−	−	D	−	−
Catalase	+	+	D	w or −	NR
Amylase	−	−	−	−	NR
Gelatinase	−	−	D	d	NR
Lipase	−	+	NR	NR	+
Phosphatase	+	NR	D	w	NR
Acid from carbohydrates	+[c]	−	−	−	−
Carbohydrates fermented	+[c]	−	−	−	−
Temperature range, °C	≤4–30	4–41	2–43	10–43	20–35
Water-soluble, brown pigment	+	NR	D	−	NR
mol% G + C of DNA	46	54.9	45–50	45	54.8

[a]For symbols see standard definitions; NR, not reported; w, weakly positive.

[b]This includes the members of the genus *Oceanospirillum* as described in this book.

[c]Carbohydrate oxidation/fermentation tests were done in PSS media (Hylemon et al., 1973). Acidification took up to two weeks to occur.

TABLE BXII.γ.92. Differentiation of *Neptunomonas* from some other marine naphthalene-degrading bacteria[a,b]

Characteristic	*Neptunomonas*	*Cycloclasticus*	*Marinobacter*	*Pseudoalteromonas*	*Vibrio*
Indigo from indole[c]	+	−	+	d	−
Utilization of phenanthrene[c]	− or w	+	−[d]	d	+
Na^+ requirement	+	+	D	+	+[e]
Growth on marine broth 2216	+	− or w	+	+	+
Utilization of carbohydrates	+	−	−	+	+
D-glucose	+	−	−	+	+
D-fructose	+	−	−	d	D
Utilization of amino acids	+	+[f]	+[g]	+	+
Acid from D-glucose	+ or w	NG	−	−	+
mol% G + C	46	39	57.3–57.7[d]	37–50	38–51

[a]For symbols see standard definitions; NG, no growth in test medium; w, weakly positive.

[b]Marine bacteria are defined here as requiring Na^+ ions for growth. Taxa that have not been fully described are not considered here.

[c]The results for these phenotypes are only given for naphthalene-degrading strains. Otherwise, characteristics are generalized to encompass the whole genus.

[d]For these features we used the results of Spröer et al. (1998), which differ from those obtained previously (Gauthier et al., 1992).

[e]All *Vibrio* strains require Na^+ except *V. cholerae*.

[f]*Cycloclasticus* utilizes only L-glutamate.

[g]*Marinobacter* utilizes only L-proline and L-glutamate.

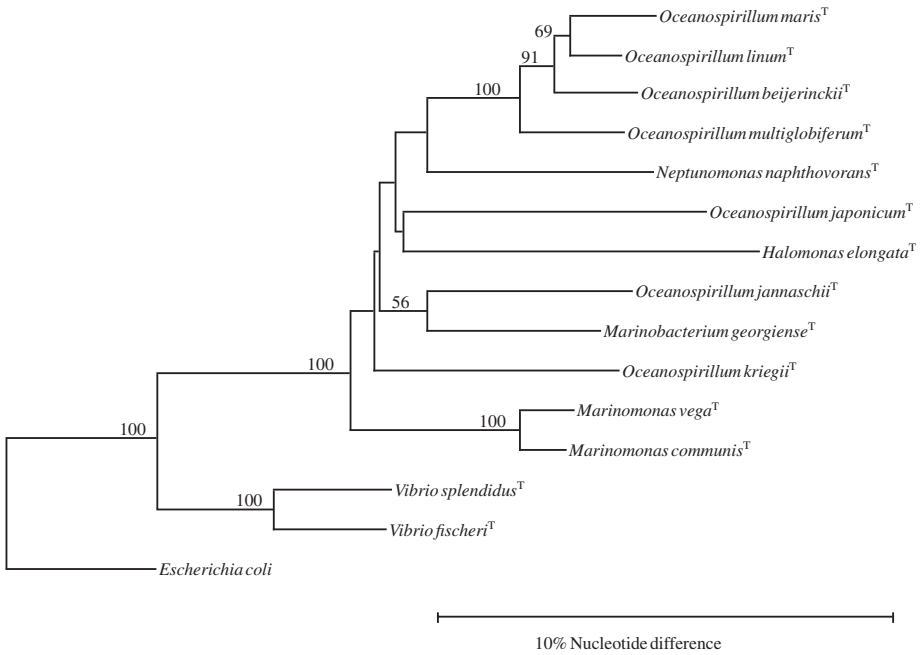

FIGURE BXII.γ.111. Dendrogram showing the relationship between *Neptunomonas* and its close relatives. The dendrogram was created in TreeCon using Jukes-Cantor distance with the Kimura 2–parameter correction. Numbers at branch nodes represent bootstrap values for 100 replications. Numbers below 50 are not shown. (Reproduced with permission from B. Hedlund et al., Applied and Environmental Microbiology *65:* 251–259, 1999 ©American Society for Microbiology, Washington, D.C.)

Family II. **Alcanivoraceae** *fam. nov.*

PETER N. GOLYSHIN, SHIGEAKI HARAYAMA, KENNETH N. TIMMIS AND MICHAIL M. YAKIMOV

Al.ca.ni.vo.ra'.ce.ae. M.L. masc. n. *Alcanivorax* type genus of the family; *-aceae* ending to denote family; M.L. masc. pl. n. *Alcanivoraceae* the *Alcanivorax* family.

Rods 0.6–0.8 × 1.6–2.5 μm in size, depending on the substrate used for growth. Some isolates are capable of anaerobic growth. Reduce nitrate to nitrite. **Principal carbon and energy sources are linear-chain alkanes and their derivatives**, with carbon chain length between 9 and 20. Only a few simple organic compounds are used as carbon and energy source (formate, acetate, propionate, methyl-pyruvate, and α-ketoglutarate). **Moderately halophilic**, NaCl content optimal for growth is between 3 and 10%. Strains of all recognized species and recent isolates are mesophilic, with the temperature optima about 25–30°C. Some isolates produce glucose lipid surfactants.

Type genus: **Alcanivorax** Yakimov, Golyshin, Lang, Moore, Abraham, Lünsdorf and Timmis 1998, 346.

Genus I. **Alcanivorax** *Yakimov, Golyshin, Lang, Moore, Abraham, Lünsdorf and Timmis 1998, 346^VP*

PETER N. GOLYSHIN, SHIGEAKI HARAYAMA, KENNETH N. TIMMIS AND MICHAIL M. YAKIMOV

Al.ca.ni.vo'.rax. M.L. masc. n. *alcanum* alkane, aliphatic hydrocarbon; L. adj. *vorax* voracious, gluttonous; M.L. masc. n. *Alcanivorax* alkane-devouring.

Rods 0.6–0.8 × 1.6–2.5 μm when grown in pyruvate-supplemented medium and 0.6–0.8 × 1.0–1.5 μm when grown on *n*-alkanes. Nonmotile. Gram negative. Colonies 2–3 days old are circular, colorless, and transparent; in older cultures, they turn opaque and light yellow if grown on alkanes. **Aerobic and microaerophilic**, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. Nitrate is reduced to nitrite, but anaerobic growth does not occur on nitrate. **Oxidase and catalase positive. Growth occurs on aliphatic hydrocarbons as sole or principal carbon sources.** Some strains produce glucose lipid surfactants that exist in two forms. **Moderately halophilic**; optimum NaCl concentration 3% (range 0.5–15%). Isolated from seawater and seawater sediments enriched with crude oil, petroleum hydrocarbons, or single aliphatic hydrocarbons.

The mol% G + C of the DNA is: 53–66.

Type species: **Alcanivorax borkumensis** Yakimov, Golyshin, Lang, Moore, Abraham, Lünsdorf and Timmis 1998, 347.

FURTHER DESCRIPTIVE INFORMATION

Some strains produce a glycine-containing biosurfactant precursor that is linked to the cell surface and increases the hydro-

phobicity of the cells and their affinity to oil droplets suspended in the water phase. Some strains produce another form—the mature extracellular glucose lipid biosurfactant, which lacks the terminal glycine residue, and which forms micelles with water-insoluble oil fractions, thereby increasing their bioavailability.

These organisms are distributed in natural marine environments around the world, and their predominance in microbial communities accidentally polluted, or experimentally spiked with oil, suggests on important role in natural petroleum biodegradation (Harayama et al., 1999).

Strains of *"Alcanivoraceae"* were isolated or detected in 16S rDNA clone libraries from different geographic locations (Table BXII.γ.93).

Genome format The genome size estimated for *Alcanivorax borkumensis* SK2T and AP1 is about 3 Mbp. Up to now, no plasmids have been detected in these bacteria.

Isolates of *Alcanivorax* are amenable to conjugative DNA transfer, and transposon mutagenesis (miniTn5-mutagenesis has been characterized by a random positioning of the inserts in the chromosome of *A. borkumensis* strain SK2T with an appropriate antibiotic marker (chloramphenicol, streptomycin/spectinomycin). Although isolates are sensitive to kanamycin, the relatively high salinity of common *Alcanivorax* growth media interferes with the use of this antibiotic marker for selection purposes.

The fluorescent *in situ* hybridization probe 5′-CCA AGA ATA CTA AGA TTC CC—complementary to rRNA between nucleotides 821-842 (*E. coli* numbering)—labeled with Cy3 to detect *"Alcanivoraceae"* in environmental samples has given satisfactory results under stringent conditions (35% (w/vol) formamide; hybridization at 46°C for 2 h). Probes that distinguish *Fundibacter* and *Alcanivorax* species hybridize to 16S rRNA between nucleotides 203 and 223 (*E. coli* numbering); these probes are 5′-GCG AGC TCA TCC ATC **T**GC A and 5′-GCG AGC TCA TCC ATC **A**GC A, respectively (mismatches are shown in boldface type).

Enrichment and Isolation Procedures

The principal sources of these organisms are seawater and seawater sediments, enriched with crude oil, petroleum hydrocarbons, or single aliphatic hydrocarbons. *n*-Hexadecane can serve as the sole carbon source for enrichment of the seawater/sediment samples; addition of nitrogen and phosphorus is required. The flat, colorless, transparent colonies of *Alcanivorax*-related strains develop on the agar surface within 3–5 d after plating. After incubation for longer than 1 week, colonies turn opaque and cream-colored.

Differentiation of the genus *Alcanivorax* from other genera

Characteristics that differentiate the genus from other related or phenotypically similar genera are given in Table BXII.γ.94.

Taxonomic Comments

The analysis of 16S rRNA gene sequencing data of both recognized and recently isolated strains belonging to the genera *Alcanivorax* and *Fundibacter* (Yakimov et al., 1998; Bruns and Berthe-Corti, 1999) suggest these bacteria belong to a distinct taxonomic group, presumably a new family, *"Alcanivoraceae"*, phylogenetically equidistant from the characterized lineages of the class *Gammaproteobacteria*, *"Alteromonadaceae"*, *Halomonas* spp., belonging to *Halomonadaceae* and members of family *Oceanospirillaceae* (Fig. BXII.γ.112).

A controversy exists regarding the taxonomic position of the

TABLE BXII.γ.93. List of strains relevant to the genus *Alcanivorax* and clones derived from 16S rRNA gene libraries from different geographic locations

Strain/clone designation	Origin	Notes
Alcanivorax borkumensis SK2, DSM 11573[T]	Isle of Borkum, North Sea	Type and only species of *Alcanivorax*
Alcanivorax jadensis T9, DSM 12178[T]	Jadebusen, North Sea	Formerly known as *Fundibacter jadensis* T9
Alcanivorax borkumensis AP1, SK, SK1, SK4A, SK7, MM1[a]	Isle of Borkum, North Sea	Isolates obtained from the enrichments along with the type strain SK2
Alcanivorax sp. ME104[a]	Mediterranean Sea, Harbor of Messina, Sicily	98% 16S rRNA gene similarity with the type strain
Alcanivorax borkumensis ME103, ME106, ME111[a]	Mediterranean Sea, Harbor of Messina, Sicily	100% 16S rRNA gene similarity with the type strain
Alcanivorax borkumensis LE4	Singapore shore	100% 16S rRNA gene similarity with the type strain
Alcanivorax sp. SCB54	California (E. Pacific)	Partially sequenced environmental clone
Alcanivorax sp. ME105, ME112[a]	Mediterranean Sea, Harbor of Messina, Sicily	95–98% 16S rRNA gene similarity with the type strain
MBIC4324–4330, 4335–4439, 4346–4351[b]	Sea of Japan	
MBIC4322, 4323, 4332, 4334[b]	Palau (S. Pacific)	
MBIC4197–4100[b]	Canada (NE Pacific)	
MBIC4064, 4066, 4067[b]	Adriatic Sea, Croatia	
MBIC4331,4168[b]	Okinawa (W. Pacific)	
MBIC4089, 4090[b]	Miyagi (Central Pacific)	
MBIC4426, 4427[b]	Shizuoka (W. Pacific)	
MBIC4103, 4104[b]	Hawaii (Central Pacific)	
"Alcanivorax profundus" MRS3300[c]	Deep area (3300m) of Mediterranean Sea located between Straits of Messina and South Ionian Sea: 36°30′N-15°50′E	98% 16S rRNA gene similarity with *Alcanivorax jadensis* strain T9
Alcanivorax borkumensis 9B1, 7B[c]	Coral Sea (S. Pacific), harbor of Noumea, New Caledonia	100% 16S rRNA gene similarity with the type strain

[a]Available in the strain collection of the Department of Microbiology of the GBF (German Research Center for Biotechnology), Mascheroder Weg 1, 38124 Braunschweig, Germany (Email pgo@GBF.de).

[b]Available in the strain collection of Marine Biotechnology Institute, 3-75-1, Kamaishi Laboratory, Heita, Kamaishi, Iwate 026-0001, Japan (Email shigeaki.harayama@kamaishi.mbio.co.jp).

[c]Available in the strain collection of the Istituto Sperimentale Talassographico CNR, Spianato S. Raineri 86, 98122 Messina, Italy (Email iakimov@its.me.cnr.it).

TABLE BXII.γ.94. Comparison of key phenotypic characteristics of *Alcanivorax* spp. and other heterotrophic marine bacteria belonging to the *Gammaproteobacteria*[a,b]

Characteristic	*Alcanivorax* spp.	*Halomonas* spp.	*Marinobacter* spp.	*Marinomonas* spp.	*Oceanospirillum* spp.
Typical Gram-negative cell wall	+	+	+	+	+
Morphology	Rods	Rods	Rods	Rods	Helical
Number and arrangement of flagella	None	Peritrichous	None or single polar	Single polar	Bipolar tufts
Optimal temperature for growth (°C)	20–30	30	32	20–25	25–32
Growth at 4°C	±	+	−	−	±
Growth at 45°C	−	NR	+	+	−
Optimal NaCl concentration (%) for growth	3–5	3–8	3–6	0.7–3.5	0.5–8
Maximal NaCl concentration (%) for growth	15	32.5	20	NR	9.75
Nitrate reduced to nitrite	+	+	+	−	−
Oxidase test (Kovacs)	+	+	+	−	+
Arginine dihydrolase activity	−	−	−	−	NR
Ornithine decarboxylase	−	+	−	−	NR
Gelatin liquefaction	−	±	−	−	−
Starch hydrolysis	−	−	−	−	−
Lysine decarboxylase	−	+	−	−	NR
Urease activity	−	±	−	−	−
Utilization as sole carbon source:					
DL-Alanine	−	+	−	+	±
L-Arginine	−	+	−	±	−
Aspartate	−	+	−	NR	−
Cellobiose	−	+	−	±	−
D-Fructose	−	+	−	+	−
D-Gluconate	−	+	−	+	NR
D-Glucose	−	+	−	+	−
L-Glutamate	−	+	+	+	±
Glycerol	−	+	−	+	−
Hexadecane	+	+	+	+	NR
p-Hydroxybenzoate	−	−	+	+	±
D-Mannitol	−	+	−	+	−
D-Mannose	−	+	−	+	−
L-Serine	−	+	−	±	−
Succinate	−	+	+	+	+
Sucrose	−	+	−	−	−
Accumulation of poly-β-hydroxybutyrate	±	+	−	−	+
Mol% G+C of the DNA	53–66	60–61	53	44–48	42–51

[a]+, Positive reaction or growth; ±, variable reaction; negative reaction or growth; NR, not reported.

[b]Modified from Yakimov et al. (1998).

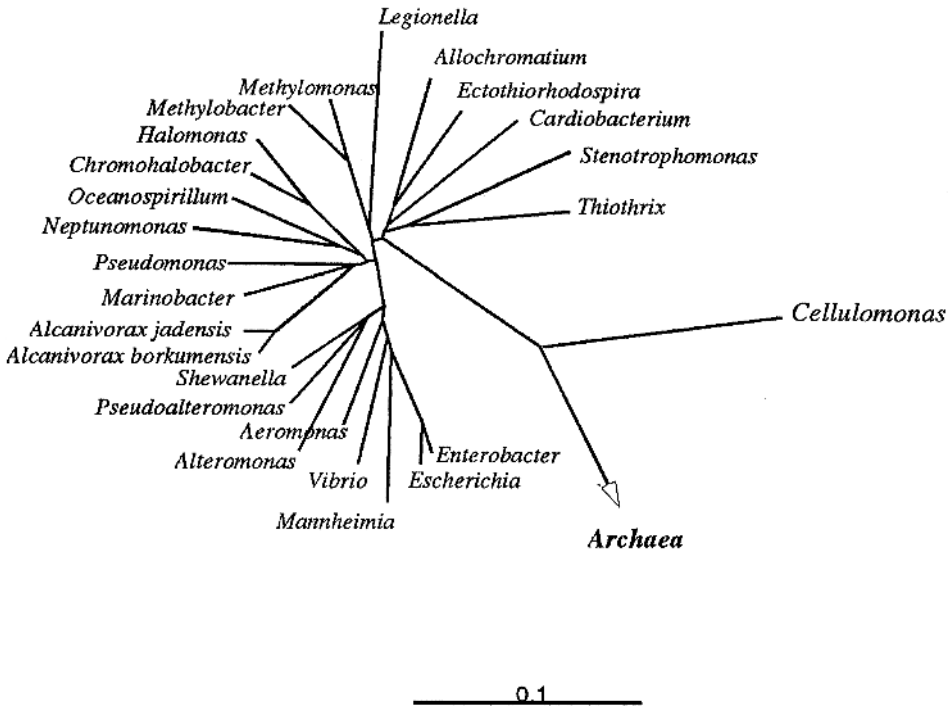

FIGURE BXII.γ.112. Phylogenetic position of species of genus *Alcanivorax* among principal genera within *Gammaproteobacteria*. Phylogenetic tree was rooted from an archaeon, *Pyrodictium occultum*, and *Cellulomonas* was used as an outgroup. The scale bar represents 0.1 fixed point mutation per sequence position. *Fundibacter jadensis* was transferred to the genus *Alcanivorax* as *Alcanivorax jadensis* (González and Whitman, 2002; Fernández-Martínez et al., 2003).

genus *Fundibacter* (see Other Organisms, below). Because two validly described genera—*Alcanivorax* (Yakimov, Golyshin, Lang, Moore, Abraham, Lünsdorf and Timmis, 1998) and *Fundibacter* (Bruns and Berthe-Corti, 1999)—share significant 16S rRNA homology (about 98%) and because no clear-cut phenotypic differentiation can be drawn between these genera, which were isolated from similar environmental sources (German North Sea, alkane-enriched aerobic pelagic sediments), it appears appropriate to assign the genus *Fundibacter* to the genus *Alcanivorax*. DNA/DNA hybridization studies between *A. borkumensis* and *F. jadensis* clearly indicate, however, that these two organisms do not belong to the same species (Steiner and Tindall, personal communication).

The sequencing of *gyrB* genes from above type cultures and a large number of recently isolated—but not validly published—*Alcanivorax* strains suggest existence of at least five genotypically distinct groups, presumably at the level of species (Fig. BXII.γ.113).

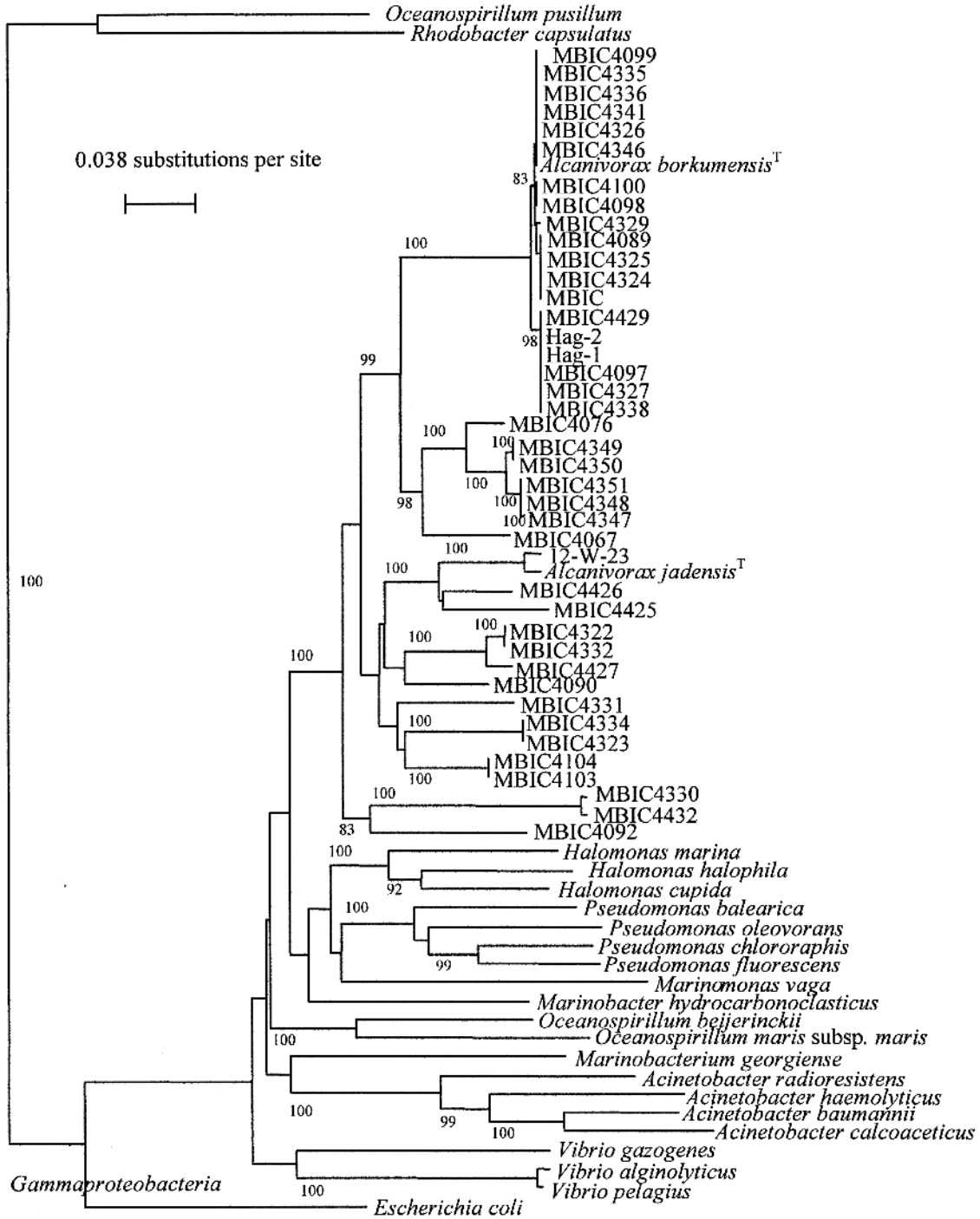

FIGURE BXII.γ.113. Phylogenetic tree derived from the similarities between the *gyrB* sequences. Bootstrap values over 80% are shown at the branch points. Strains are listed in Table BXII.γ.93. *Fundibacter jadensis* was transferred to the genus *Alcanivorax* as *Alcanivorax jadensis* (González and Whitman, 2002; Fernández-Martínez et al., 2003).

List of species of the genus Alcanivorax

1. **Alcanivorax borkumensis** Yakimov, Golyshin, Lang, Moore, Abraham, Lünsdorf and Timmis 1998, 347^{VP}
bor.ku.me' n.sis. M.L. adj. *borkumensis* from the island of Borkum, a small island in Western-Elms harbor in the North Sea, located close to the German-Dutch border.

   The characteristics are as described for the genus, with the following additional information. Optimum temperature 20–30°C; range, 10–35°C. Optimum NaCl concentration 3–10%; range, 1.0–12.5%. Reduce nitrate to nitrite, though no anaerobic growth occurs. Do not produce agarase, amylase, arginine dihydrolase, ornithine decarboxylase, lysine decarboxylase, gelatinase, or esculinase. Exhibit Tween 80 hydrolysis. Utilize aliphatic hydrocarbons (formate, acetate, propionate, methylpyruvate, α-ketoglutarate) and some short-chain fatty acids as sole or principal carbon source. Produce glucose lipids (Fig. BXII.γ.114) that exist in two forms: a glycine-containing biosurfactant precursor and the mature extracellular glucose lipid. Principal fatty acids are $C_{16:0}$, $C_{16:1}$, and $C_{18:1}$. Resistant to ampicillin, tetracycline, nalidixic acid; sensitive to streptomycin/spectinomycin, chloramphenicol, kanamycin.

   The mol% G + C of the DNA is: 53.4 (HPLC).
   Type strain: SK2, ATCC 700651, CIP 105606, DSM 11573.
   GenBank accession number (16S rRNA): Y12579.

2. **Alcanivorax jadensis** Fernández-Martínez, Pujalte, García-Martínez, Mata, Garay, and Rodríguez-Valera 2003, 337^{VP} (*Fundibacter jadensis* Bruns and Berthe-Corti 1999, 447.)
ja.den' sis. M. L. adj. *jadensis* referring to the region Jade, which forms part of the bay "Jadebusen", which belongs to the German North Sea coast.

   Rods 0.3–0.7 μm wide and 0.8–1.8 μm long. Nonmotile. Pili are present. Gram negative. Colonies are circular, 0.25–1.25 mm in diameter and ivory-pigmented. The temperature spectrum ranges from 10–40°C, with optimal growth occurring at 30°C. Shows weak halotolerance and grows at NaCl concentrations ranging from 0.5–15% (w/v); optimum, 3%. It is able to grow both aerobically and anaerobically, and reduces nitrate. It grows with the carbon sources pyruvate and acetate if a synthetic medium is used and also with tetradecane, hexadecane, and pristane if the medium is supplemented with vitamins and mineral salts. The cells are resistant to ampicillin, cefazolin, cephalotin, erythromycin, linomycin, nalidixic acid, novobiocin, ofloxacin, oxacillin, penicillin, and tetracycline. Oxidase and catalase positive. Growth occurs on aliphatic hydrocarbons as the sole or principal carbon source (tetradecane, hexadecane, and pristane). Pyruvate and acetate can be used. The organisms emulsify alkanes, but do not produce glucose lipid.
   The mol% G + C of the DNA is: 63.6 (T_m).
   Type strain: T9, ATCC 700854, DSM 12178.
   GenBank accession number (16S rRNA): AJ001150.

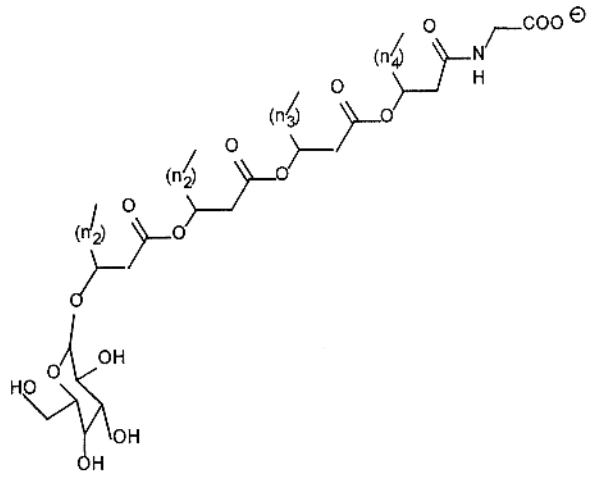

FIGURE BXII.γ.114. Cell-associated glucose lipid from *A. borkumensis* SK2^T.

Family III. **Hahellaceae** *fam. nov.*

GEORGE M. GARRITY, JULIA A. BELL AND TIMOTHY LILBURN

Ha. hel.la' ce.ae. M.L. fem. n. *Hahella* type genus of the family; *-aceae* ending to denote family; M.L. fem. pl. n. *Hahellaceae* the *Hahella* family.

The family *Hahellaceae* was circumscribed for this volume on the basis of phylogenetic analysis of 16S rDNA sequences; the family contains the genera *Hahella* (type genus) and *Zooshikella*. *Zooshikella* was proposed after the cut-off date for inclusion in this volume (June 30, 2001) and is not described here (see Yi et al. (2003)).

Aerobic or facultatively anaerobic chemoorganotrophs. Require NaCl for growth.

Type genus: **Hahella** Lee, Chun, Moon, Ko, Lee, Lee and Bae 2001a, 664^{VP}.

Genus I. **Hahella** *Lee, Chun, Moon, Ko, Lee, Lee and Bae 2001a, 664[VP]*

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Ha. hel' la. M.L. fem. n. named after Yung Chil Hah, a Korean bacteriologist who pioneered microbiological research in Korea.

Gram-negative motile rod. Facultatively anaerobic; produces acid from sugars. Reduces nitrate to nitrite. Hydrolyzes esculin and gelatin. **Produces extracellular polysaccharides and red pigment. Requires NaCl.**

The mol% G + C of the DNA is: 55.

Type species: **Hahella chejuensis** Lee, Chun, Moon, Ko, Lee, Lee and Bae 2001a, 665.

FURTHER DESCRIPTIVE INFORMATION

Analysis of 16S rDNA sequences showed that *Hahella chejuensis* is a member of the *Gammaproteobacteria* but is not closely related to any known genera (Lee et al., 2001a).

ENRICHMENT AND ISOLATION PROCEDURES

The strain was isolated directly from diluted marine sediment spread on ZoBell's medium (Lee et al., 2001a).

MAINTENANCE PROCEDURES

The strain was stored at −80° in 20% (w/v) glycerol (Lee et al., 2001a).

DIFFERENTIATION OF THE GENUS *HAHELLA* FROM OTHER GENERA

Lee et al. (2001a) provide a table of characteristics that differentiate *Hahella chejuensis* from other marine/halophilic bacteria in the *Gammaproteobacteria*.

List of species of the genus Hahella

1. **Hahella chejuensis** Lee, Chun, Moon, Ko, Lee, Lee and Bae 2001a, 665[VP]

   che.ju.en' sis. M.L. adj. *chejuensis* pertaining to Cheju Island, Republic of Korea, geographical origin of the type strain of the species.

   Young cells 0.5–0.7 × 1.6–9.0 μm; older cells 0.7–0.8 × 1.4–1.7 μm. Halophilic; produces red pigment soluble in methanol. Growth at 1–8% NaCl (optimum 2% NaCl), 10–45°C, and pH 6–10 (optimum pH 7). Grows on and produces acid from adonitol, D(+)-fructose, D(+)-glucose, inositol, D(+)-maltose, D(−)-mannitol, D(+)-mannose, D(−)-sorbitol, sucrose, and D(+)-trehalose. Does not grow on L(+)-arabinose, citrate, D(+)-galactose, D(+)-lactose, malate, malonate, D(+)-melibiose, D(+)-raffinose, L(+)-rhamnose, and D(+)-xylose. Major fatty acids octadecenoic, *cis*-9-hexadecenoic/*iso*-2-hydroxypentadecanoic, hexadecanoic, and 3-hydroxydodecanoic.

   The mol% G + C of the DNA is: 55 (T_m).

   Type strain: 96CJ10356, KCTC 2396, IMSNU 11157.

   GenBank accession number (16S rRNA): AF195410.

Family IV. **Halomonadaceae** Franzmann, Wehmeyer and Stackebrandt 1989, 205[VP] emend. Dobson and Franzmann 1996, 558

GEORGE M. GARRITY, JULIA A. BELL AND TIMOTHY LILBURN

Ha.lo.mo.na.da' ce.ae. M.L. fem. n. *Halomonas* type genus of the family; *-aceae* ending to denote family; M.L. fem. pl. n. *Halomonadaceae* the *Halomonas* family.

The family *Halomonadaceae* was circumscribed for this volume on the basis of phylogenetic analysis of 16S rDNA sequences; the family contains the genera *Halomonas* (type genus), *Carnimonas*, *Chromohalobacter*, *Cobetia*, *Deleya*, and *Zymobacter*. *Cobetia* was proposed after the cut-off date for inclusion in this volume (June 30, 2001) and is not described here (see Arahal et al. (2002a)).

Halotolerant or halophilic, except for *Zymomonas*. Motile by means of flagella. Aerobic or facultatively anaerobic chemoorganotrophs.

Type genus: **Halomonas** Vreeland, Litchfield, Martin, and Elliot 1980, 494[VP] emend. Dobson and Franzmann 1996, 557.

Genus I. **Halomonas** *Vreeland, Litchfield, Martin, and Elliot 1980, 494[VP] emend. Dobson and Franzmann 1996, 557*

RUSSELL H. VREELAND

Ha.lo.mo' nas. Gr. n. *hals, halos* salt of the sea; Gr. n. *monas* a unit, monad; M.L. fem. n. *Halomonas* salt(-tolerant) monad.

Straight or curved rod shaped cells, generally 0.6–0.8 × 1.6–1.9 μm. One species (*H. halodenitrificans*) presents coccoid cells. Species may form elongated, flexuous filaments under certain conditions. Endospores are not formed. **Gram negative. Rod shaped species are motile** by lateral, polar, or peritrichous flagella. Colonies are white or yellow, turning light brown with age. **Possess a mainly respiratory type of metabolism with oxygen as the terminal electron acceptor.** Some species are also capable of anaerobic growth in the presence of nitrate. Some species have been reported to grow under anaerobic conditions in the absence of nitrate if supplied with glucose (but not other carbohydrates or amino acids). Nitrate is reduced to nitrite; nitrogen

gas is not formed. All species tested are catalase positive; 17 of the 21 known species are also oxidase positive. Chemoorganotrophic. Carbohydrates, amino acids, polyols, and hydrocarbons can serve as sole carbon sources in mineral media. Ammonium sulfate can serve as a sole nitrogen source. **Intracellular granules are not produced. Halotolerant** (also described as slight to moderate halophiles), **able to grow in NaCl concentrations ranging from 0.1–32.5% (w/v).** The major respiratory quinone is ubiquinone 9. The major fatty acids are $C_{16:1}$, $C_{17:0\ cyc}$, $C_{16:0}$, $C_{18:1}$, and $C_{19:0\ cyc}$. In addition to having the signature sequences found in the *Halomonadaceae*, *Halomonas* species contain the following four signature bases (by *E. coli* numbering): C at position 1424, U at position 1439, A at position 1462, and C at position 1464. **Isolated from saline environments around the world**, including solar salt facilities, intertidal estuaries, the open ocean, and hypersaline lakes (Dead Sea, Israel; Organic Lake, Antarctica).

The mol% G + C of the DNA is: 52–68.

Type species: **Halomonas elongata** Vreeland, Litchfield, Martin and Elliot 1980, 495.

Further descriptive information

During exponential growth, 20 of the 21 *Halomonas* species consist of mixtures of straight and curved rods. Upon entry into stationary phase, several *Halomonas* species also form elongated, flexuous filaments of various lengths. The percentage and length of these elongated cells depends upon the type of growth medium. In complex Casamino acids medium (CAS)[1] with 8% NaCl, all of the cells may be elongated, with many filaments containing irregular loops and bends. In a chemically defined mineral salts medium (MS)[2] containing 8% NaCl, as few as 25% of the cells may be elongated, and the filaments seldom produce irregular looping or extensive bending.

Electron microscopy of thin sections from optimum growth conditions reveals a typical Gram-negative type of cell wall (Vreeland and Martin, 1980).

The number and arrangement of flagella on short cells depends upon the species examined. Cells generally possess 4–7 flagella. In some strains, the flagella are arranged laterally, while others possess only polar flagella. One biovar of this genus has both lateral and polar flagellation. Motile *Halomonas* cells describe a helix when viewed by light microscopy. Motility is generally lost rapidly under low oxygen concentrations. Flagella have not been observed on elongated cells or on cells grown in low NaCl concentrations (<0.2%).

On solid CAS or MS medium containing 8% NaCl, most *Halomonas* colonies are white to cream-colored, smooth, glistening, opaque, and ~2 mm in diameter after 24 h at 30°C. The colonies generally become yellow to light brown and spread following prolonged incubation.

Halomonas species require Na^+ for growth—at least 0.1% w/v NaCl added to CAS medium or 0.3% w/v NaCl added to MS medium—and will grow in a wide range of NaCl concentrations (0.1–32% NaCl w/v). When subjected to osmotic shock from either rapid dilution or increased NaCl concentration, *Halomonas* cells respond as osmometers and exhibit swelling or plasmolysis. Osmotically shocked *Halomonas* cells do not lose viability and soon reestablish osmotic balance and resume growth. This reestablishment of osmotic balance is mediated by an increased concentration of ectoine, which is the primary compatible solute found in *H. elongata*. The optimum NaCl concentration is 2.2–8.0% at 30°C. The Na^+ requirement of the type species can be satisfied by NaCl, $NaNO_3$, or NaBr (Vreeland and Martin, 1980). The NaCl requirement of the other species has not been tested in detail. NaCl tolerance is affected by growth temperature, but apparently not by the carbon source being used (Vreeland and Martin, 1980). In terms of the ability to promote salt tolerance, temperatures can be arranged from greatest degree of promotion to least as follows: 30°C, 23°C, 37°C, 15°C, 45°C, 4°C (Vreeland et al., 1980).

In CAS medium containing 8% NaCl, the *Halomonas* species that have been tested grow at pH values from 5.0–9.0. Alkaliphilic strains generally grow from pH 7.0–11.0 with an optimum at 9.5. The pH tolerance has not been tested on MS medium and has not been tested at different temperatures.

The physiological characteristics of all properly recognized *Halomonas* species are listed in Tables BXII.γ.95 and BXII.γ.96. Although considered mainly aerobic, some *Halomonas* species have been reported to grow on glucose under anaerobic conditions in the absence of nitrate or other terminal electron acceptors. If the glucose is replaced by other carbohydrates or an amino acid, nitrate is required for anaerobic growth.

Members of the genus are nutritionally versatile. The following compounds often serve as sole carbon sources for growth: glucose, gluconate, glycerol, fructose, mannose, sucrose, cellobiose, succinate, mannitol, alanine, glutamine, glutamate, asparagine, aspartate, lysine, histidine, phenylalanine, tyrosine, tryptophan, proline, arginine, leucine, isoleucine, valine, methionine, cysteine, serine, and threonine.

Halomonas species have been isolated from a solar salt facility on the island of Bonaire, Netherlands Antilles (Vreeland et al., 1980) from the Dead Sea and Canada (Huval et al., 1995), the Antarctic (Franzmann et al., 1987; James et al., 1990a), Great Salt Lake, Utah (Fendrich, 1988), estuaries (Hebert and Vreeland, 1987), and the Pacific Ocean (Baumann et al., 1983a). Representatives of the genus may be even more widespread in nature.

Enrichment and Isolation Procedures

Halomonas species may be isolated using a wide variety of media. Brine samples can be spread onto the surface of the medium and incubated at close to the environmental temperature and under high humidity for 2–7 d. *Halomonas* colonies are white to cream colored and easily distinguished from red-pigmented halophiles. Alternatively, the brine samples can be added directly to filter pads saturated with medium. This method has the advantage of allowing the use of small (9 mm) Petri plates, facilitating shipment to distant field areas. A disadvantage of the technique is that the white *Halomonas* colonies can be difficult to see

1. CAS medium is a modification of the medium of Abram and Gibbons, as described by Gibbons (1969), and contains (g/l of distilled water): yeast extract, 1.0; casamino acids (Difco) (not "vitamin-free"), 7.5; Proteose peptone No. 3 (Difco), 5.0; sodium citrate, 3.0; $MgSO_4 \cdot 7H_2O$, 20.0; K_2HPO_4, 7.5; and NaCl (or solar salt), 80.0. The pH is adjusted to 8.0 ± 0.1 with NaOH prior to sterilization and is 7.5 ± 0.1 after autoclaving at 121°C for 20 minutes (Vreeland et al., 1980, 1984). CAS medium is stable for several weeks when stored in the dark. It should be discarded if any crystal formation is seen.

2. MS medium is similar to that described by Vreeland and Martin (1980). It contains (g/l): $MgCl_2 \cdot 6H_2O$, 5.3; KCl 0.75; and $(NH_4)_2SO_4$, 4.1. It is supplemented with a carbon source (10–50 mM) and NaCl (2.92–198.7 g/l). Phosphate ($K_2HPO_4 \cdot 3H_2O$) is added as a sterile 10× concentrate after the medium has been autoclaved to give a final concentration of 0.5 g/l. The medium may also be supplemented with $CaCl_2$ (0.11 g/l) to enhance growth. The pH of the medium is adjusted to 7.2 with 1 N KOH prior to autoclaving and is 7.0 after sterilization.

on white filter pads; also, the pad fiber makes colonial isolation tedious.

A more direct isolation technique is to take advantage of the halotolerance of all of the species by setting up a salt-mediated selection medium. High salt brines can be mixed with low salt or distilled water, then spread onto a low salt medium. Alternatively, low salt samples can be added to high salt media to select for salt-tolerant organisms.

Maintenance Procedures

Halomonas strains can be maintained on agar slants containing 8% (w/v) NaCl. Stock cultures are transferred every 6 months, allowed to grow at 30°C for 1–7 d, and then stored in the dark at 4°C (Vreeland et al., 1980). Some media that have proven useful for growing Halomonas are HSC medium[3], MH medium[4], and AOL medium[5].

Most species of *Halomonas* have been lyophilized, but survival rates may vary with the strain and species. *Halomonas elongata* strains ATCC 33173 and 33174 have survived for up to 2 years. The growth from a fresh CAS slant or broth is suspended in 0.5 ml of a solution containing Proteose peptone No. 3 (Difco) (0.5%), yeast extract (1.0%), and NaCl (0.2%). A small amount of the suspension (0.1–0.2 ml) is transferred to a lyophilization vial, dipped into liquid N_2 for 5–10 min, and lyophilized. Preserved cultures are reconstituted by suspending the cells in a small amount of CAS lacking salt. After 15–30 min, the cells are transferred to CAS broth containing NaCl and incubated at 30°C.

Halomonas strains may also be stored frozen on ceramic Protec® or Microbank® beads at −80°C. When using ceramic beads, it is best to grow the cultures under optimum growth conditions and then add the culture to fresh medium and place it onto the pre-sterilized beads. The cultures may then be re-incubated at 37°C for several hours to overnight to allow time for the cells to attach to the beads. After this incubation, the fluid may be drawn off and the culture frozen. *Halomonas* cultures have been preserved for up to 10 years using this technique; however, the quality of the culture has been found to deteriorate slowly under these conditions (R. Vreeland, unpublished observations).

Halomonas may also be stored frozen on ceramic beads under liquid N_2. Due to the variation in survival rates for other storage methods, liquid N_2 storage is the method of choice for long-term preservation.

Procedures for Testing Special Characters

A common characteristic of all *Halomonas* species is their ability to grow at a wide variety of salt concentrations. Huval and Vreeland (unpublished studies) have determined that the phenotypic characteristics of these bacteria are often affected by the salt concentration of the growth and testing medium. These effects are particularly noticeable at the low and high salt extremes. This aspect has also been encountered by Hebert and Vreeland (1987) during their characterization of *Halomonas halodurans*. Consequently, prior to conducting any taxonomic analysis on isolates suspected of belonging to *Halomonas*, the optimum growth conditions with respect to NaCl concentration, temperature, and pH must be determined. These characteristics are best tested by use of complex media, each containing 0, 3.5, 8.0, 15.0, 20.0, or 32.0% (w/v) NaCl or solar salt. Temperature tolerance and qualitative effects of temperature on salt tolerance can be tested by incubating tubes of each salt concentration at temperatures from 4–45°C. After 2–3 weeks, all tubes without visible growth should be incubated for an additional 2–3 weeks at the optimum temperature to determine whether any apparent inhibitory conditions have actually been lethal (Vreeland, 1993).

DNA may be extracted from *Halomonas* by the method of Marmur (1961) with some modification. *Halomonas* suspensions and the sodium dodecyl sulfate (SDS) used for lysis must be heated to 50°C. Following SDS addition (2% final concentration), the lysate is held at 50°C for 15 min. Pronase is not added to the suspension. Water-saturated phenol (also at 50°C) is then added to fully denature the protein. The preparation may then be cooled to room temperature and the rest of the Marmur procedure followed. This modification is necessary since some *Halomonas* strains possess a nuclease, which, unless inactivated by heating, causes rapid breakdown of DNA following cell lysis.

All other characterization tests may be performed using conventional techniques (Holding and Collee, 1971; Vreeland, 1993). All media are supplemented with NaCl at the optimum concentration, as determined above. Uninoculated controls are necessary to ensure that any reactions detected are not artifacts caused by the NaCl.

Differentiation of the genus *Halomonas* from other genera

Halomonas is easily differentiated from such genera as *Zymomonas*, *Cellvibrio*, *Oceanospirillum*, or *Serpens* on the basis of salt tolerance, fermentative ability, flexibility, high mol% G + C value, and the ability to catabolize several carbohydrates. *Halomonas* can be quickly differentiated from other genera by its ability to survive exposure to, and to grow in, very high (20%) NaCl concentrations.

Taxonomic Comments

The genus *Halomonas* currently contains 23 recognized, validly named species, which have been included because of the simi-

3. HSC medium is prepared as two separate solutions (A and B). Solution A contains NaCl or solar salt (25%, w/v); the pH is adjusted to 11 with 1 N NaOH, and "vitamin-free" casein (Difco) is added to a concentration of 15 g/l. This mixture is incubated at 30°C overnight to allow protein acidic groups to be exposed. The pH is then adjusted to 7.9 ± 0.1 with sterile NaOH or HCl. Solution B also contains 25% (w/v) solar salt or NaCl and is supplemented with yeast extract (0.2%), sodium citrate (0.6%), $MgSO_4 \cdot 7H_2O$ (4.0%), and ferric ammonium sulfate (0.01%). This solution is adjusted to pH 7.9 with 1 N NaOH and sterilized by autoclaving. Just before use, solutions A and B are mixed 1:1 (v/v). If a solid medium is to be used, agar (4.0%) is added to solution B prior to autoclaving.

4. MH medium contains 1% yeast extract, 0.5% Proteose Peptone no. 3 (Difco), and 0.1% glucose. It may be supplemented with synthetic sea salts. The concentrations of sea salts that have been used include: 0.5, 5.0, 10, 20, and 25% (w/v). The pH of this medium is usually adjusted to 7.2 prior to sterilization. Note that the salt concentrations listed for this medium do not represent the concentration of individual salts, such as NaCl, KCl, and $MgCl_2$; consequently, media made with sea salts obtained from different suppliers may differ in terms of the actual ion contents. Given the tolerance of the various *Halomonas* species, however, this should not be a problem.

5. AOL medium (Franzmann et al., 1987) is a synthetic medium that mimics the Antarctic hypersaline lake from which several species of *Halomonas* have been isolated. It consists of NaCl, 80.0 g; $MgSO_4 \cdot 7H_2O$, 9.5 g; KCl, 5.0 g; $CaCl_2 \cdot 2H_2O$, 0.2 g; $(NH_4)_2SO_4$, 0.1 g; KNO_3, 0.1 g; and yeast extract, 1.0 g; suspended in 960 ml of distilled water. The pH is adjusted to 7.0 and the medium is sterilized by autoclaving. When the solution cools to 60°C, 20 ml of Huntner mineral base solution (Atlas, 1997) is added, followed by 1.0 ml of a filter-sterilized vitamin solution containing (mg/100 ml): cyanocobalamin, 10.0; biotin, 2.0; thiamine hydrochloride, 10.0; calcium pantothenate, 5.0; folic acid, 2.0; nicotinamide, 5.0; and pyridoxine hydrochloride, 10.0. The medium is then completed by adding 20.0 ml of a sterile phosphate solution containing 0.25 g K_2HPO_4 and 0.25 g KH_2PO_4 per 100 ml of distilled water. The medium may be solidified with 15 g/l agar. An AOL-peptone medium may also be made by adding 5.0 g/l peptone to AOL medium.

TABLE BXII.γ.95. Carbon sources utilized by *Halomonas* species[a]

Characteristic	1. *H. elongata*	2. *H. aquamarina*	3. *H. campisalis*	5. *H. cupida*	6. *H. desiderata*	7. *H. eurihalina*	8. *H. halmophila*	9. *H. halodenitrificans*	10. *H. halodurans*	11. *H. halophila*	13. *H. magadii*	14. *H. marina*	15. *H. marisflavae*	16. *H. maura*	17. *H. meridiana*	18. *H. pacifica*	19. *H. pantelleriensis*	20. *H. salina*	21. *H. subglaciescola*	22. *H. variabilis*	23. *H. venusta*
L-Arabinose	+	−	−	+	+	+	−		−	+	+	−	+	+	d	−	−	d		−	−
Cellobiose	+	−		d	D	D				+	+		+		d	−	−	d	−	−	−
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d	−	−	+
Lactose	+	−	−	d		D	−	−	+	d	+	−	+		d	−	−	d	−	−	−
Maltose	+	d	+	−	+	+	+			+		−	+	+	+	+	+	d		−	d
Mannose	+	−	−	+	D	+	+		+	+	+		+	+	+	−	+	d		−	−
Sucrose	+	d	+	d	+	+	+			+	+	−	+	+	+	−	+	d	−	−	d
Acetate	+	+	+	+	+			−	+	+	+	+		+		+	+	+	−	+	+
Citrate	+	d		+	+			+	+	+	+	+	+	+		+	+	d	−	−	+
Gluconate	+	+		+	+	+		+	−	+	+	+	+	+	+	+	+	+	−		+
Lactate	+	+	+	+	+			+		+		+		+		+		d	+	−	+
Propionate	+	+		+		+	+			+		+		+	+	+	+	d		−	+
Succinate	+	+		+	+	+	−	+	+	+	+	+			+	+		d	+	−	+
L-Alanine	+	+		+	+			+	+	+	+	+		+		+		d	+	−	+
L-Arginine	+	+		+		+	+	−		+	+	d		+	+	+	+	d	+		+
L-Histidine	+	−		+		−	+	−	−	d	+	−		+	−	+		−	−		+
L-Isoleucine	+	+		d		+	+	+		d	+	d			+	−		d	−		d
L-Lysine	+	+		+		D	+	+		d	+	d			d	+		d	+		+
L-Serine	+	−		+		D	+	+	−	+	+	+		+	d	+	−	d	−		−
L-Tryptophan	−	−		−		+		−	+	d	+	−		−	+	−		d	−		d
Glycine	+	−		d		D		+	+			−			D	+			+	−	−
L-Leucine	+	d		+		+	+	−	+	d	+	+		+	+	+		d	−		+

[a]For symbols see standard definitions.

larity of their 16S rDNA sequences and the presence of respiratory ubiquinone-9. Although the genus is currently claimed to constitute a monophyletic group of organisms, it contains at least three main evolutionary branches. Subgroup 1 is composed of *H. halmophila*, *H. elongata* (the type species), *H. eurihalina*, *H. salina*, *H. halophila*, *H. pacifica*, *H. cupida*, and *H. halodenitrificans*. The 16S rDNA sequence similarities between pairs of these species range from 93.1 to 99.2%. The second subgroup contains both biovars of *H. subglaciescola* (ACAM 21 and ACAM 12), *H. halodurans*, *H. venusta*, *H. aquamarina*, *H. meridiana*, and *H. variabilis*, and the 16S rDNA sequence similarities among these species range from 94.4 to 100%. The third subgroup is composed of a single species, *H. marina*. When 16S rDNA sequences are used to distinguish the various phylogenetic subgroups within the genus, the situation improves only marginally. At the most restrictive similarity levels (98% and above), the genus seems to contain at least 10 individual phylogenetic groups (Fig. BXII.γ.115). Unfortunately, resolution of these subgroups deteriorates rapidly below this level. In fact, sequence homology levels existing between some members of the current genus are lower than those between members of the *Halomonas* and species currently listed in a different genus (Dobson and Franzmann, 1996).

Confusion about the current taxonomy of this group has arisen because the data that have been used to describe some of these individual species have been inconsistent. For instance, in the initial descriptions of *Halomonas subglaciescola* (Franzmann et al., 1987) and *Halomonas meridiana* (James et al., 1990a), data were used to compare these species to the type species *H. elongata*. Unfortunately, these two reports had only 55 kinds of characteristics in common, and within these common tests, 15 (27%) gave different results for *H. elongata*. Moreover, 17 of the 55 (31%) test results reported by James et al. (1990a) differed from those reported by Franzmann et al. (1987) for the type strain of *H. subglaciescola*. There is presently no information on the degree to which such discrepancies would affect the taxonomic placement of the species involved.

A similar situation exists with the organisms that were originally part of genera such as *Deleya* and *Halovibrio*, and with the species *"Paracoccus halodenitrificans"*, all of which were merged with the *Halomonas* based entirely upon 16S rDNA sequence similarities and a single chemotaxonomic feature. Very few of the phenetic characters originally used to describe these taxa are equivalent to those used to describe the rest of the *Halomonas* species. In fact, over 80 of the nearly 100 phenotypic descriptors for *Deleya* involve utilization of sole carbon sources, with virtually no information about the presence or absence of specific enzyme systems or abilities. Thus, it is difficult to provide a coherent and useable phenotypic description of the genus *Halomonas* and to provide reliable identifying features for the individual species.

At the chemotaxonomic level, the genus *Halomonas* represents a reasonably coherent group of bacteria; however, they do not possess specific or unique cell components that can be considered to be taxonomically distinct markers. All of the species possess ubiquinone-9 as their major respiratory quinone. When cells are grown on AOL with peptone, the major fatty acids of the *Halomonas* species that have been examined are $C_{16:1\ \omega7c}$, $C_{16:0}$, $C_{17:0\ cyclo}$, $C_{18:1}$, and $C_{19:0\ cyclo\ 11\text{-}12}$. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, and an unidentified periodate–Schiff positive phospholipid (Franzmann and Tindall, 1990). Vreeland et al. (1984) have shown that the type strain *H. elongata* also contains cardiolipin. This particular lipid may be detected in increased concentration in cells grown in higher salt concentration.

Common molecular features among the species include the

TABLE BXII.γ.96. Additional characteristics of *Halomonas* species[a]

Characteristic	1. *H. elongata*	2. *H. aquamarina*	3. *H. campisalis*	5. *H. cupida*	6. *H. desiderata*	7. *H. eurihalina*	8. *H. halmophila*	9. *H. halodenitrificans*	10. *H. haloduran*s	11. *H. halophila*	13. *H. magadii*	14. *H. marina*	15. *H. marisflavae*	16. *H. maura*	17. *H. meridiana*	18. *H. pacifica*	19. *H. pantelleriensis*	20. *H. salina*	21. *H. subglaciescola*	22. *H. variabilis*	23. *H. venusta*
Rods/curved rods	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+
Cocci								+													
Gram reaction	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Motile	+	+	+	+	+	+	−	−	+	+	+	+	+	−	+	+	+	−	+	+	+
Pigmentation[b]	C	C		C		C	Y	W	W	C	C	C	YO	C	W	C	CP	C	W	B	C
Growth conditions:																					
0% NaCl	+	−	−	−	+	D	−	−	+	−	+		−	−	d		−	−	−	−	
0.5% NaCl			+								+		+	−							
3.5% NaCl	+		+		+	+	+	+	+	+	+		+	+	+		+	+	+	−	
8.0% NaCl	+		+		+	+	+	+	+	+	+		+	+	+		+	+	+	+	
15% NaCl	+		+		+	+	+	+	+	+	+		+	+	+		+	+	+	+	
25% NaCl	+		+		−	+	+	−	+	+	−		+	−	+		−	−	−	+	
<10°C	−	−	+	−	+	+		+	+	−	−	+	+	−	+	−	−	d	+	−	+
15°C			+								−		+	+							
25°C	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+
30°C	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+		+	+
37°C	+	+	+	+	+	D		+	+	+	+	+	+	+	d	+	+	+	−	+	+
45°C	−	−	+	−	+				−	+	+	−	−	−	d	−	−	+		−	−
Oxidase present	+	+	+	−	+	+		+	+	+	+	−	−	+	+	+	+	+	+	+	+
Nitrate reduced to nitrite	+	+	+	+	+	−		+	+	+	+	−	−	+	−	−	+	+	d	−	+
Urease produced	d	d	−		−	+		−	−	d			−	−	+		+	+	−	+	
Starch hydrolysis	−	+		−	−	d	−	−	−	−	+	−	−	−	d		−	−	−	−	−
mol% G + C content	60–61	57–58	66	60–63	66	59–65	62.9	64–66	63.2	66.7	62.2	62–64	59	64.1	58.8–59.1	67–68	65	60.7–64.2	60.9–62.9	57	52–54

[a]For symbols see standard definitions.

[b]B, light brown; C, cream-beige; CP, cream to pink; W, white; Y, yellow; YO, yellow-orange.

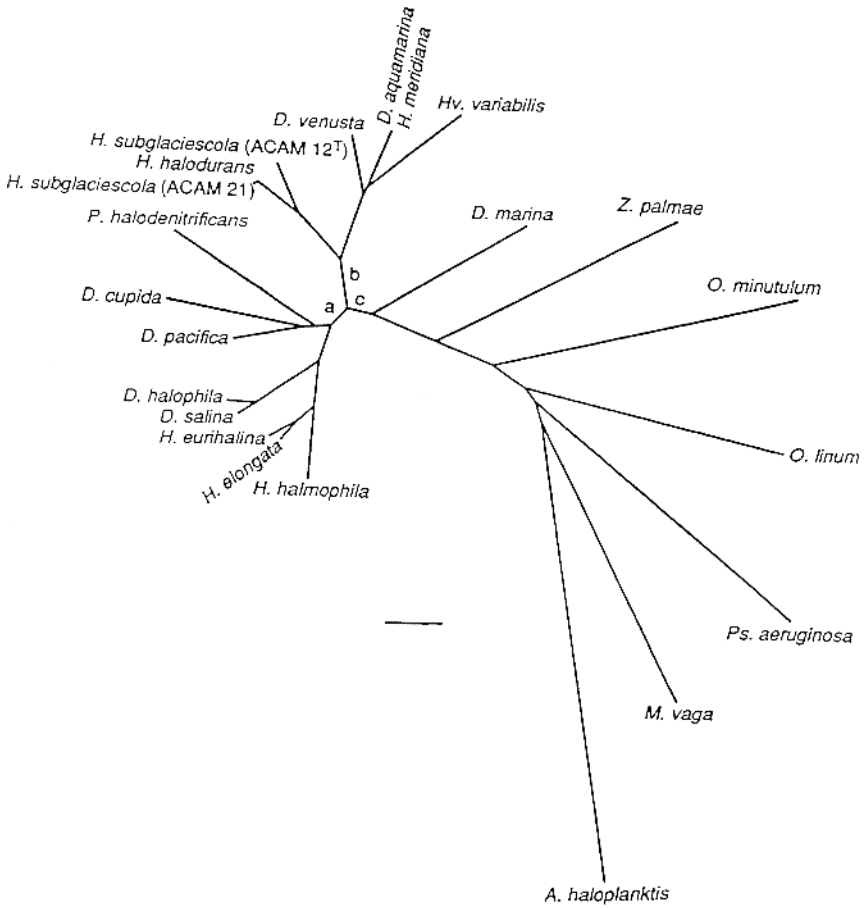

FIGURE BXII.γ.115. Distance tree containing the members of the genus *Halomonas* and other members of the class *Gammaproteobacteria*. Bar = 0.01 distance calculated by the Jukes–Cantor equation. Lower case letters a, b, and c refer to short internodes connecting the main branches of the monophyletic *Halomonas*. Abbreviations: *H.*, *Halomonas*; *O.*, *Oceanospirillum*; *Z.*, *Zymobacter*; *M.*, *Marinomonas*; *Ps.*, *Pseudomonas*; *A.*, *Alteromonas*. Redrawn and reprinted with permission from S. Dobson and P.D. Franzmann, International Journal of Systematic Bacteriology *46:* 550–558, 1996 ©International Union of Microbiological Societies.

presence of specific bases at 16 specific sites along the 16S rRNA molecule (Table BXII.γ.97). A taxonomic study of six species of *Halomonas* has recently been conducted by Huval et al. (1995). The DNA–DNA reassociation data presented in that article shows *Chromohalobacter israelensis* and *Chromohalobacter canadensis* possess 49% and 55% similarity, respectively, with the type species *Halomonas elongata*. *Halomonas halodurans* demonstrates DNA similarity of 28% with *H. elongata*. In contrast, *H. halophila* shows no detectable similarity with the type species of this genus (Table BXII.γ.98). Dobson et al. (1993) have shown that *H. aquamarina* shares a 41% DNA similarity with *H. venusta*, 16% similarity with *H. cupida*, and only 12% similarity with *H. pacifica* and *H. marina*. DNA–DNA hybridizations have not yet been conducted using any other species in the genus.

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TABLE BXII.γ.97. 16S rRNA signatures of the genus *Halomonas*[a]

Position number	Sequence of nucleotide
76–93	6 bp stem
484	A
486	C
640	G
660-[b]	A-
745	U
668-	A-
738-	U-
669-	A-
737	U
776	U
1124	U
1297	U
1298	C
1423	A
1424	C
1439	U
1462	A
1464	C

[a]Data from Dobson and Franzmann (1996).

[b]Dashes indicate that the specified nucleotide base pairs with the next one listed.

TABLE BXII.γ.98. DNA–DNA similarities between the type strains of *Halomonas elongata*, *Chromohalobacter canadensis* and strains of related species[a]

Source target DNA	% Hybridization with radiolabeled DNA	
	H. elongata ATCC 33173^T	*C. canadensis* NRCC 41227^T
H. elongata ATCC 33173^T	100	57
H. elongata ATCC 33174	72	49
H. halodurans ATCC 29868^T	28	34
H. halophila CCM 662^T	0	7
C. canadensis NRCC 41227^T	55	100
C. israelensis ATCC 43985^T	49	20

[a]Data from Huval et al. (1995).

Further Reading

Dobson, S.J. and P.D. Franzmann. 1996. Unification of the genera *Deleya* (Baumann et al. 1983), *Halomonas* (Vreeland et al. 1980), and *Halovibrio* (Fendrich 1988) and the species *Paracoccus halodenitrificans* (Robinson and Gibbons 1952) into a single genus, *Halomonas*, and placement of the genus *Zymobacter* in the family *Halomonadaceae*. Int. J. Syst. Bacteriol. *46*: 550–558.

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.

Vreeland, R.H. 1991. The Family *Halomonadaceae*. *In* Balows, Trüper, Dworkin, Harder and Schleifer (Editors), The Prokaryotes, 2nd edition, Springer-Verlag, New York. pp. 3181–3188.

Differentiation of the species of the genus *Halomonas*

Characteristics differentiating the species of the genus *Halomonas* are listed in Table BXII.γ.96.

List of species of the genus Halomonas

1. **Halomonas elongata** Vreeland, Litchfield, Martin and Elliot 1980, 495^{VP}
   e.lon′ga.ta. L. fem. part. adj. *elongata* elongated, stretched out.

   The characteristics are as described for the genus and as listed in Tables BXII.γ.95 and BXII.γ.96. Morphological features are shown in Figs. BXII.γ.116 and BXII.γ.117. Additional characteristics of the species include the ability to grow at 0.5% and 30% NaCl. Grows at pH 5.0–9.0 in complex medium with 8% NaCl at 30–37°C. Grows at 15°C. Catalase, phosphatase, lysine decarboxylase, β-galactoside, and indole are produced. Some strains may be positive for esculin hydrolysis. Hydrogen sulfide negative. Does not possess DNase or phenylalanine deaminase. Hydrolyzes Tween 20, but not Tween 80. This species has been reported to produce acid from fructose, D-glucose, lactose, D-maltose, and sucrose, but not from D-galactose, inulin, or D-mannitol. In addition to those listed in Table BXII.γ.95, the following compounds serve as sole carbon sources: esculin, D-galactose, L-rhamnose, D-salicin, D-xylose, fumarate, pyruvate, β-hydroxybutyrate, L-aspartic acid, L-glutamic acid, L-ornithine, L-valine, proline, L-asparagine, and tyrosine. The species does not utilize inulin, raffinose, trehalose, malonate, L-tryptophan, L-threonine, or methionine as sole carbon sources. Sensitive to gentamicin (10 μg), mercuric chloride (1:5000), neomycin (30 μg), streptomycin (10 μg), and

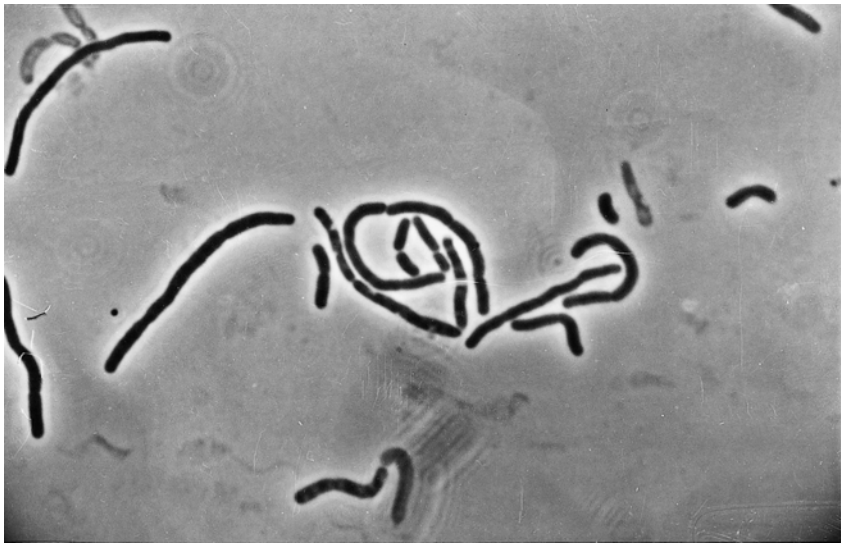

FIGURE BXII.γ.116. Phase contrast micrograph of *Halomonas elongata* strain 1H9 (ATCC 33173), showing both long and short cell forms (× 2000).

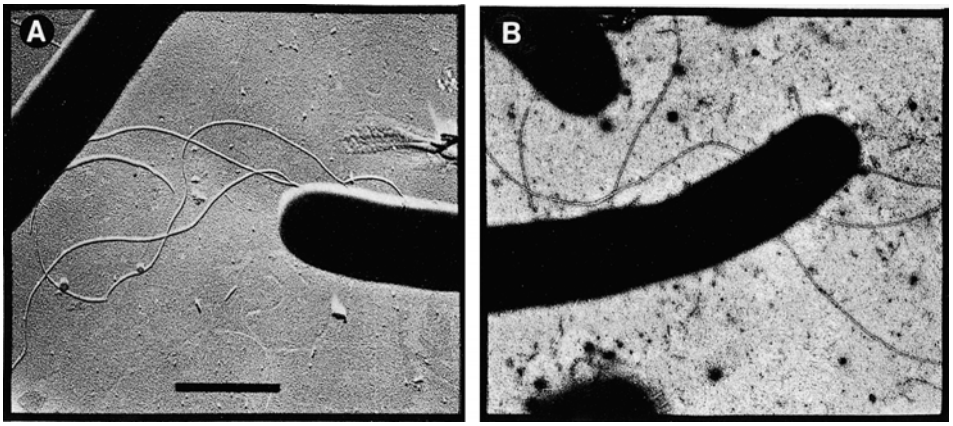

FIGURE BXII.γ.117. Electron micrographs of *Halomonas elongata*, showing flagellar arrangement. *A*, strain 1H9 (ATCC 33173); carbon shadowed. *B*, strain 1H15 (ATCC 33174); negatively stained (Bar = 1.0 μm).

chloramphenicol (30 μg). Resistant to ampicillin (10 μg), bacitracin (10 i.u.), cephalothin (30 μg), erythromycin (15 μg), nalidixic acid (30 μg), novobiocin (20 μg), vibriostat O/129 (10 μg), penicillin G (10 i.u.), and tetracycline (30 μg).

Sodium salts, such as sodium glutamate, $NaNO_3^-$, and NaBr, can substitute for NaCl. Na_2SO_3 and NaI cannot. The chloride salts of magnesium, lithium, potassium, and ammonia cannot substitute for NaCl in growth media. Possesses peptidoglycan. Based upon amino acid analyses, the peptidoglycan contains leucine in a crossbridge (Vreeland et al., 1984). Present in solar salterns, estuaries, salt marshes, and other environments containing NaCl. This species originally contained two biovars (ATCC 33174 = 1H15 [Vreeland et al., 1980] and DSM 3043 = 1H11 [Vreeland et al., 1980]). Both biovars have now been reclassified as *Chromohalobacter salexigens* (Arahal et al., 2001a).

The mol% G + C of the DNA is: 60.5 ± 0.5 (Bd, T_m).

Type strain: 1H9 of Vreeland et al., 1980, ATCC 33173, DSM 2581.

GenBank accession number (16S rRNA): M93355, X67023.

2. **Halomonas aquamarina** (ZoBell and Upham 1944) Dobson and Franzmann 1996, 556^{VP} (*Alcaligenes aquamarinus* (ZoBell and Upham 1944) Hendrie, Holding and Shewan 1974, 537; *Deleya aquamarina* (ZoBell and Upham 1944) Akagawa and Yamasato 1989, 466; *"Achromobacter aquamarinus"* ZoBell and Upham 1944, 264.)
a.qua.ma.ri' na. L. n. *aqua* water; L. adj. *marina* of the sea; L.adj. *aquamarina* pertaining to seawater.

The characteristics are as described for the genus and as listed in Tables BXII.γ.95 and BXII.γ.96. This species may or may not produce H_2S and phenylalanine deaminase. The species does not hydrolyze esculin or produce DNase or β-galactosidase. This species utilizes malate, pyruvate, β-hydroxybutyrate, caprylate, L-aspartic acid, L-glutamic acid, and proline as sole sources of carbon. The species also utilizes butyrate, isobutyrate, valerate, isovalerate, caproate, heptanoate, adipate, pimelate, azelate, sebacate, levulinate, erythritol, *meso*-inositol, *n*-propanol, *n*-butanol, isobutanol, cytosine, and uracil. This is the only *Halomonas* species known to hydrolyze starch (Table BXII.γ.96) and utilize suberate. Accumulates polyhydroxyalkanoates. Some strains of the species may utilize D-galactose, trehalose, L-ornithine, and L-valine. The species does not grow on inulin, L-rhamnose, D-salicin, D-xylose, formate, malonate, oxalacetate, D-tartrate, benzoate, L-threonine, or tyrosine as the sole carbon source. There are no data available about the sensitivity of this species to antimicrobials. Isolated from the Pacific Ocean. The similarity of the 16S rRNA sequence of this species to the other *Halomonas* species ranges from 93.6–100%.

The mol% G + C of the DNA is: 60.5 ± 0.5 (Bd, T_m).

Type strain: ATCC 14400, DSM 30161, IAM 12550, NCMB 557.

GenBank accession number (16S rRNA): M93352.

3. **Halomonas campisalis** Mormile, Romine, Garcia, Ventosa, Bailey and Peyton 2000, 949^{VP} (Effective publication: Mormile, Romine, Garcia, Ventosa, Bailey and Peyton 1999, 556.)
cam.pi.sa' lis. L. masc. n. *campus* plain, field; L. masc. n. *sal* salt; gen. *salis* of salt; L. masc. n. *campisalis* of the plain of salt, of the salt plain.

General characteristics are as described for the genus and are presented in Tables BXII.γ.95 and BXII.γ.96. Additional characteristics include the ability to grow at temperatures from 4°C to 50°C. Optimal growth occurs at 30°C. This species reproduces at pH between 6.0 and 11.0 with optimal growth at pH 9.5. The optimal salt concentration is 1.5 M with a growth range from 0.2 to 4.5 M. In addition to those listed in Table BXII.γ.95, the species utilizes *n*-acetyl-glucosamine, ethanol, D(−)-fructose, D(+)-glucosamine, glycerol, pyruvate, and yeast extract. The species does not utilize benzoic acid, D(+)-galactose, D(+)-mannose, methanol, pectin, D(−)-ribose, L-rhamnose, L(−)-sorbose, whey, and D(+)-xylose. The organism does not grow fermentatively. The species gives negative tests for arginine dihydrolase, phenylalanine deaminase, indole production, and Voges–Proskauer.

This species is susceptible to ampicillin, chloramphenicol, and rifampin. It is resistant to kanamycin, neomycin, and streptomycin. The concentrations of antibiotics used were not provided in the original description.

The species was isolated from a soil sample just below the crystalline salt surface of a salt flat south of Alkali Lake in eastern Washington State, USA.

The mol% G + C of the DNA is: 66 (T_m).
Type strain: 4A, ATCC 700597.
GenBank accession number (16S rRNA): AFO54286.

4. **Halomonas cupida** (Baumann, Baumann, Mandel and Allen 1972) Dobson and Franzmann 1996, 556^{VP} (*Deleya cupida* (Baumann, Baumann, Mandel and Allen 1972) Baumann, Bowditch and Baumann 1983a, 801; *Alcaligenes cupidus* Baumann, Baumann, Mandel and Allen 1972, 426.) *cu' pi.da.* L. adj. *cupidus* desiring.

The characteristics are as described for the genus and as listed in Tables BXII.γ.95 and BXII.γ.96. This species possesses peritrichous flagella; it has been described as utilizing 69–85 organic compounds, possibly more than any other *Halomonas* species. In addition to those listed, the species also utilizes D-galactose, D-xylose, fumarate, malate, pyruvate, β-hydroxybutyrate, benzoate, caprylate, L-aspartic acid, L-glutamic acid, proline, tyrosine, melibiose, saccharate, galacturonate, glucuronate, *N*-acetylglucosamine, butyrate, isobutyrate, valerate, isovalerate, caproate, heptanoate, pelargonate, caprate, glycolate, DL-glycerate, aconitate, erythritol, *meso*-inositol, *n*-propanol, isobutanol, benzoylformate, *p*-hydroxybenzoate (but not the *ortho* or *meta* isomers), putrescine, spermine, betaine, sarcosine, hippurate, and allantoin. The species may or may not grow on L-rhamnose, D-salicin, D-trehalose, malonate, and L-ornithine. This organism does not use formate, oxalate, D-tartrate, L-valine, or L-threonine as the sole source of carbon. The similarity of the 16S rDNA of this species to other *Halomonas* species ranges from 92.6–96.5%. Isolated from marine habitats.

The mol% G + C of the DNA is: 60–63 (Bd).
Type strain: ATCC 27124, DSM 4740.
GenBank accession number (16S rRNA): L42615.

5. **Halomonas desiderata** Berendes, Gottschalk, Heine-Dobbernack, Moore and Tindall 1997, 242^{VP} (Effective publication: Berendes, Gottschalk, Heine-Dobbernack, Moore and Tindall 1996,165.)
de.si.de.ra' ta. L. part. adj. *desiderata* wished for, the strain wished for.

Cells are motile with peritrichous flagella and are 0.4–0.6 × 1.0–2.6 μm. The colony type has not been described, but the species does not produce pigments. Requires sodium ions. Optimum pH is 9.7 and the optimum temperature for growth is 37–42°C. The characteristics are as described for the genus and as listed in Tables BXII.γ.95 and BXII.γ.96.

Facultatively anaerobic in the presence of nitrate. Obligately alkaliphilic; optimum pH 9.5. Grows at 45°C. Denitrifying. Does not grow below pH 7 or above pH 11. Does not grow at 50°C. Supplemental vitamins or amino acids are not needed. Catalase positive and phenylalanine deaminase positive. Arginine dihydrolase negative. Does not hydrolyze starch, gelatin, casein, Tween 80, Tween 60, hippurate, or pullulan. Produces poly-β-hydroxybutyrate as a reserve material but does not produce levan from sucrose. Grows on MacConkey agar, *Salmonella–Shigella* agar, and Cetrimide agar. At pH 9.7, the species is resistant to up to 1% bile salts.

Utilizes D-xylose, ribose, trehalose, glycerol, DL-β-hydroxybutyrate, and benzoate as sole sources of carbon. Does not utilize inositol. Not fermentative; indole and Voges–Proskauer negative. The major respiratory quinone is ubiquinone-9. The major fatty acids are $C_{18:1}$ (52–65% depending on the strain), $C_{16:0}$ (13–23% depending on the strain), and $C_{16:1\ \omega7c}$. Other fatty acids detected in various strains include $C_{10:0}$, $C_{10:0\ 3OH}$, $C_{12:0}$, $C_{12:0\ 3OH}$, $C_{14:0}$, $C_{17:0\ cyclo}$, $C_{18:0}$, and $C_{19:0\ cyclo}$. The major phospholipids are phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol, and an uncharacterized phosphoglycolipid. The similarity of the 16S rDNA of this species to other *Halomonas* species ranges from 89.7–94.8%. DNA–DNA hybridization data show similarities below 60–70%. Isolated from municipal sewage.

The mol% G + C of the DNA is: 66 (HPLC).
Type strain: DSM 9502.
GenBank accession number (16S rRNA): X92417.

6. **Halomonas eurihalina** (Quesada, Valderrama, Bejar, Ventosa, Gutierrez, Ruiz-Berraquero and Ramos-Cormenzana 1990) Mellado, Moore, Nieto and Ventosa 1995b, 715^{VP} (*Volcaniella eurihalina* Quesada, Valderrama, Bejar, Ventosa, Gutierrez, Ruiz-Berraquero and Ramos-Cormenzana 1990, 265.)
eu.ri.ha.li' na. Gr. adj. *euris* wide, broad; Gr. adj. *halinos* salted; M.L. adj. *eurihalina* growing at a wide range of salt concentrations.

The characteristics are as described for the genus and as listed in Tables BXII.γ.95 and BXII.γ.96. Colonies grown on MH medium with 7.5% NaCl at 32°C for 72 h are 4–5 mm in diameter, circular, convex, smooth, opaque, cream-colored with entire margins, and very mucoid. This species requires Na^+ ions. Sodium can be supplied as NaCl, Na_2SO_4, or NaBr. Under optimum conditions, the species grows at pH 5.0–9.0. Catalase positive. Phosphatase, lysine decarboxylase, and β-galactosidase negative. Does not hydrolyze esculin. Produces acid from fructose and maltose. Some strains may also produce acid from D-galactose, D-glucose, lactose, mannitol, and sucrose. Utilizes fumarate, β-hydroxybutyrate, L-valine, L-threonine, proline, and tyrosine as sole carbon sources. Some strains also utilize D-galactose, raffinose, D-salicin, D-trehalose, L-ornithine, and methionine as sole carbon sources. This species hydrolyzes gelatin and grows on KCN, MacConkey, and Cetrimide agar. Some strains of this species produce H_2S and hydrolyze Tween 20. Sensitive to chloramphenicol (30 μg). Some strains may be sensitive to gentamicin (10 μg) and neomycin (30 μg). Resistant to most other antimicrobials. Isolated from hypersaline habitats (soils and salterns) and from ocean water.

The mol% G + C of the DNA is: 59.1–65.7 (T_m).
Type strain: ATCC 49336, DSM 5720.
GenBank accession number (16S rRNA): L42620, X87218.

7. **Halomonas halmophila** (Elazari-Volcani 1940) Franzmann, Wehmeyer and Stackebrandt 1989, 205^{VP} (Effective publication: Franzmann, Wehmeyer and Stackebrandt 1988, 19) emend. Dobson, James, Franzmann and McMeekin 1990, 462 (*Flavobacterium halmophilum* Elazari-Volcani 1940, 85.)
hal.mo.phi' lum. Gr. n. *halme* brine, seawater; Gr. adj. *philos* loving; M.L. n. adj. *halmophilum* seawater loving.

The characteristics are as described for the genus and as listed in Tables BXII.γ.95 and BXII.γ.96. Does not produce acid from sugars, D-mannitol, or inulin and does not produce indole. The species is sensitive to nalidixic acid

(30 μg) and resistant to ampicillin (10 μg), gentamicin (10 μg), neomycin (30 μg), streptomycin (10 μg), and tetracycline (30 μg). This species was transferred into the genus *Halomonas* when 16S rRNA cataloging showed an S_{AB} value of 0.66 to the type species of the genus *Halomonas*. The similarity of the 16S rDNA of this species to other *Halomonas* species ranges from 92.2–97.9%. The mol% G + C content of the DNA has been reported to be 49.7. The exact site from which this bacterium was isolated is not known, but was probably somewhere in the Dead Sea.

The mol% G + C of the DNA is: 49.7 (method unknown).

Type strain: ATCC 19717, DSM 5349.

GenBank accession number (16S rRNA): M59153.

The name of this species has been incorrectly spelled as *Flavobacterium halmephilum* (Weeks, 1974) and as *Flavobacterium halmephilium* (Weeks, 1974).

8. **Halomonas halodenitrificans** (Robinson and Gibbons 1952) Dobson and Franzmann 1996, 556^{VP} (*Paracoccus halodenitrificans* (Robinson and Gibbons 1952); *Micrococcus halodenitrificans* Robinson and Gibbons 1952, 154.)
ha.lo.de.ni.tri'fi.cans. Gr. n. *hals, halis* salt; M.L. v. *denitrifico* to denitrify; M.L. part. adj. *halodenitrificans* salt (requiring) denitrifying.

This is the only member of this genus that has a coccoid morphology. The characteristics are as described for the genus and as listed in Tables BXII.γ.95 and BXII.γ.96. Most of the characteristics have been taken from the description of Kocur (1984). When grown on nutrient agar containing 6% NaCl, the colonies of this species are described as circular, entire, convex, butyrous, glistening, cream-colored, and opaque. The optimum growth temperature is 25–30°C; the growth range is 5–32°C. The species cannot grow in media containing less than 3% NaCl. Optimum growth occurs with 4.4–8.8% NaCl; slow growth occurs with up to 20% NaCl in media. Catalase positive; phosphatase is present. Hydrogen sulfide is not produced. Esculin is not hydrolyzed. Lysine decarboxylase, phenylalanine deaminase, and β-galactosidase negative. Indole and Voges–Proskauer negative. Does not hydrolyze Tween 80. Acid is not produced from either D-glucose or sorbitol. Acid production from other sugars or polyols has not been tested. L-valine, L-threonine, proline, and L-asparagine serve as sole carbon sources. Does not utilize L-aspartic acid, L-glutamic acid, or methionine as sole carbon sources. Sensitive to mercuric chloride and polymyxin B, although no concentrations have been specified. Resistant to gentamicin (10 μg), vibriostat O/129 (10 μg), penicillin G (10 i.u.), streptomycin (10 μg), tetracycline (30 μg), and chloramphenicol (30 μg). The similarity of the 16S rDNA of this species to each member of the genus *Halomonas* ranges from 91.5–95.6%. Isolated from meat-curing brines.

The mol% G + C of the DNA is: 64–66 (T_m, Bd).

Type strain: ATCC 13511, CCM 286, DSM 735, NCMB 700.

GenBank accession number (16S rRNA): L04942.

9. **Halomonas halodurans** Hebert and Vreeland 1987, 350^{VP}
ha.lo.du'rans. Gr. n. *hals, halos;* the sea, salt; L. v. *durare* to last endure; M.L. masc. adj. *Halodurans* salt tolerating.

The characteristics are as described for the genus and as listed in Tables BXII.γ.95 and BXII.γ.96. The original description of this species was based on tests using media supplemented with 2.6% NaCl. Later descriptions of this species by Hebert and Vreeland (1987) were performed at the optimum NaCl concentration for growth of the species (8.0%). Hebert and Vreeland (1987) noted several differences in the results of the phenotypic testing and attributed them to the differences in the salt content of the media. The biochemical properties listed in Tables BXII.γ.95 and BXII.γ.96 for this species are those corresponding to tests conducted with media containing 8% NaCl.

The cells are motile by single polar flagellum that is 8–10 μm in length and 12–14 nm wide. Colonies are circular, convex, smooth, entire, 2–3 mm in diameter, and nonpigmented following 48 h of growth at 20–35°C. This species does not grow in 30% NaCl. Grows at pH 6.0–8.0 in 8% NaCl.

Catalase positive; phosphatase, and lysine decarboxylase are present. Phenylalanine deaminase and β-galactosidase are absent. Hydrolyzes esculin. Tolerant to KCN. Hydrolyzes Tween 80. Hydrogen sulfide, indole, and acetyl-methyl carbinol (Voges–Proskauer) are not produced. Produces acid from D-fructose, D-galactose, D-glucose, lactose, salicin, sucrose, and D-xylose. Does not produce acid from D-mannitol, D-mannose, or L-rhamnose. Utilizes D-galactose, inulin, ribose, malonate, pyruvate, benzoate, L-ornithine, L-threonine, proline, and L-asparagine as sole sources of carbon. The species cannot use L-rhamnose, malate, β-hydroxybutyrate, or methionine as sole carbon sources. Sensitive to erythromycin (15 μg), nalidixic acid, (30 μg), streptomycin (10 μg), tetracycline (30 μg), and polymixin B (concentration unknown). The species is resistant to vibriostat O/129 (10 μg), penicillin G (10 i.u.), and chloramphenicol (30 μg). The similarity of the 16S rDNA of this species to other species of *Halomonas* ranges from 93.7–99.9%. The level of DNA–DNA homology between this species *H. elongata* and *H. canadensis* is shown in Table BXII.γ.98. Isolated from Great Bay Estuary (New Hampshire, USA).

The mol% G + C of the DNA is: 63.2 ± 1.1 (T_m, Bd).

Type strain: ATCC 29686, DSM 5160.

GenBank accession number (16S rRNA): L42619.

10. **Halomonas halophila** (Quesada, Ventosa, Ruiz-Berraquero and Ramos-Cormenzana 1984) Dobson and Franzmann 1996, 556^{VP} (*Deleya halophila* Quesada, Ventosa, Ruiz-Berraquero and Ramos-Cormenzana 1984, 290.)
ha.lo.phi'la. Gr. n. *halos* salt; Gr. adj. *philus* loving; M.L. adj.*halophila* salt loving.

The characteristics are as described for the genus and as listed in Tables BXII.γ.95 and BXII.γ.96. When grown with 7.5% sea salt for 4 days at 32°C, the colonies are circular, low convex, smooth, opaque, and cream-colored. Grows in 30% NaCl and at pH 5.0–9.0. Tests positive for catalase and phosphatase. DNase and phenylalanine deaminase negative. Produces hydrogen sulfide and hydrolyzes esculin, but does not hydrolyze Tween 80. Indole and Voges–Proskauer negative. Produces acid from D-galactose, D-glucose, and D-maltose. Some strains may also produce acid from sucrose, D-trehalose, and D-xylose. Acid is not produced from lactose and mannitol. In addition to the carbon sources listed in Table BXII.γ.95, this species also utilizes D-galactose, D-salicin, D-trehalose, D-xylose, fumarate, malate, pyruvate, L-aspartic acid, and L-glutamic acid as sole carbon sources. Some strains may also use L-orni-

thine. The species does not utilize L-rhamnose, hippurate, D-tartrate, benzoate, caprylate, or L-valine as sole carbon sources. The species is sensitive to ampicillin (10 μg), cephalothin (30 μg), erythromycin (15 μg), chloramphenicol (30 μg), polymyxin B, and rifampin A (no concentrations have been specified for the latter two anti-microbials). Resistant to bacitracin (10 i.u.) and lincomycin (concentration unknown). The similarity of the 16S rDNA of this species to each member of the *Halomonas* ranges from 93.8–99.2%. Isolated from saline soils.

The mol% G + C of the DNA is: 66.7 (T_m).

Type strain: CCM3662, DSM 4770.

GenBank accession number (16S rRNA): L42619 and M93353.

11. **Halomonas magadii** Duckworth, Grant, Jones, Meijer, Márquez and Ventosa 2000b, 1415^{VP} (Effective publication: Duckworth, Grant, Jones, Meijer, Márquez and Ventosa 2000a, 59.)
ma.ga' di.i. M.L. gen. n. *magadi* of Magadi, named for lake Magadi, a saline soda lake in Kenya.

The characteristics are as described for the genus and are listed in Tables BXII.γ.95 and BXII.γ.96. Other descriptive characters are listed below. Rods 4.0–6.0 × 0.6–0.8 μm. Catalase positive, produces circular, low convex opaque cream-colored colonies. Alkaliphilic, growth occurs between pH 7.0 and 11.0 with an optimum around 9.5. Grows at salt concentrations from 0% to 20% between 25°C and 40°C with an optimum at 37°C. Phosphatase and phenylalanine deaminase negative. Does not hydrolyze gelatin or casein. Indole negative. No anaerobic growth in the presence or absence of nitrate. Produces H_2S from cysteine. When grown in alkaline medium, the species utilizes amygdalin, esculin, D-fructose, D-fucose, D-galactose, D-melibiose, L-raffinose, D-ribose, salicin, starch, trehalose, D-xylose, adonitol, D-glucuronolctone, D-glucosamine, dulcitol, erythritol, ethanol, glycerol, D-mannitol, *m*-inositol, propanol, D-sorbitol, *N*-acetylglucosamine, propionide, quinate, D-saccharate, salicylate, suberate, tartrate, L-asparagine, DL-aspartic acid, DL-phenylalanine, L-glutamine, α-aminovalerate, aconitate, α-ketoglutarate, butyrate, caprylate, fumarate, DL-glycerate, D-glucuronate, glutamate, *p*-hydroxybenzoate, hippurate, DL-malate, malonate, oxalate, L-ornithine, L-leucine, L-methionine, L-proline, L-threonine, L-valine, and pyruvate. Sarcosine, ethionine, creatine, DL-aminobutyrate, Tween 80, inulin, and L-rhamnose are not used. Sensitive to erythromycin (5 mg), streptomycin (10 mg), polymyxin (300 IU), sulphafurazole (100 mg), chloramphenicol (25 mg), and oleandomycin (5 mg). Not sensitive to gentamicin (10 mg), kanamycin (30 mg), fusidic acid (10 mg), tetracycline (25 mg), rifampicin (2 mg), bacitracin (10 IU), neomycin (30 mg), novobiocin (5 mg), and vancomycin (30 mg). Listed as insensitive to sulphamethoxazole, nitrofurantoin, trimethoprim, ampicillin, penicillin G, and methicillin, but no concentrations were specified.

The major polar lipids are phosphatidylglycerol, phosphatidyl glycerol phosphate, diphosphatidylglycerol, phosphatidylethanolamine, and a small amount of an unidentified glycolipid. Cells also contain ubiquinones 6 and 9.

The mol% G + C of the DNA is: 62 (T_m).

Type strain: 21M1, NCIMB 13595.

GenBank accession number (16S rRNA): X92150.

12. **Halomonas marina** (Cobet, Wirsen and Jones 1970) Dobson and Franzmann 1996, 556^{VP} (*Deleya marina* (Cobet, Wirsen and Jones 1970) Baumann, Bowditch and Baumann 1983a,801; *Pseudomonas marina* (Cobet, Wirsen and Jones 1970) Baumann, Baumann, Mandel and Allen 1972, 423; *"Arthrobacter marinus"* Cobet, Wirsen and Jones 1970, 159.)
ma.ri' na. L. adj. *marina* of the sea.

The characteristics are as described for the genus and as listed in Tables BXII.γ.95 and BXII.γ.96. The cells possesses polar flagella. Baumann et al. (1983a) note that this species has flagella that tend to bend back toward the cell, giving arrangements that appear to be lateral or peritrichous. The species has been described as utilizing 35–47 organic compounds as sole sources of carbon. In addition to those listed in Table BXII.γ.95, this species utilizes D-galactose, fumarate, malate, pyruvate, β-hydroxybutyrate, L-aspartic acid, L-glutamic acid, L-proline, butyrate, valerate, isovalerate, caproate, heptanoate, pelargonate, caprate, DL-glycerate, α-ketoglutarate, aconitate, *n*-butanol, sarcosine, and creatine. Some strains may also utilize malonate and L-ornithine as sole carbon sources. The species does not use L-rhamnose, ribose, D-salicin, D-trehalose, D-xylose, hippurate, D-tartrate, benzoate, L-valine, or L-threonine. The similarity of the 16S rDNA of this species to other *Halomonas* species is only 92.3–94.8%. Isolated from marine habitats.

The mol% G + C of the DNA is: 63 (Bd).

Type strain: C 25374, DSM 4741.

GenBank accession number (16S rRNA): M93354.

13. **Halomonas marisflavae** Yoon, Choi, Lee, Kho, Kang, and Park 2001a, 1176^{VP}
ma.ris.fla' vae. L. gen. neut. n. *maris* of the sea; L. neut. adj. *flavum* yellow; L. gen. neut. n. *marisflavae* of the Yellow Sea, Korea.

The characteristics are as described for the genus and are listed in Tables BXII.γ.95 and BXII.γ.96 The species produces rod or oval shaped cells that are 0.9–1.3 × 1.7–2.3 μm following 3 days growth at 28°C. Cells are motile with a single polar flagellum. Colonies are yellow-orange, smooth, glistening, circular, and slightly irregular on Tryptic Soy Agar (TSA) and on Marine Agar (MA). The colonies are described as being convex on TSA and concave on MA. Optimal growth occurs between 0.5% and 12% NaCl (w/v) and at 25–30°C. The optimal pH for growth is 7.0–8.0. Grows anaerobically on MA medium although the exact conditions for this characteristic were not specified. Catalase positive. Hydrolyzes esculin and gelatin, but not casein, hypoxanthine, Tween 80, tyrosine, or xanthine. Indole and arginine deaminase negative. Assimilates and produces acid from D-glucose, arabinose, mannose, mannitol, gluconate, malate, and citrate. The species is also described as producing acid from glycerol, erythritol, ribose, D-xylose, galactose, D-fructose, arbutin, salicin, maltose, melibiose, trehalose, gentiobiose, D-turanose, D-lyxose, and D-furanose. Also produces a weak acid reaction from adonitol, mannitol, and xylitol.

The predominant respiratory quinone is ubiquinone-9. The major fatty acids are $C_{18:1}$, $C_{16:0}$, $C_{16:1\ \omega7c}$ and/or $C_{15:0\ iso\ 2OH}$.

Isolated from the Yellow Sea, Korea.

The mol% G + C of the DNA is: 59 (HPLC).

Type strain: SW32, JCM 10873, KCCM 80003.

GenBank accession number (16S rRNA): AF251143.

14. **Halomonas maura** Bouchotroch, Quesada, Del Moral, Llamas and Bejar 2001, 1630^{VP}
mau′ra. L. adj. *maurus* northwest African.

The characteristics are as described for the genus and are listed in Tables BXII.γ.95 and BXII.γ.96. This species produces one of the longest rods (6.0–9.0 × 0.5–0.7 μm) in the genus. It and *Halomonas eurihalina* are the only species known to produce large amounts of exopolysaccharide. Colonies are circular, convex, mucoid, cream-colored, and 2–3 mm in diameter after 24 hours growth at 32°C. Colonies expand to over 5 mm in diameter after 72 hours. Grows in salt concentrations between 1% and 15% with optimal growth occurring between 7.5 and 10% (w/v). The salt concentrations given actually reflect the percentage of total sea salts and not specifically NaCl. However, the original description of this species states that the organism is able to grow optimally in medium containing 1.2M NaCl (7.0% w/v) and 0.2M $MgSO_4 \cdot 7H_2O$ (4.5%). The description also states that $MgCl_2 \cdot 6H_2O$ may be substituted for magnesium sulfate. The species is capable of anaerobic growth in the presence of nitrate but not fumarate. Catalase positive. Does not produce acids from sugars. Reduces selenite and oxidizes gluconate. The species produces H_2S from peptones and hydrolyzes Tween 20. Indole, methyl-red, and Voges–Proskauer tests are negative. The species does not utilize esculin, tyrosine, DNA, gelatin, casein, lecithin, and Tween 80, nor does it hydrolyze blood, produce phenylalanine deaminase, grow on cetrimide agar or under anaerobic conditions on normal medium. In addition, the type strain of the species produces phosphatase and grows on MacConkey and KCN agars and is ONPG negative.

In addition to the compounds listed in Table BXII.γ.95, the species utilizes D-galactose, *myo*-inositol, and D-sorbitol as sole sources of carbon and energy. The species does not utilize formate, D-salicin, or L-sorbose. The type strain differs somewhat in these characters in that it also utilizes citrate, ethanol, fumarate, D-fructose, glycerol, D-rhamnose, and D-ribose. The type strain also does not utilize lactose or D-trehalose. The species also utilizes the amino acids listed in Table BXII.γ.95, as well as L-ornithine and L-valine, as sole sources of carbon and nitrogen. The organism does not utilize cysteine or L-tryptophan in this manner. As a whole, the species is susceptible to amoxycillin, ampicillin, cephalothin, cefoxitin, chloramphenicol, nalidixic acid, nitrofurantoin, polymixin, rifampicin, and trimethoprim-sulfamethoxazole. The species is resistant to tetracycline and Vibriostat O/129. The type strain is also sensitive to gentamicin, penicillin, sulfonamide, and tobramycin while being resistant to erythromycin. Unfortunately, no antibiotic concentrations were provided in the original species description. These data may be provided by Bouchotroch et al. (1999) which was cited by Bouchotroch et al. (2001).

The major fatty acids (up to 81% total) of the type strain are $C_{18:1\ \omega7c}$, $C_{16:1\ \omega7c}/C_{15:0\ iso\ 2OH}$, and $C_{16:0}$. The bacterium produces exo-polysaccharide designated EPS S-31 which is composed of neutral sugars glucose, mannose, and galactose in a ratio of 1:4:2.5. EPS S-31 emulsifies crude oil.

The species was isolated from a solar saltern in Asilah Morocco.

The mol% G + C of the DNA is: 62.2–64.1 (T_m).
Type strain: S-31, CECT 5298, DSM13445.
GenBank accession number (16S rRNA): AJ271864.

15. **Halomonas meridiana** James, Dobson, Franzmann and McMeekin 1990b, 470^{VP} (Effective publication: James, Dobson, Franzmann and McMeekin 1990a, 277.)
me.ri.di.a′na. L. adj. *meridiana* of the south.

The characteristics are as described for the genus and as listed in Tables BXII.γ.95 and BXII.γ.96. The colonies produced by this species are smooth, circular, convex, and white to off-white, with entire margins. Yellow colonies may be produced on media containing alanine or pyruvate. The cells may possess either lateral or polar flagella. The species grows in media of pH 6.0–9.0; some strains may grow at pH 5.0. Catalase positive. Test results for phosphatase, DNase, lysine decarboxylase, phenylalanine deaminase, and β-galactosidase are all negative. Most strains have been reported to hydrolyze Tween 80 and Tween 20. Produces acid from D-fructose, maltose, and sucrose. Some strains may produce acid from D-galactose, D-glucose, lactose, and mannitol. Utilizes D-xylose, fumarate, β-hydroxybutyrate, L-valine, proline, and L-asparagine as sole sources of carbon. Most strains also utilize D-galactose and D-rhamnose. Some strains may utilize raffinose, D-salicin, D-trehalose, L-ornithine, L-threonine, and methionine as sole carbon sources. Does not use malonate. Resistant to most antibiotics, including ampicillin (10 μg), bacitracin (10 i.u.), cephalothin (30 μg), erythromycin (10 μg), mercuric chloride 1:5000 (200 mg/l), nalidixic acid (30 μg), novobiocin (20 μg), vibriostat O/129 (10 μg), penicillin G (10 i.u.), streptomycin (10 μg), and tetracycline (30 μg). Some strains show sensitivity to gentamicin (10 μg), neomycin (30 μg), and chloramphenicol (30 μg). The similarity of the 16S rDNA of this species to each member of the *Halomonas* ranges from 93.6–100%. Isolated from Antarctic lakes.

The mol% G + C of the DNA is: 58.8–59.1 ± 0.8 (T_m).
Type strain: ACAM 246, ATCC 49692, DSM 5425, UQM 3352.
GenBank accession number (16S rRNA): M93356.

16. **Halomonas pacifica** (Baumann, Baumann, Mandel and Allen 1972) Dobson and Franzmann 1996, 556^{VP} (*Deleya pacifica* (Baumann, Baumann, Mandel and Allen 1972) Baumann, Bowditch and Baumann 1983a, 801; *Alcaligenes pacificus* Baumann, Baumann, Mandel and Allen 1972, 426.)
pa.ci′fic.a. M.L. adj. *pacifica* pertaining to the Pacific ocean.

The characteristics are as described for the genus and as listed in Tables BXII.γ.95 and BXII.γ.96. In addition, the species has been shown to utilize fumarate, malate, pyruvate, β-hydroxybutyrate, benzoate, L-aspartic acid, L-glutamic acid, L-ornithine, proline, *N*-acetylglucosamine, butyrate, isobutyrate, valerate, isovalerate, caproate, heptanoate, glutarate, DL-glycerate, 2,3-butyleneglycol, benzoate, *p*-hydroxybenzoate (but not the *ortho* and *meta* isomers), phenylacetate, δ-aminovalerate, DL-kynurenine, kynurenate, anthranilate, benzylamine, putrescine, spermine, histamine, butylamine, betaine, sarcosine, and nicotinate as carbon sources. Some strains may use caprylate as well. The species does not use D-galactose, L-rhamnose, ribose, D-salicin, D-trehalose, D-xylose, malonate, D-tartrate, L-valine, or L-threonine as sole carbon sources. The similarity of the 16S rDNA of this species to each member of the *Halomonas* ranges from 93.2–96.6%. Isolated from marine habitats.

The mol% G + C of the DNA is: 67–68 (Bd).
Type strain: ATCC 27122, DSM 4742.
GenBank accession number (16S rRNA): L42616.

17. **Halomonas pantelleriensis** Romano, Nicolaus, Lama, Manca and Gambacorta 1997, 601^{VP} (Effective publication: Romano, Nicolaus, Lama, Manca and Gambacorta 1996, 332.)
pan.tel.le.ri.en′ sis. from the place of isolation, Pantelleria Island in the south of Sicily, Italy.

The characteristics are as described for the genus and as listed in Tables BXII.γ.95 and BXII.γ.96. Colonies are cream to pink, making this the only species of the genus with pink colonies. Colonies are smooth, granulate, entire, circular, raised, and 0.11 mm in diameter. The species produces PHB. In defined medium, the species requires biotin or thiamine (50 μg/l) in addition to the carbon sources listed in Table BXII.γ.95. Optimum growth occurs in media containing 10% NaCl, 33–35°C, pH 9.0. The species is alkalophilic and grows at a pH range of 7.5–11.0. Catalase and α-glucosidase positive; β-galactosidase negative. Decomposes tyrosine. Hydrolyzes hippurate, but does not attack casein, gelatin or L- or D-*N′*-benzoylarginine-*p*-nitroanilide. The species gives a weak reaction for phenylalanine deaminase and is not sensitive to lysozyme. In addition to the substrates listed in Table BXII.γ.95, the species also utilizes fructose, D-xylose, galactose, trehalose, salicin, sorbitol, glycerol, pyruvate, heptanoate, and valine. The organism cannot utilize raffinose, ethanol, or valerate. The species is sensitive to chloramphenicol (30 μg) and erythromycin (30 μg). It is insensitive to lincomycin (15 μg), gentamicin (10 μg), ampicillin (25 μg), tetracycline (50 μg), penicillin (10 μg), neomycin (30 μg), bacitracin (10 μg), and novobiocin (30 μg). The similarity of the 16S rDNA of this species to other members of the *Halomonas* ranges from 93.1–95.8%. This species represents a distinct phylogenetic line within the larger genus, and is most closely related to *H. marina* and *H. halodenitrificans*. Isolated from hard sand on the island of Pantelleria, south of Sicily, Italy.
The mol% G + C of the DNA is: 65.02 (HPLC).
Type strain: ATCC 700273, DSM 9661.
GenBank accession number (16S rRNA): X93493.

18. **Halomonas salina** (Valderrama, Quesada, Bejar, Ventosa, Gutierrez, Ruiz-Berraquero and Ramos-Cormenzana 1991) Dobson and Franzmann 1996, 556^{VP} (*Delaya salina* Valderrama, Quesada, Bejar, Ventosa, Gutierrez, Ruiz-Berraquero and Ramos-Cormanzana 1991, 382.)
sa.li′ na. L. adj. *salina* salted, saline.

The characteristics are as described for the genus and as listed in Tables BXII.γ.95 and BXII.γ.96. Of all of the *Halomonas* species, *H. salina* is currently the easiest to distinguish at the phenotypic level. The species has several characteristics that are not shared by the other halomonads. It forms coccoid cells when grown in low salts, but produces typical elongated filaments when grown in optimum (7.5% total sea salts) and higher salts, but not in 30% salts. The cells are nonmotile, produce a capsule, and accumulate poly-β-hydroxybutyrate. The colonies produced after 48 h at 32°C on MH medium are small (1–2 mm in diameter), convex, smooth, glossy, and translucent yellow to cream and have entire margins. This is also the only *Halomonas* species known to reduce selenite and to require Mg^{2+}, in addition to Na^{+}. Unlike other *Halomonas* species, *H. salina* can only use cations supplied as either Cl^{-} or SO_4^{2-} salts. Members of the species grow at pH 6.0–9.0. Some strains also grow at pH 5.0. Catalase positive. Some strains also possess DNase, phenylalanine deaminase, and β-galactosidase. Some strains produce hydrogen sulfide, are KCN tolerant and hydrolyze Tween 80 and Tween 20. Some strains grow on MacConkey and cetrimide agars. Does not possess phosphatase, lysine decarboxylase, or lecithinase. Some strains may produce acid from D-mannitol and sucrose. Acid is not produced from other sugars or poly-alcohols. The members of this species utilize D-salicin, fumarate, malate, and L-aspartic acid as sole sources of carbon. Some strains may also utilize D-galactose, inulin, raffinose, L-rhamnose, sorbose, D-trehalose, D-xylose, formate, pyruvate, D-tartrate, β-hydroxybutyrate, and L-glutamic acid. The species does not utilize oxalate, benzoate, or caprylate as sole carbon sources. Sensitive to ampicillin (10 μg), cephalothin (30 μg), erythromycin (15 μg), nalidixic acid (15 μg), penicillin G (10 i.u.), and chloramphenicol (30 μg). Some strains are also sensitive to gentamicin (10 μg), streptomycin (10 μg), and tetracycline (30 μg). Isolated from hypersaline habitats such as soils, salt ponds, salt lakes, and from the sea.
The mol% G + C of the DNA is: 60.7–64 (T_m).
Type strain: ATCC 49509, DSM 5928.
GenBank accession number (16S rRNA): L42617, X87217.

19. **Halomonas subglaciescola** Franzmann, Burton and McMeekin 1987, 32^{VP}
sub.gla′ ci.es.co.la. L. pref. *sub* below; L. n. *glacies* ice; L. suff. *cola* to dwell; M.L. adj. *subglaciescola* dwelling below ice.

The characteristics are as described for the genus and as listed in Tables BXII.γ.95 and BXII.γ.96. When motile, the cells are peritrichously flagellated. On solid media, the colonies produced are white to cream. Yellow colonies may form on media enriched with alanine or hydroxy-L-proline. This species cannot be grown on simple media, such as CAS or MH. Grows at temperatures as low as −5°C. The species has two biovars. Biovar I is motile and produces long filamentous cells >10 μm long. Biovar II is nonmotile and produces cells <10 μm long. The type strain belongs to Biovar I. Some characteristics of the species are listed in Tables BXII.γ.95 and BXII.γ.96. The species grows at pH 5.0–9.0 and does not grow above 25°C. The species does not grow in the presence of 30% NaCl. Catalase and phenylalanine deaminase positive. Phosphatase, lysine decarboxylase, and β-galactosidase negative. Does not produce indole or hydrogen sulfide; does not hydrolyze esculin. The species does not produce acid from either adonitol or sorbitol. There is no data available for acid production from other compounds. This species has been reported to grow on L-threonine and proline as primary carbon sources. It does not utilize sucrose, L-aspartic acid, L-glutamic acid, methionine, or L-asparagine as sole carbon sources. Sensitive to mercuric chloride (200 mg/l). Some strains are sensitive to Vibriostat O/129 (10 μg), penicillin G (10 i.u.), and chloramphenicol (30 μg). Resistant to gentamicin (10 μg), neomycin (30 μg), streptomycin (10 μg), and tetracycline (30 μg). The similarity of the 16S rDNA of both biovars of this species to other *Halomonas* species ranges from 93.7–96.1% for Biovar I and from 93.6–99.9% for Biovar II. The 16S rDNA sequence of Biovar II shows only 98.2% similarity to that of Biovar I. However, Biovar II shows 99.9% sequence similarity to *Halomonas halodurans*, indicating that Biovar II is likely a variant of the later species. Isolated from Antarctic hypersaline lakes.

The mol% G + C of the DNA is: 60.9–62.9 ± 1.0 (method unknown).

Type strain: ATCC 43668, DSM 4683, UQM 2926.

GenBank accession number (16S rRNA): M93358.

20. **Halomonas variabilis** (Fendrich 1989) Dobson and Franzmann 1996, 556^{VP} (*Halovibrio variabilis* Fendrich 1989, 205.) *va.ri.a' bi.lis.* L. adj. *variabilis* changeable, variable; referring to variation of the cell diameter with changing salt concentrations.

The characteristics are as described for the genus and as listed in Tables BXII.γ.95 and BXII.γ.96. The cells are curved rods (described as vibrion shaped). Coccoid bodies may form in older cultures. Cells have monotrichous flagellation. Colonies are circular, entire, smooth, slimy, raised, and produce a light brown pigment. The cells grow in NaCl concentrations from 1.2–4.9 M, with an optimum salt concentration of 1.0 M. The optimum pH for growth is 7.5; minimum, 6.5; maximum, 8.4. Temperature range for growth: 15–37°C; optimum, 33°C. Minimum doubling time, 7.5 h. The similarity of the 16S rDNA of this species to other *Halomonas* species ranges from 91.5–96.8%. Isolated from the North Arm of the Great Salt Lake Utah (USA).

The mol% G + C of the DNA is: 57.0 (method unknown).

Type strain: DSM 3051.

GenBank accession number (16S rRNA): M93357, X90483.

21. **Halomonas venusta** (Baumann, Baumann, Mandel and Allen 1972) Dobson and Franzmann 1996, 556^{VP} (*Delaya venusta* (Baumann, Baumann, Mandel and Allen 1972) Baumann, Bowditch and Baumann 1983a, 801; *Alcaligenes venustus* Baumann, Baumann, Mandel and Allen 1972, 426.) *ve.nus' tus.* L. adj. *venustus* lovely, beautiful.

The characteristics are as described for the genus and as listed in Tables BXII.γ.95 and BXII.γ.96. The cells possess peritrichous flagella. The species utilizes 52–76 organic compounds. Also utilized, in addition to compounds listed in Table BXII.γ.95, are: galacturonate, glucuronate, *N*-acetylglucosamine, butyrate, isobutyrate, valerate, isovalerate, caproate, heptanoate, caprate, glutarate, glycolate, α-ketoglutarate, aconitate, *meso*-inositol, propyleneglycol, 2,3-butyleneglycol, *n*-propanol, *n*-butanol, isobutanol, L-mandelate, benzoylformate, benzoate, *o*-, *m*-, and *p*-hydroxybenzoate, phenylate, quinate, δ-aminovalerate, anthranilate, ethanolamine, putrescine, betaine, sarcosine, acetamide, and allantoin. The similarity of the 16S rDNA of this species to other *Halomonas* species ranges from 93.2–98%.

The mol% G + C of the DNA is: 58.7 (Bd).

Type strain: ATCC 27125, DSM 4743.

GenBank accession number (16S rRNA): L42618.

Genus II. **Carnimonas** *Garriga, Ehrmann, Arnau, Hugas and Vogel 1998, 684^{VP}*

MARGARITA GARRIGA, MATTHIAS A. EHRMANN, JACINT ARNAU, MARTA HUGAS AND RUDI F. VOGEL

Car.ni' mo.nas. L. gen. n. *carnis* of meat; Gr. n. *monas* a unit, monad; *Carnimonas* a monad of meat.

Straight or slightly curved rods, 0.5–0.6 × 1.0–1.7 µm, occurring singly or in pairs. Gram negative. Does not form spores. **Nonmotile. Oxidase and catalase positive. Aerobic, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. Moderately halotolerant.** Optimum temperature for growth is 28–30°C. **No growth occurs at 5°C or 37°C.** Chemoorganotrophic. **Acid, but no gas, is produced from D-glucose, D-xylose, and various other carbohydrates. β-Galactosidase (ONPG) activity occurs.** The main respiratory quinone is ubiquinone-9. Main components in the polar lipid composition are diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylethanolamine. Major fatty acids are $C_{16:0}$, $C_{16:1}$, $C_{18:1}$, and $C_{19:0cyc}$. Belongs to the class *Gammaproteobacteria*. Forms dark spots on the surface of raw, cured meat products.

The mol% G + C of the DNA is: 56.

Type species: **Carnimonas nigrificans** Garriga, Ehrmann, Arnau, Hugas and Vogel 1998, 685.

FURTHER DESCRIPTIVE INFORMATION

On tryptone soy agar, colonies appear white, convex, shiny, and circular. No pigmentation is visible in any culture media. Optimum temperature for growth is 28–30°C. No growth occurs at 5°C or 37°C. Growth occurs in the presence of 8% NaCl, but not at higher levels.

Indole production, reduction of nitrate, and the Voges–Proskauer reaction are negative. All strains produce acid from glucose, xylose, melibiose, maltose, and saccharose. No gas is produced from glucose. Additional physiological data are provided in Table BXII.γ.99.

Garriga et al. (1998) have reported that the main polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylethanolamine. In addition, there are three unidentified components. The fatty acid profile contains major amounts of several saturated and unsaturated straight chain fatty acids and only minor fractions of 3-hydroxylated fatty acids. The main fatty acids are palmitic acid ($C_{16:0}$), which comprises 40% of total fatty acids, and the cyclopropanic acid $C_{19:0\ cyc}$, which comprises 21.07%. Oleic acid ($C_{18:1\ \omega9t}$) comprises 7.45% and a $C_{18:1\ \omega7c/\omega9t/\omega12t}$ group comprises 12.9%. Moreover, a $C_{16:1}$ fatty acid comprises 6.7%, whereas only minor amounts of $C_{12:1\ 3OH}$ (1.85%) and traces of $C_{16:0\ 3OH}$ (0.46%) are found. No match was found after comparison with the microbial ID, TSBA library (Newark, DE, USA).

No pathogenic effect on mice is detectable 8 d after intraperitoneal injection of up to 1.9×10^{10} CFU of *Carnimonas nigrificans*. Under similar conditions, the bacteria used as controls (*Escherichia coli* HM-42, *Pseudomonas aeruginosa* HS-116, *Staphylococcus aureus* HS-93) show a concentration-dependent lethality.

The presence of dark spots on the surface of raw, cured meat products was first described by Hugas and Arnau (1987). In 1993, Arnau and Garriga identified a Gram-negative bacterium (now *C. nigrificans*) as being responsible for this defect. The defect, a rust-like color turning to black within hours, can be reproduced on comminuted pork meat containing salt (40 g/kg) and dex-

trose (20 g/kg) after inoculation of an overnight culture and storage in an aerobic environment at 30°C. The browning effect is increased by some amino acids, i.e., glycine, L-arginine, L-glutamine, and L-monosodium glutamate, when added (20 g/kg) to a meat mixture containing salt and dextrose. *N*-Acetyl-L-cysteine, L-cysteine, potassium metabisulfite, and propyl-3,4,5-trihydroxybenzoate (5 g/kg) are useful in the prevention of this defect (Arnau and Garriga, 2000). The browning produced by *C. nigrificans* with dextrose and amino acids and the inhibitory properties of some of the substances studied show similarities to the Maillard reaction. However, important differences exist in the temperature pattern, the oxygen effect, and carbohydrates involved.

Enrichment and Isolation Procedures

The isolation of *C. nigrificans* from raw, cured meat products must be done at the beginning of browning; no recovery is possible later. The organism is capable of growing on Cetrimide agar and MacConkey agar; consequently, these selective media could be used for isolation procedures. Incubation should be performed under aerobic conditions at 30°C for 2–3 days. After isolation, *C. nigrificans* grows well on the usual nutrient media (e.g., tryptone soy broth, brain–heart infusion broth).

Maintenance Procedures

Cultures in routine use can be maintained in tryptone soy broth or agar at 4°C. Cultures may be preserved for long-term storage in tryptone soy broth with 20% glycerol at −80°C.

Procedures for Testing Special Characters

Comparative sequence analysis of 16S rRNA gene sequences has revealed a diagnostic sequence that can be used as a target site for specific amplification. The sequence of the oligonucleotide used as specific primer for *C. nigrificans* strain CTCBS1 is 5′-TAA CGT CCT TCA TGC CGG-3′ (binding position 469–486 in the *E. coli* numbering system). This primer (bs1) has been checked for its specificity against more than 10,000 16S rRNA sequences by using the probe-checking software provided by the Ribosomal Database Project (Maidak et al., 1996). PCR and cycle conditions for the species-specific reaction with primer bs1 and universal primer 616V are as follows: one initial cycle 94°C (120 s), followed by 32 cycles of 94°C (45 s), 50°C (90 s), and 72°C (120 s). Master mixes are prepared with reaction buffer containing 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, pH 8.3, 200 nM dNTP, 0.5 pmol each primer, and Taq DNA polymerase (Boehringer, Mannheim, Germany).

Specific amplification of a 480 bp fragment using primer bs1 and 616V occurs only in *C. nigrificans* strains.

Differentiation of the genus *Carnimonas* from other genera

The closest relatives of *Carnimonas nigrificans* are *Zymobacter palmae* and the genus *Halomonas* (at least *Chromohalobacter*). Some physiological and morphological properties, e.g., relationship to oxygen, growth temperature, hydrolysis of starch, oxidase reaction, as well as flagellation and motility, are useful in differentiating *Carnimonas* from these organisms (Table BXII.γ.99 and Table BXII.γ.100).

The mol% G + C content of 56, the presence of ubiquinone 9 as the major respiratory lipoquinone, and the lack of $C_{19:0\ cyc}$ in the non-polar lipid profile are sufficient to exclude these organisms from *Oceanospirillum*. The mol% G + C content of *Carnimonas nigrificans* CTCBS1 differs by at least 4.2% from those of *Halomonas* and *Chromohalobacter*, and there are distinct differences in the fatty acid profiles of these organisms; the levels of $C_{16:0}$ in the profiles of members of the genus *Halomonas* range from 15.5 to 32%, whereas the level in *Carnimonas nigrificans* CTCBS1 is 40%. Additionally, in contrast to *Halomonas*, no $C_{17:0\ cyc}$ could be detected in *Carnimonas nigrificans* CTCBS1.

Taxonomic Comments

The phylogenetic tree, based on 16S rDNA sequences, is shown in Fig. BXII.γ.118. The topology of all organisms illustrated in the tree is based on maximum likelihood analysis of 16S rDNA sequences (Fig. BXII.γ.118) and is consistent with a tree previously published by Dobson and Franzmann (1996). The closest relatives of strain *Carnimonas nigrificans* are *Zymobacter palmae* with 93.3% sequence similarity and, to a lesser extent, species of the genus *Halomonas* (at least 91.9%) and *Chromohalobacter* (91.5%).

The phylogenetic tree shows that the closest relationship is

TABLE BXII.γ.99. Features differentiating the genus *Carnimonas* from other genera[a]

Characteristic	*Carnimonas*	*Chromohalobacter*	*Halomonas*	*Oceanospirillum*	*Zymobacter*
Cells are rod-shaped	+	+	+	D	+
Cells are helical	−	−	−	D	−
Relationship to oxygen:					
Aerobic	+	+	+	+	−
Facultatively anaerobic	−	−	−	−	+
Acid production from hexoses	+	+	+	−	+
Motile	−	+	D	+	+
Oxidase	+	−	D	+	−
Growth at 37°C	−	+	+	+	+
Growth with 0% NaCl	+	−	D	−	−
Tolerates 15% NaCl	−	+	+	−	−
Hydrolysis of:					
Esculin	+	−	D	−	
Starch	+	−	−	−	−
Violet to brown colonies on culture media	−	+	−	−	−
Habitat:					
Raw, cured meat	+	−	−	−	−
Palm sap	−	−	−	−	+
Coastal sea water	−		−	+	−
Solar salt facilities, intertidal estuaries, and hypersaline lakes	−	+	+	−	−

[a] Symbols: see standard definitions.

TABLE BXII.γ.100. Other characteristics of the genus *Carnimonas*[a]

Characteristic	Reaction or result
α-Glucosidase (PNPG), phenylalanine deaminase	+
Arginine dihydrolase, urease, DNase, lecithinase	−
Nitrate reduction	−
Hydrolysis of gelatin, casein, Tween 80	−
Acid production from:	
Amygdalin, esculin, galactose, melibiose, sucrose, salicin	+
L-Arabinose	−
Fructose, mannose, mannitol, melezitose, raffinose, ribose, trehalose	d
Utilization of2:[b]	
Adipate, L-arabinose, caprate malate, mannitol, *N*-acetylglucosamine	−
Citrate, mannose, maltose, gluconate	+

[a]Symbols: see standard definitions.

[b]On media containing vitamins and yeast extract.

to *Zymobacter palmae* ATCC 51623^T. *Zymobacter palmae* is the single species of the genus *Zymobacter* and the closest phylogenetic relative to strain CTCBS1 (Okamoto et al., 1993). The G + C content of its DNA is 55.8 mol%, in the range of that determined for CTCBS1. The profiles of the major fatty acids of the two strains are similar; however, a significant amount (5%) of a $C_{12:0}$ fatty acid that might be unique to *Zymobacter palmae* is not detected in strain CTCBS1 or the other closely related genera.

Further Reading

Arnau, J. and M. Garriga. 1993. Black spot in cured meat-products. Fleischwirtschaft *73*: 1412–1413.

Arnau, J. and M. Garriga. 2000. The effect of certain amino acids and browning inhibitors on the 'black spot' phenomenon produced by *Carnimonas nigrificans*. J. Sci. Food Agric. *80*: 1655–1658.

Garriga, M., M.A. Ehrmann, J. Arnau, M. Hugas and R.F. Vogel. 1998. *Carnimonas nigrificans* gen. nov., sp. nov., a bacterial causative agent for black spot formation on cured meat products. Int. J. Syst. Bacteriol. *48*: 677–686.

Hugas, M. and J. Arnau. 1987. Aparicíon de manchas de color en la corteza y grasa del jamón durante el post-salado. *In* Arnau, Hugas and Monfort (Editors), Jamón Curado, Aspectos Técnicos, Institut de Recerca i Tecnología Agroalimentáries, Monells, Spain. pp. 179–182.

List of species of the genus Carnimonas

1. **Carnimonas nigrificans** Garriga, Ehrmann, Arnau, Hugas and Vogel 1998, 685^{VP}
nig.rif' i.cans. L. adj. *niger* black; L. v. *facere* to make; M.L. n. *nigrificans* black-making.

Characteristics are the same as for the genus. Colonies are nonpigmented, white, convex, shiny, and circular. Produce acid from glucose, fructose, maltose, xylose, melibiose, and saccharose. Hydrolyze esculin and starch, but not gelatin, casein, or DNA. Voges–Proskauer negative. Arginine dihydrolase, urease, lecithinase, and phenylalanine deaminase negative. Do not produce indole.

The mol% G + C of the DNA is: 56 ± 0.3 (T_m).

Type strain: CTCBS1, CECT 4437, CIP 105703, NCIMB 13550.

GenBank accession number (16S rRNA): Y13299.

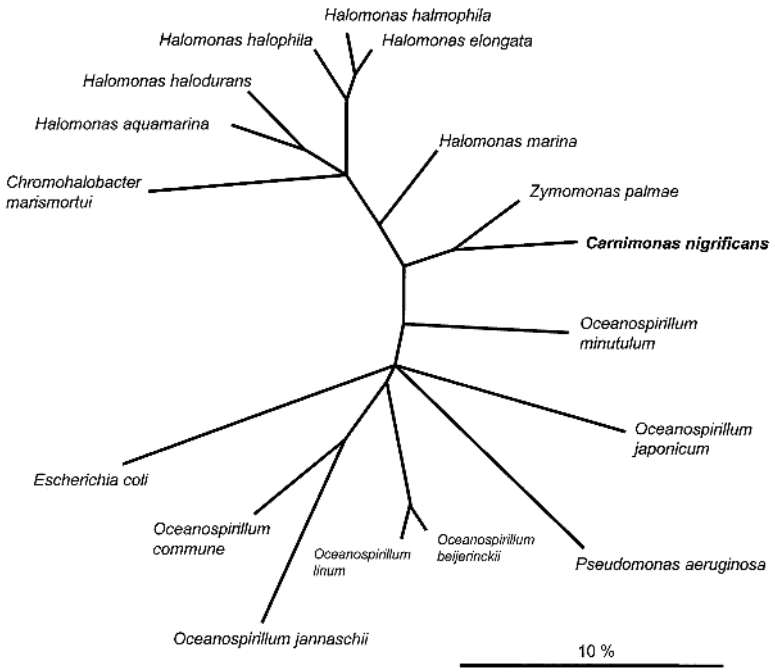

FIGURE BXII.γ.118. Phylogenetic relationships of *Carnimonas nigrificans* and selected representatives of the class *Gammaproteobacteria*.

Genus III. **Chromohalobacter** *Ventosa, Gutierrez, García and Ruiz-Berraquero 1989, 384[VP] emend. Arahal, García, Ludwig, Schleifer and Ventosa 2001a, 1446)*

ANTONIO VENTOSA

Chro.mo.ha' lo.bac'ter. Gr. n. *chroma* color; Gr. n. *halos* the sea, salt; M.L. n. *bacter* rod; M.L. masc. n. *Chromohalobacter* colored salt rod.

Gram-negative, straight or sometimes slightly curved, rods (0.6–1.2 × 1.5–4.2 μm). Motile by polar or peritrichous flagella. Cells occur singly, in pairs, and in short chains. Spores are not formed. **Moderately halophilic.** Salt is required for growth. **The optimum salt concentration for growth is between 8 and 10%.** May grow at salt concentrations up to 30%. The broader ranges of temperature and pH observed for growth are 5–45°C (optimal 30–37°C) and pH 5.0–10.0 (optimal pH 7.5), respectively. **Aerobic.** Chemoorganotrophic. Catalase positive. **Oxidase negative. Most strains reduce nitrates.** Phenylalanine deaminase test is negative. Gelatin, starch, Tween 80, esculin, DNA, and tyrosine are not hydrolyzed. Acid is produced aerobically from D-glucose and other sugars. Carbohydrates, amino acids, and some polyols can serve as sole carbon sources. Colonies are cream to brown-yellow pigmented.

The mol% G + C of the DNA is: 62–66.

Type species: **Chromohalobacter marismortui** (ex Elazari-Volcani 1940) Ventosa, Gutierrez, García and Ruiz-Berraquero 1989, 384.

FURTHER DESCRIPTIVE INFORMATION

The genus *Chromohalobacter* was created by Ventosa et al. (1989) to accommodate seven moderately halophilic isolates obtained during the course of an extensive taxonomic study of bacteria isolated from salterns in the Mediterranean coast (Ventosa et al., 1982). These isolates showed phenotypic and chemotaxonomic features very similar to those of *"Chromobacterium marismortui"*, a species originally described by Elazari-Volcani (1940) based on strains isolated from the Dead Sea. This species was described in the 7th edition of *Bergey's Manual of Determinative Bacteriology* (Breed et al., 1957), and in the 8th edition it was included in the genus *Chromobacterium* as a species *incertae sedis* since it did not produce violacein and lacked the typical flagellar arrangement of the genus *Chromobacterium* (Buchanan and Gibbons, 1974). It was not included in the Approved Lists of Bacterial Names (Skerman et al., 1980). Until recently, the genus *Chromohalobacter* was represented by a single species, *Chromohalobacter marismortui*, which was phylogenetically closely related to species of the genus *Halomonas* and included in the family *Halomonadaceae* (Mellado et al., 1995b). Very recent studies based on 16S rDNA gene sequence comparison and other phenotypic and molecular data supported the conclusion that two species previously described as members of the genus *Halomonas*, *Halomonas canadensis* and *Halomonas israelensis*, should be placed in the genus *Chromohalobacter*, as *Chromohalobacter canadensis* and *Chromohalobacter israelensis*, respectively (Arahal et al., 2001a). Finally, a study of two strains previously assigned to the species *Halomonas elongata*, strains DSM 3043 and ATCC 33174, showed that they represent a different species of the genus *Chromohalobacter*, and named them as *Chromohalobacter salexigens* (Arahal et al., 2001b).

All species currently described as members of the genus *Chromohalobacter* have a specific requirement for Na^+. They grow optimally in media containing 8–10% salt and are defined as moderately halophilic microorganisms (Ventosa et al., 1998). Few studies have been carried out with the type species *C. marismortui*; however, *C. canadensis* and *C. israelensis* have been extensively used for physiological studies. These species were formerly designated as strain Ba_1 (Rafaeli-Eshkol, 1968), isolated from the Dead Sea (*C. canadensis*) and strain NRCC 41277, isolated by Matheson et al. (1976) as a medium contaminant (*C. israelensis*). More detailed information about their physiological and biochemical features can be found in a recent review (Ventosa et al., 1998).

To cope with the high external salinity, halophilic bacteria need to balance their cytoplasm with the osmotic pressure exerted by the external medium. However, in *C. canadensis* and *C. israelensis*, as in many other moderate halophiles, the sum of the apparent intracellular concentration of Na^+ and K^+ ions is much lower than the external concentration (Matheson et al., 1976; Goldberg and Gilboa, 1978). They accumulate intracellular organic compounds, called compatible solutes, such as glycine betaine (Rafaeli-Eshkol and Avi-Dor, 1968; Shkedy-Vinkler and Avi-Dor, 1975). However, the most wide spread compatible solutes found in halophilic bacteria, and in *Chromohalobacter* in particular, are ectoine and its β-hydroxy derivative, hydroxyectoine (Ventosa et al., 1998). During recent years, several studies have focused on the osmoregulatory mechanisms of *C. salexigens* DSM 3043. It was selected as an excellent model organism because it displays one of the widest ranges of salt tolerance found in nature. In order to maintain its internal osmolarity and generate turgor in environments with high salinities, *C. salexigens* accumulates compounds such as glycine betaine, choline or choline-*O*-sulfate, when present externally. However, the main osmoadaptation mechanism is the *de novo* synthesis of ectoine and hydroxyectoine (Cánovas et al., 1996, 1997, 1998b, 1999; Nieto et al., 2000). Genes involved in the biosynthesis of ectoine and the oxidation of choline to betaine have been recently characterized (Cánovas et al., 1998a, 2000). The ectoine synthesis genes of *C. salexigens* and *Halomonas elongata* have lower sequence homology values than was expected from two closely related halophilic microorganisms (Nieto et al., 2000).

The genome size, determined by pulsed-field gel electrophoresis, of three strains of *C. marismortui* (including the type strain) ranged from 1770 to 2295 kb and that of *C. israelensis* was estimated to be 2490 kb (Mellado et al., 1998). Several plasmids have also been reported (Ventosa et al., 1994; Mellado et al., 1995a; Vargas et al., 1995a). The basic replicon of the narrow-host-range plasmid pCM1 from *C. marismortui* has been sequenced and characterized in detail (Mellado et al., 1995a). Cloning and shuttle vectors useful for the genetic manipulation of these bacteria have been reported (Mellado et al., 1995c; Vargas et al., 1995a). Conjugation is the only genetic transfer mechanism that has been described for this genus (Vargas et al., 1997).

ENRICHMENT AND ISOLATION PROCEDURES

Selective procedures for the enrichment and isolation of species of the genus *Chromohalobacter* have not been reported. They can be isolated from hypersaline environments by direct plating on complex media supplemented with a salt mixture and incubation at 30–37°C for 7–10 d. The isolation medium described by Ventosa et al. (1982) can be used.

Maintenance Procedures

For short-term storage, the species of the genus *Chromohalobacter* can be maintained on complex medium containing 10% salts. An appropriate medium is described by Ventosa et al. (1989). Long-term storage of members of this genus can be carried out by freeze-drying, storage at −80°C, or storage in liquid nitrogen.

Differentiation of the genus *Chromohalobacter* from other genera

The genus *Chromohalobacter* belongs to the family *Halomonadaceae* and is closely related to the genus *Halomonas* (Fig. BXII.γ.119), as well as to *Zymobacter* and *Carnimonas*. Since the genus *Halomonas* is taxonomically heterogeneous and contains greater than 20 species, it is very difficult to differentiate these two genera based on phenotypic or chemotaxonomic characteristics. Typical features of species of the genus *Chromohalobacter* are their optimal growth in media containing 8–10% salt and characteristic results for the biochemical tests reported in the genus description. Species of the genus *Chromohalobacter* can be phylogenetically differentiated from other related genera by comparison of 16S rRNA gene sequences.

Differential features of the four currently recognized species of the genus *Chromohalobacter* are given in Table BXII.γ.101.

Acknowledgments

I am grateful to Drs. R.H. Vreeland and J.T. Staley for the critical reading of this article and to Dr. D.R. Arahal for help with the phylogenetic analysis.

Further Reading

Arahal, D.R., M.T. García, W. Ludwig, K.H. Schleifer and A. Ventosa. 2001. Transfer of *Halomonas canadensis* and *Halomonas israelensis* to the genus *Chromohalobacter* as *Chromohalobacter canadensis* comb. nov and *Chromohalobacter israelensis* comb. nov. Int. J. Syst. Evol. Microbiol. *51*: 1443–1448.

Mellado, E., E.R.B. Moore, J.J. Nieto and A. Ventosa. 1995. Phylogenetic inferences and taxonomic consequences of 16S ribosomal DNA-sequence comparison of *Chromohalobacter marismortui*, *Volcaniella eurihalina*, and *Deleya salina* and reclassification of *Volcaniella eurihalina* as *Halomonas eurihalina* comb. nov. Int. J. Syst. Bacteriol. *45*: 712–716.

Ventosa, A., M.C. Gutierrez, M.T. García and F. Ruiz-Berraquero. 1989. Classification of *"Chromobacterium marismortui"* in a new genus, *Chromohalobacter* gen. nov. as *Chromohalobacter marismortui*, comb. nov., nov. rev. Int. J. Syst. Bacteriol. *39*: 382–386.

Ventosa, A., J.J. Nieto and A. Oren. 1998. Biology of moderately halophilic aerobic bacteria. Microbiol. Mol. Biol. Rev. *62*: 504–544.

List of species of the genus Chromohalobacter

1. **Chromohalobacter marismortui** (ex Elazari-Volcani 1940) Ventosa, Gutierrez, García and Ruiz-Berraquero 1989, 384^{VP} *ma.ris.mor' tu.i.* L. gen. n. *maris* of the sea; L. adj. *mortuus* dead; M.L. gen. n. *marismortui* of the Dead Sea.

   See Table BXII.γ.101 and generic description for many features. Cells are Gram negative, rod shaped, and sometimes slightly curved, the length varies with the concentration of salt; at 10% salts, the cells are 0.6–1.0 × 1.5–4.0 μm; at higher and lower salt concentrations, the cells are longer. Cells occur singly or in pairs. Motile by means of peritrichous flagella. Spores are not formed. On solid, complex media containing 10% salts, colonies are circular, convex, smooth, entire, and concentrically ringed with dark brown centers followed by bluish brown, grayish brown, and

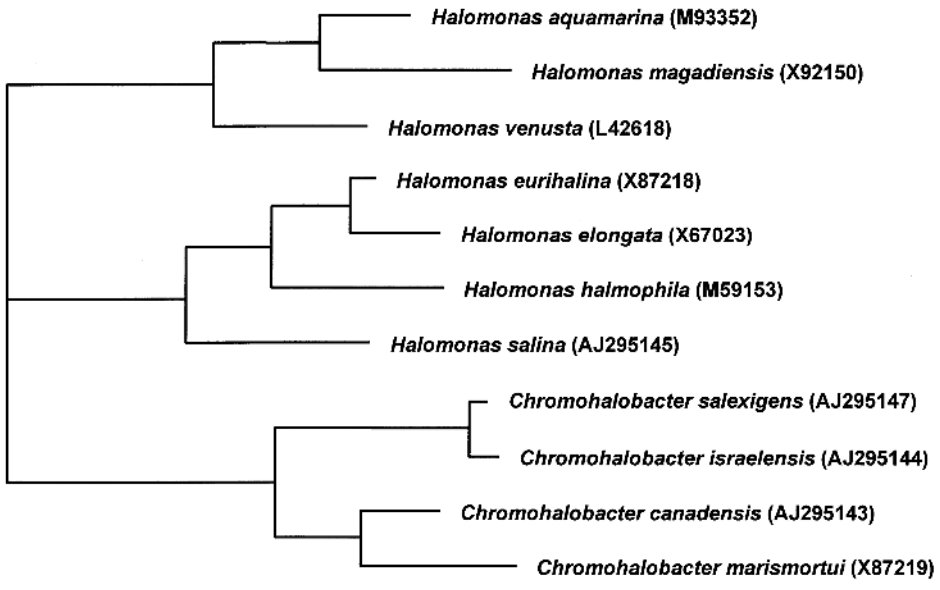

FIGURE BXII.γ.119. Phylogenetic tree derived from analysis of the 16S rRNA gene sequences of the type strain of species of the genus *Chromohalobacter* and some representative related species of the genus *Halomonas*. Figures in parentheses indicate the accession numbers of 16S rRNA gene sequence data. Bar = 5% sequence difference.

TABLE BXII.γ.101. Differential characteristics of species of the genus *Chromohalobacter*[a]

Characteristic	1. *C. marismortui*	2. *C. canadensis*	3. *C. israelensis*	4. *C. salexigens*
Indole production	−	+	+	−
H_2S production	−	−	−	+
Simmons citrate	+	−	−	+
Hydrolysis of casein	−	−	−	+
Urease	−	−	−	+
Lysine decarboxylase	−	+	+	−
Ornithine decarboxylase	−	+	+	−
Acid production from:				
Arabinose	−	+	+	+
Maltose	+	−	+	+
Sucrose	+	−	+	+
D-Trehalose	+	−	−	−
Growth on:				
D-Cellobiose	−	+	+	−
Sucrose	+	−	+	+

[a]For symbols see standard definitions.

yellow rings. They produce a yellow pigment and a violet-blue pigment which is not violacein. Production of pigment is favored by suboptimal growth temperatures, glycerol, and a salt concentration of 10%. In liquid medium containing 10% salts, turbidity and a pellicle are produced; usually brown-to-yellow pigments are also produced.

Grows in media containing 1–30% salts, with an optimum at about 10% salts. Growth occurs at 5–45°C (optimum temperature is 37°C) and pH 5–10 (optimal growth at pH 7.5) on solid media containing 10% salts. Acid is produced without gas under aerobic conditions in marine oxidation–fermentation medium supplemented with 10% salts and D-glucose, D-galactose, maltose, lactose, D-arabinose, D-xylose, sucrose, trehalose, glycerol, and D-mannitol. Generally, nitrate is reduced to nitrite (except the type strain). Gelatin, casein, starch, esculin, tyrosine, Tween 80, and DNA are not hydrolyzed. Phosphatase, Voges–Proskauer, β-galactosidase, phenylalanine deaminase, and arginine dehydrolase tests are negative.

The following compounds are utilized as sole carbon and energy sources: dulcitol, D-fucose, D-galactose, D-gluconate, D-glucose, glutamate, *meso*-inositol, maltose, D-mannitol, D-mannose, pyruvate, D-ribose, sucrose, D-sorbitol, and D-xylose. The following compounds are not utilized as sole carbon and energy sources: *N*-acetylglucosamine, amygdalin, DL-α-aminobutyrate, butyrate, cellobiose, citrate, esculin, *p*-hydroxybenzoate, hippurate, inulin, malonate, melibiose, oxalate, raffinose, salicylate, salicin, and D-tartrate. The following compounds are utilized as sole carbon, nitrogen, and energy sources: L-alanine, DL-arginine, L-glutamine, L-ornithine, L-proline, putrescine, and L-serine. The following compounds are not utilized as sole carbon, nitrogen, and energy sources: L-allantoin, betaine, creatine, ethionine, L-isoleucine, L-leucine, phenylalanine, sarcosine, L-threonine, and L-valine.

Isolated from the Dead Sea and marine salterns.

The mol% G + C of the DNA is: 62.1–64.9 (T_m).

Type strain: ATCC 17056, CCM 3518, DSM 6770, NCIMB 8731.

GenBank accession number (16S rRNA): X87219.

Additional Remarks: Other sequences: strain A-65 (X87220), strain A-100 (X87221), strain A-492 (X87222), and strain T1093 (U78719).

2. **Chromohalobacter canadensis** (Huval, Latta, Wallace, Kushner and Vreeland 1996) Arahal, García, Ludwig, Schleifer and Ventosa 2001a, 1447^{VP} (*Halomonas canadensis* Huval, Latta, Wallace, Kushner and Vreeland 1995, 1130.)
ca.na.den′sis. M.L. n. *canadensis* pertaining to Canada, the country where the type strain was isolated.

See Table BXII.γ.101 and generic description for many features. Cells are Gram negative, straight or curved rods with rounded ends, 0.6–1.2 × 2.0–3.8 μm; cells occur singly, in pairs, or in short chains. Pleomorphic cells are not present. Both culture age and salt concentration affect chain length. Motile by a single polar flagellum. Spores are not formed. On solid complex media with 8% salts, colonies are translucent and convex with a smooth glistening surface, produce a nondiffusible white pigment, and have a diameter of 2–6 mm after 7 days of growth at 30°C.

Grows in media containing 3–25% NaCl from 15 to 30°C; grows in media with 8–32% NaCl at 45°C. Optimal growth at 7.5% NaCl. Growth occurs at 5–45°C (optimum temperature is 30°C) and from pH 5 to 9 on solid media containing 8% NaCl. Acid is produced from mannose, lactose, glycerol, and cellobiose but not from sucrose. Nitrate is reduced to nitrite. Phenylalanine deaminase and Voges–Proskauer tests are negative. Gelatin, starch, DNA, esculin, and agar are not hydrolyzed.

The following compounds are utilized as sole carbon and energy sources: acetate, citrate, DL-malate, succinate, lactate, D-glucose, xylose, L-arabinose, D-fructose, D-mannitol, *meso*-inositol, ethanol, mannose, esculin, glycerol, and gluconic acid. The following compounds are not utilized as sole carbon and energy sources: butyrate, isobutyrate, propionate, tartrate, benzoate, maltose, melibiose, L-sorbose, D-ribose, D-sorbitol, *n*-propanol, *n*-butanol, ethylene glycol, *n*-hexadecane, pyridine-1-oxide, formaldehyde, formamide, *N,N*-dimethylacetamide, and ethylenediamine. Isolated as a contaminant on Sehgal and Gibbons medium containing 25% NaCl.

The mol% G + C of the DNA is: 62 (T_m).

Type strain: ATCC 43984, DSM 6769, CECT 5385, CCM 4919, CIP 105571, NCIMB 13767, NRCC 41227.

GenBank accession number (16S rRNA): AJ295143.

3. **Chromohalobacter israelensis** (Huval, Latta, Wallace, Kushner and Vreeland 1996) Arahal, García, Ludwig, Schleifer

and Ventosa 2001a, 1447^{VP} (*Halomonas israelensis* Huval, Latta, Wallace, Kushner and Vreeland 1996, 1189.)
is.ra.el.en′sis. M.L. n. *israelensis* an inhabitant of Israel.

See Table BXII.γ.101 and generic description for many features. Gram negative. Cells are straight rods with rounded ends, 0.6–0.9 × 1.5–4.2 μm, occurring singly and occasionally in pairs or short chains. Pleomorphic cells are not present. On solid complex medium with 8% NaCl colonies are flat with entire margins, produce a nondiffusible cream pigment, and have 2–6 mm in diameter after 7 days.

Grows in media containing 3.5–20% NaCl (optimum at 8% NaCl). Growth occurs at 15–45°C (optimum temperature is 30°C) and from pH 5 to 9. Acid is produced without gas from mannose, lactose, glycerol, cellobiose, and sucrose. Nitrate is reduced to nitrite. Phenylalanine deaminase, Voges–Proskauer, and methyl red tests are negative. Gelatin, starch, esculin, and DNA are not hydrolyzed.

The following compounds are utilized as sole carbon and energy sources: cellobiose, lactose, mannose, esculin, glycerol, acetate, starch, gluconic acid, and sucrose.

The mol% G + C of the DNA is: 65 (T_m).

Type strain: ATCC 43985, DSM 6768, CECT 5287, CCM 4920, CIP 106853, NCIMB 13766.

GenBank accession number (16S rRNA): AJ295144.

4. **Chromohalobacter salexigens** Arahal, García, Vargas, Cánovas, Nieto and Ventosa 2001b, 1460^{VP}
sal.ex′i.gens. L. n. *sal* salt; L. v. *exigo* to demand; M.L. part. adj. *salexigens* salt-demanding.

See Table BXII.γ.101 and generic description for many features. Cells are Gram-negative rods, 0.7–1.0 × 2.0–3.0 μm; cells occur singly or in pairs. Motile. Spores are not formed. On solid, complex media containing 10% salts, colonies are cream, opaque, and circular and less than 2 mm in diameter; spreading may occur after extended incubation. In liquid medium containing 10% salts, a homogeneous turbidity is produced.

Grows in media containing 0.9–25% salts, and optimal growth at 7.5–10% salts. Growth occurs at 15–45°C (optimum temperature is 37°C) and from pH 5 to 10 on liquid media containing 10% salts (optimal growth at pH 7.5). Acid is produced from L-arabinose, D-fructose, D-galactose, glycerol, D-glucose, lactose, maltose, D-mannose, sucrose, and D-xylose, but not from trehalose. Nitrate is reduced to nitrite but nitrite is not reduced. Gelatin, starch, esculin, DNA, and Tween 80 are not hydrolyzed. Methyl red positive. Indole and acetoin are not produced. Phenylalanine deaminase, lysine decarboxylase, and ornithine decarboxylase are not produced.

The following compounds are utilized as sole carbon and energy sources: L-arabinose, erythritol, D-fructose, D-galactose, D-glucose, maltose, D-mannitol, D-mannose, D-sorbitol, sucrose, D-trehalose, D-ribose, D-xylose, ethanol, glycerol, *meso*-inositol, dulcitol, acetate, α-aminovalerate, α-ketoglutarate, citrate, fumarate, DL-glycerate, glutamate, malate, malonate, propionate, D-saccharate, succinate, and D-tartrate. The following compounds are not utilized as sole carbon and energy sources: adonitol, cellobiose, L-fucose, α-lactose, D-melibiose, D-raffinose, L-rhamnose, galactosamine, gluconolactone, inulin, DL-α-aminobutyrate, butyrate, caprylate, lactate and oxalate. The following compounds are utilized as sole sources of carbon, nitrogen and energy: L-arginine, L-asparagine, betaine, glycine, L-glutamine, L-lysine, L-ornithine, L-proline, and L-serine. The following compounds are not utilized as sole sources of carbon, nitrogen and energy: L-alanine, creatine, L-methionine, putrescine, sarcosine, L-threonine, and L-valine.

Isolated from salterns.

The mol% G + C of the DNA is: 64.2–66.0 (T_m).

Type strain: ATCC BAA-138, CECT 5384, CCM 4921, CIP 106854, DSM 3043, NCIMB 13768.

GenBank accession number (16S rRNA): AJ295146.

Additional Remarks: Other sequence: AJ295147 (*C. salexigens* ATCC 33174).

Genus IV. **Zymobacter** *Okamoto, Taguchi, Nakamura, Ikenaga, Kuraishi and Yamasato 1995, 418^{VP} (Effective publication: Okamoto, Taguchi, Nakamura, Ikenaga, Kuraishi and Yamasato 1993, 336)*

TOMOYUKI OKAMOTO, HIROSHI KURAISHI AND KAZUHIDE YAMASATO

Zy.mo.bac′ter. Gr. n. *zyme* leaven, ferment; M.L. n. *bacter* masc. equivalent of Gr. neut. n. *bakterion* rod♂ sc. n. *Zymobacter* the fermenting rod.

Rod-shaped cells with rounded ends, 1.3–2.4 × 0.7–0.9 μm; usually single. **Motile by peritrichous flagella** that are nonsheathed. Gram negative. **Facultatively anaerobic.** Chemoorganotrophic. **Grow on and ferment 1 mol of glucose or the hexose moiety of maltose to produce approximately 2 mol each of ethanol and CO_2, with trace amounts of acids.** Ferment hexoses, α-linked di- and trisaccharides, and sugar alcohols. Growth occurs at pH 4.7–8.1. Catalase positive. **The predominant cellular fatty acids are oleic acid, cyclopropanic acid of $C_{19:0}$, and palmitic acid. The hydroxylated acid is characteristically $C_{12:0\ 3OH}$. The quinone system is ubiquinone-9.**

The mol% G + C of the DNA is: 55.4–56.2 (HPLC).

Type species: **Zymobacter palmae** Okamoto, Taguchi, Nakamura, Ikenaga, Kuraishi and Yamasato 1995, 418 (Effective publication: Okamoto, Taguchi, Nakamura, Ikenaga, Kuraishi and Yamasato 1993, 336.)

FURTHER DESCRIPTIVE INFORMATION

The morphology of the cells is depicted in Fig. BXII.γ.120. The cells can possess as many as 20 peritrichous flagella.

When grown on MY agar[1], the colonies are round, entire, smooth, opaque, and milky white. Colonies of similar size develop under aerobic and anaerobic growth conditions. When the organism is grown in MY broth, the medium is slightly turbid, becoming cloudy with flocculent cells during the late log phase growth, then transparent due to sedimentation of cells during the stationary phase. No pellicle is formed. Growth is better in static cultures than in shaken cultures. *Zymobacter* requires nic-

1. MY agar (per liter): yeast extract (Difco), 10.0; maltose, 20.0; KH_2PO_4, 2.0; NaCl, 5.0; agar (Difco), 15.0; pH 6.0.

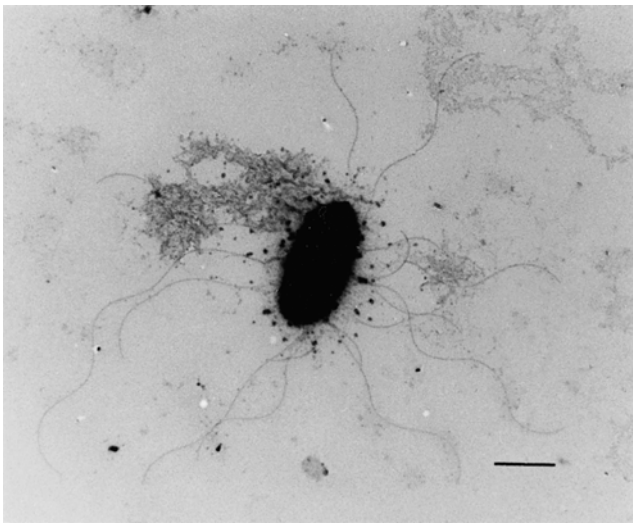

FIGURE BXII.γ.120. Electron micrograph of peritrichous flagella of *Zymobacter palmae* T109^T. Bar = 1 μm.

otinic acid for growth. Growth does not occur in the absence of a sugar or sugar alcohol.

Growth occurs at 15–37°C (optimum, 30°C) and pH 4.7–8.1 (optimum, 6.0). The organisms are neither halophilic nor halotolerant, but they are considerably tolerant to ethanol and produce 5.8% ethanol from maltose after 6 d of fermentation.

The organism produces more than 0.5% (w/v) ethanol in the culture broth from glucose, fructose, maltose, sucrose, melibiose, raffinose, sorbitol, mannitol, and—depending on the strain—mannose and galactose. The specific growth rate on glucose is higher than that on maltose at sugar concentrations less than 7.5% (w/v). Growth is poor at glucose concentrations greater than 15% (w/v). The specific growth rate on maltose is substantially similar over a concentration range of 2.5–20% (w/v) (Table BXII.γ.102). Growth is initiated at 50% (w/v) maltose, but not at 25% (w/ v) glucose; this divergence may be due to the differing osmotic pressures exerted by these sugars.

The genera *Zymomonas*, *Saccharobacter*, and *Zymobacter* are unique among bacteria in terms of their energy-yielding metabolism, which produces ethanol, CO_2, and small amounts of other products. *Zymomonas* has been studied as a potential bacterium for the industrial production of ethanol, but its spectrum of fermentable sugars is limited to glucose, fructose, and sucrose (Ingram et al., 1998). *Zymobacter* (and *Saccharobacter*) may be preferable to *Zymomonas* in this regard, as it has a wider profile of fermentable sugars, especially maltose and raffinose.

Palmitic acid ($C_{16:0}$) comprises 46–54% of the whole cell fatty acids. Oleic acid ($C_{18:1\ \omega9c}$) $C_{19:0\ cyc}$ account for 30–42% (Table BXII.γ.103).

Enrichment and Isolation Procedures

Okamoto et al. (1993) have used a two-step procedure to first isolate ethanol-tolerant bacteria and then select maltose-fermenting, ethanol-producing isolates to obtain *Zymobacter* strains. The composition of the isolation medium is (g/l): yeast extract (Difco), 10.0; glucose, 20.0; KH_2PO_4, 2.0; Bacto agar (Difco), 15.0 g; pH 6.0. Ethanol is added to a concentration of 5% (v/v). To prevent multiplication of fungi and yeasts, the antibiotic Kabicidine (Wako Pure Chemical Industries, Osaka, Japan) is added to a concentration of 100 mg/l. Diluted samples are plated onto the medium and incubated at 30°C for 4 d. Isolates are incubated at 30°C for 4 d in MY medium, and those that produce concentrations of ethanol in excess of 2% are selected. Four strains have been obtained from palm sap from among 500 samples of plant sap, fermented foods, and alcoholic beverages collected from tropical and subtropical regions of Brazil, Indonesia, Japan, Thailand, and other countries.

Maintenance Procedures

Zymobacter cultures can be maintained by serial transfer every 2 weeks on MY agar and stored at 4°C. *Zymobacter* can be preserved for many years by lyophilization, L-drying, or freezing with 50% glycerol at −80°C.

Procedures for Testing Special Characters

Characterization procedures for *Zymobacter* strains have been described by Okamoto et al. (1993). Conventional taxonomic features can be determined through standard methods. Test media that cannot support the growth must be supplemented with nicotinic acid (1 mg/l) and/or yeast extract (10.0 g/l), as well as with glucose or maltose. Flagellation is observed when cells are grown at 20°C for 12 h in brain–heart infusion broth (Difco) supplemented with nicotinic acid (1 mg /l), maltose (20.0 g/l), and liver infusion (10% (v/v); pH 6.0. The liver infusion is prepared by gently boiling 100 g of sliced cattle liver in 250 ml water for 30 min and filtering through cloth. Cells can be shadowed with platinum and visualized by transmission electron microscopy. The basal liquid medium for testing growth-factor requirement and fermentation of carbon compounds is composed of (g/l): K_2HPO_4, 7.0; KH_2PO_4, 2.0; $MgSO_4 \cdot 7H_2O$, 0.1; $(NH_4)_2SO_4$, 1.0; pH 6.0. Maltose is added when assessing growth-factor requirements, and nicotinic acid is added when evaluating the fermentation of carbon compounds.

Differentiation of the genus *Zymobacter* from other genera

Table BXII.γ.104 indicates the salient features that differentiate *Zymobacter* from other phylogenetically related genera. Characteristics that distinguish *Zymobacter* from *Halomonas* and *Chromohalobacter* are ethanol and CO_2 production from glucose, growth in relationship to oxygen, halophilic property, and habitat.

TABLE BXII.γ.102. Specific growth rate of *Zymobacter palmae* T109^T as affected by the initial concentration of glucose and maltose

	Specific growth rate μ (h^{-1}) at a sugar concentration of (%):							
Sugar	2.5	5	7.5	10	12.5	15	17.5	20
Glucose	0.47	0.43	0.38	0.26	0.19	0.08	0.07	0.05
Maltose	0.2	0.24	0.25	0.29	0.26	0.26	0.2	0.2

Phenotypically and ecologically, *Zymobacter* is similar to the ethanol-producing genera *Zymomonas* and *Saccharobacter*, both of which occur in sugar-containing niches, such as plant juices. The features that differentiate *Zymobacter* from these genera are highlighted in Table BXII.γ.105. *Zymobacter* is clearly differentiated from these two genera by flagellation, sugar fermentation, vitamin requirements for growth, osmotic tolerance, mol% G + C of DNA, and ubiquinone system.

Taxonomic Comments

Zymobacter belongs to the family *Halomonadaceae*. The 16S rRNA gene of *Zymobacter* palmae T109^T (accession number D14555) is

TABLE BXII.γ.103. Cellular fatty acids of *Zymobacter palmae*[a]

Fatty Acid	Range, %	Mean, %
$C_{12:0}$	5–6	5
$C_{14:0}$	3–4	4
$C_{16:0}$	46–54	51
$C_{16:1}$	<0.5	<0.5
$C_{18:0}$	4-6	5
$C_{18:1\ \omega9c}$	8–21	11
$C_{19:0\ cyc}$	21–26	24
$C_{10:0\ 3OH}$	<0.5	<0.5
$C_{12:0\ 3OH}$	8–13	12

[a]From an examination of four strains. The percentages of fatty acid compositions were calculated on the basis of total non-hydroxylated acids.

TABLE BXII.γ.104. Differential characteristics of the genus *Zymobacter* and other genera in the family *Halomonadaceae*[a]

Characteristics	*Zymobacter*	*Halomonas*	*Chromohalobacter*
Motility	+	D	+
Flagella:			
Peritrichous	+	+	+
Polar	−	+	−
Mol% G + C of DNA	55.4–56.2	52–68	62.1–64.9
Growth in relationship to oxygen:			
Facultatively anaerobic	+	−	−
Aerobic	−	+[b]	+
Glucose fermented to ethanol/CO_2	+	−	−
Halophilic	−	+	+
Habitat:			
Palm sap	+	−	−
Saline environment	−	+	+

[a]Symbols: see standard definitions.

[b]Some strains can grow anaerobically in the presence of nitrate.

TABLE BXII.γ.105. Differential characteristics of *Zymobacter palmae* and other ethanol-producing bacteria[a]

Characteristics	*Zymobacter palmae*	*Zymomonas mobilis*	*Saccharobacter fermentatus*
Cell diameter, μm	0.7–0.9	1.0–1.4	0.5–0.9
Cell length, μm	1.3–2.4	2.0–6.0	1.0–1.9
Motility	+	− (usually)	+
Flagellar arrangement:			
Peritrichous	+	−	+
Polar, 1–4 flagella	−	+	−
Utilization of citrate	−	−	+
Arginine dihydrolase	−	−	+
Phenylalanine deaminase	−		+
β-galactosidase	−		+
Utilization of:			
L-Aarabinose	−	−	+
D-Xylose	−	−	+
Mannose	+	−	
Maltose	+	−	+
Sucrose	+	d	+
Melibiose	+	−	+
L-Rhamnose	−	−	+
Trehalose	−	−	+
Lactose	+	−	+
Starch	−	−	+
Growth factor requirement:			
Nicotinic acid	+	−	
Biotin	−	+	
Pantothenate	−	+	
Osmotic tolerance to:			
Glucose	<25%	40%	35%
Maltose	50%		
Mol% G + C of DNA	55.4–56.2	47.5–49.5	63.3–63.8
Ubiquinone system	Ubiquinone-9	Ubiquinone-10	
Characteristic non-hydroxylated acids, %			
$C_{16:0}$	51	12	
$C_{18:1}$ (oleic)	11		
$C_{18:1}$ (vaccenic)		69[b]	
$C_{19:0}$ cyc	24		

[a]Symbols: see standard definitions.

[b]Data from Carey and Ingram (1983).

TABLE BXII.γ.106. The signature characteristics of *Zymobacter palmae* and related bacteria

Position(s)	*Zymobacter palmae*[a]	*Halomonas*[b]	*Chromohalobacter marismortui*[c]	*Oceanospirillum linum*
76–93	6-bp stem	6-bp stem	6-bp stem	6-bp stem
484	A	A	A	G
486	C	C	C	U
640	G	G	G[d]	G
660[e]	A	A	A	A
745	U	U	U	U
668[f]	A	A	A	G
738	U	U	U	C
669[g]	A	A	A	U
737	U	U	U	A
776	U	U	U	G
1124	U	U	G[h]	U
1297	U	U	U	G
1298	C	C	C	U
1423	A	A	A	G
1424	U	C	C	U
1439	C	U	U	C
1462	G	A	A[i]	G
1464	U	C	C[j]	C

[a]*Zymobacter palmae* T109^T (D14555).

[b]Data from Dobson and Franzmann, 1996.

[c]*Chromohalobacter marismortui* ATCC 17056^T (X87219).

[d]G for strains T1093 (U78719), A-65 (X87220), and A-492 (X87222).

[e]Nucleotides at 660 and 745 form a base pair.

[f]Nucleotides at 668 and 738 form a base pair.

[g]Nucleotides at 669 and 737 form a base pair.

[h]G for strains ATCC 17056 (X87219), A-65 (X87220), A-100 (X87221), and A-492 (X87222); U for strain T1093 (U78719).

[i]Strains T1093 (U78719), A-65 (X87220), and A-492 (X87222).

[j]Strains T1093 (U78719), A-65 (X87220), and A-492 (X87222).

89.3–92.5% similar to that of *Halomonas* and 91.1% similar to that of *Chromohalobacter* (Dobson and Franzmann, 1996).

Zymobacter belongs to the family *Halomonadaceae*, which is in the class *Gammaproteobacteria*, and includes the marine genera *Halomonas* and *Chromohalobacter*. In contrast with these genera, *Zymobacter* is of terrestrial origin, is neither halophilic nor halotolerant, is facultatively anaerobic, and produces ethanol and CO_2. The nucleic acid similarity among the 16S rRNAs of these genera is about 90%, and, like *Halomonas* and *Chromohalobacter*, *Zymobacter* demonstrates the 15 signature sequences necessary to place it in the family *Halomonadaceae* (Dobson and Franzmann, 1996) (Table BXII.γ.106). *Zymobacter* (type strain of *Zymobacter palmae*) differs from *Chromohalobacter* and *Halomonas* at 16S rRNA positions 1424, 1439, 1462, and 1464 (*E. coli* numbering system), at which *Halomonas* has the sequences described as characteristic of its genera (Dobson and Franzmann, 1996) (Table BXII.γ.106). The 16S rRNA similarity of *Zymobacter* to another marine genus, *Oceanospirillum*, is 88.7–89.7%—similar to that between *Zymobacter* and *Halomonas* and *Chromohalobacter*. However, *Oceanospirillum* is phylogenetically distinct; it lacks the signature sequences of the *Halomonadaceae* (Dobson and Franzmann, 1996). Chemotaxonomically, the *Halomonadaceae* share the ubiquinone-9 respiratory system, whereas *Oceanospirillum* uses ubiquinone-8 (Sakane and Yokota, 1994). Because the genus *Zymobacter* and the species *Zymobacter palmae* were established in light of the taxonomic features of four strains isolated from a limited source (palm sap from the southern region of Japan), a precise circumscription of the attributes of this genus and species requires the isolation of strains from more varied sources.

The phenotypic features of *Zymobacter* are similar to those of the genera *Zymomonas* and *Saccharobacter*. All of these organisms are Gram negative, facultatively anaerobic, inhabit plant juice and the like, and produce ethanol and CO_2 from carbohydrates. Phylogenetically, *Zymobacter* is distinct from *Zymomonas*, which be-

TABLE BXII.γ.107. Other characteristics of *Zymobacter palmae*[a]

Characteristics	Reaction or result
Catalase	+
Oxidase	−
Nicotinic acid required for growth	+
Methyl red test	+
Voges–Proskauer test	+
Utilization of citrate (Koser, Christensen)	−
Nitrate reduction	−
Indole production	−
Gelatin liquefaction	−
Hydrolysis of starch	−
Phenylalanine deaminase	−
Arginine dihydrolase	−
Lysine decarboxylase	−
Ornithine decarboxylase	−
α-Glucosidase	+
β-Galactosidase	−
Utilization of carbon sources:	
L-Arabinose	−
D-Xylose	−
D-Glucose	+
D-Fructose	+
D-Mannose	d
D-Galactose	d
Maltose	+
Sucrose	+
Melibiose	+
L-Rhamnose	−
Trehalose	−
Cellobiose	−
Lactose	−
Raffinose	+
Sorbitol	+
Mannitol	+
Dextrin	−
Inulin	−

[a]Symbols: see standard definitions.

longs to the *Alphaproteobacteria*. Like *Zymobacter*, *Saccharobacter* is peritrichously flagellated, but it has a higher mol% G + C (63.5 versus 55.8). The generic description of *Saccharobacter* lacks a description of chemotaxonomic characteristics and phylogenetic analysis. The phylogenetic relationship and exact differentiation of *Zymobacter* from *Saccharobacter* requires detailed comparative study.

List of species of the genus Zymobacter

1. **Zymobacter palmae** Okamoto, Taguchi, Nakamura, Ikenaga, Kuraishi and Yamasato 1995, 418[VP] (Effective publication: Okamoto, Taguchi, Nakamura, Ikenaga, Kuraishi and Yamasato 1993, 336.)
pal′mae. L. gen. n. *palmae* of palm.

The characteristics are as given for the genus. See also Tables BXII.γ.104, BXII.γ.105, and BXII.γ.107. Isolated from palm sap in Okinawa Prefecture, Japan.
The mol% G + C of the DNA is: 55.4–56.2 (HPLC).
Type strain: T109, ATCC 51623, IAM 14233.
GenBank accession number (16S rRNA): D14555, AF211871.

Order IX. **Pseudomonadales** Orla-Jensen 1921, 270[AL]

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Pseu.do.mon.a.da′les. M.L. fem. n. *Pseudomonas* type genus of the order; *-ales* suffix to denote order; M.L. fem. n. *Pseudomonadales* the *Pseudomonas* order.

The order *Pseudomonadales* was circumscribed for this volume on the basis of phylogenetic analysis of 16S rRNA sequences; the order contains the families *Pseudomonadaceae* and *Moraxellaceae*.

Aerobic chemoorganotrophs with respiratory metabolism. Most are motile by means of flagella. *Azomonas* spp. and *Azotobacter* spp. fix nitrogen; *Azotobacter* spp. form cysts; *Moraxella* spp. inhabit the mucosa of animals and man.

Type genus: **Pseudomonas** Migula 1894, 237[AL] (Nom. Cons., Opin. 5 of the Jud. Comm. 1952, 121.)

Family I. **Pseudomonadaceae** Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith 1917, 555[AL]

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Pseu.do.mon.a.da′ce.ae. M.L. fem. n. *Pseudomonas* type genus of the family; *-aceae* ending to denote family; M.L. fem. pl. n. *Pseudomonadaceae* the *Pseudomonas* family.

The family *Pseudomonadaceae* was circumscribed for this volume on the basis of phylogenetic analysis of 16S rRNA sequences; the family contains the genera *Pseudomonas* (type genus), *Azomonas*, *Azotobacter*, *Cellvibrio*, *Mesophilobacter*, *Rhizobacter*, and *Rugamonas*. *Serpens* is also included.

Aerobic chemoorganotrophs with respiratory metabolism. Most are motile by means of flagella. *Azomonas* and *Azotobacter* fix nitrogen; *Azotobacter* forms cysts.

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Genus I. **Pseudomonas** *Migula 1894, 237[AL] (Nom. Cons., Opin. 5 of the Jud. Comm. 1952, 121)**

NORBERTO J. PALLERONI

Pseu.do′mo.nas or Pseu.do.mo′nas. Gr. adj. *pseudes* false; Gr. n. *monas* a unit, monad; M.L. fem. n. *Pseudomonas* false monad.

Straight or slightly curved rods but not helical, 0.5–1.0 × 1.5–5.0 μm. **Most of the species do not accumulate granules of polyhydroxybutyrate**, but accumulation of polyhydroxyalkanoates of monomer lengths higher than C_4 may occur when growing on alkanes or gluconate. Do not produce prosthecae and are not surrounded by sheaths. No resting stages are known. Gram negative. **Motile by one or several polar flagella**; rarely nonmotile. In some species lateral flagella of short wavelength may also be formed. **Aerobic, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor; in some cases nitrate can be used as an alternate electron acceptor**, allowing growth to occur anaerobically. **Xanthomonadins are not produced.** Most, if not all, species fail to grow under acid conditions (pH 4.5 or lower). Most species do not require organic growth factors. Oxidase positive or negative. Catalase positive. Chemoorganotrophic. **Strains of the species include in their composition the hydroxylated fatty acids $C_{10:0\ 3OH}$ and $C_{12:0}$, and $C_{12:0\ 2OH}$, and ubiquinone Q-9.** Widely distributed in nature. Some species are pathogenic for humans, animals, or plants.

**Editorial Note:* The literature search for the chapter on *Pseudomonas* was completed in January, 2000. During the course of unavoidable publication delays, a number of new species were described or reclassified after the chapter was completed. It was not possible to include these species in the text of to include their characteristics in the comparative tables. The reader is encouraged to consult the studies listed in the Further Reading section and the *International Journal of Systematic and Evolutionary Microbiology* (2000–2003).