12 The Order *Halanaerobiales*, and the Families *Halanaerobiaceae* and *Halobacteroidaceae*

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Abstract
The order *Halanaerobiales*, families *Halanaerobiaceae* and *Halobacteroidaceae*, consists of obligatory anaerobic, moderately halophilic bacteria that require NaCl concentrations between 0.5 and 3.4 M for optimal growth. Representatives have been isolated from anaerobic sediments of salt lakes worldwide, from brines associated with oil reservoirs, and also from fermented salted foods. Some species are thermophilic or alkaliphilic. Although phylogenetically affiliated with the low G+C branch of the *Firmicutes*, the cells show a Gram-negative wall structure, and most species stain Gram-negative. Some representatives of the *Halobacteroidaceae* produce endospores. Most species ferment carbohydrates to acetate, ethanol, H_2, CO_2, and other fermentation products. Within the *Halobacteroidaceae*, a greater metabolic diversity is found, with some species displaying a homoacetogenic metabolism; growth by anaerobic respiration using different electron acceptors including nitrate, trimethylamine *N*-oxide, selenate, arsenate, or Fe(III); or chemolithoautotrophic growth on hydrogen and elemental sulfur.

Taxonomy, Historical and Current

Bottom sediments of hypersaline lakes and lagoons may support a rich community of anaerobic halophilic bacteria, as the solubility of oxygen in hypersaline brines is low and the amounts of organic matter available are often high (Oren 1988). It is therefore surprising that the first records of the isolation of obligatory anaerobic fermentative bacteria growing at salt concentrations of 10–20 % and higher were published only in the early 1980s, when *Halanaerobium praevalens* was isolated from the bottom sediments of Great Salt Lake, Utah (Zeikus 1983; Zeikus et al. 1983) and *Halobacteroides halobius* and *Sporohalobacter lortetii* were discovered in Dead Sea sediments (Oren 1983; Oren et al. 1984b). *Halanaerobium praevalens* probably resembles "Bacteroides halosmophilus," isolated by Baumgartner (1937) from solar salt and from salted anchovies. Unfortunately no cultures of that isolate have been preserved.

Order *Halanaerobiales* corrig. Rainey and Zhilina 1995, 879^{VP} (Validation List no. 55); Effective Publication: Rainey, Zhilina, Boulygina, Stackebrandt, Tourova and Zavarzin 1995, 193.

Hal.an.ae.ro.bi.a'les. N.L. neut. n. *Halanaerobium*, type genus of the order; suff. *–ales*, ending denoting an order; N.L. fem. pl. n. *Halanaerobiales*, the *Halanaerobiaceae* order.

Cells are rod-shaped and generally stain Gram-negative. Endospores are produced by some species. Strictly anaerobic. Oxidase negative and generally catalase negative. Most species ferment carbohydrates to products including acetate, ethanol, H_2, and CO_2. Some species may grow fermentatively on amino acids, and others have a homoacetogenic metabolism or may grow by anaerobic respiration on nitrate, trimethylamine *N*-oxide, selenate, arsenate, or Fe(III). Chemolithoautotrophic growth on H_2 and elemental sulfur may also occur. Moderately

Dedicated to the memory of George A. Zavarzin (1933–2011), a pioneer of research on anaerobic halophilic microorganisms.

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halophilic. NaCl concentrations between 0.5 and 3.4 M are required for optimal growth, and no growth is observed below 0.3–1.7 M NaCl, depending on the species.

The mol% G+C of the DNA varies between 27 and 45.

Type Genus: *Halanaerobium*

The order *Haloanaerobiales* was created in 1995, based on 16S rRNA sequence comparisons. These resulted in a reclassification of the species of the former family *Haloanaerobiaceae* over two families: the *Haloanaerobiaceae* and the newly created family *Halobacteroidaceae* (Rainey et al. 1995). Physiologically the group is coherent, to the extent that, as yet, no aerobes or non-halophiles are known to cluster phylogenetically within the order.

The genus *Halanaerobium* (originally named *Haloanaerobium* and corrected in accordance with Rule 61 of the Bacteriological Code) (Oren 2000) is now the largest genus within the order (nine species and two subspecies). Based on 16S rRNA sequence comparisons (Rainey et al. 1995), a number of species formerly classified in other genera were transferred to this genus: the former *Halobacteroides acetoethylicus* (Rengpipat et al. 1988a) was reclassified as *Halanaerobium acetethylicum* (Oren 2000; Patel et al. 1995; Rainey et al. 1995), and the former *Haloincola saccharolyticus* (originally described under the name *Haloincola saccharolytica*) (Zhilina et al. 1992b) was renamed as *Halanaerobium saccharolyticum*, with two subspecies, *saccharolyticum* and *senegalense* (Cayol et al. 1994a; Oren 2000; Rainey et al. 1995). The genera *Halobacteroides*, *Acetohalobium*, *Halanaerobacter*, and *Sporohalobacter*, previously classified within the family *Halanaerobiaceae*, were transferred to the *Halobacteroidaceae* (Rainey et al. 1995).

At the time of writing (March 2012), 30 species had been described. The family *Halanaerobiaceae* currently has 4 genera with 12 species; the family *Halobacteroidaceae* contains 11 genera with 18 species (see *Figs. 12.1* and *12.2* and *Tables 12.1*, *12.2*, *12.3*, *12.4*, *12.5*, *12.6*, and *12.7*).

The group was earlier reviewed by Kivistö and Karp (2011); Lowe et al. (1993); Ollivier et al. (1994), and Oren (1986a, 1990, 1993a, b, 2006).

Phylogenetic Structure of the Family and Its Genera

Figure 12.3 shows a neighbor-joining phylogenetic tree of the type strains of the 31 species and subspecies of the order *Halanaerobiales*. It may be noted that *Halobacteroides elegans* does not cluster with *Halobacteroides halobius*, the type species of the genus, but with the species of the genus *Halanaerobacter*, suggesting that reclassification of *H. elegans* may be recommended. The family is associated with the low-G+C branch of the *Firmicutes*. The group forms a coherent cluster close to the bifurcation point that separates the *Actinobacteria* and the *Bacillus/Clostridium* group (Rainey et al. 1995; Tourova et al. 1995). The deep branching justifies classification in a separate order (Rainey et al. 1995). The order *Halanaerobiales* has been used as a paradigm to demonstrate the application of 16S rRNA gene sequencing and DNA-DNA hybridization in bacterial taxonomy (Tourova 2000). Two families were described: the *Halanaerobiaceae* (Oren et al. 1984a) and the *Halobacteroidaceae* (Rainey et al. 1995).

The species share a low content of G+C in their DNA, generally between 29 mol% and 34 mol%. Exceptions are the thermophilic *Halothermothrix orenii* with a G+C content of 37.9 mol% and the atypical, non-fermentative anaerobic respirer *Halarsenatibacter silvermanii* with 45 mol%.

Genome Analysis

At the time of writing (March 2012), three complete genome sequences of members of the *Halanaerobiales* had been published: the type strain of *Halanaerobium praevalens* (Ivanova et al. 2011), the thermophilic *Halothermothrix orenii* (Mijts and Patel 2001; Mavromatis et al. 2009), and a haloalkaliphilic hydrogen-producing strain known as "*Halanaerobium hydrogenoformans*," earlier designated as "*Halanaerobium sapolanicus*" (Brown et al. 2011). This organism is not currently available from culture collections. Except for the genome sequence, little information is available about it beyond the fact that it was isolated from the alkaline hypersaline and sulfide-rich Soap Lake, Washington, USA, that it grows optimally at pH 11, 7 % NaCl, and 33 °C and that it produces acetate, formate, and H_2 (*Table 12.8*).

The three genomes are 2.3–2.6 Mbp in length and each contains four identical or nearly identical copies of the 16S rRNA gene. Analysis of the *H. orenii* gene showed a few features characteristic for Gram-negative bacteria such as a pathway for lipid A biosynthesis, outer membrane secretion proteins, and two copies of the chaperone OmpH, a periplasmic protein that helps to transport proteins to the outer membrane. There also are a number of sporulation-related genes. The main sporulation regulator Spo0A of bacilli and clostridia is present, but sporulation was never shown in this organism. Genes coding for the biosynthesis of organic osmotic solutes were not detected except for the finding of a gene for sucrose phosphate synthase, suggesting that sucrose can be formed and may possibly act as an osmotic solute (Chua et al. 2008; Mavromatis et al. 2009).

Comparative analysis of the three *Halanaerobiales* genomes did not show an unusually high content of acidic amino acids or a low content of basic amino acids in the encoded proteins. The apparent excess of acidic amino acids in the bulk protein of *Halanaerobium praevalens*, *H. saccharolyticum*, *Halobacteroides halobius*, *Sporohalobacter lortetii*, and *Natroniella acetigena* reported earlier (Detkova and Boltyanskaya 2006; Oren 1986b) is therefore due to the high content of glutamine and asparagine in their proteins, which yield glutamate and aspartate upon acid hydrolysis. The proteins of the *Halanaerobiales*, which are active in the presence of high intracellular KCl concentrations, do thus not possess the typical acidic signature of the

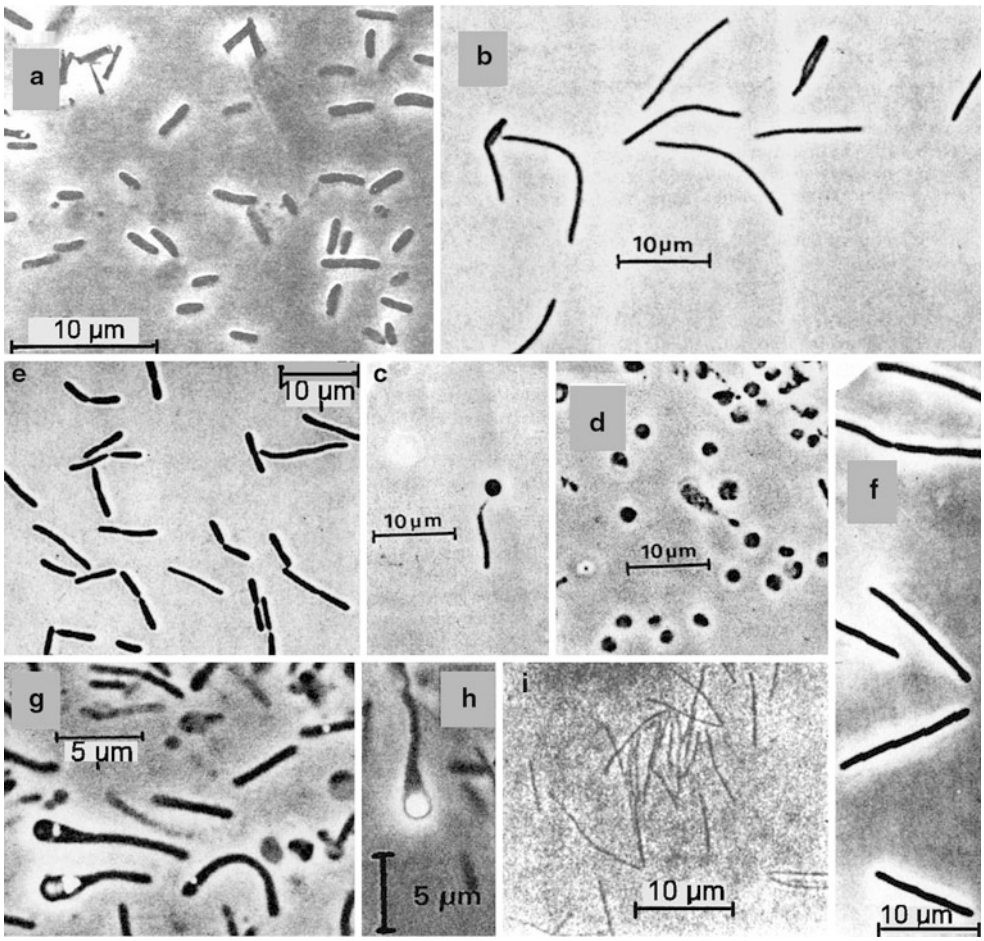

Fig. 12.1
Phase-contrast micrographs of members of the *Halanaerobiales*: (a) *Halanaerobium alcaliphilum*; (b–d) young, senescent, and old cells of *Halobacteroides halobius*; (e) *Acetohalobium arabaticum*; (f) *Natroniella acetigena*; (g) *Sporohalobacter lortetii*; (h) *Orenia marismortui*; (i) *Halothermothrix orenii*. Figures were derived from Tsai et al. (1995), Oren et al. (1984b), Zavarzin et al. (1994), Zhilina et al. (1996), Oren (1983), and Cayol et al. (1994b), respectively; reproduced with permission

"halophilic" proteins of the Archaea of the order *Halobacteriales* or of the extremely halophilic bacterium *Salinibacter* (Elevi Bardavid and Oren 2012).

Phages

No phages active on strains of *Halanaerobiales* have yet been described.

Phenotypic Analyses

General Comments

Members of the *Halanaerobiales* display a Gram-negative type of cell wall with an outer membrane and periplasmic space (*Fig. 12.2*). *Meso*-diaminopimelic acid was detected in the peptidoglycan of *Halanaerobium saccharolyticum* subsp. *saccharolyticum* (Zhilina et al. 1992b). Most species also show a negative Gram-stain reaction; however, *Halanaerobium tunisiense* and *Halanaerocella petrolearia* stain Gram-positive (Gales et al. 2011; Hedi et al. 2009).

Heat-resistant endospores are produced by a number of species of *Halobacteroidaceae*, including *Sporohalobacter lortetii* (Oren 1983), the three *Orenia* species (Mouné et al. 2000; Oren et al. 1987; Zhilina et al. 1999), and *Natroniella acetigena* (Zhilina et al. 1996). When initially isolated, *Acetohalobium arabaticum* produced spores, but sporulation was not observed during subsequent transfers (Zavarzin et al. 1994). Special conditions may be required for induction of endospore formation. Growth on solid media or in nutrient-poor liquid media enhances sporulation in certain species (Oren 1983; Oren et al. 1987). A phenotypic test which may be correlated with the phylogenetic position of the *Halanaerobiaceae* within the *Firmicutes* and with the ability to form endospores is the

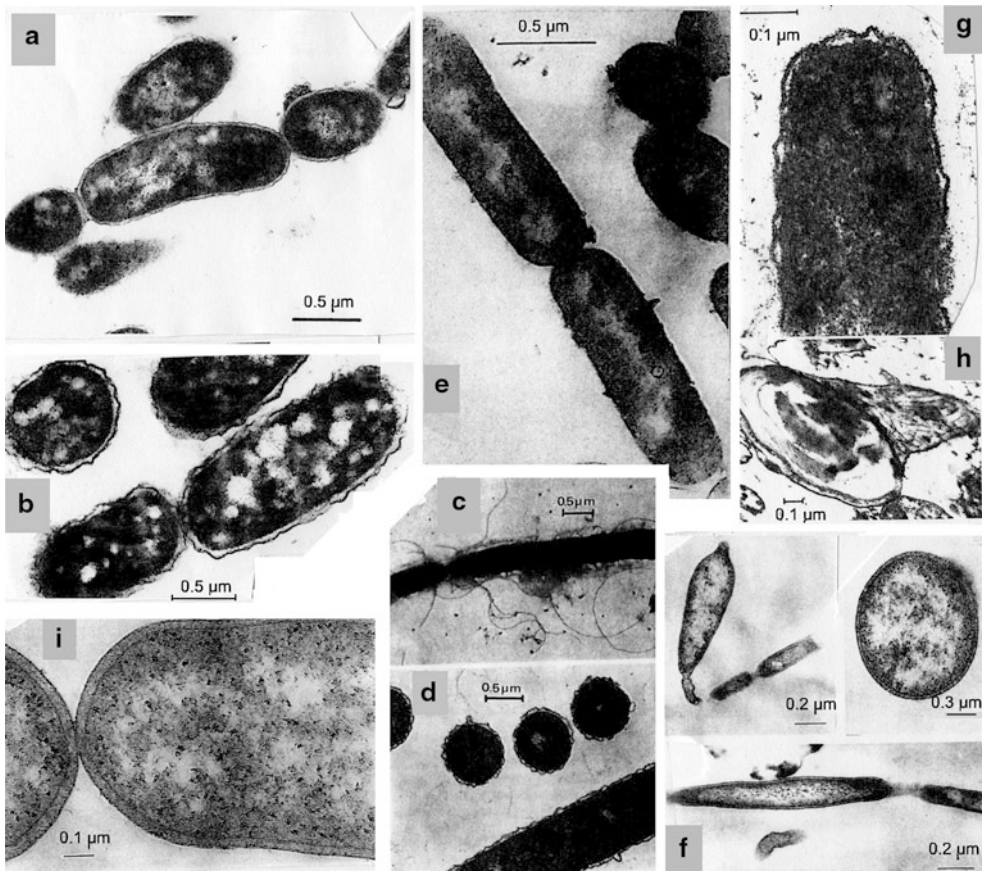

Fig. 12.2
Electron micrographs of members of the *Halanaerobiales*: (a) *Halanaerobium lacusrosei*; (b) *Halanaerobium saccharolyticum*; (c,d) *Halobacteroides halobius*; (e) *Acetohalobium arabaticum*; (f) *Halothermothrix orenii*; (g,h) *Sporohalobacter lortetii*; (i) *Halanaerobium saccharolyticum* subsp. *senegalense*. Figures were derived from Cayol et al. (1995), Zhilina et al. (1992b), Oren et al. (1984b), Zavarzin et al. (1994), Cayol et al. (1994b), Oren (1983), and Cayol et al. (1994a), respectively; reproduced with permission

hydrolysis of the D-isomer of *N*′-benzoyl-arginine-*p*-nitroanilide (BAPA). Three representatives of the *Halanaerobiales* (*Halobacteroides halobius*, *Halanaerobium praevalens*, *Orenia marismortui*) were found to hydrolyze D-BAPA, while L-BAPA was not hydrolyzed. *Sporohalobacter lortetii* degraded neither of the BAPA stereoisomers (Oren et al. 1989).

All members of the *Halanaerobiales* are strict anaerobes. They are oxidase negative, and most species lack catalase, *Halarsenatibacter silvermanii* being the only known exception. All members of the *Halanaerobiaceae* and most members of the *Halobacteroidaceae* obtain their energy by fermenting simple sugars (*Tables 12.9* and *12.10*). *Halanaerobacter chitinivorans* uses chitin, and *Halocella cellulosilytica* degrades cellulose. Fermentation products typically include acetate, H_2, and CO_2. Some strains produce in addition butyrate, lactate, propionate, and/or formate. *Halarsenatibacter silvermanii* lives by dissimilatory reduction of arsenate to arsenite, Fe(III) to Fe(II), and elemental sulfur to sulfide. Chemoautotrophic growth occurs with sulfide as the electron donor and arsenate as the electron acceptor (Switzer Blum et al. 2009). Within the family *Halobacteroidaceae*, the metabolic diversity is much greater than within the *Halanaerobiaceae*. Thus, there are species that ferment amino acids, either alone or by using the Stickland reaction. For example, *Halanaerobacter salinarius* and *Halanaerobacter chitinivorans* can use serine as an electron donor using the Stickland reaction while reducing glycine betaine, with the formation of acetate, trimethylamine, CO_2, and NH_3 (Mouné et al. 1999). *Sporohalobacter lortetii* is primarily an amino acid fermenter, and sugars are poorly used (Oren 1983). Anaerobic respiration also occurs, using different electron acceptors: *Selenihalanaerobacter shriftii* oxidizes glycerol or glucose by anaerobic respiration with nitrate, trimethylamine *N*-oxide, or selenate as electron acceptor (Switzer Blum et al. 2001a).

Acetohalobium arabaticum (neutrophilic), *Natroniella acetigena* (alkaliphilic), and *Fuchsiella alkaliacetigena* (alkaliphilic) have a homoacetogenic metabolism, producing acetate as the main end product of their energy metabolism. *Acetohalobium arabaticum* grows on H_2 + CO_2 or on carbon monoxide as a lithoautotroph, on trimethylamine as a methylotroph, and on other substrates (formate, glycine betaine, lactate, pyruvate, histidine, aspartate, glutamate, and

Table 12.1

Comparison of selected characteristics of members of the genus *Halanaerobium*

Character	*Halanaerobium praevalens*[a]	*Halanaerobium alcaliphilum*[b]	*Halanaerobium acetethylicum*[c,d,e]	*Halanaerobium* salsuginis[f]	*Halanaerobium* saccharolyticum *subsp. saccharolyticum*[e,g]
Earlier name/ basonym	*Haloanaerobium praevalens*	*Haloanaerobium alcaliphilum*	*Halobacteroides acetoethylicus*	*Haloanaerobium salsugo*	*Haloincola saccharolytica*; *Haloanaerobium saccharolyticum* subsp. *saccharolyticum*
Type strain	DSM 2228	DSM 8275	DSM 3532	ATCC 51327	DSM 6643
Cell size	0.9–1.1 × 2.0–2.6 μm	0.8 × 3.3–5 μm	0.4–0.7 × 1–1.6 μm	0.3–0.4 × 2.6–4 μm	0.5–0.7 × 1–1.5 μm
Morphology	Rods	Rods	Rods	Rods	Rods
Motility	–	+, peritrichous	+, peritrichous	–	+, peritrichous
		flagella	flagella		flagella
Endospores	–	–	–	–	–
Spheroplasts	–	NR	–	NR	–
Gas vesicles	NR	NR	NR	NR	NR
NaCl range	2–30 %	2.5–25 %	5–22 %	6–24 %	3–30 %
NaCl optimum	13 %	10 %	10 %	9 %	10 %
pH range	6.0–9.0	5.8–10.0	5.4–8.0	5.6–8.0	6.0–8.0
pH optimum	7.0–7.4	6.7–7.0	6.3–7.4	6.1	7.5
Temperature range	5–50 °C	25–50 °C	15–45 °C	22–51 °C	15–47 °C
Temperature optimum	37 °C	37–40 °C	34 °C	40 °C	37–40 °C
Doubling time	4 h	3.3 h	7.5 h	9 h	3.9 h
Carbohydrates utilized	+	+	+	+	+
End products of fermentation	Acetate, butyrate, propionate, H_2, CO_2	Acetate, butyrate, lactate, H_2, CO_2	Acetate, ethanol, H_2, CO_2	Acetate, ethanol, H_2, CO_2	Acetate, H_2, CO_2
Major fatty acids	14:0 16:0 16:1	NR	14:0 16:0 16:1	14:0 16:0 16:1 $17:0_{cyc}$	15:1 16:0 16:1
G+C content of DNA (mol%)	30.3[m]	31.0	32.0	34.0	31.3
Sample source and site	Sediment, Great Salt Lake, Utah, USA	Sediment, Great Salt Lake, Utah, USA	Filter material, offshore oil rig, Gulf of Mexico	Petroleum reservoir fluid, Oklahoma, USA	Sediment, Lake Sivash, Crimea
Character	***Halanaerobium saccharolyticum* subsp. *senegalense*[e,h]**	***Halanaerobium congolense*[i]**	***Halanaerobium lacusrosei*[j]**	***Halanaerobium kushneri*[k]**	***Halanaerobium fermentans*[l]**
Earlier name/ basonym	*Haloincola saccharolytica* subsp. *senegalensis*	*Haloanaerobium congolense*	*Haloanaerobium lacusroseus*	*Haloanaerobium kushneri*	*Haloanaerobium fermentans*
Type strain	DSM 7379	DSM 11287	DSM 10165	ATCC 700103	JCM 10494
Cell size	0.4–0.6 × 2–5 μm	0.5–1 × 2–4 μm	0.4–0.6 × 2–3 μm	0.5–0.8 × 0.7–3.3 μm	1.0–1.2 × 2.7–3.3 μm
Morphology	Rods	Rods	Rods	Rods	Rods
Motility	+, peritrichous flagella	–	+, peritrichous flagella	+, peritrichous flagella	+, peritrichous flagella
Endospores	–	–	NR	–	–
Spheroplasts	NR	NR	NR	NR	NR

Table 12.1 (continued)

Character	*Halanaerobium saccharolyticum* subsp. *senegalense*[e,h]	*Halanaerobium congolense*[i]	*Halanaerobium lacusrosei*[j]	*Halanaerobium kushneri*[k]	*Halanaerobium fermentans*[l]
Gas vesicles	NR	NR	NR	NR	NR
NaCl range	5–25 %	4–24 %	7.5–34 %	9–18 %	7–25 %
NaCl optimum	7.5–12.5 %	10 %	18–20 %	12 %	10 %
pH range	6.3–8.7	6.3–8.5	NR	6.0–8.0	6–9
pH optimum	7.0	7.0	7.0	6.5–7.5	7.5
Temperature range	20–47 °C	20–45 °C	20–50 °C	20–45 °C	15–45 °C
Optimum temperature	40 °C	42 °C	40 °C	35–40 °C	35 °C
Doubling time	4.2 h	2.5 h	2.4 h	7.3 h	NR
Carbohydrates utilized	+	+	+	+	+
End products of fermentation	Acetate, H_2, CO_2	Acetate, H_2, CO_2	Acetate, ethanol, H_2, CO_2	Acetate, ethanol, H_2, CO_2	Acetate, ethanol, formate, lactate, H_2, CO_2
Major fatty acids[1]	14:0 16:0 15:1 16:1	NR	NR	14:0 16:0 16:1	NR
G+C content of DNA (mol%)	31.7	34	32	32.4–36.9	33.3
Sample source and site	Sediment, Lake Retba, Senegal	Offshore field, Congo	Sediment, Lake Retba, Senegal	Petroleum reservoir fluid, Oklahoma	Fermented puffer fish ovaries, Japan

Data taken from:
[a]Zeikus et al. (1983)
[b]Tsai et al. (1995)
[c]Rengpipat et al. (1988a)
[d]Patel et al. (1995)
[e]Rainey et al. (1995)
[f]Bhupathiraju et al. (1994)
[g]Zhilina et al. (1992b)
[h]Cayol et al. (1994a)
[i]Ravot et al. (1997)
[j]Cayol et al. (1995)
[k]Bhupathiraju et al. (1999)
[l]Kobayashi et al. (2000a)
[m]Based on the genome sequence
NR not reported

asparagine) as an organotroph. *Fuchsiella* can also grow chemolithoautotrophically (Kevbrin et al. 1995; Zavarzin et al. 1994; Zhilina and Zavarzin 1990a, b; Zhilina et al. 1996, 2012).

Several species (*Halanaerobium saccharolyticum*, *Halanaerobacter lacunarum*, *Halobacteroides halobius*, *Halobacteroides elegans*) can use methanethiol as the sole source of assimilatory sulfur for growth and reduce elemental sulfur to sulfide (Kevbrin and Zavarzin 1992a; Zhilina et al. 1992a, b, 1997). *Acetohalobium arabaticum* slowly reduces sulfur to sulfide, but this was not accompanied by growth enhancement (Kevbrin and Zavarzin 1992b; Zavarzin et al. 1994). *Natroniella acetigena* can grow chemolithoautotrophically by oxidizing H_2, using elemental sulfur as electron acceptor (Sorokin et al. 2011). *Halanaerobium congolense* uses thiosulfate and elemental sulfur as electron acceptors. Addition of thiosulfate or sulfur increased the growth yield sixfold and threefold, respectively, and growth rates were enhanced (Ravot et al. 1997). Thiosulfate reduction was also observed in *Orenia marismortui* and in *Halanaerobium congolense* (Oren et al. 1987; Ravot et al. 2005).

High concentrations of Na^+, K^+, and Cl^-, high enough to be at least isotonic with the medium, were measured inside the cells of *Halanaerobium praevalens*, *Halanaerobium acetethylicum*, *Halobacteroides halobius*, and *Natroniella acetigena* (Detkova and Pusheva 2006; Oren 1986b; Oren et al. 1997; Rengpipat et al. 1988b). No organic osmotic solutes have been detected in the anaerobic halophilic bacteria (Oren 1986b; Oren et al. 1997; Rengpipat et al. 1988b), except in the case of *Orenia salinaria*, found to accumulate glycine betaine when grown in medium containing yeast extract (Mouné et al. 2000). The intracellular enzymatic machinery appears to be well adapted to function in the presence of high salt concentrations. The enzymes tested (including glyceraldehyde-3-phosphate dehydrogenase,

Table 12.2

Comparison of selected characteristics of members of the monospecific genera *Halocella*, *Halothermothrix*, and *Halarsenatibacter* (family *Halanaerobiaceae*)

Character	*Halocella cellulosilytica*[a]	*Halothermothrix orenii*[b]	*Halarsenatibacter silvermanii*[c]
Earlier name	*Halocella cellulolytica*		
Type strain	DSM 7362	OCM 544	ATCC BAA-1651
Cell size	0.4–0.6 × 3.8–12 μm	0.4–0.6 × 10–20 μm	0.5 × 3 μm
Morphology	Rods	Rods	Curved rods
Motility	+, peritrichous flagella	+, peritrichous flagella	+, paired subpolar flagella
Endospores	–	–	–
Spheroplasts	+	NR	–
Gas vesicles	NR	NR	–
NaCl range	5–20 %	4–20 %	20–35 %
NaCl optimum	15 %	10 %	35 %
pH range	5.5–8.5	5.5–8.2	8.7–9.8
pH optimum	7.0	6.5–7.0	9.4
Temperature range	20–50 °C	45–68 °C	28–55 °C
Optimum temperature	39 °C	60 °C	44 °C
Carbohydrates utilized	+	+	–
End products of fermentation	Acetate, ethanol, lactate, H_2, CO_2	Acetate, ethanol, H_2, CO_2	Not fermentative; reduces arsenate, Fe(III), and sulfur
Major fatty acids[1]	14:0 16:0 $15:0_{anteiso}$	14:0 $15:0_{iso}$ 16:0	$15:0_{iso}$ 18:0 $17:0_{iso}$ 16:0
G+C content of DNA (mol%)	29.0	38	45.2
Sample source and site	Sediment, Lake Sivash, Crimea	Sediment, hypersaline lake, Tunisia	Sediment, Searles Lake, California, USA

Data taken from:
[a]Simankova et al. (1993)
[b]Cayol et al. (1994b)
[c]Switzer Blum et al. (2009)
NR not reported

NAD-linked alcohol dehydrogenase, pyruvate dehydrogenase, and methyl viologen-linked hydrogenase from *Halanaerobium acetethylicum*, the fatty acid synthetase complex of *Halanaerobium praevalens*, hydrogenase and CO dehydrogenase of *Acetohalobium arabaticum*, CO dehydrogenase of *Natroniella acetigena*) function better in the presence of molar concentrations of salts than in salt-free medium (Detkova and Boltyanskaya, 2006; Oren and Gurevich 1993; Pusheva and Detkova 1996; Pusheva et al. 1992; Rengpipat et al. 1988b; Zavarzin et al. 1994).

The Properties of the Genera and Species of *Halanaerobiales*

Information on the phenotypic properties of the genera and species of the *Halanaerobiales*, as summarized below, was derived from Cayol et al. 2009; Mesbah 2009; Oren 2009b, c, d, e, f; Oren et al. 2009; Rainey 2009; Zavarzin 2009; Zavarzin and Zhilina 2009a, b; and Zhilina et al. 2009 and from the original species descriptions.

Family *Halanaerobiaceae* corrig.

Oren, Paster and Woese 1984, 503^{VP} (Validation List no. 16) (Effective Publication: Oren, Paster and Woese 1984a, 79).

Hal.an.ae.ro.bi.a.ce'ae N.L. neut. n. *Halanaerobium*, type genus of the family; suff. *–aceae*, ending to denote a family; N.L. fem. pl. n. *Halanaerobiaceae*, the *Halanaerobium* family.

Cells are rod-shaped and stain Gram-negative. Endospore formation never observed. Strictly anaerobic. Oxidase and catalase negative. Carbohydrates are fermented to products including acetate, ethanol, H_2, and CO_2. Moderately halophilic. NaCl concentrations between 1.7 and 2.6 M are required for optimal growth, and no growth is observed below 0.3–1.7 M NaCl, depending on the species.

Type Genus: *Halanaerobium*

Table 12.3
Comparison of selected characteristics of members of the genus *Halobacteroides*

Character	*H. halobius*[a]	*H. elegans*[b,c]
Earlier name		*Halobacteroides halobius*
Type strain	ATCC 35273	DSM 6639
Cell size	0.5–0.6 × 10–20 μm	0.3–0.5 × 2–10 μm
Morphology	Flexible rods	Curved rods
Motility	+, peritrichous flagella	+, peritrichous flagella
Endospores	−[d]	+
Spheroplasts	+	+
Gas vesicles	NR	NR
NaCl range	7–19 %	10–30 %
NaCl optimum	9–15 %	10–15 %
pH range	NR	6.5–8.0
pH optimum	NR	7.0
Temperature range	30–47 °C	28–47 °C
Optimum temperature	37–42 °C	40 °C
Doubling time	1 h	2 h
Carbohydrates utilized	+	+
End products of fermentation	Acetate, ethanol, H_2, CO_2	Acetate, ethanol, H_2, CO_2
Major fatty acids[1]	14:0 16:0 16:1	14:0 16:0 16:1
G+C content of DNA (mol%)	30.7	30.5
Sample source and site	Sediment, Dead Sea	Cyanobacterial mat, Lake Sivash, Crimea

Data taken from:
[a]Oren et al. (1984b)
[b]Zhilina et al. (1997)
[c]Based on 16S rRNA sequence comparison, *Halobacteroides elegans* does not cluster with *Halobacteroides halobius* but with the species of the genus *Halanaerobacter*, suggesting that reclassification of *H. elegans* may be recommended
[d]Similar isolates were recovered from anaerobic sediments following pasteurization, suggesting that heat-stable endospores may be formed (Oren 1987)
NR not reported

Genus *Halanaerobium* corrig.

Zeikus, Hegge, Thompson, Phelps and Langworthy 1984, 503[VP] (Validation List no. 16), Emend. Rainey, Zhilina, Boulygina, Stackebrandt, Tourova and Zavarzin 1995, 197 (Effective publication: Zeikus, Hegge, Thompson, Phelps and Langworthy 1983, 232).

Hal.an.ae.ro'bium. Gr. n. *hals halos*, salt; Gr. pref. *an*, not; Gr. n. *aer*, air; Gr. n. *bios*, life; N.L. neut. n. *Halanaerobium*, salt organism which grows in the absence of air.

Cells rod-shaped, nonmotile or motile by peritrichous flagella, generally staining Gram-negative. Strictly anaerobic, chemoorganotrophic with fermentative metabolism. Carbohydrates are fermented with production of acetate, H_2, and CO_2; in some species, ethanol, formate, propionate, butyrate, and lactate are found in addition. Thiosulfate and elemental sulfur may be used as electron acceptors in certain species. Halophilic, growing optimally at NaCl concentrations around 1.7–2.5 M and requiring a minimum of 0.3–1.7 M NaCl for growth. Neutral or slightly alkaline pH values are preferred. Endospore formation never observed.

Type Species: *Halanaerobium praevalens*

The main features of members of the genus *Halanaerobium*, updated for March 2012, are listed in *Table 12.1*.

Genus *Halocella*

Simankova, Chernych, Osipov and Zavarzin 1994, 182[VP] (Validation List no. 48) (Effective publication: Simankova, Chernych, Osipov and Zavarzin 1993, 389).

Ha.lo.cel'la. Gr. n. *hals halos*, salt; L. fem. n. *cella*, a storeroom and in biology a cell; N.L. fem. n. *Halocella*, salt cell.

Cells are straight or slightly curved rods, non-sporulating, and motile by means of peritrichous flagella. Cell wall of Gram-negative structure. Obligately anaerobic. Moderately halophilic. Ferment carbohydrates, including cellulose, producing acetate, ethanol, lactate, H_2, and CO_2. Peptides and amino acids are not utilized.

Type Species: *Halocella cellulosilytica*

Genus *Halothermothrix*

Cayol, Ollivier, Prensier, Guezennec and Garcia 1994b, 538[VP]

Ha.lo.ther'mo.thrix. Gr. n. *hals halos*, salt; Gr. adj. *thermos*, hot; Gr. fem. n. *thrix*, hair; N.L. fem. n. *Halothermothrix*, a thermophilic (fermentative) hair-shaped halophile.

Long rod-shaped bacteria with cells that are 0.4–0.6 × 10–20 μm, occurring mainly singly. Motile by peritrichous flagella. Non-sporulating. Gram stain-negative. Strictly anaerobic. Chemoorganotrophic; ferment carbohydrates to acetate, ethanol, H_2, and CO_2. NaCl and yeast extract are required for growth. Thermophilic.

Type Species: *Halothermothrix orenii*

Genus *Halarsenatibacter*

Switzer Blum, Han, Lanoil, Saltikov, White, Tabita, Langley, Beveridge, Jahnke and Oremland 2010, 1985[VP] (Validation List no. 135) (Effective publication: Switzer Blum, Han, Lanoil, Saltikov, White, Tabita, Langley, Beveridge, Jahnke and Oremland 2009, 1958).

Hal.ar.se.na.ti.bac'ter. Gr. n. *hals halos*, salt; N.L. n. *arsenas -atis*, arsenate; N.L. masc. n. *bacter*, rod; N.L. masc. n. *Halarsenatibacter*, halophilic arsenate-utilizing rod.

Gram-negative, motile, strictly anaerobic, slightly curved rods (3.0 by 0.5 μm). Motility achieved by a pair of flagella located along the side of the organism. Extremely halophilic, growing between 20 % and 35 % salt with an optimum at salt saturation. Alkaliphilic. A limited number of organic substrates support growth, including a few sugars and organic acids but not fatty acids or amino acids. Fermentative growth or

Table 12.4

Comparison of selected characteristics of members of the genus *Halanaerobacter*

Character	*H. chitinivorans*[a]	*H. lacunarum*[b,c]	*H. salinarius*[d]	*H. jeridensis*[e]
Earlier name	*Haloanaerobacter chitinovorans*	*Halobacteroides lacunaris*		
Type strain	OCG 229	DSM 6640	DSM 12146	DSM 23230
Cell size	0.5 × 1.4–8 μm	0.5–0.6 × 0.7–1 μm	0.3–0.4 × 5–8 μm	1.2 × 2.5–6 μm
Morphology	Flexible rods	Slightly curved rods	Flexible rods	Rods
Motility	+, peritrichous flagella	+, peritrichous flagella	+, peritrichous flagella	+
Endospores	–	–	–	–
Spheroplasts	+	+	+	–
Gas vesicles	NR	NR	NR	–
NaCl range	3–30 %	10–30 %	5–30 %	6–30 %
NaCl optimum	12–18 %	15–18 %	14–15 %	15 %
pH range	NR	6.0–8.0	5.5–8.5	6–9.6
pH optimum	7.0	6.5–7.0	7.4–7.8	8.3
Temperature range	23–50 °C	25–52 °C	10–50 °C	30–60 °C
Optimum temperature	30–45 °C	35–40 °C	45 °C	45 °C
Doubling time	2.5 h	2.9 h	2.3 h	NR
Carbohydrates utilized	+	+	+	+
End products of fermentation	Acetate, isobutyrate, H_2, CO_2; trimethylamine from glycine betaine in the Stickland reaction	Acetate, ethanol, H_2, CO_2	Acetate, ethanol, propionate, formate, H_2, CO_2; trimethylamine from glycine betaine in the Stickland reaction	Lactate, ethanol, acetate, H_2, CO_2
Major fatty acids	16:0 16:1	16:0 16:1	NR	$16:1_{cis9}$ 16:0
G+C content of DNA (mol%)	34.8	32.4	31.6	33.3
Sample source and site	Sediment, saltern pond, California, USA	Silt, Lake Chokrak, Kerch Peninsula	Sediment, saltern pond, France	Sediment, Chott el Djerid, Tunisia

Data taken from:
[a]Liaw and Mah (1992)
[b]Zhilina et al. (1992a)
[c]Rainey et al. (1995)
[d]Mouné et al. (1999)
[e]Mezghani et al. (2012)
NR not reported

microaerophilic growth not observed. Growth is by dissimilatory (respiratory) reduction of arsenate to arsenite, Fe(III) to Fe(II), and elemental sulfur to sulfide. Chemoautotrophic growth occurs with sulfide as the electron donor and arsenate as the electron acceptor. Catalase positive.

Type Species: *Halarsenatibacter silvermanii*

The main features of members of the monospecific genera *Halocella*, *Halothermothrix*, and *Halarsenatibacter*, updated for March 2012, are listed in *Table 12.2*.

Family *Halobacteroidaceae*

Zhilina and Rainey 1995, 879^{VP} (Validation List no. 55) (Effective publication: Rainey, Zhilina, Boulygina, Stackebrandt, Tourova and Zavarzin 1995, 193).

Ha.lo.bac.te.ro.i.da.ce'ae. N.L. masc. n. *Halobacteroides*, type genus of the family; suff. *–aceae*, ending to denote a family; N.L. fem. pl. n. *Halobacteroidaceae*, the *Halobacteroides* family.

Table 12.5
Comparison of selected characteristics of members of the genus *Orenia*

Character	*O. marismortui*[a,b]	*O. salinaria*[c]	*O. sivashensis*[d]
Basonym	*Sporohalobacter marismortui*		
Type strain	ATCC 35420	ATCC 700911	DSM 12596
Cell size	0.6 × 3–13 μm	1 × 6–10 μm	0.5–0.75 × 2.5–10 μm
Morphology	Rods	Rods	Flexible rods
Motility	+, peritrichous flagella	+, peritrichous flagella	+, peritrichous flagella
Endospores	+	+	+
Spheroplasts	+	+	+
Gas vesicles	–	–	+
NaCl range	3–18 %	2–25 %	5–25 %
NaCl optimum	3–12 %	5–10 %	7–10 %
pH range	NR	5.5–8.5	5.5–7.8
pH optimum	NR	7.2–7.4	6.3–6.6
Temperature range	25–50 °C	10–50 °C	Up to 50 °C
Optimum temperature	36–45 °C	40–45 °C	40–45 °C
Doubling time	40 min	NR	3.5 h
Carbohydrates utilized	+	+	+
End products of fermentation	Acetate, ethanol, butyrate, formate, H_2, CO_2	Acetate, ethanol, formate, lactate, H_2, CO_2	Acetate, ethanol, formate, butyrate, H_2, CO_2
Major fatty acids	14:0 16:0 16:1 18:0	NR	NR
G+C content of DNA (mol%)	29.6	33.7	28.6
Sample source and site	Sediment, Dead Sea	Sediment, saltern pond, France	Cyanobacterial mat, hypersaline lagoon, Lake Sivash, Crimea

Data taken from:
[a]Oren et al. (1987)
[b]Rainey et al. (1995)
[c]Mouné et al. (2000)
[d]Zhilina et al. (1999)
NR not reported

Cells are rod-shaped and stain Gram-negative. Endospores produced by some species. Strictly anaerobic. Oxidase and generally catalase negative. Most species ferment carbohydrates to products including acetate, ethanol, H_2, and CO_2. Some species may grow fermentatively on amino acids; others have a homoacetogenic metabolism or grow by anaerobic respiration while reducing nitrate, trimethylamine *N*-oxide, selenate, or arsenate or chemolithoautotrophically on H_2 and elemental sulfur. Moderately halophilic. NaCl concentrations between 1.7 and 2.5 M are required for optimal growth, and no growth is observed below 0.3–1.7 M NaCl, depending on the species.

Type Genus: *Halobacteroides*

Genus *Halobacteroides*

Oren, Weisburg, Kessel and Woese 1984, 355[VP] (Effective publication: Oren, Weisburg, Kessel and Woese 1984, 68).

Ha.lo.bac.te.ro'i.des. Greek n. *hals halos*, salt; N.L. masc. n. *bacter*, a staff or rod; L. suff. –oides (from Gr. suff. *eides*, from Gr. n. *eidos*, that which is seen, form, shape, figure; N.L. masc. n. *Halobacteroides*, rod-like salt organism.

Cells are long, thin, often flexible rods and motile by peritrichous flagella, staining Gram-negative. Endospores may be formed. Strictly anaerobic, chemoorganotrophic with fermentative metabolism. Carbohydrates are fermented with production of acetate, ethanol, H_2, and CO_2. Halophilic, growing optimally at NaCl concentrations around 1.7–2.6 M and requiring a minimum of 1.2–1.7 M NaCl for growth.

Type Species: *Halobacteroides halobius*

The main features of members of the genus *Halobacteroides*, updated for March 2012, are listed in Table 12.3.

Genus *Halanaerobacter*

Liaw and Mah 1996, 362[VP] (Validation List no. 56), Emend. Rainey, Zhilina, Boulygina, Stackebrandt, Tourova and Zavarzin 1995, 197; Emend. Mouné, Manac'h, Hirschler, Caumette, Willison and Matheron 1999, 109 (Effective publication: Liaw and Mah 1992, 265).

Table 12.6
Comparison of selected characteristics of members of the genus *Natroniella*

Character	*N. acetigena*[a]	*N. sulfidigena*[b]
Type strain	DSM 9952	DSM 22104
Cell size	1–1.2 × 6–15 μm	0.3–0.5 × 3–30 μm
Morphology	Rods	Flexible rods
Motility	+, peritrichous flagella	+, peritrichous flagella
Endospores	+	–
Spheroplasts	+	+
Gas vesicles	–	–
NaCl range	10–26 %	1.4–4 M Na^+
NaCl optimum	12–15 %	3 M Na^+
pH range	8.1–10.7	8.1–10.6
pH optimum	9.7–10.0	10.0
Temperature range	28–42 °C	Up to 41 °C
Optimum temperature	37 °C	35 °C
Carbohydrates utilized	–	+
End products of fermentation	Acetate	No fermentative growth observed; chemolithoautotroph or acetate-dependent sulfur respiration
Major fatty acids	14:0 16:1	14:0 16:0 16:1 $16:1_{ald}$
G+C content of DNA (mol%)	31.9	31.3–32.0
Sample source and site	Sediment, Lake Magadi, Kenya	Sediment, soda lakes, Wadi Natrun, Egypt, and Kulunda Steppe, Russia

Data taken from
[a]Zhilina et al. (1996)
[b]Sorokin et al. (2011)
NR not reported

Hal.an.ae.ro.bac'ter. Gr. n. *hals halos*, salt; Gr. pref. *an*, not; Gr. n. *aer aeros*, air; N.L. masc. n. *bacter*, rod; N.L. masc. n. *Halanaerobacter*, salt rod which grows in the absence of air.

Cells are rod-shaped or slightly curved, flexible, and motile by means of peritrichous flagella. Gram-stain-negative. Strictly anaerobic. Chemoorganotrophic with fermentative metabolism; some strains can utilize amino acids in the Stickland reaction or with hydrogen as electron donor. Carbohydrates are fermented with production of acetate, H_2, and CO_2. In some species, ethanol, propionate, formate, and isobutyrate are also formed. Elemental sulfur can be used as electron acceptor in certain species. Halophilic; optimal growth occurs at NaCl concentrations around 2.0–3.0 M. Cells require a minimum of 0.5–1.6 M NaCl for growth. Neutral to slightly alkaline pH values required for optimal growth. Mesophilic to slightly thermotolerant. Endospores not observed. Short degenerate cells and spheroplasts occur in stationary phase.

Type Species: *Halanaerobacter chitinivorans*

The main features of members of the genus *Halanaerobacter*, updated for March 2012, are listed in Table 12.4.

Genus *Orenia*

Rainey, Zhilina, Boulygina, Stackebrandt, Tourova and Zavarzin 1995, 880[VP] (Validation List no. 55) (Effective publication: Rainey, Zhilina, Boulygina, Stackebrandt, Tourova and Zavarzin 1995, 197).

O.re'ni.a. N.L. fem. n. *Orenia*, named after Aharon Oren, an Israeli microbiologist.

Rods, 2.5–13 μm in length with rounded ends. Gram-stain-negative. Motile by peritrichous flagella. Spores are round, terminal, or subterminal. Gas vesicles detected in some species. Forms spheroplasts. Strictly anaerobic. Halophilic; optimum NaCl concentration for growth 3–12 %; no growth below 2 % or above 25 %. Mesophilic to slightly thermophilic. Chemoorganotrophic. End products of glucose fermentation include H_2, CO_2, lactate, acetate, butyrate and ethanol.

Type Species: *Orenia marismortui*

The main features of members of the genus *Orenia*, updated for March 2012, are listed in Table 12.5.

Genus *Natroniella*

Zhilina, Zavarzin, Detkova and Rainey 1996, 1189[VP] (Validation List no. 59); Emend. Sorokin, Detkova and Muyzer 2011, 94 (Effective publication: Zhilina, Zavarzin, Detkova and Rainey 1996b, 324).

Na.tro.ni.el'la. N.L. n. *natron* (arbitrarily derived from the Arabic n. *natrun* or *natron*) soda, sodium carbonate; N.L. fem. n. *Natroniella*, organism growing in soda deposits.

Flexible rods, motile by peritrichous flagella. Spores may be formed. Cell wall has Gram-negative structure. Strictly anaerobic. Possesses a respiratory type of homoacetogenic metabolism. Extremely alkaliphilic, developing in soda brines at pH 9–10. Halophilic, growing at 1.7–4.4 M NaCl. Obligately dependent on Na^+, Cl^-, and CO_3^{2-} ions. Mesophilic. Chemoorganotrophic: some organic acids, amino acids, and alcohols are fermented. Acetate is the product of fermentation. Some representatives have obligate sulfur-dependent respiratory metabolism and are able to grow autotrophically or with acetate as an electron donor with sulfur serving as an electron acceptor.

Type Species: *Natroniella acetigena*

The main features of members of the genus *Natroniella*, updated for March 2012, are listed in Table 12.6.

Genus *Acetohalobium*

Zhilina and Zavarzin 1990, 470[VP] (Validation List no. 35) (Effective publication: Zhilina and Zavarzin 1990b, 747).

A.ce.to.ha.lo'bi.um. L. n. *acetum*, vinegar; Gr. n. *hals halos*, salt; Gr. n. *bios*, life; N.L. neut. n. *Acetohalobium*, acetate-producing organism living in salt.

Table 12.7

Comparison of selected characteristics of members of the monospecific genera *Acetohalobium*, *Sporohalobacter*, *Fuchsiella*, *Halarsenatibacter*, *Halanaerobaculum*, *Halonatronum*, *Selenihalanaerobacter*, and *Halanaerocella* (family *Halobacteroidaceae*)

Character	*Acetohalobium arabaticum*[a]	*Sporohalobacter lortetii*[b,c]	*Fuchsiella alkaliacetigena*[d]	*Halanaerobaculum tunisiense*[e]
Basonym		*Clostridium lortetii*	–	
Type strain	DSM 5501	ATCC 35059	VKM B-2667	DSM 19997
Cell size	0.7–1 × 2–5 μm	0.5–0.6 × 2.5–10 μm	0.2–0.5 × 10–30 μm	0.7–1 × 4–13 μm
Morphology	Curved rods	Rods	Flexible rods	Rods
Motility	+, 1–2 subterminal flagella	+, peritrichous flagella	+, peritrichous flagella	–
Endospores	Rare	+	+	–
Spheroplasts	NR	–	+	–
Gas vesicles	NR	+	–	–
NaCl range	10–25 %	4–15 %	0–14 %	14–30 %
NaCl optimum	15–18 %	8–9 %	7–8.5 %	20–22 %
pH range	5.8–8.4	NR	8.5–10.7	5.9–8.4
pH optimum	7.4–8.0	NR	8.8–9.3	7.2–7.4
Temperature range	NR–47 °C	25–52 °C	25–45 °C	30–50 °C
Optimum temperature	38–40 °C	37–45 °C	40 °C	42 °C
Doubling time	NR	8 h	85 h	2.1 h
Carbohydrates utilized	–	Weak	Only uses lactate, pyruvate, glutamate, ethanol, and propanol	+
End products of fermentation	Acetate	Acetate, propionate, isobutyrate, isovalerate, H_2, CO_2	Acetate (from H_2 + CO_2)	Acetate, lactate, butyrate, H_2, CO_2
Major fatty acids	16:0 16:1	16:0 16:1	14:0 $15:0_{anteiso}$ 16:0	16:1 16:0 14:0 $12:0_{3\text{-}OH}$ 10:0
G+C content of DNA (mol%)	33.6	31.5	32.0	34.3
Sample source and site	Sediment, Lake Sivash, Crimea	Sediment, Dead Sea	Sediment, soda lake, Altai, Russia	Sediment, Chott el Djerid, Tunisia

Character	*Halonatronum saccharophilum*[f]	*Selenihalanaerobacter shriftii*[g]	*Halanaerocella petrolearia*[h,i]
Type strain	DSM 13868	ATCC BAA-73	DSM 22693
Cell size	0.4–0.6 × 3.5–10 μm	0.6 × 2–6 μm	0.8–1.2 × 8–15 μm
Morphology	Flexible rods	Rods	Flexible rods
Motility	+, peritrichous flagella	–	–
Endospores	+	–	–
Spheroplasts	+	–	+
Gas vesicles		–	–
NaCl range	3–17 %	10–24 %	6–26 %
NaCl optimum	7–12 %	21 %	15 %
pH range	7.7–10.3	6–8.5	6.2–8.8
pH optimum	8–8.5	7.2	7.3
Temperature range	18–60 °C	16–42 °C	25–47 °C
Optimum temperature	36–55 °C	38 °C	40–45 °C
Doubling time	2.5 h	4.3 h (nitrate); 8.9 h (selenate)	3.5 h
Carbohydrates utilized	+	+	+

Table 12.7 (continued)

Character	*Halonatronum saccharophilum*[f]	*Selenihalanaerobacter shriftii*[g]	*Halanaerocella petrolearia*[h,i]
End products of fermentation	Acetate, ethanol, formate, H_2, CO_2	Acetate, CO_2 (with reduction of selenate or nitrate)	Acetate, ethanol, formate, lactate, H_2, CO_2
Major fatty acids	NR	NR	16:1 16:0 14:0
G+C content of DNA (mol%)	34.4	31.2	32.7
Sample source and site	Sediment, Lake Magadi, Kenya	Sediment, Dead Sea	Hypersaline oil reservoir, Gabon

Data taken from
[a]Zhilina and Zavarzin (1990b)
[b]Oren (1983)
[c]Oren et al. (1987)
[d]Zhilina et al. (2012)
[e]Hedi et al. (2009)
[f]Zhilina et al. (2001)
[g]Switzer Blum et al. (2001)
[h]Gales et al. (2011)
[i]Name not yet validly published
NR not reported

Rod-shaped cells. Motile with 1–2 subterminal flagella. Multiplication by binary fission is by constriction rather than septation. Gram-negative wall structure. Thermoresistant endospores formed by some strains. Strictly anaerobic. Possess a respiratory type of homoacetogenic metabolism. Extremely halophilic, growing at 1.7–4 M NaCl. Neutrophilic. Mesophilic-Metabolism variable; lithoheterotrophic, utilizing H_2, formate, and carbon monoxide; methylotrophic, utilizing methylamines and betaine; or chemoorganotrophic, fermenting some amino acids and organic acids. Acetate is the end product with all substrates utilized.

Type Species: *Acetohalobium arabaticum*

Genus *Sporohalobacter*

Oren, Pohla and Stackebrandt 1988, 136^{VP} (Validation List no. 24) (Effective publication: Oren, Pohla and Stackebrandt 1987, 239).

Spo.ro.ha.lo.bac'ter. Gr. n. *spora*, seed; Greek n. *hals halos*, salt; N.L. n. *bacter* a staff or rod; N.L. masc. n. *Sporohalobacter* spore-producing salt rod.

Gram-negative rod-shaped cells, motile by peritrichous flagella. Halophilic, growing optimally at 1.4–1.5 M NaCl and requiring minimum 0.7 M NaCl for growth. Temperature optimum about 40 °C. Strictly anaerobic. Ferments amino acids with production of acetate, propionate and other acids, H_2, and CO_2. Sugars poorly used. Endospores produced. Gas vesicles are attached to the endospores in the single species described.

Type Species: *Sporohalobacter lortetii*

Genus *Fuchsiella*

Zhilina, Zavarzina, Panteleeva, Osipov, Kostrikina, Tourova and Zavarzin 2012, 1671^{VP}.

Fuch.si.el'la. N.L. gem. dim. n. *Fuchsiella*, named in the honor of Prof. Georg Fuchs (Freiburg, Germany), who made a most serious contribution to our understanding of multiple pathways of CO_2 assimilation by microorganisms.

Gram-negative, spore-forming rods, motile by peritrichous flagella. Obligatory anaerobic. Obligately alkaliphilic and natronophilic. Performing homoacetogenic metabolism of a restricted number of compounds. Able to grow chemolithoautotrophically with H_2 + CO_2. Few organic compounds are metabolized with external electron acceptors.

Type Species: *Fuchsiella alkaliacetigena*

Genus *Halanaerobaculum*

Hedi, Fardeau, Sadfi, Boudabous, Ollivier and Cayol 2009, 923^{VP} (Effective publication: Hedi, Fardeau, Sadfi, Boudabous, Ollivier and Cayol 2009, 317).

Hal.an.ae.ro.ba'cu.lum. Gr. n. *hals halos*, salt; Gr. pref. *an-*, not; Gr. n. *aer aeros*, air; L. neut. n. *baculum*, stick; N.L. neut. n. *Halanaerobaculum*, salt stick not living in air.

Cells are Gram-negative, nonmotile, non-sporulating rods appearing singly, in pairs, or occasionally as long chains, halophilic, obligate anaerobes. Metabolize only carbohydrates. Grow at NaCl concentrations ranging from 14 to 30 %. The end products from glucose fermentation are butyrate, lactate, acetate, H_2, and CO_2.

Type Species: *Halanaerobaculum tunisiense*

Genus *Halonatronum*

Zhilina, Garnova, Tourova, Kostrikina and Zavarzin 2001, 263^{VP} (Validation List no. 79) (Effective publication: Zhilina, Garnova, Tourova, Kostrikina and Zavarzin 2001a, 70).

Ha.lo.na.tro'num. Gr. n. *hals halos* salt; N.L. n. *natron* (arbitrarily derived from the Arabic n. *natrun* or *natron*) soda,

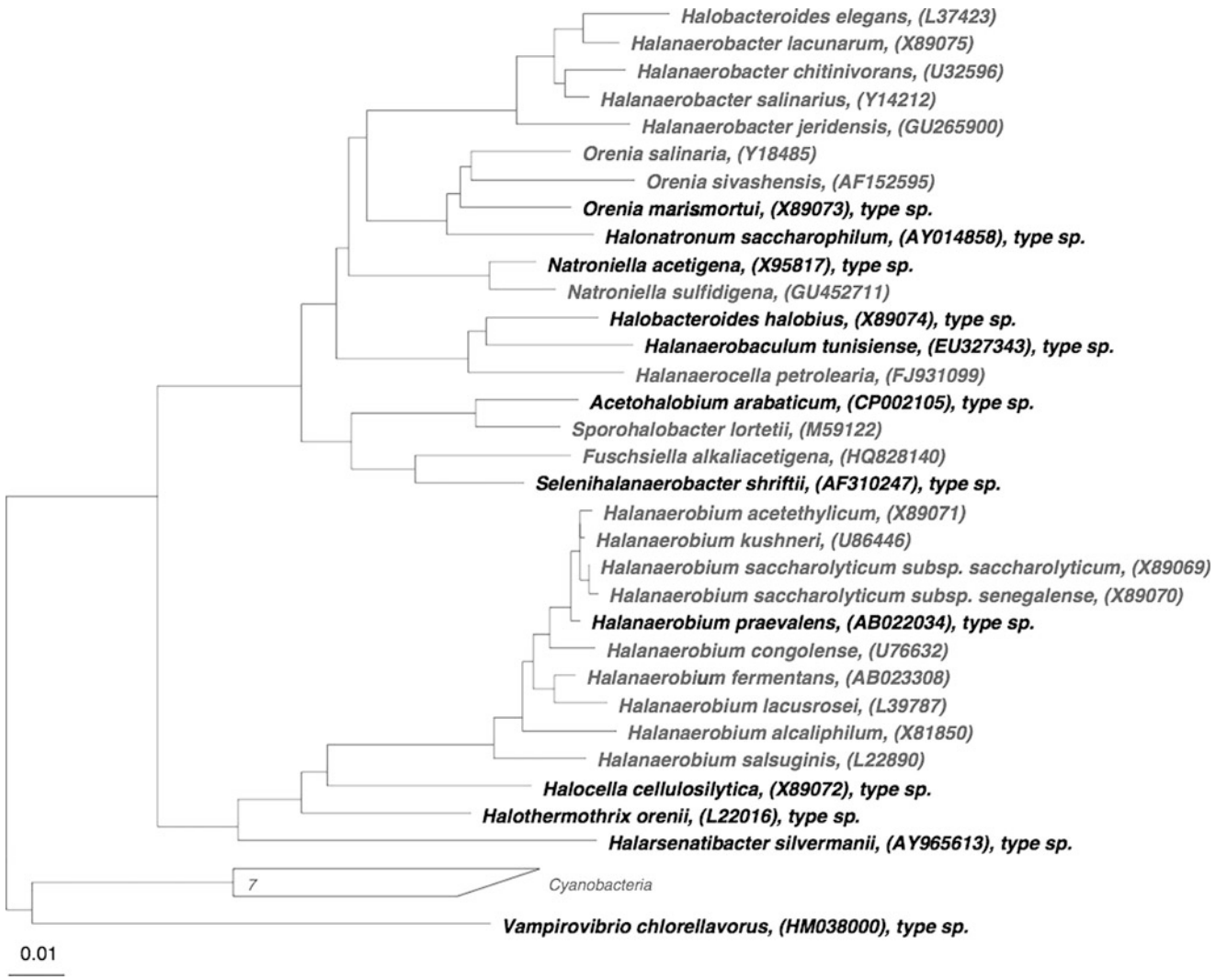

Fig. 12.3

Neighbor-joining genealogy reconstruction of the 31 species and subspecies of the order *Halanaerobiales* present in the LTP_106 (Yarza et al. 2010). The tree was reconstructed by using a subset of sequences 767 type strains of Bacteria and Archaea to stabilize the tree topology. In addition, a 40 % conservational filter for the whole bacterial domain was used to remove hypervariable positions. Numbers in triangles denote number of taxa included. The bar indicates 1 % sequence divergence

sodium carbonate; N.L. neut. n. *Halonatronum*, an organism growing with salt and soda.

Cells are rod-shaped, flexible, and motile by peritrichous flagella. The cell wall has a Gram-negative structure. Strictly anaerobic, chemoorganotrophic with fermentative metabolism. Carbohydrates, including soluble polysaccharides, are fermented to acetate, ethanol, formate, H_2, and CO_2. Halophilic and alkaliphilic. Endospores produced.

Type Species: *Halonatronum saccharophilum*

Genus *Selenihalanaerobacter*

Switzer Blum, Stolz, Oren and Oremland 2001, 1229^{VP} (Validation List no. 81) (Effective publication: Switzer Blum, Stolz, Oren and Oremland 2001, 217).

Se.le.ni.hal.an.ae.ro.bac'ter. N.L. n. *selenium* (from Gr. n. *selênê*, the moon), selenium, element 34; Gr. n. *hals halos*, salt; Gr. pref. *an*, not; Gr. n. *aer aeros*, air; N.L. masc. n. *bacter*, a staff or rod; N.L. masc. n. *Selenihalanaerobacter*, the salty anaerobic selenium rod.

Gram-negative rod-shaped cells, nonmotile. Halophilic, growing optimally at 3.6 M NaCl and requiring minimum 1.7 M NaCl for growth. Temperature optimum about 38 °C. Strictly anaerobic. Grows by anaerobic respiration on organic electron donors, using selenate and other electron acceptors. Fermentative growth not observed. Endospores not produced.

Type Species: *Selenihalanaerobacter shriftii*

Genus *Halanaerocella*

(Effective publication: Gales, Chehider, Joulian, Battaglia-Brunet, Cayol, Postec, Borgomano, Neria-Gonzalez, Lomans, Ollivier and Alazard 2011, 570; the name is yet to be validated).

Table 12.8

Properties of the sequenced genomes of members of the *Halanaerobiales*

Property	*Halanaerobium praevalens* DSM 2228[Ta]	*"Halanaerobium hydrogenoformans"*[b]	*Halothermothrix orenii* DSM 9562[Tc]
Accession number	CP002175	CP002304	CP001098
Genome length (bp)	2,309,262	2,613,116	2,578,146
G+C content	30.3 mol%	33.1 mol%	37.9 mol%
Extrachromosomal elements	0	0	0
% Coding bases	89.2 %	NR	88.6 %
Number of predicted genes	2,180	NR	2,451
Predicted protein-coding genes	2,110	2,295	2,366
% of proteins with putative function	77.7 %	NR	80.6 %
% assigned to COGs	80.7 %	NR	76.6 %
Number of 16S rRNA genes	4	4	4

Data taken from
[a]Ivanova et al. (2011), [b]Brown et al. (2011), [c]Mavromatis et al. (2009), *NR* not reported

Hal.an.ae.ro.cel'la. Gr. h. *hals halos*, salt; Gr. pref. *an*, not; Gr. n. *aer*, air; L. fem. n. *cella*, a store-room and in biology a cell; N.L. fem. n. *Halanaerocella*, salt cell not living in air.

Cells stain Gram-positive, nonmotile, non-sporulating rods occurring singly, in pairs, or occasionally as long chains. Obligate anaerobe metabolizing only carbohydrates. The end products from glucose fermentation are lactate, ethanol, acetate, formate, H_2, and CO_2.

The Type Species is *Halanaerocella petrolearia*.

The main features of members of the monospecific genera *Acetohalobium*, *Halanaerobacter*, *Sporohalobacter*, *Fuchsiella*, *Halanaerobaculum*, *Halonatronum*, *Selenihalanaerobacter*, and *Halanaerocella* (updated for March 2012), are listed in *Table 12.7*.

Isolation, Enrichment, and Maintenance Procedures

Any anoxic reducing medium containing high salt concentrations (5–25 %) and containing a suitable carbon source is a potential enrichment and growth medium for members of the *Halanaerobiales*. A variety of such media have been used for isolation and cultivation. *Table 12.11* presents a selection. Most species grow as fermenters on simple sugars. Although most species are not extremely sensitive to molecular oxygen, strict anaerobic techniques should be used, including boiling the media under nitrogen or nitrogen-CO_2 (80:20) and adding reducing agents such as cysteine, dithionite, or ascorbate + thioglycollate to the boiled media. Protocols for the preparation of media were compiled by Oren (2006); details can be found in the original species descriptions. For the enrichment of thermophiles such as *Halothermothrix*, the incubation temperature should be adjusted to that of the natural environment. More specialized media have been designed for the cultivation of amino acid fermenting, homoacetogenic, selenate- and arsenate-respiring members, and other atypical organisms belonging to the order. For the isolation of *Selenihalanaerobacter*, selenate is the preferred electron acceptor, because nitrate and trimethylamine *N*-oxide also enable anaerobic growth of a variety of facultative anaerobes belonging to other orders.

The formation of heat-resistant endospores has been exploited in a selective enrichment procedure for *Halobacteroides halobius*-like bacteria, based on negative selection by pasteurization of the inoculum for 10–20 min at 80–100 °C (Oren 1987). In view of the number of endospore-forming genera within the family (*Halobacteroides*, *Orenia*, *Sporohalobacter*, *Acetohalobium*, *Natroniella*), such an enrichment strategy could be useful for the isolation of other novel members.

Maintenance

Many species of *Halobacteroidaceae*, notably the species of the genera *Halobacteroides* (Oren et al. 1984b; Zhilina et al. 1997), *Orenia* (Oren et al. 1987; Mouné et al. 2000; Zhilina et al. 1999), *Haloanaerobacter* (Liaw and Mah 1992; Zhilina et al. 1992; Mouné et al. 1999), *Halonatronum* (Zhilina et al. 2001), and *Natroniella* (Zhilina et al. 1996), easily undergo autolysis, generating spherical degeneration forms (*Fig. 12.1 c, d*). Lysis starts at the end of the exponential growth phase, especially at relatively high growth temperatures. One possibility to avoid death of such cultures is the use of media with a reduced nutrient content and lower growth temperatures (15–25 °C). Weekly transfers may then suffice to maintain viable cultures. Long-term preservation is by freezing anaerobic suspensions in 20 % glycerol at −80 °C (Rengpipat et al. 1988a), by lyophilization, or by storage in liquid nitrogen.

Table 12.9
Substrates used by the type strains of carbohydrate-fermenting species of *Halanaerobiaceae*

Substrate	*Halanaerobium praevalens*	*Halanaerobium alcaliphilum*	*Halanaerobium acetethylicum*	*Halanaerobium salsuginis*	*Halanaerobium saccharolyticum* subsp. *saccharolyticum*	*Halanaerobium saccharolyticum* subsp. *senegalense*	*Halanaerobium congolense*	*Halanaerobium lacusrosei*	*Halanaerobium kushneri*	*Halanaerobium fermentans*	*Halothermothrix orenii*	*Halocella cellulosilytica*
L-Arabinose	NR	–	NR	+	+	–	–	–	+	–	+	–
Cellobiose	–	–	+	–	+	+	NR	+	+	+	+	+
Chitin	–	NR	–	–	–	NR	NR	NR	–	NR	NR	NR
Erythritol	NR	–	NR	NR	+	NR	NR	NR	NR	NR	NR	NR
Fructose	+	+	+	+	+	+	+	+	+	+	+	–
Galactose	–	–	–	+	+	–	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+
N-acetyl- glucosamine	+	+	+	+	+	NR	NR	NR	NR	+	NR	NR
Glycerol	–	–	–	–	+	+	NR	+	+	-	–	NR
Lactose	–	–	+	+	+	+	–	+	–	+	–	NR
Maltose	NR	+		+	+	+	+	+	+	+	–	NR
Mannitol	NR	–	NR	–	+	+	NR	+	+	NR	–	NR
D-Mannose	+	+	+	+	+	+	+	+	+	+	+	+
Melibiose	NR	NR	NR	NR	+	NR	NR	–	NR	NR	+	NR
Pectin	+	–	–	–	–	–	NR	NR	–	–	NR	NR
Pyruvate	–	+	+	+	+	NR	NR	NR	+	–	NR	NR
Raffinose	NR	Slight	NR	+	–	+	NR	NR	NR	+	NR	NR
Rhamnose	NR	–	NR	+	–	–	–	–	NR	–	–	NR
D-Ribose	NR	–	NR	+	+	+	+	+	NR	+	+	–
L-Sorbose	–	NR	–	+	–	–	NR	NR	–	–	–	NR
Starch	–	–	–	–	–	NR	NR	+	–	–	+	+
Sucrose	–	+	+	+	+	+	+	+	+	+	–	+
Trehalose	NR	–	NR	+	+	NR	+	NR	NR	NR	NR	NR
D-Xylose	–	–	+	+	+	–	–	+	–	–	+	–

NR not reported

Table 12.10

Substrates used by the type strains of carbohydrate-fermenting species of *Halobacteroidaceae*

Substrate	*Halobacteroides halobius*	*Halobacteroides elegans*	*Halanaerobacter chitinivorans*	*Halanaerobacter lacunarum*	*Haloanaerobacter salinarius*	*Halanaerobacter jeridensis*	*Orenia marismortui*	*Orenia salinaria*	*Orenia sivashensis*	*Halanaerobaculumtunisiense*	*Halonatronum saccharophilum*	*Halanaerocella petrolearia*
L-Arabinose	–	NR	NR	–	–	–	–	NR	–	NR	NR	NR
Cellobiose	–	Weak	+	Weak	NR	v	–	+	+	+	–	+
Chitin	NR	–	+	–	–	NR	NR	NR	–	NR	NR	NR
Erythritol	NR	NR	NR	–	NR	NR	NR	NR	–	NR	NR	NR
Fructose	+	+	+	+	+	+	+	+	–	–	+	+
Galactose	+	Weak	NR	NR	+	+	–	–	–	+	NR	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+
N-acetyl-glucosamine	NR	–	+	–	+	NR	–	–	+	NR	+	NR
Glycerol	–	NR	–	–	NR	+	–	NR	–	NR	NR	–
Lactose	–	NR	NR	–	–	–	–	NR	–	NR	NR	+
Maltose	+	Weak	+	+	+	+	+	+	+	+	+	+
Mannitol	–	Weak	NR	+	+	+	NR	+	+	–	–	+
D-Mannose	+	+	+	+	+	+	+	–	+	+	–	+
Melibiose	–	NR	NR	–	NR	NR	NR	NR	–	NR	NR	NR
Pectin	NR	NR	–	–	–	NR	NR	NR	–	NR	NR	NR
Pyruvate	+	Weak	–	Weak	–	+	–	–	+	+	–	+
Raffinose	+	–	–	–	+	+	–	–	–	NR	NR	–
Rhamnose	+	NR	NR	–	–	–	–	NR	–	–	NR	–
D-Ribose	–	–	NR	Weak	NR	+	+	NR	+	–	–	–
L-Sorbose	–	NR	NR	NR	–	–	NR	NR	–	+	NR	–
Starch	+	+	Weak	+	–	+	+	–	+	+	+	NR
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	NR	+	NR	+	+	+	NR	+	+	NR	–	NR
D-Xylose	–	NR	NR	–	–	+	–	NR	–	–	NR	+

NR not reported

Ecology

Species belonging to the *Halanaerobiales* can probably be found in any hypersaline anaerobic environment where simple sugars are available or other substrates metabolized by the members of the order. Representatives have been isolated from Great Salt Lake, Utah (Tsai et al. 1995; Zeikus et al. 1983); Salton Sea, California (Shiba 1991; Shiba and Horikoshi 1988; Shiba et al. 1989); Searles Lake, California (Switzer Blum et al. 2009), the Dead Sea (Oren 1983; Oren et al. 1984b, 1987; Switzer Blum et al. 2001); and a hypersaline sulfur spring on the shore of the Dead Sea (Oren 1989); from the alkaline (pH 10.2) hypersaline lake in Magadi, Kenya—shown to harbor a varied anaerobic community, including cellulolytic, proteolytic, saccharolytic, and homoacetogenic bacteria (Shiba and Horikoshi 1988; Zhilina and Zavarzin 1994; Zhilina et al. 1996, 2001)—Big Soda Lake, Nevada (Shiba and Horikoshi 1988; Shiba et al. 1989), soda lakes in Russia (Sorokin et al. 2011; Zhilina et al. 2012), and hypersaline lakes and lagoons in the Crimea (Simankova et al. 1993; Zhilina and Zavarzin 1990b; Zhilina et al. 1991, 1992b) and Senegal (Cayol et al. 1994a, 1995); and from the hypersaline lakes in Tunisia (Cayol et al. 1994b; Hedi et al. 2009; Mezghani et al. 2012) and saltern evaporation ponds in California (Liaw and Mah 1992) and France (Mouné et al. 1999, 2000). Brines associated with oil wells and petroleum reservoirs also yielded a number of interesting species (Bhupathiraju et al. 1991, 1993, 1994, 1999; Gales et al. 2011; Ravot et al. 1997; Rengpipat et al. 1988a). They may also be present in salted fermented foods (Kobayashi et al. 2000a, b). 16S rRNA sequences of yet uncultured organisms affiliated with the *Halanaerobiales* are often recovered in clone libraries prepared from DNA extracted from anaerobic hypersaline environments such as sediments of saltern evaporation ponds (Mouné et al. 2003; Sørensen et al. 2005) and also from anaerobic brines in the depths of the Red Sea (Eder et al. 2001).

The ability to use glycerol, glucosylglycerol, trehalose, cellulose, and chitin may be of particular ecological importance. The first three compounds are accumulated at high concentrations as organic osmotic solutes by aerobic photosynthetic halophilic microorganisms inhabiting salt lakes: glycerol in the green unicellular alga *Dunaliella* and glucosylglycerol and trehalose in a variety of cyanobacteria. Such compounds may then be available to the anaerobic bacterial community in the bottom sediments of these lakes. *Halanaerobium saccharolyticum* and *Halanaerobium lacusrosei* ferment glycerol (Cayol et al. 1994a, 1995; Zhilina et al. 1992). Glycerol oxidation by anaerobic halophiles may be markedly improved through interspecies hydrogen transfer when grown in coculture with H_2-consuming sulfate-reducing bacteria (Cayol et al. 1995). *Halanaerobium saccharolyticum* was isolated from a cyanobacterial mat dominated by *Coleofasciculus* (*Microcoleus*) *chthonoplastes*, covering the bottom of a hypersaline lagoon in the Crimea; its ability to use glucosylglycerol, the osmotic solute produced by the cyanobacteria, may be of great ecological importance (Zhilina and Zavarzin 1991). The same organism also degrades trehalose, produced by other cyanobacteria for similar purposes (Zhilina et al. 1992b).

The hypersaline lagoons of the Crimea also contain large masses of dead macroalgae (*Cladophora*). Such environments show high cellulolytic activity. The optimum salt concentration for cellulose decomposition was 15 %, and decomposition was possible up to 25 % salt (Siman'kova and Zavarzin 1992). The cellulose-degrading *Halocella cellulosilytica* was isolated from this habitat (Simankova et al. 1993), and its cellulase complex was characterized in part (Bolobova et al. 1992). Another biopolymer that may be available in large quantities in hypersaline lakes is chitin, derived from the brine shrimp *Artemia* and from larvae of the brine fly which are often abundant in such environments. Evolution of gas bubbles was observed from the sediment of a Californian saltern containing massive amounts of dead brine shrimp. Two strains of *Halanaerobacter chitinivorans* were isolated from this saltern, of which only one grew on chitin (Liaw and Mah 1992). Another substrate that may be available abundantly in hypersaline environments is glycine betaine. This compound is produced as an osmotic solute by the most halophilic among the cyanobacteria and by halophilic anoxygenic photosynthetic bacteria such as *Halorhodospira* species. Glycine betaine is fermented to acetate and trimethylamine by *Halanaerobium alcaliphilum* isolated from Great Salt Lake (Tsai et al. 1995), and by the (non-saccharolytic) *Acetohalobium arabaticum*. The latter species produces only minor amounts of trimethylamine as most is converted to acetate (Zhilina and Zavarzin 1990b). Glycine betaine can also be used as an electron acceptor in the Stickland reaction by *Halanaerobacter salinarius* and *Halanaerobacter chitinivorans*, with H_2 or serine as electron donor (Mouné et al. 1999).

Quantitative data on the occurrence of members of the *Halanaerobiales* in hypersaline anoxic environments are scarce. *Halanaerobium praevalens* was reported to be present in Great Salt Lake surface sediment in numbers of up to 10^8 per mL sediment (Zeikus 1983; Zeikus et al. 1983), while 10^3–10^5 *Halobacteroides* cells were counted per mL of Dead Sea sediment (Oren et al. 1984b). Up to 10^7–10^9 anaerobic halophilic cellulolytic bacteria were enumerated per mL sediment in lagoons of the Arabat strait (Siman'kova and Zavarzin 1992), and up to 4.6×10^3 anaerobic halophiles were counted in anaerobic brines associated with an oil reservoir in Oklahoma (Bhupathiraju et al. 1991, 1993). The few data available prove that these anaerobic halophiles may form a significant component of the ecosystem in anaerobic hypersaline sediments.

Pathogenicity, Clinical Relevance

All members of the *Halanaerobiales* are moderately halophilic and do not grow at low salt concentrations. Accordingly, no pathogens are found within the group.

Sensitivity to antibiotics was tested in some species. Within the family *Halanaerobiaceae*, *Halanaerobium salsuginis*, *H. kushneri*, and *H. lacusrosei* were reported to be sensitive to penicillin,

Table 12.11

Media for the growth of members of selected members of the *Halanaerobiales* (all values in g/L, unless stated otherwise). Additional information can be found in the original species description papers and in the website of the Deutsche Sammlung von Mikroorganismen und Zellkulturen: http://www.dsmz.de

Compound	*Halanaerobium species*	*Halanaerobium salsuginis*	*Halanaerobium acetethylicum*	*Halobacteroides* spp., *Halanaerobium saccharolyticum*	*Halothermothrix orenii*	*Halocella cellulosilytica*
NaCl	130	120	100	100–150	100	150
$MgSO_4 \cdot 7H_2O$	8.8	0.2				
$MgCl_2 \cdot 6H_2O$			0.4	0.33	2.0	3.3
KCl	1.0	0.1		0.33	4.0	0.33
NH_4Cl		1.0	0.9	0.33	1.0	0.33
$CaCl_2 \cdot 2H_2O$		0.2		0.33	0.2	0.33
KH_2PO_4		0.1	0.75	0.33	0.3	0.33
K_2HPO_4			1.5			
$FeSO_4 \cdot 7H_2O$			3 mg			
$NaHCO_3$				1.5[a]	5.0[a]	2.5
Na_2CO_3						
$Na_2S \cdot 9H_2O$	0.5[a]		1.0[a]	0.5[a]	0.2[a]	0.5[a]
Glucose	5.0[a]	2.5[a]	5.0[a]	5.0[a]	10[a]	
Chitin						
Microcrystalline cellulose or cellobiose						5.0 or 5.0[a]
Trimethylamine HCl or glycine betaine						
Na-acetate					1.0	
Ethanol						
Yeast extract	10	1.0	3.0[a]			2.0
Trypticase	10		10		0.5	
Peptone				5.0		
Casamino acids	1.0		1.0			
Nutrient broth						
L-Glutamic acid						
Vitamin solution[b]	10 mL[a]	10 mL	5 mL	10 mL		10 mL
Trace element solution	5 mL[c]	10 mL[c]	9 mL[d]	1 mL[e]	1 mL[c]	1 mL[e]
Thioglycolate-ascorbate solution[g]		25 mL[a]				
Cysteine HCl	0.5					
Na dithionite					10 mg[a]	
Resazurin	0.5 mg		1 mg	2 mg	1 mg[a]	2 mg
NaOH 2 N		10 mL[a]				
PIPES-di-K[h]	1.5					
Final pH[i]	7.1–7.3	9.0	7.2–7.4	7.5	7.0	7.0

Table 12.11 (continued)

	Halobacteroides halobius, Orenia marismortui	*Acetohalobium arabaticum*	*Halanaerobacter chitinivorans*	*Sporohalobacter lortetii*	*Natroniella acetigena*
NaCl	140	150	100	105	15.7
$MgSO_4 \cdot 7H_2O$			9.6		
$MgCl_2 \cdot 6H_2O$	20.3	4.0	7.0	10[c]	0.1
KCl	3.7	0.33	3.8	0.75	0.2
NH_4Cl	7.35	0.33	1.0		1.0
$CaCl_2 \cdot 2H_2O$		0.33	0.5	3.7	
KH_2PO_4		0.33	0.4		0.2
K_2HPO_4					
$FeSO_4 \cdot 7H_2O$				2 mg	
$NaHCO_3$	5.0[a]	4.5[a]	3.0[a]		38.3[a]
Na_2CO_3			1.0[a]		68.3
$Na_2S \cdot 9H_2O$		0.5[a]	0.5[a]		1.0[a]
Glucose			5.0[a] or		
Chitin			5.0		
Microcrystalline cellulose or cellobiose					
Trimethylamine HCl or glycine betaine		2.4[a] or 4.5[a]			
Na acetate					
Ethanol					5 mL[a]
Yeast extract	5.0	0.05[a]	1.0	2.0	0.2
Trypticase					
Peptone					
Casamino acids				2.0	
Nutrient broth				2.0	
L-Glutamic acid				4.0	
Vitamin solution[b]		10 mL[a]		10 mL	10 mL
Trace element solution		10 mL[c]	1 mL[c]	10 mL[c]	1 mL[f]
Thioglycolate-ascorbate solution[g]					
Cysteine HCl			0.5[c]	0.5[c]	
Na dithionite					
Resazurin	1 mg	1 mg	1 mg	1 mg	0.5 mg
PIPES-di-K[h]	40 mM				
Final pH[i]	6.5–7.0	7.6–8.0	7.2	6.5	9.7-10.0

[a]Add separately from sterile anoxic solutions

[b]Vitamin solution containing per liter: biotin, 2 mg; folic acid, 2 mg; pyridoxine HCl, 10 mg; thiamine.HCl·$2H_2O$, 5 mg; riboflavin, 5 mg; nicotinic acid, 5 mg; D-Ca pantothenate, 5 mg; vitamin B_{12}, 0.1 mg; *p*-aminobenzoic acid, 5 mg; lipoic acid, 5 mg

[c]Trace element solution containing per liter: nitrilotriacetic acid, 1.5 g; $MgSO_4 \cdot 7H_2O$, 3 g; $MnSO_4 \cdot 2H_2O$, 0.5 g; NaCl, 1 g; $FeSO_4 \cdot 7H_2O$, 100 mg; $CoSO_4 \cdot 7H_2O$, 180 mg; $CaCl_2 \cdot 2H_2O$, 100 mg; $ZnSO_4 \cdot 7H_2O$, 180 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $KAl(SO_4)_2 \cdot 12H_2O$, 20 mg; H_3BO_3, 10 mg; $Na_2MoO_4 \cdot 2H_2O$, 10 mg; $NiCl_2 \cdot 6H_2O$, 25 mg; $Na_2SeO_3 \cdot 5H_2O$, 0.3 mg. First dissolve the nitrilotriacetic acid and adjust to pH 6.5, then add the other minerals. Adjust the final pH to 7.0 with KOH

[d]Trace element solution containing per liter: nitrilotriacetic acid, 12.8 g; $FeCl_2 \cdot 4H_2O$, 200 mg; $MnCl_2 \cdot 4H_2O$, 100 mg; $CoCl_2 \cdot 6H_2O$, 170 mg; $CaCl_2 \cdot 2H_2O$, 100 mg; $ZnCl_2$, 100 mg; $CuCl_2$, 20 mg; H_3BO_3, 10 mg; $Na_2MoO_4 \cdot 2H_2O$, 10 mg; $NiCl_2 \cdot 6H_2O$, 26 mg; NaCl, 1 g; $Na_2SeO_3 \cdot 5H_2O$, 20 mg. First dissolve the nitrilotriacetic acid and adjust to pH 6.5 with KOH

[e]Trace element solution containing per liter: 25 % HCl, 10 mL; $FeCl_2 \cdot 4H_2O$, 1.5 g; $ZnCl_2$, 70 mg; $MnCl_2 \cdot 4H_2O$, 100 mg; H_3BO_3, 6 mg; $CoCl_2 \cdot 6H_2O$, 190 mg; $CuCl_2 \cdot 2H_2O$, 2 mg; $NiCl_2 \cdot 6H_2O$, 24 mg; $Na_2MoO_4 \cdot 2H_2O$, 36 mg; pH 6.0. First dissolve the $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts, and adjust the volume to 1 L

[f]Trace element solution containing per liter: Na_2EDTA, 5.2 g; $FeCl_2 \cdot 4H_2O$, 1.5 g; $ZnCl_2$, 70 mg; $MnCl_2 \cdot 4H_2O$, 100 mg; H_3BO_3, 6 mg; $CoCl_2 \cdot 6H_2O$, 190 mg; $CuCl_2 \cdot 2H_2O$, 2 mg; $NiCl_2 \cdot 6H_2O$, 24 mg; $Na_2MoO_4 \cdot 2H_2O$, 36 mg; pH 6.0

[g]0.5 g of Na-thioglycolate and 0.5 g of Na-ascorbate in 25 mL H_2O, sterilized by filtration

[h]PIPES = piperazine-*N*,*N*′-*bis*-ethane-sulfonic acid (sesquisodium salt or dipotassium salt have been used in different protocols)

[i]To be adjusted with sterile anoxic HCl, NaOH, or Na_2CO_3 (recommended for *Acetohalobium arabaticum*)

chloramphenicol, and tetracycline. An alkaliphilic member of the genus *H. alcaliphilum* resists low concentrations of antibiotics but is inhibited by 200 μg/mL penicillin, 400 μg/mL cycloserine, and 1,000 μg/mL streptomycin. *Halocella cellulosilytica* is inhibited by streptomycin, penicillin, vancomycin, rifampicin, and bacitracin; *Halarsenatibacter silvermanii* is sensitive to vancomycin, kanamycin, penicillin, and tetracycline.

Members of the *Halobacteroidaceae* tested for antibiotics sensitivity include *Halobacteroides halobius* (forming large spheres in the presence of penicillin, also sensitive to chloramphenicol and bacitracin), *Halanaerobacter chitinivorans* (inhibited by chloramphenicol, but not by 100 μg/mL cycloserine, penicillin, streptomycin, or tetracycline), *H. salinarius* (sensitive to chloramphenicol, erythromycin, kanamycin, and tetracycline), *Orenia marismortui* (sensitive to penicillin, bacitracin, novobiocin, erythromycin, polymyxin, and chloramphenicol, but not to streptomycin), *O. salinaria* (sensitive to chloramphenicol, erythromycin, and tetracycline but resistant to kanamycin), and *Fuchsiella alkaliacetigena* (sensitive to vancomycin, novobiocin, and rifampicin).

Application

Use in Food Fermentations

Halanaerobium fermentans was isolated from "fugunoko nukaduke," a traditional Japanese food prepared from fermented salted puffer fish ovaries. Puffer fish ovaries are salted for at least 6 months, and the ovaries are then fermented naturally with rice bran, fish sauce, and koji for several years. *H. fermentans* may be one of the main bacteria involved in the fermentation process (Kobayashi et al. 2000a). Halophilic anaerobes identified as *Halanaerobium praevalens* (based on 16S rRNA sequence and DNA-DNA hybridization) or *H. alcaliphilum*, producing acetate, butyrate, and propionate, were isolated from canned Swedish fermented herrings ("surströmming") (Kobayashi et al. 2000b). Members of the genus *Halanaerobium* may thus be involved in the manufacturing of traditional fermented food products.

Industrial Fermentation for Hydrogen and Acetate

The use of anaerobic halophilic bacteria in the industrial fermentation of complex organic matter and the production of organic solvents has been proposed (Lowe et al. 1993; Wise 1987), but any such applications are still in an experimental stage. Recently it was proposed to use *Halanaerobium saccharolyticum* subsp. *saccharolyticum* and subsp. *senegalense* for the industrial production of hydrogen from glycerol formed as by-product of the biodiesel industry. The highest H_2 yield (1.6 mol H_2/mol glycerol) was obtained with *H. saccharolyticum* subsp. *senegalense* grown at 15 % salt. *H. saccharolyticum* subsp. *saccharolyticum* produced less H_2 (0.6 mol/mol glycerol) but also yielded 1,3-propanediol (up to 0.49 mol/mol glycerol) as a valuable by-product (Kivistö et al. 2010). *Halocella cellulosilytica* is a cellulose degrader (Simankova et al. 1993), but its biotechnological potential for cellulose degradation at high salt concentrations has not yet been exploited.

Enhanced Oil Recovery

Several species of *Halanaerobium* (*H. salsuginis*, *H. acetethylicum*, *H. kushneri*, *H. congolense*) and *Halanaerocella petrolearia* were isolated from brines associated with oil reservoirs (Bhupathiraju et al. 1994, 1999; Gales et al. 2011; Ravot et al. 1997; Rengpipat et al. 1988a). Such bacteria may be applied for microbially enhanced oil recovery from oil reservoirs by plugging of porous reservoirs and by anaerobically metabolizing nutrients with the production of useful products such as gases, biosurfactants, and polymers under the environmental conditions that exist in the reservoirs (Bhupathiraju et al. 1991).

Treatment of Saline Wastewater

Treatment of saline wastewater in an anaerobic packed bed reactor inoculated with *Halanaerobium lacusrosei* was explored, using model wastewaters with glucose as carbon source applying a gradual increase in salinity from 0 to 5 % or from 3 to 10%. Glucose removal at 70 % efficiency was claimed at 3 % salt (Kapdan and Erten 2007; Kapdan and Boylan 2009). As *H. lacusrosei* does not grow below 6 % salt and has its optimum at 20 % salt (Cayol et al. 1995), it is not clear to what extent the glucose degradation observed was indeed effected by *Halanaerobium*.

Nitrosubstituted aromatic compounds such as nitrobenzene, nitrophenols, 2,4-dinitrophenol, and 2,4-dinitroaniline are reduced to the amino derivatives by *Halanaerobium praevalens* and by *Orenia marismortui* (Oren et al. 1991).

Enzymes

Several enzymes from members of the *Halanaerobiales* have been cloned, purified, and characterized. One such enzyme is the rhodanese-like protein (thiosulfate: cyanide sulfurtransferase; EC 2.8.1.1) of *Halanaerobium congolense* (Ravot et al. 2005). *Halothermothrix orenii* has become a popular object of such studies because of the prospect of enzymes that function both at high salinity and at high temperature. A few such enzymes have been crystallized to study their structure: α-amylase AmyA (EC 3.2.1.1) (optimum activity at 65 °C in 5 % NaCl, with significant activity at 25 % NaCl) (Li et al. 2002; Mijts and Patel 2002), α-amylase AmyB (Tan et al. 2008), ribokinase (EC 2.7.1.15) (Kori et al. 2012), sucrose

phosphate synthase (EC 2.4.1.14) (Chua et al. 2008; Huynh et al. 2005), fructokinase (EC 2.7.1.4) (Chua et al. 2010), and class II 5-enopyruvylshikimate-3-phosphate synthase (EC 2.5.1.19). The latter protein, a key enzyme in the synthesis of aromatic amino acids, when expressed in *Arabidopsis* plants bestowed resistance to glyphosate herbicides (Tian et al. 2012).

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