17 The Family *Halomonadaceae*

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Abstract

The family *Halomonadaceae*, within the class *Gammaproteobacteria*, consists mostly of marine and moderately halophilic microorganisms that are phenotypically rather diverse. As of January 2014, this family contains ten genera and 106 validly published species names and, therefore, constitutes the largest group of halophilic bacteria. In this chapter, the historical and current taxonomy have been reviewed along with molecular and phenotypic analyses. In addition, isolation and preservation procedures were considered, as well as the ecological habitats where the members of the family *Halomonadaceae* can be found and their clinical relevance as human pathogens. The increasing interest of this group of microorganisms due to its biotechnological and environmental applications has also been addressed.

Introduction

The family *Halomonadaceae*, within the class *Gammaproteobacteria*, consists mostly of marine and moderately halophilic microorganisms that are phenotypically rather diverse. Because of this apparent lack of a core of differential phenotypic traits, many of its current species were previously assigned to other genera such as *Deleya* (now extinct), *Alcaligenes*, *Pseudomonas*, *Halovibrio*, *Volcaniella*, etc. Reorganizations among these species started by the mid 1990s with the aid of 16S rRNA gene sequence comparison. In the meanwhile, new genera and species descriptions within the family *Halomonadaceae* have been reported, and the increasing number of species led some authors to review its phylogeny (Arahal et al. 2002c; de la Haba et al. 2010a, 2012) and phenotypic features (Mata et al. 2002).

A Subcommittee on the Taxonomy of the *Halomonadaceae*, a member of the International Committee on Systematic of Prokaryotes, was constituted more than 10 years ago (Vreeland and Ventosa 2003) and can be taken as a sign of the increasing interest in this group of organisms.

Species of the genera *Halomonas* and *Chromohalobacter* have been largely studied as model organisms of halophilism. Some of their representatives are among the most halophilic bacteria (Ventosa et al. 1998) and are adapted to a wide range of saline concentrations, even wider than extreme halophiles. Another

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source of interest for the study of this group of organisms has been their potential in biotechnological applications. These include the production of compatible solutes, extracellular enzymes (adapted to saline stress), and exopolysaccharides among others.

Taxonomy, Historical and Current

Short Description of the Family

Ha.lo.mo.na.da'ce.ae. M.L. fem. n. *Halomonas*, type genus of the family; suff. *-aceae*, ending to denote a family; M.L. fem. pl. n. *Halomonadaceae*, the *Halomonas* family. The description of the family is identical to that given by Franzmann et al. (1988) and emended by Dobson and Franzmann (1996), Ntougias et al. (2007) and Ben Ali Gam et al. (2007).

The family *Halomonadaceae* belongs, together with the *Alcanivoraceae*, the *Hahellaceae*, the *Litoricolaceae*, the *Oceanospirillaceae*, the *Oleiphilaceae* and the "*Saccharospirillaceae*" to the order *Oceanospirillales*, within the class *Gammaproteobacteria* (Garrity et al. 2005a) that consists mainly of marine species.

The family *Halomonadaceae* was originally proposed by Franzmann et al. (1988) and it was later emended by Dobson and Franzmann (1996), Ntougias et al. (2007) and Ben Ali Gam et al. (2007). At the start of 2014, this family included ten recognized genera: *Halomonas* (type genus), *Aidingimonas*, *Carnimonas*, *Chromohalobacter*, *Cobetia*, *Halotalea*, *Kushneria*, *Modicisalibacter*, *Salinicola*, and *Zymobacter* (Parte 2014). *Table 17.1* contains relevant taxonomic information on these genera and their species.

As mentioned before, some of the current species were isolated and described many years before the proposal of the genera *Halomonas* (Vreeland et al. 1980), *Chromohalobacter* (Ventosa et al. 1989) or *Cobetia* (Arahal et al. 2002a): *Pseudomonas beijerinckii* (basonym of *Chromohalobacter beijerinckii*), "*Chromobacterium marismortui*" (now *Chromohalobacter marismortui*), "*Arthrobacter marinus*" (earlier synonym of *Cobetia marina*), "*Achromobacter aquamarinus*" (*Halomonas aquamarina*), *Flavobacterium halmophilum* (basonym of *Halomonas halmophila*) and "*Micrococcus halodenitrificans*" (*Halomonas halodenitrificans*) are the oldest examples. In 1972, Baumann and coworkers published an extensive taxonomic study of Gram-negative, nonfermentative marine bacteria, including four organisms assigned at that time to the genus *Alcaligenes*, namely *Alc. aestus*, *Alc. cupidus*, *Alc. pacificus* and *Alc. venustus* (Baumann et al. 1972). About one decade later, Baumann et al. (1983) proposed the creation of the genus *Deleya* to accommodate those four marine species as well as *Pseudomonas marina*.

The genera *Halomonas* and *Deleya* served as the basis for the creation of the family *Halomonadaceae* (Franzmann et al. 1988). At that time these genera contained four and six species, respectively. A chemotaxonomic study (Franzmann and Tindall 1990) of members of the family *Halomonadaceae* concluded that on the basis of respiratory quinone, polar lipid, and fatty acid compositions, no clear distinction existed at the genus level. Additionally, it was concluded that *Alcaligenes aquamarinus* (currently *Halomonas aquamarina*) and *Halovibrio variabilis* (*Halomonas variabilis*) were members of the family *Halomonadaceae* and could perhaps be accomodated within existing genera of the family.

Only a few months earlier Ventosa et al. (1989) proposed *Chromohalobacter* as a new genus with a single species, *C. marismortui*, on the basis of a subculture of "*Chromobacterium marismortui*," isolated from the Dead Sea (Elazari-Volcani 1940), and seven moderately halophilic isolates from a Mediterranean saltern in Spain that were found to be very closely related to it. Later, in the phylogenetic study of Mellado et al. (1995b) it was concluded that this genus belongs to the family *Halomonadaceae*.

The genus *Zymobacter*, with its single species *Z. palmae*, was created by Okamoto et al. (1993) and placed later in the family *Halomonadaceae* (Dobson and Franzmann 1996).

By then, 16S rRNA phylogenetic analyses were used as definitive evidence of the lack of correlation in the taxonomic arrangements within the family *Halomonadaceae* (Dobson et al. 1993). Mellado et al. (1995b) proposed the reclassification of *Volcaniella eurihalina* as *Halomonas eurihalina* and pointed out the heterogeneity of the *Halomonas-Deleya* complex. Dobson and Franzmann (1996) transferred all species of the genus *Deleya* to the genus *Halomonas* together with *Halovibrio variabilis* and *Paracoccus halodenitrificans*. In one way, this simplification stopped the confusion of the naming within the *Halomonas-Deleya* complex, but the resulting genus, *Halomonas*, contained (and still does) very different species and it is considered too heterogeneous. The genus *Halomonas* was expanded to 15 species, with few characters in common, while the only two other genera recognized at that time, *Chromohalobacter* and *Zymobacter*, contained one each. Meanwhile, the genus *Carnimonas* was created by Garriga et al. (1998) to accommodate one single species, *C. nigrificans* isolated from cured meat products, and later it was included into the family *Halomonadaceae* (Arahal et al. 2002c). The same year, the genus *Cobetia* was created by Arahal et al. (2002a) to accommodate the species *Halomonas marina*.

More recently, other five additional genera belonging to this family have been described: *Halotalea* (Ntougias et al. 2007), *Modicisalibacter* (Ben Ali Gam et al. 2007), *Kushneria* (Sánchez-Porro et al. 2009), *Aidingimonas* (Wang et al. 2009), and *Salinicola* (Anan'ina et al. 2007). Besides, the genera *Halomonas*, *Chromohalobacter*, and *Cobetia* were expanded to include new species since new descriptions were carried out. Moreover, several reclassifications have taken place among genera of the family *Halomonadaceae*: the species *Halomonas canadensis* and *H. israelensis* (Arahal et al. 2001a) were transferred to the genus *Chromohalobacter*; the species *H. avicenniae*, *H. indalinina*, and *H. marisflavi* to the genus *Kushneria* (Sánchez-Porro et al. 2009); and the species *H. salaria* and *Chromohalobacter salarius* to the

Table 17.1

Validly published genera and species names of the family *Halomonadaceae* (as to 31 January 2014). Basonyms/synonyms of microorganisms that have been transferred to other genera are not included. For genera/species whose descriptions have been emended only the most recent reference is included

Genus and species name	Reference	Type strain designation(s)
Aidingimonas		
A. halophila	Wang et al. (2009)	YIM 90637 = CCTCC AB 207002 = KCTC 12885
Carnimonas		
Car. nigrificans[a]	Garriga et al. (1998)	CTCBS1 = ATCC BAA-78 = CECT 4437 = CIP 105703
Chromohalobacter		
Chr. beijerinckii	Peçonek et al. (2006)	ATCC 19372 = CCUG 49679 = CIP 106957 = DSM 7218 = JCM 13305 = JCM 21422 = LMG 2148 = NBRC 103041 = NCCB 35008 = NCIMB 9041 = NRRL B-3153
Chr. canadensis	Arahal et al. (2001a)	ATCC 43984 = CECT 5385 = CCM 4919 = CIP 105571 = DSM 6769 = LMG 19547 = NCIMB 13767 = NRCC 41227
Chr. israelensis	Arahal et al. (2001a)	Ba1 = ATCC 43985 = CECT 5287 = CCM 4920 = CIP 106853 = DSM 6768 = LMG 19546 = NCIMB 13766
Chr. japonicus	Sánchez-Porro et al. (2007)	43 = CCM 7416 = CECT 7219
Chr. marismortui[a]	Ventosa et al. (1989)	CCM 3518 = ATCC 17056 = DSM 6770 = JCM 21220 = LMG 3935 = NBRC 103155
Chr. nigrandesensis	Prado et al. (2006)	LTS-4 N = CECT 5315 = DSM 14323
Chr. salexigens	Arahal et al. (2001b)	ATCC BAA-138 = CECT 5384 = CCM 4921 = CIP 106854 = DSM 3043 = NCIMB 13768 = 1H11
Chr. sarecensis	Quillaguamán et al. (2004a)	LV4 = ATCC BAA-761 = CCUG 47987 = DSM 15547
Cobetia		
Cob. amphilecti	Romanenko et al. (2013)	CCUG 49560 = KMM 1561 = NRIC 0815
Cob. crustatorum	Kim et al. (2010b)	JO1 = JCM 15644 = KCTC 22486
Cob. litoralis	Romanenko et al. (2013)	CCUG 49563 = KMM 3880 = NRIC 0814
Cob. marina[a]	Arahal et al. (2002a)	219 = ATCC 25374 = CCUG 49558 = CCUG 49558 = CECT 4278 = CIP 104765 = DSM 4741 = LMG 2217 = NBRC 102605 = NCIMB 1877
Cob. pacifica	Romanenko et al. (2013)	CCUG 49562 = KMM 3879 = NRIC 0813
Halomonas[b]		
H. alimentaria	Yoon et al. (2002)	YKJ-16 = DSM 15356 = KCCM 41042 = JCM 10888
H. alkaliantarctica	Poli et al. (2007)	CRSS = ATCC BAA-848 = DSM 15686
H. alkaliphila	Romano et al. (2006)	18bAG = ATCC BAA-953 = DSM 16354
H. almeriensis	Martínez-Checa et al. (2005)	M8 = CECT 7050 = LMG 22904
H. andesensis	Guzmán et al. (2010)	LC6 = CCUG 54844 = DSM 19434 = LMG 24243
H. anticariensis	Martínez-Cánovas et al. (2004a)	FP35 = CECT 5854 = LMG 22089
H. aquamarina	Dobson and Franzmann (1996)	ZoBell and Upham 558 = ATCC 14400 = CCUG 16157 = CIP 105454 = DSM 30161 = IAM 12550 = LMG 2853 = NCIMB 557
H. arcis	Xu et al. (2007)	AJ282 = CGMCC 1.6494 = JCM 14607 = LMG 23978
H. axialensis	Kaye et al. (2004)	Althf1 = ATCC BAA-802 = CECT 5812 = DSM 15723
H. beimenensis	Wang et al. (2012)	NTU-107 = BCRC 17999 = JCM 16084 = KCTC 22876
H. boliviensis	Quillaguamán et al. (2004b)	LC1 = ATCC BAA-759 = DSM 15516
H. campaniensis	Romano et al. (2005)	5AG = ATCC BAA-966 = DSM 15293
H. campisalis	Mormile et al. (1999)	4A = ATCC 700597 = CIP 106639
H. caseinilytica	Wu et al. (2008b)	AJ261 = CGMCC 1.6773 = JCM 14802
H. cerina	González-Domenech et al. (2008b)	SP4 = CECT 7282 = LMG 24145
H. cibimaris	Jeong et al. (2013)	10-C-3 = JCM 16914 = KACC 14932
H. cupida	Dobson and Franzmann (1996)	79 = ATCC 27124 = CCUG 16075 = CIP 103199 = DSM 4740 = JCM 20632 = LMG 3448 = NBRC 102219
H. daqiaonensis	Qu et al. (2011)	YCSA28 = CGMCC 1.9150 = NCCB 100305 = MCCC 1B00920
H. daqingensis	Wu et al. (2008a)	DQD2-30 = CGMCC 1.6443 = LMG 23896
H. denitrificans	Kim et al. (2007)	M29 = DSM 18045 = KCTC 12665

Table 17.1 (continued)

Genus and species name	Reference	Type strain designation(s)
H. desiderata	Berendes et al. (1996)	FB2 = CIP 105505 = DSM 9502 = LMG 19548
H. elongata[a]	Vreeland et al. (1980)	1H9 = ATCC 33173 = CIP 104264 = DSM 2581 = NBRC 15536 = JCM 21044 = LMG 9076
H. eurihalina	Mellado et al. (1995b)	F9-6 = ATCC 49336 = CIP 106091 = DSM 5720
H. flava	Chen et al. (2011)	YIM 94343 = CCTCC AB 2010382 = KCTC 23356
H. fontilapidosi	González-Domenech et al. (2009)	5CR = CECT 7341 = LMG 24455
H. gomseomensis	Kim et al. (2007)	M12 = DSM 18042 = KCTC 12662
H. gudaonensis	Wang et al. (2007b)	SL014B-69 = CGMCC 1.6133 = LMG 23610
H. halmophila	Dobson et al. (1990)	ACAM 71 = ATCC 19717 = CIP 105455 = DSM 5349 = NBRC 15537 = JCM 21222 = LMG 4023 = NCIMB 1971
H. halocynthiae	Romanenko et al. (2002)	KMM 1376 = DSM 14573 = CIP 107736
H. halodenitrificans	Dobson and Franzmann (1996)	ATCC 13511 = CIP 105456 = DSM 735 = CCM 286 = CECT 5012 = IAM 13950 = KCTC 5069 = NBRC 14912
H. halophila	Dobson and Franzmann (1996)	F5-7 = ATCC 49969 = CCM 3662 = CIP 103512 = DSM 4770 = JCM 20791 = LMG 6456 = NBRC 102604
H. hamiltonii	Kim et al. (2010a)	W1025 = DSM 21196 = KCTC 22154
H. hydrothermalis	Kaye et al. (2004)	Slthf2 = ATCC BAA-800 = CECT 5814 = DSM 15725
H. ilicicola	Arenas et al. (2009)	SP8 = CCM 7522 = CECT 7331 = DSM 19980
H. janggokensis	Kim et al. (2007)	M24 = KCTC 12663 = DSM 18043
H. jeotgali	Kim et al. (2011)	Hwa = JCM 15645 = KCTC 22487
H. johnsoniae	Kim et al. (2010a)	T68687 = DSM 21197 = KCTC 22157
H. kenyensis	Boltyanskaya et al. (2007)	AIR-2 = DSM 17331 = VKM B-2354
H. koreensis	Lim et al. (2004)	SS20 = JCM 12237 = KCTC 12127
H. korlensis	Li et al. (2008)	XK1 = CGMCC 1.6981 = DSM 19633
H. kribbensis	Jeon et al. (2007)	BH843 = DSM 17892 = KCTC 12584
H. lutea	Wang et al. (2008a)	YIM 91125 = CCTCC AB 206093 = KCTC 12847
H. magadiensis	Duckworth et al. (2000)	21 MI = CIP 106823 = CIP 106874 = DSM 15367 = NCIMB 13595
H. maura	Bouchotroch et al. (2001)	S-31 = ATCC 700995 = CECT 5298 = DSM 13445
H. meridiana	James et al. (1990)	ACAM 246 = ATCC 49692 = CIP 104043 = DSM 5425 = NBRC 15608 = UQM 3352
H. mongoliensis	Boltyanskaya et al. (2007)	Z-7009 = DSM 17332 = VKM B-2353
H. muralis	Heyrman et al. (2002)	LMG 20969 = CIP 108825 = DSM 14789
H. nanhaiensis	Long et al. (2013)	YIM M 13059 = JCM 18142 = CCTCC AB 2012911
H. neptunia	Kaye et al. (2004)	Eplume1 = ATCC BAA-805 = CECT 5815 = DSM 15720
H. nitroreducens	González-Domenech et al. (2008a)	11S = CECT 7281 = LMG 24185
H. olivaria	Amouric et al. (2014)	TYRC17 = DSM 19074 = CCUG 53850B
H. organivorans	García et al. (2004)	G-16.1 = CCM 7142 = CECT 5995
H. pacifica	Dobson and Franzmann (1996)	62 = ATCC 27122 = CIP 103200 = DSM 4742 = JCM 20633 = LMG 3446 = NBRC 102220
H. pantelleriensis	Romano et al. (1996)	AAP = ATCC 700273 = CIP 105506 = DSM 9661 = LMG 19550
H. qijiaojingensis	Chen et al. (2011)	YIM 93003 = CCTCC AB 208133 = KCTC 22228
H. ramblicola	Luque et al. (2012)	RS-16 = CECT 7896 = LMG 26647
H. rifensis	Amjres et al. (2011)	HK31 = CECT 7698 = LMG 25695
H. sabkhae	Kharroub et al. (2008)	5-3 = CECT 7246 = DSM 19122 = LMG 24084
H. saccharevitans	Xu et al. (2007)	AJ275 = CGMCC 1.6493 = JCM 14606 = LMG 23976
H. salifodinae	Wang et al. (2008b)	BC7 = CGMCC 1.6774 = JCM 14803
H. salina	Dobson and Franzmann (1996)	F8-11 = ATCC 49509 = CIP 106092 = DSM 5928 = JCM 21221
H. shengliensis	Wang et al. (2007a)	SL014B-85 = CGMCC 1.6444 = LMG 23897
H. sinaiensis	Romano et al. (2007)	ALO Sharm = ATCC BAA-1308 = DSM 18067
H. smyrnensis	Poli et al. (2013)	AAD6 = DSM 21644 = JCM 15723
H. stenophila	Llamas et al. (2011)	N12 = CECT 7744 = LMG 25812

Table 17.1 (continued)

Genus and species name	Reference	Type strain designation(s)
H. stevensii	Kim et al. (2010a)	S18214 = DSM 21198 = KCTC 22148
H. subglaciescola	Franzmann et al. (1987)	ACAM 12 = ATCC 43668 = CIP 104042 = DSM 4683 = NBRC 14766 = JCM 21045 = LMG 8824 = UQM 2926
H. subterranea	Xu et al. (2007)	ZG16 = CGMCC 1.6495 = JCM 14608 = LMG 23977
H. sulfidaeris	Kaye et al. (2004)	Esulfide1 = ATCC BAA-803 = CECT 5817 = DSM 15722
H. taeanensis	Lee et al. (2005)	BH539 = DSM 16463 = KCTC 12284
H. titanicae	Sánchez-Porro et al. (2010)	BH1 = ATCC BAA-1257 = CECT 7585 = JCM 16411 = LMG 25388
H. variabilis	Dobson and Franzmann (1996)	isolate III = ATCC 49240 = CIP 105504 = DSM 3051 = IAM 14440 = JCM 21223 = NBRC 102410
H. ventosae	Martínez-Cánovas et al. (2004b)	Al12 = CECT 5797 = DSM 15911
H. venusta	Dobson and Franzmann (1996)	86 = ATCC 27125 = CCUG 16063 = CIP 103201 = DSM 4743 = JCM 20634 = LMG 3445 = NBRC 102221
H. vilamensis	Menes et al. (2011)	SV325 = DSM 21020 = LMG 24332
H. xianhensis	Zhao et al. (2012)	A-1 = CGMCC 1.6848 = JCM 14849
H. xinjiangensis	Guan et al. (2010)	TRM 0175 = CCTCC AB 208329 = KCTC 22608
H. zhanjiangensis	Chen et al. (2009)	JSM 078169 = CCTCC AB 208031 = DSM 21076 = KCTC 22279
H. zincidurans	Xu et al. (2013)	B6 = CGMCC 1.12450 = JCM 18472
Halotalea		
Halot. alkalilenta[a]	Ntougias et al. (2007)	AW-7 = CECT 7134 = DSM 17697
Kushneria		
K. aurantia[a]	Sánchez-Porro et al. (2009)	A10 = CCM 7415 = CECT 7220
K. avicenniae	Sánchez-Porro et al. (2009)	MW2a = CCM 7396 = CECT 7193 = CIP 109711
K. indalinina	Sánchez-Porro et al. (2009)	CG2.1 = CECT 5902 = CIP 109528 = DSM 14324 = LMG 23625
K. marisflavi	Sánchez-Porro et al. (2009)	SW32 = CIP 107103 = DSM 15357 = JCM 10873 = KCCM 80003
K. sinocarnis	Zou and Wang (2010)	Z35 = CCTCC AB 209027 = DSM 23229 = NRRL B-59197
Modicisalibacter		
M. tunisiensis[a]	Ben Ali Gam et al. (2007)	LIT2 = CCUG 52917 = CIP 109206
Salinicola		
S. halophilus	de la Haba et al. (2010b)	CG4.1 = CECT 5903 = LMG 23626
S. peritrichatus	Huo et al. (2013)	DY22 = CGMCC 1.12381 = JCM 18795
S. salarius	de la Haba et al. (2010b)	M27 = DSM 18044 = KCTC 12664
S. socius[a]	Anan'ina et al. (2007)	SMB35 = DSM 19940 = VKM B-2397
Zymobacter		
Z. palmae[a]	Okamoto et al. (1993)	T109 = ATCC 51623 = DSM 10491 = IAM 14233 = JCM 21091 = NBRC 102412

Abbreviations of culture collections are: *ACAM* Australian Collection of Antarctic Microorganisms, *ATCC* American Type Culture Collection, *BCRC* Bioresource Collection and Research Center, *CCM* Czech Collection of Microorganisms, *CCTCC* China Center for Type Culture Collection, *CCUG* Culture Collection University of Göteborg, *CECT* Colección Española de Cultivos Tipo, *CIP* Collection de L'Institut Pasteur, *CGMCC* China General Microbiological Culture Collection Center, *DSM* Deutsche Sammlung von Mikroorganismen und Zellkulturen, *IAM* Institute of Applied Microbiology, *JCM* Japan Collection of Microorganisms, *KACC* Korean Agricultural Culture Collection, *KCCM* Korean Culture Center of Microorganisms, *KCTC* Korean Collection for Type Cultures, *KMM* Collection of. Marine Microorganisms, *LMG* Belgian Co-ordinated Collections of Microorganisms, *MCCC* Marine Culture Collection of China, *NBRC*, NITE Biological Resource Center, *NCCB* Netherlands Culture Collection of Bacteria, *NCIMB* The National Collection of Industrial, Marine and Food Bacteria, *NRCC* National Research Council of Canada, *NRIC*, *NODAI* Research Institute Culture Collection, *NRRL* Agricultural Research Service Culture Collection, *UQM* Australian Collection of Microorganisms, *VKM* All-Russian Collection of Microorganisms

[a]Type species of the genus

[b]Type genus of the family

genus *Salinicola* (de la Haba et al. 2010b). In 2013, Romanenko et al. (2013) classified *H. halodurans* as a later heterotypic synonym of *Cobetia marina*, invalidating the former species name.

As of January 2014 there are 106 validly published species names within the family *Halomonadaceae*, 79 belonging to the genus *Halomonas*, eight to *Chromohalobacter*, five to *Cobetia*, five to *Kushneria*, four to *Salinicola*, and the genera *Aidingimonas*, *Carnimonas*, *Halotalea*, *Modicisalibacter*, and *Zymobacter* with a single species each one. The list of articles in press of the International Journal of Systematic and Evolutionary Microbiology (http://ijs.sgmjournals.org/content/early/recent)

includes the description of a new *Halomonas* species for which the name *H. huangheensis* has been proposed.

A valuable effort to address the taxonomy of the group from an entirely phenotypic point of view was the study of Mata et al. (2002). In that article, they presented a detailed phenotypic characterization of the type strains of all *Halomonas* species recognized at that time and the intraspecific variation of four of those species by studying 87 additional strains. The authors compared the reactions of 234 morphological, physiological, biochemical, nutritional and antimicrobial susceptibility tests. Part of the nutritional characterization was obtained by using a miniaturized (Biolog) identification system. It was the first time that such a method was employed so extensively among halomonads. In addition to the new data that were presented in their paper, some differences were observed between their results and those from the original species descriptions. Numerical analyses demonstrated the phenotypic heterogeneity of the *Halomonas* species (Mata et al. 2002). An important conclusion of the study of Mata et al. (2002) is that phenotypic traits can be selected according to their usefulness for distinguishing *Halomonas* species.

In 2007, the International Committee on Systematics of Prokaryotes–Subcommittee on the Taxonomy of the *Halomonadaceae*, following Recommendation 30b of the Bacteriological Code (1990 Revision) (Lapage et al. 1992), published the minimal standards for describing new taxa within this family (Arahal et al. 2007). This paper evaluates many different approaches to ensure that a rich polyphasic characterization is given and must be considered as guidelines for authors to prepare descriptions of novel taxa. Although a list of traits that are required and recommended is provided, the manuscript does not attempt to limit the characterization of new isolates to the features that are indicated in the text. Moreover, current or yet to be described species may show new and interesting characteristics not listed in the paper of Arahal et al. (2007) and such features may prove to be of taxonomic importance. More recently, the Subcommittee on the Taxonomy of *Halomonadaceae*, in an open meeting held in Storrs, Connecticut, USA (June 2013), agreed that the current minimal standards document are still adequate in combination with Notes on the characterization of prokaryote strains for taxonomic purposes (Tindall et al. 2010), but the following additions were suggested: (i) It was stressed that it is highly desirable to base the description of new species on more than one strain. (ii) A good database of sequences of suitable genes for multilocus sequence analysis is now available (de la Haba et al. 2012), and inclusion of multilocus sequence analysis in the minimal standards is highly desirable. (iii) Fatty acid analysis should be added as required rather than recommended in the recommended standards for describing new taxa of the family *Halomonadaceae* (Oren and Ventosa 2013).

Phylogenetic Structure of the Family and Its Genera

Seven main phylogenetic studies have been conducted on the *Halomonadaceae*. In the first one (Franzmann et al. 1988), which was the basis for the proposal of this family, the method employed was the 16S rRNA oligonucleotide cataloguing technique. Later, Dobson et al. (1993) obtained the 16S rRNA sequences of *Deleya aquamarina* (now *Halomonas aquamarina*), *Deleya halophila* (*Halomonas halophila*), *Deleya marina* (*Cobetia marina*), *Halomonas elongata*, *Halomonas meridiana*, *Halovibrio variabilis* (*Halomonas variabilis*) and *Halomonas subglaciescola* and analyzed them together with the sequences of *Halomonas halmophila* and other species belonging to the *Gammaproteobacteria*. They showed that the level of 16S rRNA sequence similarity among members of the family *Halomonadaceae* is 100–92.6 % and that the phylogenetic grouping did not correspond to the taxonomic assignment of the species analyzed, suggesting the unification into a single genus. They also proposed a number of characteristic sequence signatures of the members of the family *Halomonadaceae* that have been readapted in other studies (Dobson and Franzmann 1996; Arahal et al. 2002c; Ntougias et al. 2007; Ben Ali Gam et al. 2007), as new members have been described. Currently, the 16S rRNA sequence signature characteristics of the family *Halomonadaceae* are defined by the following positions: 484 (A or G), 486 (C or U), 640 (A or G), 660 (A), 668 (A), 669 (A), 737 (U), 738 (U), 745 (U), 776 (U), 1124 (U or G), 1297 (U), 1298 (C), 1423 (A), 1424 (C or U), 1439 (U or C), 1462 (A or G) and 1464 (C or U). However, fullsequence analyses are much more informative than signatures alone since the latter have to be redefined on the basis of present and future new species.

Mellado et al. (1995b) conducted a phylogenetic study on six new 16S rRNA sequences corresponding to *Chromohalobacter marismortui* (four strains), *Volcaniella eurihalina* (now *Halomonas eurihalina*), *Deleya salina* (*Halomonas salina*), and close relatives. They proposed the reclassification of *Volcaniella eurihalina* as *Halomonas eurihalina* but highlighted the need of a polyphasic approach to determine the natural taxonomic position of members of the *Halomonadaceae*, especially for the genus *Halomonas* since its heterogeneity was (and still is) too large for a single genus.

Further studies by Dobson and Franzmann (1996) determined another seven 16S rRNA sequences corresponding to the type strains of *Halomonas subglaciescola*, *Deleya cupida* (currently *Halomonas cupida*), *Deleya pacifica* (*Halomonas pacifica*), *Deleya salina* (*Halomonas salina*), *Deleya venusta* (*Halomonas venusta*), *Halomonas halodurans* (*Cobetia marina*), and *Halomonas eurihalina*. On the basis of their results, they proposed the unification of the genera *Deleya*, *Halomonas* and *Halovibrio* and the species *Paracoccus halodenitrificans* into the genus *Halomonas*.

Arahal et al. (2002c) evaluated the phylogenetic status of the family *Halomonadaceae* using 16S and 23S rRNA gene sequences. In addition to the new sequences determined in their study, 18 for the 23S rRNA and 7 for the 16S rRNA, the sequences were compared to more than 16,000 full or almost full rRNA sequences. By that time, the number of those sequences that could be ascribed to the family *Halomonadaceae* exceeded 70 (including many sequences from environmental clones and poorly characterized isolates). In addition, several

treeing methods were used to elucidate the most stable branchings. A good agreement between the 16S rRNA- and the 23S rRNA-based trees was obtained. According to this study, the genus *Halomonas* was formed by two well-defined phylogenetic groups (containing five and seven species, respectively) as well as six species that could not be assigned to any of the above-mentioned groups. Group 1 comprised *Halomonas elongata* (type species of the genus), *Halomonas eurihalina*, *Halomonas halmophila*, *Halomonas halophila*, and *Halomonas salina*, all bearing a 98.2 % average sequence (16S rRNA or 23S rRNA) similarity. Group 2 included the species *Halomonas aquamarina*, *Halomonas meridiana*, *Halomonas magadiensis*, *Halomonas variabilis*, *Halomonas venusta*, *Halomonas halodurans* (now *Cobetia marina*), and *Halomonas subglaciescola*, and exhibited a 97.6 % mean 23S rRNA sequence similarity (97.4 % in the case of the 16S rRNA sequences). The species *Halomonas pacifica*, *Halomonas halodenitrificans*, *Halomonas cupida*, *Halomonas desiderata*, *Halomonas campisalis*, and *Halomonas pantelleriensis*, not only did not clearly fall into either of the two groups mentioned above but also shared relatively low values of sequence similarity with them or even between themselves (91.7–96.7 %; Arahal et al. 2002c). With respect to the genus *Chromohalobacter*, the four species described in those days within this genus formed a group closely related to *Halomonas*. The average rRNA sequence similarity of species of *Chromohalobacter* was 98.6 % (for the 23S rRNA) and 98.5 % (for the 16S rRNA). Within this group fell the sequence of *Pseudomonas beijerinckii*, which was later reclassified as *Chromohalobacter beijerinckii* (Peçonek et al. 2006). When the *Chromohalobacter* sequences were compared to those of other halomonads, values below 95 % (generally accepted as a good borderline for genus separation) were obtained in all cases. Similar low values were obtained for the sequence of *Halomonas marina*, which forms a deeper branch of the *Halomonas*-*Chromohalobacter* group. Indeed, according to this and other data, this organism was proposed as the type species of the new genus *Cobetia* (Arahal et al. 2002a). Finally, in this study, the sequences of *Zymobacter palmae* and *Carnimonas nigrificans* showed a deeper branching in the tree. Their 16S rRNA sequence similarity was 93.5 % and even lower values were obtained when comparing any of the two with the other members of the family (Arahal et al. 2002c).

More recently, de la Haba et al. (2010a) updated the comparative analysis based on 23S and 16S rRNA gene sequences of Arahal et al. (2002c) including the 49 novel species that had been described since 2002. A total of 28 new complete 23S rRNA sequences were obtained in this study. Additionally, following the recommended minimal standards for the description of new members of the family *Halomonadaceae*, seven already-sequenced 16S rRNA genes of type strains were resequenced to resolve undetermined positions and to reach the established quality standards. In that sense, some suggestions were included in the paper about the recommended sequences to be used for future comparative phylogenetic analysis. In general, there was excellent agreement between the phylogenies based on both rRNA genes, but the 23S rRNA gene showed higher resolution in the differentiation of species of the family *Halomonadaceae* due to the slower evolutionary rate for the 16S rRNA gene. As previously reported by Arahal et al. (2002c), the genus *Halomonas* resulted to be not monophyletic and comprised two clearly separated phylogenetic groups that now contained larger numbers of species. Group 1, representing *Halomonas sensu stricto*, was formed by *Halomonas elongata* (the type species of the genus), *H. eurihalina*, *H. caseinilytica*, *H. halmophila*, *H. sabkhae*, *H. almeriensis*, *H. halophila*, *H. salina*, *H. organivorans*, *H. koreensis*, *H. maura* and *H. nitroreducens*. The mean 16S rRNA gene sequence similarity for this group was 97.8 %, whereas a lower value was obtained with the 23S rRNA gene sequences (97.0 %). Group 2, included the 16 species *Halomonas aquamarina*, *H. meridiana*, *H. axialensis*, *H. magadiensis*, *H. hydrothermalis*, *H. alkaliphila*, *H. venusta*, *H. boliviensis*, *H. neptunia*, *H. variabilis*, *H. sulfidaeris*, *H. subterranea*, *H. janggokensis*, *H. gomseomensis*, *H. arcis* and *H. subglaciescola*. This group displays mean similarities of 97.4 % and 97.5 % for the 16S and 23S rRNA gene sequences, respectively. Similarity values between groups 1 and 2 were low enough as to suggest that they could constitute two different genera, however, neither chemotaxonomic nor more general phenotypic studies have permitted their separation. The other 27 species at that time assigned to the genus *Halomonas* did not appear to be included clearly in either of these phylogenetic groups. One of these species, *H. salaria*, formed a separate cluster with the species *Chromohalobacter salarius* and *Salinicola socius*, which was confirmed by further studies proposing the transference of the first two species to the genus *Salinicola* (de la Haba et al. 2010b). An important finding in the paper of de la Haba et al. (2010a) is the fact that the type species of *Halomonas halodurans* and *Cobetia marina* shared 100 % sequence similarity (16S and 23S rRNA) and, according to their data, there was not sufficient evidence to determine whether they were members of the same or different species. A recent publication has demonstrated that, actually, they belong to the same genospecies (Romanenko et al. 2013). Concerning the genus *Chromohalobacter*, all the species described until then clustered together (the mean 16S and 23S rRNA gene sequence similarity of this group was 98.0 % and 97.8 %, respectively) with the only exception being *Chromohalobacter salarius*, as discussed previously (de la Haba et al. 2010a). Finally, the phylogenetic distinctness of the remaining genera at that time included in the family *Halomonadaceae* (*Carnimonas*, *Cobetia*, *Halotalea*, *Kushneria*, *Modicisalibacter*, *Salinicola*, and *Zymobacter*) was confirmed in the mentioned paper, being stable in the trees produced from all methods of analysis.

In the meanwhile, 26 new species have been proposed within the genus *Halomonas*, some of them belonging to the group 1 (*H. beimenensis*, *H. sinaiensis*, *H. smyrnensis*, and *H. stenophila*), others to the group 2 (*H. alkaliantarctica*, *H. andesensis*, *H. cibimaris*, *H. hamiltonii*, *H. jeotgali*, *H. johnsoniae*, *H. nanhaiensis*, *H. olivaria*, *H. stevensii*, *H. titanicae*, *H. vilamensis*, and *H. zhanjiangensis*) and others that cannot be assigned to any of these two groups (*H. daqiaonensis*, *H. flava*, *H. fontilapidosi*, *H. ilicicola*, *H. qijiaojingensis*, *H. ramblicola*, *H. rifensis*,

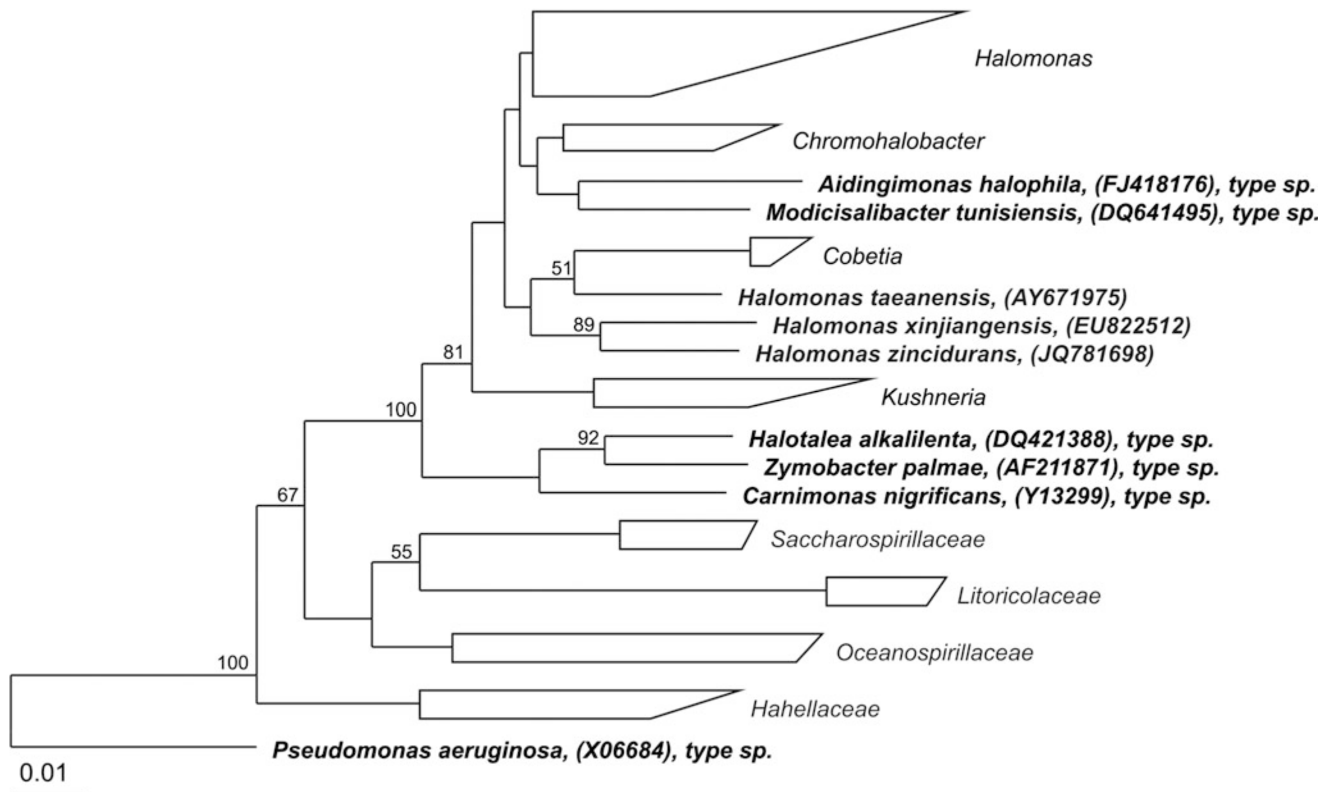

Fig. 17.1

Phylogenetic reconstruction of the family *Halomonadaceae* based on 16S rRNA and created using the neighbour-joining algorithm with the Jukes-Cantor correction. The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). 1,000 resampling boostrap values over 50 % are shown. Scale bar indicates estimated sequence divergence

H. xianhensis, *H. xinjiangensis*, and *H. zincidurans*) (Fig. 17.1). Besides, four novel species of the genus *Cobetia*, and one of the genera *Kushneria* and *Salinicola*, respectively, have been described, all of them forming a monophyletic branch with the other relatives of their respective genera (Fig. 17.1). Additionally, the new genus *Aidingimonas*, not included in the study of de la Haba et al. (2010a), clustered together with the genus *Modicisalibacter* (Fig. 17.1), but they share ≤95 % 16S rRNA similarity values among them and with respect to the other genera of the family *Halomonadaceae*.

From the phylogenetic point of view, some well-defined relationships can be observed within members of the *Halomonadaceae*. These groups, as defined above, are stable regardless of the methodology employed. Other relations may become better defined once more in-between sequences become available.

The most recent phylogenetic study conducted within the family *Halomonadaceae* was carried out by de la Haba et al. (2012). A multilocus sequence analysis (MLSA) of 52 representative species was performed for the first time in a moderately halophilic bacterial group with the purpose of investigating in detail the phylogenetic relationships of species from the family *Halomonadaceae* and helping clarify the current classification of this complex and dynamic family. A total of six loci were selected for the analysis, the 16S and 23S rRNA and the following four protein-encoding genes (housekeeping genes): *atpA* (F1-ATP synthase, α subunit), *gyrB* (DNA gyrase, B subunit), *rpoD* (RNA polymerase, β subunit) and *secA* (protein translocase, SecA subunit). Different nucleotide substitution models and tree-constructing algorithms were compared. The average pairwise sequence similarity values for 16S rRNA, 23S rRNA, *atpA*, *gyrB*, *rpoD* and *secA* were 94.0 %, 93.1 %, 86.8 %, 79.7 %, 79.6 % and 76.6 %, respectively, indicating that the *secA* gene had the highest theoretical discriminatory power, although some halomonads *secA* gene sequences were identical, suggesting gene flow. In any case, the six genes studied were not always sufficient to correctly assign a new strain to the genus *Halomonas* since there was a large overlap between the intrageneric and intergeneric sequence similiarities, that is, halomonads sequences could be more similar to those from species belonging to other genera of the family than to those from species of the genus *Halomonas*. This overlap was mainly due to the enormous variability within the genus *Halomonas*, suggesting that the genus should be divided into two or more genera. Besides, the overlap problem also lies with the huge sequence similarity of the pair *Halomonas halodurans*-*Cobetia marina*, whose taxonomic status has been recently revised by

Romanenko et al. (2013) and concluded that they are member of the same species. With respect to the phylogenetic trees, the different methods produced variable results, with those generated from the maximum-likelihood and neighbour-joining algorithms being more similar than those obtained by maximum-parsimony methods. Except *atpA* gene, the other five genes studied showed a consistent evolutionary history (with some exceptions probably due to lateral gene transfer events, and other intrinsic and extrinsic factors, such as the size of the dataset and the taxa included); therefore *atpA* gene may not be useful as an individual gene phylogenetic marker within the family *Halomonadaceae*. The *gyrB*-based tree was the only that formed monophyletic branches for the different genera of the family, including the genus *Halomonas*. Although there were some exceptions, in general, the two groups defined within this genus by Arahal et al. (2002c) and de la Haba et al. (2010a) (group 1 or *Halomonas sensu stricto* and group 2) could be clearly distinguished in the phylogenetic trees for each gene. Concatenation of the six loci enhanced the phylogenetic reconstruction and optimized the taxonomic resolution by adding more informative data and minimizing the weight of recombination events. Trees resulting from the six-gene concatenation demonstrated a monophyletic and well-supported separation of the different genera, including the genus *Halomonas*. The only exceptions were the pairs *Halomonas halodurans*-*Cobetia marina* (mentioned above, with concatenated sequence similarity of 99.7 %) and *Halomonas muralis*–*M. tunisiensis* (with concatenated sequence similarity of 90.8 %). With regard to the intrageneric groups, the six-gene concatenations resolved *Halomonas* groups 1 and 2 monophyletically, demonstrating to be a very good tool for the delineation of taxonomic relationships on a broad scale, including intrageneric and intergeneric relationships, at the level of the family *Halomonadaceae*. In order to simplify the MLSA approach within this family, de la Haba et al. (2012) attempted to reduce the number of genes to be analyzed but retaining the resolution obtained with the six concatenated gene sequences. With this idea the general use of the individual and concatenated 16S rRNA, *gyrB* and *rpoD* genes is suggested for future taxonomic studies using MLSA within the family *Halomonadaceae*. This proposal has been recently endorsed and recommended within the minimal standards by the ICSP-Subcommittee on the Taxonomy of the *Halomonadaceaae* (Oren and Ventosa 2013). Finally, the paper of de la Haba et al. (2012) analyze the phylogeny of this family within the domain *Bacteria* by comparing the sequences obtained in this study with those of 445 bacterial species with sequenced genomes available from the GenBank/EMBL/DDBJ databases. The results showed that the family *Halomonadaceae* constituted a robust and monophyletic branch within the domain *Bacteria* for three of the six analysed genes, 16S rRNA, *gyrB* and *rpoD*. Besides, according to *Figs. 17.1*, *17.2*, and *17.3* the family is related to families *Saccharospirillaceae*, *Litoricolaceae*, *Oceanospirillaceae*, and *Hahellaceae*.

Molecular Analyses

DNA-DNA Hybridization Studies

DNA–DNA hybridization data between type strains of species contained in the family *Halomonadaceae* are widely available. Actually, the vast majority of descriptions include results of DNA-DNA hybridization (DDH). As indicated in the recomended minimal standards for this family, DDH studies remains essential when novel species are described (Arahal et al. 2007), and only in those proposals based on a single isolate that possess less than 97 % 16S rRNA gene sequence similarity with its closest relative can DDH data be considered redundant.

With respect to DDH between strains belonging to the same species within the family *Halomonadaceae* data are very limited, mainly due to the fact that the majority of the species have been described based on a single isolate.

Multilocus Sequence Analysis (MLSA)

With the objective to overcome the limitations attached to DNA-DNA hybridization studies (time-consuming, expensive, lack of uniformity and reproducibility problems, etc.) and to 16S rRNA gene sequence based taxonomy (high levels of conservation, microheterogeneity among the different copies, lateral gene transfer, etc.), additional rRNA and protein-encoding genes (called housekeeping genes) have been suggested as phylogenetic markers (Stackebrandt et al. 2002; Zeigler 2003; Arahal et al. 2007; Tindall et al. 2010) to perform multilocus sequence analysis (MLSA).

Although some authors (Arahal et al. 2002c; Lee et al. 2005; de la Haba et al. 2010a; Okamoto et al. 2004; González-Domenech et al. 2010) had previously used some housekeeping genes (23S rRNA, *gyrB*, *ectBC*, *narH*, *nirS*, and *nosZ*) for studying the family *Halomonadaceae*, the first MLSA study was conducted by de la Haba et al. (2012) based on six phylogenetic markers: 16S rRNA, 23S rRNA, *atpA*, *gyrB*, *rpoD*, and *secA* genes. However, the correlation of DDH values against the MLSA data could not be determined and, therefore, this approach cannot be taken yet as an alternative to DDH for species circumscription.

DNA Fingerprinting Methods

Determination of inter- and intraspecies relatedness by rapid DNA typing methods (AFLP, RAPD, rep-PCR, BOX-PCR, PFGE, ribotyping of ribosomal ribonucleic operons, ARDRA) has not been widely applied to the *Halomonadaceae* except for the studies by Mellado et al. (1998) and Llamas et al. (2002), who applied PFGE, Garriga et al. (1998), who used RAPD, Heyrman et al. (2002), who reported rep-PCR, and Li et al. (2008) who carried out BOX-PCR genomic fingerprinting.

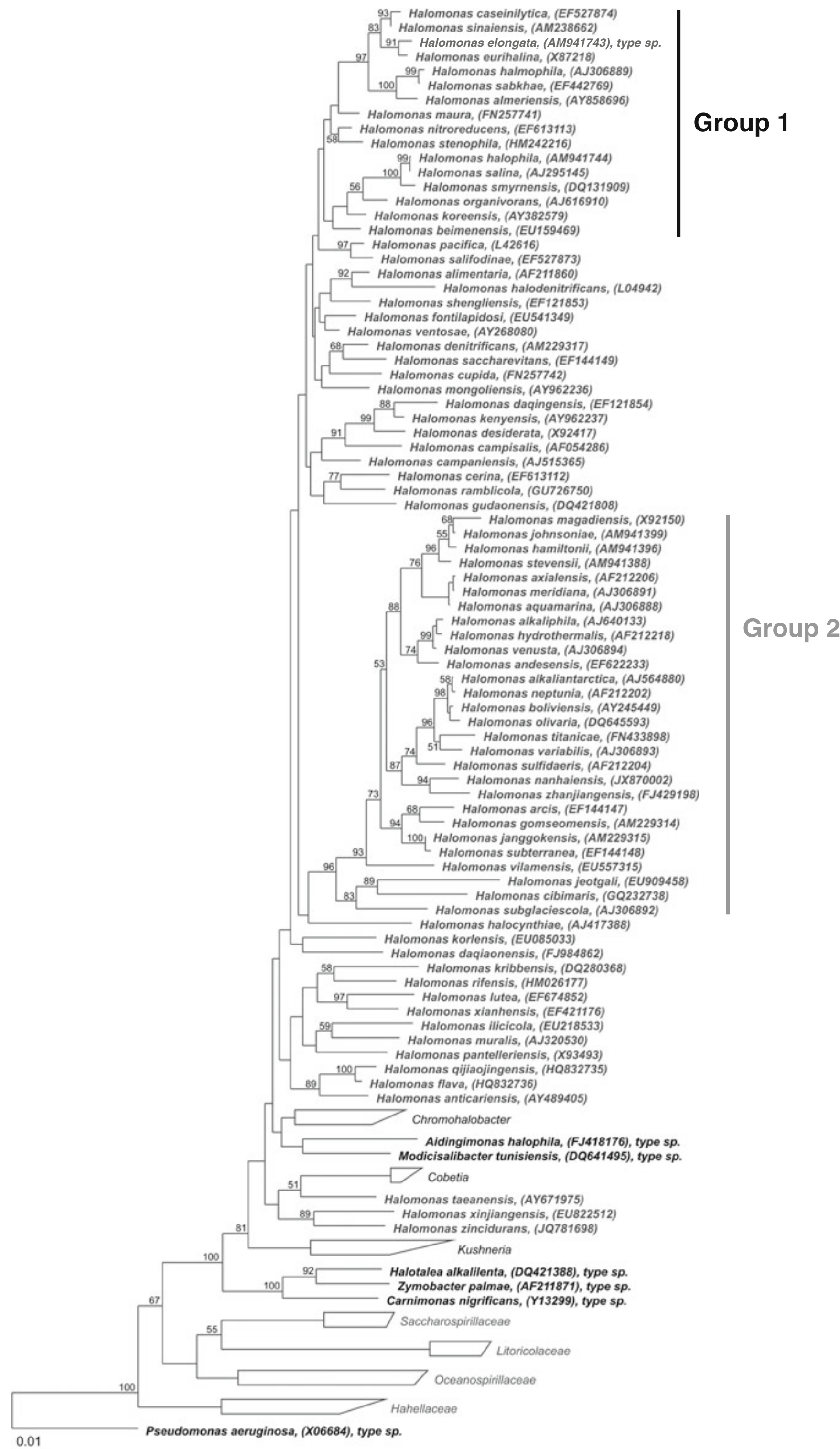

Fig. 17.2

Phylogenetic distribution of the genus *Halomonas* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). 1,000 resampling boostrap values over 50 % are shown. Scale bar indicates estimated sequence divergence

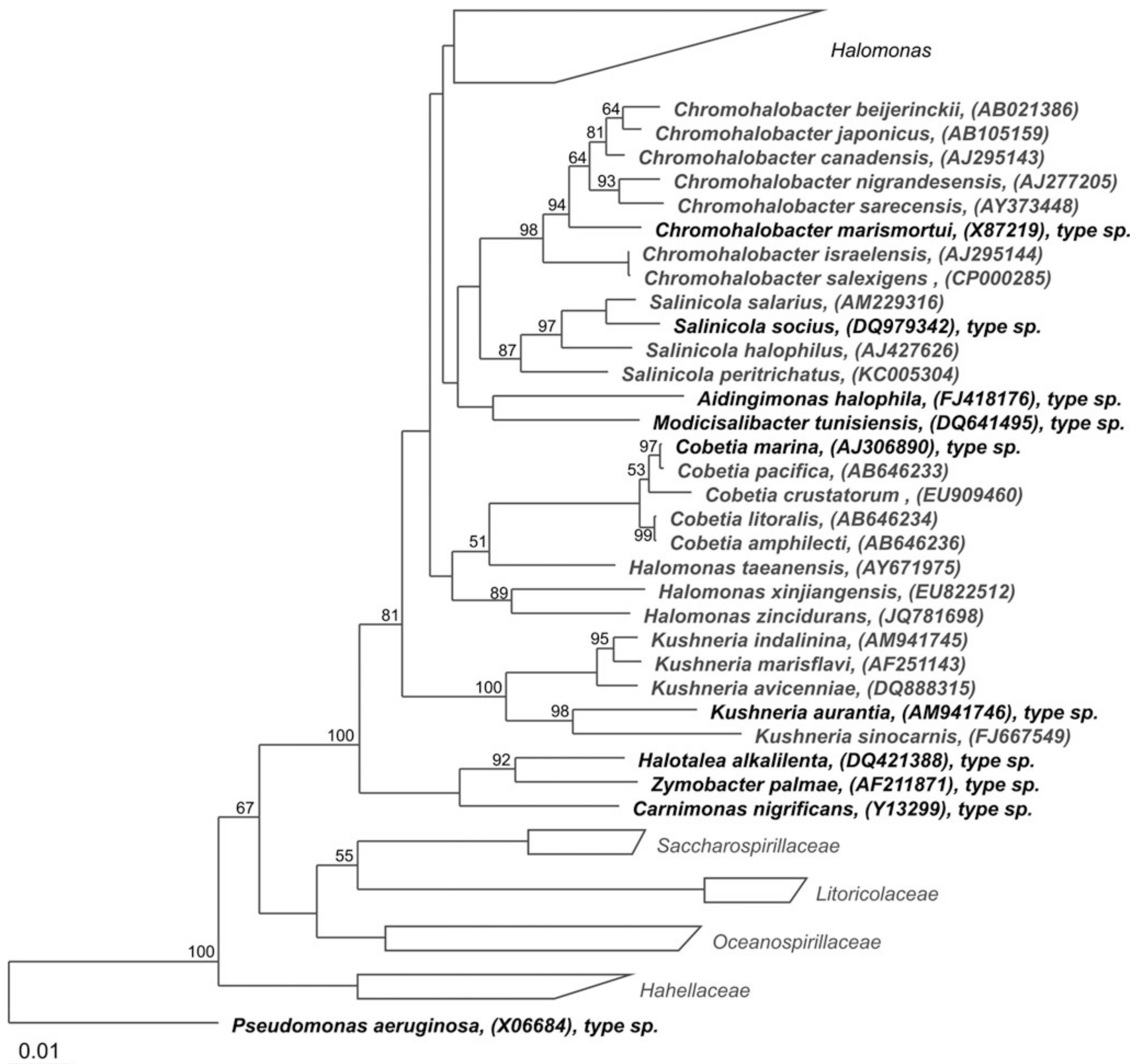

Fig. 17.3

Phylogenetic distribution of genera within the family *Halomonadaceae* other than *Halomonas* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence datasets and alignments were usedaccording to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). 1,000 resampling boostrap values over 50 % are shown. Scale bar indicates estimated sequence divergence

Genes Sequenced and Characterized

Several genes from different members of the family *Halomonadaceae* have been sequenced and characterized, mainly from *Halomonas elongata* (Göller et al. 1998; Grammann et al. 2002; Kraegeloh et al. 2005; Schwibbert et al. 2011) and *Chromohalobacter salexigens* (Cánovas et al. 1998, 2000; Copeland et al. 2011), but also from *Cobetia marina* (Kraiwattanapong et al. 1999), *Halomonas eurihalina* (Llamas et al. 2003), *Halomonas halodenitrificans* (Sakurai and Sakurai 1998; Sakurai et al. 2006), *Halomonas maura* (Llamas et al. 2006), *Halomonas meridiana* (Coronado et al. 2000b), *Halomonas organivorans* (Moreno et al. 2011), *Halomonas salina* (Sripo et al. 2002), and *Zymobacter palmae* (Raj et al. 2002).

Genome Sizes and Plasmid

Before the genomic era, estimations of the genome size of 11 *Halomonas* and *Chromohalobacter* strains were carried out by using pulsed-field gel electrophoresis (Mellado et al. 1998; Llamas et al. 2002; Quesada et al. 2004). Additionally, the presence of plasmids (and megaplasmids) in strains of *Halomonas*, *Chromohalobacter* and *Cobetia* has been investigated (Fernández-Castillo et al. 1992; Vargas et al. 1995; Mellado et al. 1995a; Llamas et al. 1997; Argandoña et al. 2003).

Genome Comparison

As of January 2014, complete or draft genome sequences were available for type strains of the following species: *Carnimonas nigrificans* (JAGO00000000), *Chromohalobacter salexigens* (CP000285), *Halomonas anticariensis* (ASTJ00000000, AUAB00000000), *Halomonas boliviensis* (AGQZ00000000), *Halomonas elongata* (FN869568), *Halomonas halocynthiae* (AUDZ00000000), *Halomonas jeotgali* (AMQY00000000), *Halomonas lutea* (ARKK00000000), *Halomonas smyrnensis* (AJKS00000000), *Halomonas stevensii* (AJTS00000000), *Halomonas titanicae* (AOPO00000000), *Halomonas zhanjiangensis* (ARIT00000000), and *Kushneria aurantia* (ARNK00000000). Although they have not been sequenced yet, the type species of *Halomonas halodenitrificans*, *Halotalea alkalilenta*, and *Zymobacter palmae* are part of the Genomic Encyclopedia of Type Strains, Phase I: the 1,000 microbial genomes (KMG) project.

Additional sequenced strains are: *Halomonas* sp. 23_GOM-1509m (JADJ01000000), *Halomonas* sp. A3H3 (CBRE000000000), *Halomonas* sp. BJGMM-B45 (AVBC00000000), *Halomonas* sp. GFAJ-1 (AHBC00000000), *Halomonas* sp. HAL1 (AGIB00000000), *Halomonas* sp. HTNK1 (Gi05412), *Halomonas* sp. KM-1 (BAEU00000000), and *Halomonas* sp. TD01 (AFQW00000000).

A summary of the genome characteristics of the sequenced species of the family *Halomonadaceae* is presented in ➲ *Table 17.2*.

Phenotypic Analyses

Halomonadaceae Franzmann et al. (1989), emend. Dobson and Franzmann (1996), Ntougias et al. (2007), and Ben Ali Gam et al. (2007)

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

The members of the family *Halomonadaceae* cannot be defined by a reasonably large number of common-to-all features. This phenotypic heterogeneity of the family is also a handicap for the identification at the genus or species level unless a sufficient number of characters are determined. Cells are Gram-negative, straight or curved, rod-shaped. They are either slight or moderate halophiles or halotolerant, except species of the genus *Zymobacter* (Okamoto et al. 1993), growing in the presence of high concentrations of sugars. Aerobic or facultatively anaerobic, chemoorganotrophs (Dobson and Franzmann 1996; Garrity et al. 2005b). Mata et al. (2002) reviewed in depth the phenotypic features of the genus *Halomonas*, and confirmed the enormous diversity among the species of this genus. In addition, they reported a large number of traits not analyzed previously for all strains and found tests that are useful for distinguishing the species of the genus *Halomonas*.

Genotypic diversity within the *Halomonadaceae* is also huge, as indicated for the genomic DNA G+C content, which ranges from 52.0 to 74.3 mol% (Martínez-Cánovas et al. 2004b; Arahal and Ventosa 2006).

From the chemotaxonomic point of view, the fatty acid profile is available for most species described within this family whereas the analysis of respiratory lipoquinones or polar lipids has been addressed for only some of them. There are a set of features shared for the vast majority of the species belonging to the *Halomonadaceae*. The major respiratory lipoquinone is ubiquinone 9 (Q9), although ubiquinone 8 (Q8) and ubiquinone 10 (Q10) are also present in several species. With regards to polar lipid composition all the members possess phosphatidylethanolamine and phosphatidylglycerol (Franzmann and Tindall 1990), except for the species *Aidingimonas halophila*, which contains diphosphatidylglycerol instead of phosphatidylglycerol (Wang et al. 2009). The fatty acid profile notably varies depending on the growing media and conditions, but in general, the main fatty acids are $C_{16:0}$, $C_{18:1}\omega7c$, $C_{16:1}\omega7c$, $C_{19:0}$ cyclo $\omega8c$, $C_{17:0}$ cyclo, and $C_{12:0}$ 3-OH.

➲ *Table 17.3* shows the phenotypic and chemotaxonomic features, as well as the DNA G+C content comparison among the genera of the family *Halomonadaceae*. The type genus of the family is *Halomonas* (Vreeland et al. 1980).

Halomonas Vreeland et al. (1980), emend. Dobson and Franzmann (1996)

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.

Gram-negative, straight or curved, rod-shaped cells, generally 0.6–0.8 × 1.6–1.9 µm, except the species *H. halodenitrificans* that presents coccoid cells. Some species may produce poly-β-hydroxyalcanoates and/or exopolysaccharides. Endospores are not formed. Motile by means of peritrichous, lateral or polar flagella or nonmotile. Colonies are white to yellow, turning light brown with age. Slight to moderate halophiles, that are able to grow in NaCl concentrations ranging from 0.1 % to 32.5 % (w/v). Possess a mainly respiratory type of metabolism with oxygen as the terminal electron acceptor, but some species are also capable of anaerobic growth in the

Table 17.2
Genome characteristics of some members of the family *Halomonadaceae*

Strain	Accession number	Genome size (Mb)	Predicted ORFs	G+C content (mol %)	References
Carnimonas nigrificans ATCC BAA-78[T]	JAGO01000000	2.7	2,500	56.0	Unpublished
Chromohalobacter salexigens 1H11[T]	CP000285	3.7	3,412	63.9	Copeland et al. (2011)
Halomonas anticariensis FP35[T] *Halomonas anticariensis* DSM 16096[T]	ASTJ00000000 AUAB00000000	5.1 5.0	4,652 4,807	58.5 58.5	Tahrioui et al. (2013a) Unpublished
Halomonas boliviensis LC1[T]	AGQZ00000000	4.2	3,915	54.7	Guzmán et al. (2012)
Halomonas elongata DSM 2581[T]	FN869568	4.1	3,556	63.6	Schwibbert et al. (2011)
Halomonas halocynthiae DSM 14573[T]	AUDZ00000000	2.9	2,772	53.8	Unpublished
Halomonas jeotgali Hwa[T]	AMQY00000000	2.8	2,636	62.9	Unpublished
Halomonas lutea DSM 23508[T]	ARKK00000000	4.5	4,368	59.1	Unpublished
Halomonas smyrnensis AAD6[T]	AJKS00000000	3.6	3,326	67.9	Sogutcu et al. (2012)
Halomonas sp. 23_GOM-1509m	JADJ01000000	5.4	5,025	54.6	Unpublished
Halomonas sp. A3H3	CBRE000000000	5.6	5,279	55.6	Unpublished
Halomonas sp. BJGMM-B45	AVBC00000000	4.8	4,209	58.5	Unpublished
Halomonas sp. GFAJ-1	AHBC00000000	3.6	3,347	53.9	Phung et al. (2012)
Halomonas sp. HAL1	AGIB00000000	4.4	4,212	54.1	Lin et al. (2012)
Halomonas sp. HTNK1	Gi05412	4.4	4,221	53.0	Unpublished
Halomonas sp. KM-1	BAEU00000000	5.0	4,685	64.1	Kawata et al. (2012)
Halomonas sp. TD01	AFQW00000000	4.1	3,889	52.6	Cai et al. (2011)
Halomonas stevensii S18214[T]	AJTS00000000	3.7	3,523	60.2	Kim et al. (2012)
Halomonas titanicae BH1[T]	AOPO00000000	5.3	3,314	54.6	Sánchez-Porro et al. (2013)
Halomonas zhanjiangensis DSM 21076[T]	ARIT00000000	4.1	3,739	54.5	Unpublished
Kushneria aurantia DSM 21353[T]	ARNK00000000	3.8	3,598	62.8	Unpublished

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). Some species reduce nitrate to nitrite; nitrogen gas is not formed. Catalase positive and most of them are also oxidase positive. Chemoorganotrophic. Carbohydrates, organic acids, polyols, and amino acids, can be used as sole carbon and energy sources or as sole carbon, nitrogen and energy sources (Vreeland et al. 1980; Dobson and Franzmann 1996; Vreeland 2005).

The major respiratory lipoquinone is ubiquinone 9 (Vreeland 2005), with the exception of *H. alkaliphila* that mainly possess ubiquinone 8 and ubiquinone 6 (Romano et al. 2006). The major fatty acids are $C_{16:1}\omega7c$, $C_{17:0}$ cyclo, $C_{16:0}$, $C_{18:1}\omega7c$, and $C_{19:0}$ cyclo $\omega8c$ (Vreeland 2005). The predominant polar lipids are phosphatidylethanolamine and phosphatidylglycerol.

DNA G+C content ranges between 52.0 and 74.3 mol% (Martínez-Cánovas et al. 2004b; Arahal and Ventosa 2006), demonstrating the enormous heterogeneity within this genus.

Halomonas is the type genus of the family *Halomonadaceae*. The species *Halomonas elongata* is the type species of the genus.

Aidingimonas Wang et al. (2009)

Ai.ding.i.mo'nas. N.L. n. *Aiding* a lake located in Xinjiang province of north-west China; L. fem. n. *monas*, *monad* a unit, a monad; N.L. fem. n. *Aidingimonas* a monad from Aiding Lake.

Cells are Gram-negative, facultatively anaerobic, non-endospore-forming, short rods. Non-motile, without flagella. Moderately halophilic. Positive for catalase activity. Negative for oxidase activity and nitrate reduction. Ubiquinone 9 is present. Major fatty acids are $C_{19:0}$ cyclo $\omega8c$ and $C_{16:0}$.

Table 17.3
Phenotypic and chemotaxonomic characteristics and DNA G+C content of genera of the family *Halomonadaceae*

Characteristic	*Aidingimonas*	*Carnimonas*	*Chromohalobacter*	*Cobetia*	*Halomonas*	*Halotalea*	*Kushneria*	*Modicisalibacter*	*Salinicola*	*Zymobacter*
Oxidase	–	+	D	–	D	+	–	–	D	–
Motility	–	–	+	D	D	+	D	+	+	+
Acid production from:										
L-arabinose	+	–	ND	–	D	–	D	ND	ND	ND
D-fructose	+	+	ND	+	D	+	+	ND	+	ND
D-glucose	+	+	+	+	D	+	+	ND	+	ND
Lactose	+	ND	+	–	D	–	D	ND	+	ND
Maltose	+	+	+	D	D	+	D	ND	+	ND
D-mannitol	–	+	ND	D	D	–	D	ND	–	ND
D-mannose	ND	+	+	+	D	+	D	ND	+	ND
D-melezitose	–	–	ND	–	D	ND	–	ND	–	ND
Sucrose	–	+	D	D	D	–	D	ND	–	ND
Hydrolysis of:										
DNA	ND	–	–	D	D	–	–	ND	D	ND
Starch	–	+	–	–	D	–	–	ND	D	–
Casein	–	–	D	D	D	–	–	ND	D	ND
Aesculin	–	+	–	D	D	ND	D	–	–	ND
Indole production	–	–	D	–	–	–	–	–	–	–
Methyl red	–	ND	D	–	D	–	D	ND	+	+
Voges-Proskauer	V	–	–	–	D	–	–	ND	–	+
Citrate utilization	+	+	D	–	D	+	+	–	+	–
Arginine dihydrolase	+	–	ND	–	D	–	–	–	ND	–
Lysine decarboxylase	–	ND	D	–	–	–	–	ND	–	–
Ornithine decarboxylase	–	ND	D	–	D	–	D	ND	–	–
H_2S production	–	ND	D	–	D	–	–	–	D	ND
Nitrate reduction	–	–	D	–	D	–	D	+	D	–
Phosphatase	+	ND	D	+	D	ND	+	+	+	ND
Urease	+	–	D	D	D	–	–	–	D	ND
Lecithinase	ND	–	ND	V	D	ND	ND	ND	ND	ND
Phenylalanine deaminase	–	–	–	–	D	–	D	ND	–	–

ONPG	–	+	ND	+	D	ND	+	–	–	–
O/F (D-glucose)	ND	ND	ND	O	D	ND	O	ND	F	F
Growth on:										
MacConkey agar	ND	+	ND	+	+	ND	D	ND	ND	ND
Cetrimide agar	ND	+	ND	+	D	ND	ND	ND	ND	ND
Utilization of:										
Starch	ND	ND	+	–	D	ND	–	ND	D	ND
L-arabinose	+	–	ND	–	D	ND	+	ND	ND	ND
Aesculin	ND	ND	+	D	D	ND	–	ND	D	ND
D-fructose	+	ND	D	+	D	+	+	+	D	ND
D-glucose	+	ND	+	+	D	+	+	+	D	ND
Lactose	+	ND	+	–	D	–	D	ND	D	ND
Maltose	+	+	D	+	D	+	+	–	D	ND
D-mannose	+	+	+	D	D	ND	+	–	D	ND
Ribose	+	ND	D	D	D	ND	+	–	D	ND
Sucrose	+	ND	+	D	D	+	D	ND	D	ND
D-xylose	ND	ND	+	–	D	+	D	ND	D	ND
Glycerol	+	ND	+	+	D	+	+	ND	+	ND
myo-inositol	+	ND	D	D	D	ND	–	ND	D	ND
D-mannitol	+	–	+	D	D	+	D	ND	+	ND
Sorbitol	+	ND	D	–	D	+	D	ND	+	ND
Gluconate	ND	+	+	D	D	ND	+	ND	ND	ND
Propionate	ND	ND	D	+	D	ND	–	ND	+	ND
Succinate	+	ND	D	+	D	+	+	ND	–	ND
Polar lipids	DPG, PE	DPG, PG, PE	DPG, PG, PE	PE, PG	PE, PG	ND	PG, DPG, PE	ND	PG, PE	ND
Respiratory quinones	Q9	Q9	Q9, Q8	Q9, Q8	Q9, Q8	Q9	Q9, Q8, Q10	ND	Q9, Q10, Q8	Q9
Fatty acids	$C_{19:0}$ cyclo, $C_{16:0}$	$C_{16:0}$, $C_{16:1}$, $C_{18:1}$, $C_{19:0}$ cyclo	$C_{16:0}$, $C_{19:0}$ cyclo, $C_{18:1}$, $C_{17:0}$ cyclo, $C_{12:0}$ 3-OH	$C_{16:1}$, $C_{16:0}$, $C_{12:0}$ 3-OH $C_{18:1}$, $C_{17:0}$ cyclo,	$C_{16:1}$, $C_{17:0}$ cyclo, $C_{16:0}$, $C_{18:1}$, $C_{19:0}$ cyclo	$C_{18:1}$, $C_{16:0}$, $C_{19:0}$ cyclo, $C_{12:0}$ 3-OH, $C_{16:1}$/iso-$C_{15:0}$ 2-OH	$C_{16:0}$, $C_{18:1}$, $C_{19:0}$ cyclo, $C_{12:0}$ 3-OH, $C_{17:0}$ cyclo	$C_{16:0}$, $C_{18:1}$, $C_{16:1}$, $C_{19:0}$ cyclo, $C_{17:0}$	$C_{16:1}$, $C_{16:0}$, $C_{18:1}$, $C_{19:0}$ cyclo, $C_{12:0}$ 3-OH	$C_{16:0}$, $C_{19:0}$ cyclo, $C_{18:1}$, $C_{12:0}$ 3-OH
DNA G+C content (mol%)	57.2–57.5	56.0	56.1–66.0	61.4–64.2	52.0–74.3	64.4	59.0–61.7	53.7	58.8–63.6	55.4–56.2

Data from original descriptions and from Franzmann and Tindall (1990) and Arahal and Ventosa (2006). + positive, – negative, *V* variable among different studies, *D* different results for species belonging to the same genus, *ONPG* ortho-nitrophenyl-β-D-galactopyranoside, *O* oxidative, *F* fermentative, *ND* no data available, *DPG* diphosphatidylglycerol, *PE* phosphatidylethanolamine, *PG* phosphatidylglycerol

The polar lipid pattern consists of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides, two unknown phospholipids, two unknown phosphoglycolipids and one unknown glycolipid. The DNA G+C content is 57.2–57.5 mol% (Wang et al. 2009).

The only species within this genus is *Aidingimonas halophila*, which was isolated from a salt lake in Xinjiang province, northwest China. Cell size ranges between 0.1–0.3 × 0.7–1.5 μm. It forms colourless to yellow brown colonies, flat and opaque with slightly irregular edges. Growth occurs at 10–45 °C, at pH 5.0–10.0 and in 1–25 % (w/v) NaCl, with optimal growth at 37 °C, pH 7.0–8.0 and 5–10 % NaCl. Does not contain poly-β-hydroxybutyrate granules or produce exopolysaccharide. Growth occurs under anoxic conditions in the presence of nitrate ion as electron acceptor. The Voges–Proskauer test is variable. Indole and H_2S are not produced. Milk peptonization and coagulation and the methyl red test are negative. Gelatin, aesculin, casein, starch, and Tweens 40, 60 and 80 are not hydrolysed, but positive for hydrolysis of Tween 20 and urea. ONPG, phenylalanine deaminase and lysine and ornithine decarboxylase tests are negative, but positive for arginine dihydrolase. Citrate and other substrates can be utilized as a sole carbon or nitrogen and energy sources. Acid is produced from different carbohydrates and organic acids. The type strain is YIM 90637^T, with a DNA G+C content of 57.5 mol% (Wang et al. 2009).

Carnimonas Garriga et al. (1998)

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 endospores. Nonmotile. Oxidase and catalase positive. Aerobic, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. Slightly or moderate halophile. No growth occurs in the presence of more than 8 % (w/v) NaCl. 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, melibiose, maltose and sucrose. β-galactosidase (ONPG) activity occurs. Forms dark spots on the surface of raw, cured meat products. 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 $C_{19:0}$ cyclo. The G+C content of the DNA is 56.0 mol% (Garriga et al. 1998; 2005).

The type and the single species within this genus is *Carnimonas nigrificans*. In the species description a total of nine strains, CTCBS1^T to CTCBS9 were reported to be isolated from cured meat products. Colonies are non-pigmented, white, convex, shiny and circular. Aesculin and starch are hydrolysed. Gelatin, casein and DNA are not hydrolysed. Voges-Proskauer negative. Arginine dihydrolase, urease, lecithinase and phenylalanine deaminase negative. Indole is not produced. Nitrate is not reduced (Garriga et al. 1998; 2005).

Chromohalobacter Ventosa et al. (1989) emend. Arahal et al. (2001a)

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 (0.4–1.2 × 0.8–6.1 μm). Motile by polar or peritrichous flagella. Cells occur singly, in pairs, and in short chains. Colonies are cream to brown-yellow pigmented, with the exception of *C. nigrandesensis* that shows black pigmentation. Endospores 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 0–45 °C (optimal 30–37 °C) and pH 5.0–10.0 (optimal pH 7.5), respectively. Aerobic. Chemoorganotrophic. Catalase positive. Oxidase negative, with the exception of *C. beijerinckii* (Peçonek et al. 2006) and *C. sarecensis* (Quillaguamán et al. 2004a). Some strains reduce nitrates, but H_2S and urease are not produced, with the exception of *C. nigrandesensis* (Prado et al. 2006) and *C. salexigens* (Arahal et al. 2001b). Phenylalanine deaminase test is negative. Starch, Tween 80, aesculin, DNA, and tyrosine are not hydrolyzed. The species *C. japonicus* is the only able to hydrolyse gelatin (Sánchez-Porro et al. 2007). Acid is produced aerobically from D-glucose and other carbohydrates. Carbohydrates, amino acids, and some polyols can serve as sole carbon or nitrogen sources (Arahal et al. 2001a; Ventosa 2005).

Predominant fatty acids are $C_{16:0}$, $C_{19:0}$ cyclo $\omega 8c$, and $C_{18:1}$ $\omega 7c$; $C_{17:0}$ cyclo, and $C_{12:0}$ 3-OH are present in smaller amounts. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and two unknown phospholipids are major to moderate compounds in the polar lipid profile. The quinone system consists of the major compound Q-9 (>95 %) and small amounts of Q-8 (Peçonek et al. 2006; Sánchez-Porro et al. 2007). The DNA G+C base composition ranges from 56.1 to 66.0 mol%.

The type species of the genus is *Chromohalobacter marismortui*, previously named as "*Chromobacterium marismortui*", which was originally isolated from the Dead Sea (Ventosa et al. 1989).

Cobetia Arahal et al. (2002b) emend. Romanenko et al. (2013)

Co.be'ti.a. N.L. fem. n. *Cobetia* named after A. B. Cobet, who originally described the type species as "*Arthrobacter marinus*".

Gram-negative, straight, rod-shaped cells that are 1.6–4.0 × 0.8–1.2 μm, and occur singly and in pairs. Strains are non-motile or motile by means of a single polar flagellum and/or two to

seven lateral flagella. Some strains can produce fimbria-like structures and capsules. Colonies are round, bright, smooth and cream pigmented. Poly-β-hydroxyalkanoate is accumulated. Oxidase negative. Aerobic; unable to grow anaerobically in the presence of nitrate or arginine. Sodium ions are not essential for growth. Most strains can grow without addition of NaCl to the medium, but optimal growth occurs in the presence of 5–6 % (w/v) salts and they can considered as slightly halophiles. Good growth is also obtained up to 20 % (w/v) but not at higher salinities. Hydrolyses tyrosine, but not gelatine, starch or Tween 80. Hydrolysis of casein, DNA and aesculin is strain-dependent (negative reaction for most strains). Negative for chitin hydrolysis and Simmons' citrate test. ONPG positive. Phosphatase positive. Negative for phenylalanine deaminase, methyl red, indole production, and nitrate reduction. H_2S is not produced. Acid is produced from glucose and other carbohydrates (Arahal et al. 2002a; Romanenko et al. 2013).

Polar lipids include phosphatidylethanolamine, phosphatidylglycerol, unknown phospholipids, unknown lipids, an unknown aminolipid and phosphatidic acid. The major fatty acids are $C_{16:1}$ $\omega 7c$, $C_{16:0}$, $C_{12:0}$ 3-OH, $C_{18:1}$ $\omega 7c$ and $C_{17:0}$ cyclo. The DNA G+C content varies between 61.4 and to 64.2 mol% (Kim et al. 2010b; Romanenko et al. 2013).

The species *Cobetia marina* is the type of the genus and strain DSM 4741^T is the type strain of this species. Strain DSM 5160, formerly the type strain of the species *Halomonas halodurans*, has been proposed as member of *C. marina* based on phylogenetic analysis and DNA-DNA hybridization and chemotaxonomic characteristics (Romanenko et al. 2013).

Halotalea Ntougias et al. (2007)

Ha.lo.ta.le'a. Gr. n. *hals halos* salt; L. fem. n. *talea* a staff, rod; N.L. fem. n. *Halotalea* rod-shaped cells living in saline conditions.

Cells are Gram-negative, rods, motile by peritrichous flagella and forming small, non-pigmented pale yellow colonies. Endospores are not formed. Strictly aerobic. Halotolerant and alkalitolerant. Sugar-tolerant. Oxidase- and catalase-positive. Chemo-organotrophic. Ubiquinone-9 is present in the respiratory chain. The major fatty acids are $C_{18:1}$ $\omega 7c$, $C_{16:0}$, $C_{19:0}$ cyclo $\omega 8c$, $C_{12:0}$ 3-OH and $C_{16:1}$ $\omega 7c$/iso-$C_{15:0}$ 2-OH. The DNA G+C content is 64.4 mol% (Ntougias et al. 2007).

The type species is *Halotalea alkalilenta*, which tolerates up to 15 % (w/v) NaCl, with an optimum salt concentration of 0–3 % (w/v) NaCl. Tolerates up to 45 % and 60 % w/v (+)-D-glucose and maltose, respectively. Grows at pH 5–11, with optimum at pH 7. The temperature range for growth is 5–45 °C, with an optimum temperature of 32–37 °C. Negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, phenylalanine deaminase, nitrate reduction, H_2S production from L-cysteine, indole production, methyl red and Voges-Proskauer. Acid is produced from (+)-D-glucose and other carbohydrates. Hydrolyses Tween 20, but does not hydrolyse casein, DNA, gelatin, starch, Tween 80 or urea. The type strain is AW-7^T, isolated from olive mill waste (alkaline alpeorujo) obtained from the premises of the Toplou Monastery in the region of Sitia, Crete, Greece (Ntougias et al. 2007).

Kushneria Sánchez-Porro et al. (2009)

Kush.ne'ri.a. N.L. fem. n. *Kushneria* from the name Kushner, honouring Dr Donn J. Kushner, a Canadian microbiologist who carried out pioneering studies on halophilic micro-organisms.

Cells are Gram-negative, motile rods (0.5–2.0 × 1.7–5.0 μm). Endospores are not formed. Strictly aerobic. Colonies are yellow-orange to cream. Moderately halophilic; Na^+ is required for growth. The optimal NaCl range supporting the growth is 0.5–12 % (w/v). Mesophilic (able to grow from 4 to 42° C, optimum at 25–37° C). Growth occurs at pH 4.5–10.0 (optimally at pH 7.0–8.0). Chemo-organotrophic. Catalase-positive and oxidase-negative. Aesculin and gelatin are hydrolysed, with the exception of *Kushneria sinocarnis* that does not possess gelatinase activity (Zou and Wang 2010). Casein, starch, Tween 80, DNA, and tyrosine are not hydrolysed. Indole and H_2S production, and Voges-Proskauer test are negative. Phosphatase is produced, but urease, arginine dihydrolase, and lysine decarboxylase are not. Except *K. indalinina* (Cabrera et al. 2007) and *K. sinocarnis* (Zou and Wang 2010), the other species do not reduce nitrate to nitrite. The major respiratory quinone is Q9. Major fatty acids are $C_{16:0}$, $C_{18:1}$ $\omega 7c$, $C_{19:0}$ cyclo $\omega 8c$, $C_{12:0}$ 3-OH, and $C_{17:0}$ cyclo. Polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and unidentified phospholipids and glycolipids. The DNA G+C content is 59.0–61.7 mol% (Yoon et al. 2001; Cabrera et al. 2007; Soto-Ramírez et al. 2007; Sánchez-Porro et al. 2009; Zou and Wang 2010).

The type species of the genus is *Kushneria aurantia*, with the type strain A10^T isolated from the leaf surface of *Avicennia germinans* (black mangrove).

Modicisalibacter Ben Ali Gam et al. (2007)

Mo'di.ci.sa'li.bac'ter. L. adj. *modicus* moderate, limited; L. n. *sal, salis* salt; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Modicisalibacter* a moderately halophilic rod.

Cells are Gram-negative, non-endospore-forming, motile rods. Moderately halophilic. Strictly aerobic and require Na^+ for growth. Mesophilic, growing well at 15–45 °C, oxidase-negative and reduce nitrate. Predominant fatty acids are $C_{16:0}$, $C_{18:1}$ $\omega 7c$, $C_{16:1}$ $\omega 7c$, $C_{19:0}$ cyclo $\omega 8c$ and $C_{17:0}$. Contents of $C_{19:0}$ cyclo $\omega 8c$ and $C_{17:0}$ differ significantly from those of other members of the *Halomonadaceae*. The DNA G+C content is 53.7 mol% (Ben Ali Gam et al. 2007).

The genus contains a single species, namely *Modicisalibacter tunisiensis*, whose cells are approximately 1.0–4.0 μm long × 0.6–1.0 μm wide. Colonies on marine agar are circular, smooth, convex and 2–3 mm in diameter after 48 h of incubation at

37 °C. Cells grow at 4–45 °C, with optimum at 37 °C. The pH range for growth is 5–10, with an optimum at pH 7.2. Growth occurs in the range 0.1–25 % (w/v) NaCl and optimally at 10 % (w/v) NaCl. Catalase reaction is positive. ONPG hydrolysis is negative. Citrate is not utilized. Urease and arginine dihydrolase are not produced. Gelatin, alginate and aesculin are not hydrolysed. H_2S and indole are not produced. D-glucose, D-fructose, tryptone, peptone and Casamino acids are utilized. The type strain is LIT2^T, which was isolated from a sample of oilfield-water injection collected in the Sidi Litayem area near Sfax, Tunisia (Ben Ali Gam et al. 2007).

Salinicola Anan'ina et al. (2008)

Sa.li.ni.co'.la. L. fem. pl. n. *salinae* salterns, salt-works; N.L. suff. *-cola*, derived from *incola*, inhabitant; N.L. masc. n. *Salinicola*, inhabitant of salterns.

Cells are Gram-negative, non-endospore-forming rods (0.5–1.3 × 1.0–3.2 μm). Motile by means of a single lateral/polar flagellum or by peritrichous flagella (Huo et al. 2013). Colonies are circular, smooth, convex and creamy yellow-coloured. Aerobic and chemoorganotrophic. Moderately halophilic that grows in the range of 0–30 % (w/v) NaCl with the optimum of 0.5–20 % (w/v) NaCl, at pH 4.5–10.0 (optimum pH 5.0–8.0). Mesophilic, growing in the range 4–45 °C (optimal growth temperature is 25–37 °C). Exopolysaccharides are not produced, but the species *Salinicola salarius* and *S. socius* produce poly-β-hydroxyalkanoate (de la Haba et al. 2010b). Respiration on fumarate, nitrate and nitrite is negative. Shows positive reaction in the oxidation/fermentation of D-glucose. Catalase reaction is positive, oxidase reaction is negative, except for *S. salarius* (Kim et al. 2007). Tween 20 is hydrolysed. Tyrosine and aesculin are not hydrolysed. β-Galactosidase is not produced. Phosphatase activity is present. For all species, except *S. peritrichatus*, methyl red test is positive and Voges-Proskauer is negative (de la Haba et al. 2010b; Huo et al. 2013). Indole is not produced. Lysine- and ornithine-decarboxylases and phenylalanine deaminase are negative. Gluconate is not oxidized. Selenite is not reduced. The predominant respiratory lipoquinone is ubiquinone with nine isoprene units (Q9). The major fatty acids are $C_{16:1}$ $\omega7c$, $C_{16:0}$, $C_{18:1}$ $\omega7c$, $C_{19:0}$ cyclo $\omega8c$, and $C_{12:0}$ 3-OH. The DNA G+C content ranges between 58.8 and 63.6 mol% (Anan'ina et al. 2007; Kim et al. 2007; Aguilera et al. 2007; de la Haba et al. 2010b; Huo et al. 2013).

The type species of the genus is *Salinicola socius*, isolated from the microbial community obtained from the soil of salt mines (Berezniki, Perm region of Russia), which grows on naphthalene as a sole carbon and energy source. The other three species comprising the genera are *S. halophilus* (formerly *Chromohalobacter salarius*), *S. peritrichatus*, and *S. salarius* (basonym of *Halomonas salaria*). The species name *S. zeshunii* (Cao et al. 2013) has been effectively, but not validly published.

Zymobacter Okamoto et al. (1995)

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

Non-endospore forming rod-shaped cells with rounded ends, 1.3–2.4 × 0.7–0.9 μm; usually single. Motile by as many as 20 peritrichous flagella that are non-sheathed. Gram-negative. Facultatively anaerobic. Chemoorganotrophic. Grow on and ferment 1 mol of glucose or hexose moiety of maltose to produce approximately 2 mol each of ethanol and CO_2, with a trace amount of acids. Ferments hexoses, α-linked di- and tri-saccharides and sugar alcohols. Growth initiates at pH values of 4.7–8.1. Catalase-positive and oxidase-negative. The major cellular fatty acids are $C_{16:0}$, $C_{19:0}$ cyclo, $C_{18:1}$ $\omega9c$, and $C_{12:0}$ 3-OH. Quinone system is ubiquinone-9. The DNA G+C base composition ranges from 55.4 to 56.2 mol% (Okamoto et al. 1993, 2005).

Currently, the genus include a single species, *Zymobacter palmae*, with the type strain T109^T isolated from palm sap. Colonies are round, entire, smooth, opaque, and milky white. Colonies of similar size develop under aerobic and anaerobic growth conditions. Growth is better in static cultures than in shaken cultures. Requires nicotinic 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, pH 6.0). The organisms are neither halophilic nor halotolerant, but they are considerably tolerant to ethanol and produce ethanol mainly from maltose (5.8 % ethanol after 6 days of fermentation), but also from glucose, fructose, sucrose, melibiose, raffinose, sorbitol, mannitol, and –depending on the strain– mannose and galactose. Methyl red, Voges-Proskauer and α-glucosidase are positive. The following tests are negative: indole production, utilization of citrate, nitrate reduction, chromogenicity, gelatin liquefaction, hydrolysis of starch, phenylalanine deaminase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and β-galactosidase (Okamoto et al. 1993, 2005).

Isolation and Maintenance Procedures

Isolation

Halomonads may be isolated following standard microbiological techniques on complex defined media with a suitable salinity and incubated at room temperature for 1–7 days.

Since not all species show the same requirements (or tolerance) of salinity (➋ *Table 17.4*), differences are expected to occur depending on the final salt content of the media. With the only exception of *Zymobacter palmae*, all species are able to grow at salinities in the range of 5–10 % but not necessarily at their optimum over the whole range. Media for the selective isolation of moderately halophilic bacteria can be prepared with higher salt contents (for instance 20 %) to prevent the growth of

non-adapted competitors. The selectivity of such media can be increased by lowering the concentration of Mg^{2+} to prevent the growth of extremely halophilic archaea (Ventosa et al. 1982). However, not all the species within the family *Halomonadaceae* are able to grow at such high salinities.

The growth of species of *Aidingimonas*, *Chromohalobacter*, some *Halomonas* (such as *H. alkaliantarctica*, *H. alkaliphila*, *H. almeriensis*, *H. anticariensis*, *H. aquamarina*, *H. beimenensis*, *H. campaniensis*, *H. cerina*, *H. cibimaris*, *H. cupida*, *H. daqiaonensis*, *H. daqingensis*, *H. denitrificans*, *H. eurihalina*, *H. fontilapidosi*, *H. gomseomensis*, *H. gudaonensis*, *H. halmophila*, *H. halodenitrificans*, *H. halophila*, *H. ilicicola*, *H. janggokensis*, *H. jeotgali*, *H. korlensis*, *H. lutea*, *H. maura*, *H. olivaria*, *H. organivorans*, *H. pantelleriensis*, *H. sabkhae*, *H. shengliensis*, *H. sinaiensis*, *H. smyrnensis*, *H. stenophila*, *H. subglaciescola*, *H. taeanensis*, *H. variabilis*, *H. ventosae*, *H. vilamensis*, and *H. xinjiangensis*), and the species *Kushneria aurantia*, *K. indalinina*, *K. sinocarnis*, *Modicisalibacter tunisiensis*, *Salinicola halophilus*, and *S. salarius* will be favored in media with a salt content of 7.5–10 %. Some of them will yield reasonable growth at 15 % salts or even at 20 %. At these concentrations, growth of extremely halophilic *Archaea* can occur, but they are easily distinguished by their red pigmentation. Most of the above-mentioned species have a minimum requirement for NaCl of 0.5–5 %.

Other species (*Cobetia amphilecti*, *Cob. crustatorum*, *Cob. litoralis*, *Cob. marina*, *Cob. pacifica*, *Halomonas alimentaria*, *H. boliviensis*, *H. campisalis*, *H. elongata*, *H. flava*, *H. hamiltonii*, *H. hydrothermalis*, *H. koreensis*, *H. kribbensis*, *H. mongoliensis*, *H. muralis*, *H. nitroreducens*, *H. qijiaojingensis*, *H. ramblicola*, *H. rifensis*, *H. saccharevitans*, *H. salina*, *H. stevensii*, *H. xianhensis*, *H. zincidurans*, *K. avicenniae*, *K. marisflavi*, and *Salinicola socius*) are also moderately halophilic, but their optimum salinity for growth is lower (around 5 %). Most of these species show no requirement of salts or require only as little as 0.5–1 %.

The slightly halophilic species (*Halomonas andesensis*, *H. arcis*, *H. axialensis*, *H. caseinilytica*, *H. desiderata*, *H. halocynthiae*, *H. johnsoniae*, *H. kenyensis*, *H. magadiensis*, *H. meridiana*, *H. nanhaiensis*, *H. neptunia*, *H. pacifica*, *H. salifodinae*, *H. subterranea*, *H. sulfidaeris*, *H. titanicae*, *H. venusta*, *H. zhanjiangensis*, *Halotalea alkalilenta* and *S. peritrichatus*) grow optimally at around 3 % NaCl. However, these species are halotolerant of up to 15–20 % NaCl (25 % in the case of *H. neptunia* and *H. titanicae* and 10 % in the case of *H. kenyensis*).

Carnimonas nigrificans shows only a limited level of halotolerance. For its isolation, two selective media have been proposed (Garriga et al. 1998), cetrimide agar and MacConkey agar, although it may grow in other media such as tryptone soy agar. Although the organism is not pigmented, it produces black spots on the surface of cured meat products (the isolation source). This coloration seems to be the consequence of nonenzymatic reactions of substances derived from the meat surface.

On the contrary, *Zymobacter palmae* is neither halophilic nor halotolerant, but other special features of this organism can be used for its isolation. Thus, Okamoto et al. (1993) first selected ethanol-tolerant bacteria using media with 5 % (v/v) ethanol and then tested isolates for production of ethanol from maltose. *Zymobacter palmae* was found to produce ethanol from the fermentation of a variety of sugars: hexoses, α-linked di- and tri-saccharides, and sugar alcohols (fructose, galactose, glucose, mannose, maltose, melibiose, saccharose, raffinose, mannitol and sorbitol).

Regarding pH, most species are neutrophilic and therefore the pH of media can be adjusted to around 7.2–7.5. Exceptions to this rule are *H. alkaliantarctica*, *H. alkaliphila*, *H. campaniensis*, *H. campisalis*, *H. daqingensis*, *H. desiderata*, *H. kenyensis*, *H. korlensis*, *H. magadiensis*, *H. mongoliensis*, and *H. pantelleriensis* that grow optimally at pH 9.0 or 9.5 or *Cobetia crustatorum*, *Salinicola peritrichatus*, and *Zymobacter palmae*, with optimal growth at pH 5 or 6 (➋ *Table 17.4*).

Finally, although all halomonads are mesophilic, some differences in their behavior towards temperature can be used for selective isolation. *Aidingimonas halophila*, *Chromohalobacter japonicus*, *C. marismortui*, *C. salexigens*, *Cobetia amphilecti*, *C. litoralis*, *C. marina*, *C. pacifica*, *Halomonas alkaliphila*, *H. beimenensis*, *H. campaniensis*, *H. desiderata*, *H. elongata*, *H. flava*, *H. fontilapidosi*, *H. halophila*, *H. ilicicola*, *H. kenyensis*, *H. lutea*, *H. magadiensis*, *H. meridiana*, *H. mongoliensis*, *H. organivorans*, *H. qijiaojingensis*, *H. ramblicola*, *H. rifensis*, *H. sabkhae*, *H. smyrnensis*, *H. titanicae*, *H. xinjiangensis*, *Halotalea alkalilenta*, *Kushneria aurantia*, *K. sinocarnis*, *Modicisalibacter tunisiensis*, *Salinicola peritrichatus*, and *S. socius* grew well at 37 °C while the rest grew only suboptimally (or not at all). Temperatures around 30 °C fit all known species so far. In the lower limit most species show no apparent growth below 4–15 °C (20 °C in the case of *H. anticariensis*, *H. flava*, *H. magadiensis*, *H. qijiaojingensis*, *Kushneria aurantia*, and *Z. palmae*; 25 °C in the case of *H. ilicicola*, *H. rifensis*, and *H. sinaiensis*; 30 °C in the case of *H. sabkhae*), but *Chromohalobacter sarecensis*, *H. boliviensis*, *H. subglaciescola* are capable of growing at 0 °C, and *H. axialensis*, *H. neptunia*, *H. sulfidaeris* at −1 °C. With respect to the upper limit, the species *H. alkaliphila*, *H. beimenensis*, *H. campisalis*, *H. daqingensis*, *H. denitrificans*, *H. mongoliensis*, *H. olivaria*, *H. sabkhae*, *H. sinaiensis*, *H. ventosae*, and *H. xinjiangensis* are able to grow up to 50 °C, although the most thermotolerant species is *H. kenyensis*, growing up to 55 °C (➋ *Table 17.4*).

Maintenance and Preservation

A variety of media have been described for the routine maintenance of halomonads strains in the laboratory. In many cases the same media served also for the isolation of the strains, whereas in others different formulations were employed. Some of these formulations, for instance the Artificial Organic Lake (AOL) medium of Franzmann et al. (1987), are intended to mimic the chemical composition of the environment from where the organisms are isolated. Once that isolation has been achieved many authors find it advantageous, especially if preparation of the isolation

Table 17.4
Physiological features of member of the family *Halomonadaceae*

Species	Salt range (%, w/v)											Temperature (°C)							pH							
	0	0.5	1	2	3	3.5	5	10	15	20	25	4–5	10	15	20	35	37	45	5	6	7	8	9	10	11	12
A. halophila	–	–	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	–	–
Car. nigrificans	+	+	+	+	+	+	+	+	–	–	–	–	ND	ND	ND	ND[a]	–	–	ND	ND	ND	ND	ND	ND	ND	ND
Chr. beijerinckii	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+[b]	+	–	+	+	+	+	–	–	–	–
Chr. canadensis	–	–	–	–	+	+	+	+	+	+	+	–	–	+	+	+[b]	+	+	+	+	+	+	+	–	–	–
Chr. israelensis	–	–	–	–	–	+	+	+	+	+	–	–	–	+	+	+[b]	+	+	+	+	+	+	+	–	–	–
Chr. japonicus	–	–	–	–	–	–	+	+	+	+	+	–	–	+	+	+	+	–	–	+	+	+	+	–	–	–
Chr. marismortui	–	–	–	+	+	+	+	+	+	+	+[c]	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–
Chr. nigrandesensis	–	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+[b]	+	+	+	+	+	+	+	+	–	–
Chr. salexigens	–	–	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	–	–	–
Chr. sarecensis	+	+	+	+	+	+	+	+	+	+	+	+[d]	+	+	+	+	+	+	+	+	+	+	+	+	–	–
Cob. amphilecti	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	–[e]	+	+	+	+	+	+	–	–
Cob. crustatorum	–	–	–	–	–	+	+	+	–	–	–	+	+	+	+[f]	–[g]	–	–	+	+	+	+	+	+	–	–
Cob. litoralis	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	–[e]	+	+	+	+	+	+	–	–
Cob. marina	–	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	–	–
Cob. pacifica	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	–[e]	+	+	+	+	+	+	+	–
H. alimentaria	–	ND	+	+	+	+	+	+	+	+	–	+	+	+	+	+[b]	+	+	+	+	+	ND	ND	ND	ND	ND
H. alkaliantarctica	–	–	–	+	+	+	+	+	+	+	–	–	+	+	+	+[b]	+	–	–	–	+	+	+	+	–	–
H. alkaliphila	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+[h]	–	–	–	+	+	+	–	–
H. almeriensis	–	–	–	–	–	–	+	+	+	+	+	–	–	+	+	+	+	–	–	+	+	+	+	+	–	–
H. andesensis	–	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	–	+	+	+	+	+	+	–
H. anticariensis	–	+	+	+	+	+	+	+	+	–	–	–	–	–	+	+	+	+	–	+	+	+	+	–	–	–
H. aquamarina	–	+	+	+	+	+	+	+	+	+	–	–	–	+	+	+	+	–	+	+	+	+	+	+	–	–
H. arcis	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+[b]	+	+	–	+	+	+	+	+	–	–
H. axialensis	–	+	+	+	+	+	+	+	+	+	–	+[i]	+	+	+	+[b]	–	–	+	+	+	+	+	+	+	+
H. beimenensis	+	+	+	+	+	+	+	+	+	–	–	–	–	+	+	+	+	+[h]	–	+	+	+	+	–	–	–
H. boliviensis	+	+	+	+	+	+	+	+	+	+	+	+[d]	+	+	+[f]	+[b]	+	+	–	+	+	+	+	+	+	ND
H. campaniensis	+	+	+	+	+	+	+	+	+	–	–	–	+	+	+	+	+	–	–	–	+	+	+	+	–	–
H. campisalis	–	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+[b]	+	+[h]	–	–	–	+	+	+	+	–
H. caseinilytica	–	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+[b]	+	+	+	+	+	+	+	–	–	–
H. cerina	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+[b]	+	+	+	+	+	+	+	+	–	–
H. cibimaris	–	–	–	–	+	+	+	+	+	–	–	–	–	+	+[f]	+[b]	–	–	–	+	+	+	+	–	–	–
H. cupida	+	+	+	+	+	+	+	+	+	–	–	–	–	+	+	+	+	–	+	+	+	+	+	+	–	–

H. daqiaonensis	–	–	–	+	+	+	+	+[j]	+	–	–	+	+	+	+	+[b]	+	–[e]	–	+	+	+	+	–	–	–
H. daqingensis	–	–	+	+	+	+	+	+	+	–	–	–	+	+	+	+[b]	+	+[h]	–	–	–	+	+	+	–	–
H. denitrificans	–	–	–	+	+	+	+	+	+	+	–	+	+	+	+[f]	+	+	+[h]	–	–	+	+	+	+	–	–
H. desiderata	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	–	–	+	+	+	+	+	–
H. elongata	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+[b]	+	+	+	+	+	+	+	+	–	–
H. eurihalina	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–
H. flava	–	+	+	+	+	+	+	+	+	+	–[k]	–	–	–	+	+	+	–[e]	–	+	+	+	+	–	–	–
H. fontilapidosi	–	–	–	–	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	–	–	–
H. gomseomensis	–	–	+	+	+	+	+	+	+	+	–	+	+	+	+[f]	+[b]	+	+	–	+	+	+	+	+	–	–
H. gudaonensis	–	–	+	+	+	+	+	+	+	+	–	–	+	+	+	+[b]	+	–	–	–	–	+	+	–	–	–
H. halmophila	–	–	–	–	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	–	–
H. halocynthiae	–	+	+	+	+	+	+	+	+	–	–	–	+	+	+[f]	+	–	–	+	+	+	+	+	+	+	–
H. halodenitrificans	–	–	–	–	+	+	+	+	+	+	–	+	+	+	+[f]	+[b]	+	–	+	+	+	+	+	+	–	–
H. halophila	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+[b]	+	+	+	+	+	+	+	+	–	–
H. hamiltonii	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	–	–	+	+	+	+	+	–	–
H. hydrothermalis	–	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+[b]	+	–	+	+	+	+	+	+	+	+
H. ilicicola	–	–	–	+	+	+	+	+	+	–	–	–	–	–	–[l]	+	+	–	–	+	+	+	+	–	–	–
H. janggokensis	–	–	+	+	+	+	+	+	+	+	–	+	+	+	+[f]	+[b]	+	+	–	+	+	+	+	+	–	–
H. jeotgali	–	–	–	–	–	–	+	+	+	+	+	–	+	+	+[f]	–[g]	–	–	+	+	+	+	+	+	–	–
H. johnsoniae	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	–	–	–	+	+	+	+	–	–
H. kenyensis	–	+	+	+	+	+	+	+	–	–	–	–	+	+	+	+	+	+[m]	–	–	–	+	+	+	–	–
H. koreensis	–	–	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	–	+	+	+	+	+	–	–
H. korlensis	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+[b]	+	–	–	+	+	+	+	+	–	–
H. kribbensis	–	–	+	+	+	+	+	+	–	–	–	–	+	+	+	+	+	–	–	+	+	+	+	–	–	–
H. lutea	–	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–
H. magadiensis	+	+	+	+	+	+	+	+	+	+	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	–
H. maura	–	–	+	+	+	+	+	+	+	+	–	–	+	+	+	+[b]	+	+	+	+	+	+	+	+	–	–
H. meridiana	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+[b]	+	+	+	+	+	+	+	+	–	–
H. mongoliensis	–	–	+	+	+	+	+	+	–	–	–	–	–	+	+	+	+	+[h]	–	–	–	+	+	+	–	–
H. muralis	+	+	+	+	+	+	+	+	+	–	–	–	+	+	+[f]	+	–	–	–	+	+	+	+	+	–	–
H. nanhaiensis	–	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+[b]	+	–[e]	–	+	+	+	+	+	–	–
H. neptunia	–	+	+	+	+	+	+	+	+	+	+	+[i]	+	+	+	+[b]	–	–	+	+	+	+	+	+	+	+
H. nitroreducens	–	–	–	–	+	+	+	+	+	+	–	+	+	+	+	+[b]	+	+	+	+	+	+	+	+	–	–
H. olivaria	+	+	+	+	+	+	+	+[j]	+	+	+	+	+	+	+	+	+	+[h]	+	+	+	+	+	+	+	–
H. organivorans	–	–	–	+	+	+	+	+	+	+	+[c]	–	–	+	+	+	+	+	–	+	+	+	+	+	–	–
H. pacifica	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–
H. pantelleriensis	–	–	+	+	+	+	+	+	+	–	–	–	+	+	+	+	+	+	–	+	+	+	+	+	+	–

Table 17.4 (continued)

Species	Salt range (%, w/v)											Temperature (°C)							pH							
	0	0.5	1	2	3	3.5	5	10	15	20	25	4–5	10	15	20	35	37	45	5	6	7	8	9	10	11	12
H. qijiaojingensis	–	+	+	+	+	+	+	+	+	+	–[k]	–	–	–	+	+	+	–[e]	–	+	+	+	+	–	–	–
H. ramblicola	–	–	+	+	+	+	+	+[j]	+	+	+[c]	+	+	+	+	+[b]	+	–[e]	+	+	+	+	+	+	–	–
H. rifensis	–	+	+	+	+	+	+	+[j]	+	+	–	–	–	–	–[l]	+[b]	+	+	+	+	+	+	+	+	–	–
H. sabkhae	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	+[g]	+	+[h]	–	+	+	+	+	–	–	–
H. saccharevitans	–	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+[b]	+	+	–	+	+	+	+	+	–	–
H. salifodinae	–	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+[b]	+	+	–	+	+	+	+	–	–	–
H. salina	–	–	–	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–
H. shengliensis	+	+	+	+	+	+	+	+	+	–	–	–	+	+	+	+[b]	+	–	–	–	–	+	+	–	–	–
H. sinaiensis	+	+	+	+	+	+	+	+	+	+	+[c]	–	–	–	–[l]	+	+	+[h]	–	+	+	+	+	–	–	–
H. smyrnensis	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	–[e]	–	+	+	+	+	–	–	–
H. stenophila	–	–	–	–	+	+	+	+	+	–	–	–	–	+	+	+[b]	+	–	–	+	+	+	–	–	–	–
H. stevensii	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	–	–	–	+	+	+	+	+	–
H. subglaciescola	–	+	+	+	+	+	+	+	+	+	–	+[d]	+	+	+[f]	+	+	+	+	+	+	+	+	+	–	–
H. subterranea	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+[b]	+	+	–	+	+	+	+	+	–	–
H. sulfidaeris	–	+	+	+	+	+	+	+	+	+	–	+[i]	+	+	+	+	–	–	+	+	+	+	+	+	–	–
H. taeanensis	–	–	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+	–	–
H. titanicae	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+[b]	+	–	–	+	+	+	+	–	–	–
H. variabilis	–	–	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	–	–	+	+	+	+	–	–	–
H. ventosae	–	–	–	–	+	+	+	+	+	ND	ND	–	–	+	+	+	+	+[h]	–	+	+	+	+	+	–	–
H. venusta	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–
H. vilamensis	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+[b]	+	–[e]	+	+	+	+	+	+	–	–
H. xianhensis	–	+	+	+	+	+	+	+[j]	+	+	+	–	+	+	+	+[b]	+	–[e]	–	+	+	+	+	–	–	–
H. xinjiangensis	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+	+	+	+[h]	–	+	+	+	+	–	–	–

H. zhanjiangensis	–	–	+	+	**+**	**+**	**+**	+	+	+	–	+	+	+	**+**[f]	**+**[b]	+	–	–	+	**+**	**+**	+	+	–	–
H. zincidurans	–	+	+	+	+	+	**+**	+	+	–	–	+	+	+	+	**+**	+	–	+	+	**+**	+	+	–	–	–
Halot. alkalilenta	**+**	**+**	**+**	**+**	**+**	+	+	+	+	–	–	+	+	+	+	**+**[b]	**+**	+	+	+	**+**	+	+	+	+	–
K. aurantia	–	–	–	–	–	–	+	**+**	+	–	–	–	–	–	+	+	**+**	–	–	+	**+**	**+**	–	–	–	–
K. avicenniae	+	+	+	+	+	+	**+**	+	+	+	+	–	–	+	+	**+**	+	–	+	+	**+**	**+**	+	–	–	–
K. indalinina	–	–	ND	ND	+	+	+	**+**	+	+	+	–	–	+	+	**+**	+	–	+	+	**+**	+	+	–	–	–
K. marisflavi	–	**+**	**+**	**+**	**+**	**+**	**+**	**+**	+	+	+	+	+	+	**+**[f]	**+**[b]	+	–	+	+	**+**	**+**	+	+	–	–
K. sinocarnis	–	–	–	–	–	–	+	**+**	**+**	+	+[c]	+	+	+	+	+	**+**	–	+	+	**+**	+	–	–	–	–
M. tunisiensis	–	–	+	+	+	+	+	**+**	+	+	+	–	–	+	+	+	**+**	+	+	+	**+**	+	+	+	–	–
S. halophilus	–	–	–	–	+	+	+	**+**	+	+	+	–	–	+	+	**+**	+	+	+	+	**+**	+	+	–	–	–
S. peritrichatus	+	**+**	**+**	**+**	+	+	+	+	+	+	–	+	+	+	+	**+**	**+**	–[e]	**+**	**+**	**+**	+	+	–	–	–
S. salarius	+	+	+	+	+	+	+	**+**	**+**	**+**	+	–	+	+	**+**[f]	**+**[b]	+	+	+	+	**+**	**+**	+	+	–	–
S. socius	–	+	+	+	**+**	**+**	**+**	**+**	+	+	+[c]	–	–	+	+	**+**[b]	**+**	–	+	**+**	**+**	**+**	+	–	–	–
Z. palmae	+	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	–	–	–	+[n]	**+**[b]	+	–	+[o]	**+**	+	+	+	+	–	–

Data from the original description of the species and from Mata et al. (2002)

A Aidingimonas, Car *Carnimonas*, Chr *Chromohalobacter*, Cob *Cobetia*, H *Halomonas*, Halot *Halotalea*, K *Kushneria*, M *Modicisalibacter*, S *Salinicola*, Z *Zymobacter*

+ growth, – not growth, *ND* no data available. Optimal values are show with + symbol in bold

[a]Available information very limited: species was described as unable to grow at 5° C and 37° C and it grows optimally at 28–30 °C

[b]Optimal temperature for growth 30 °C

[c]Also grows with 30 % (w/v) of salts

[d]Also grows at 0 °C

[e]Grows at 40–42 °C

[f]Optimal temperature for growth 25 °C

[g]Grows at 30 °C

[h]Also grows at 50 °C

[i]Also grows at –1 °C

[j]Optimal growth at 7–8 % (w/v) of salts

[k]Grows at 23 % (w/v) of salts

[l]Grows at 25 °C

[m]Also grows at 55 °C

[n]Grows at 21 °C

[o]Minimal pH for growth 3.0

medium is too laborious, to employ a more general medium that permits the growth of both fresh isolates and reference strains. Thus, one of the most commonly employed media is MH, for moderately halophilic bacteria (Ventosa et al. 1982).

MH medium (Ventosa et al. 1982)	
Yeast extract	10 g
Proteose peptone	5 g
Glucose	1 g
NaCl	81 g
$MgCl_2 \cdot 6H_2O$	7 g
$MgSO_4 \cdot 7H_2O$	9.6 g
$CaCl_2 \cdot 2H_2O$	0.36 g
KCl	2 g
$NaHCO_3$	0.06 g
NaBr	0.026 g
Distilled water q.s.	1 L

Adjust pH to 7.2 with 1 M KOH or NaOH. Add agar (20 g liter^{-1}) for preparation of solid media. Adjust total saline content (commonly 10 %) to any other desired value by lowering or raising proportionally the amounts of salts.

In 2002 Mata et al. employed MH medium at 7.5 % salt content to maintain 104 *Halomonas* strains (including 21 type strains). For the vast majority of the strains the pH was adjusted to 7.2–7.5, except for two of the strains, *H. magadiensis* NCIMB 13595^T and *H. campisalis* ATCC 700597^T, for which the pH was adjusted to 9. All the *Halomonas* species described from 2002 up to now can be mantained in the same medium at pH 7.2–7.5, with the exception of *H. alkaliantarctica*, *H. alkaliphila*, *H. campaniensis*, *H. daqingensis*, *H. desiderata*, *H. kenyensis*, *H. korlensis*, *H. mongoliensis*, and *H. pantelleriensis* that grow optimally at pH 9.0 or 9.5.

For the species of the family *Halomonadaceae* with a lower salt requirement, commercial media such as Marine Agar (MA) can be satisfactorily employed (Yoon et al. 2001; Heyrman et al. 2002; Romanenko et al. 2002, 2013; Kim et al. 2010b). Alternatively, other commercial media such as trypticase soy agar (TSA) can also be employed by adding NaCl (or a mixture of salts) or even no salts (for the non-halophilic species) (Garriga et al. 1998). With regards to the species *Zymobacter palmae* (non-halophile) the MY medium is recommended (Okamoto et al. 1993), which consisted of 1 % Bacto yeast extract (Difco), 2 % maltose, 0.2 % KH_2PO_4 and 0.5 % NaCl, pH 6.0. The species *Cobetia crustatorum* and *Salinicola peritrichatus* are also slightly acidophiles (with optimal growth at pH 5 or 6), but they can be mantained in MH medium at 7.5 % salts.

Cultures on agar slants can be sealed and stored at 4–10 °C. Although viability may last for much longer periods it is recommended that they be transferred regularly every 1–3 months.

For long-term preservation, lyophilization is advised. Prior to the vacuum drying, actively growing cells can be suspended on protecting fluids such as 5 % inositol solution, and then the vials can be frozen by immersion into liquid nitrogen.

Cryopreservation at −80 °C or under liquid nitrogen is also possible. To enhance survival of the cells they have to be suspended with a cryoprotectant such as 20 % glycerol solution. Such prepared vials can also be stored at −20 °C for middle-term preservation. However, since the quality of the culture may diminish faster (especially after frequent freeze-thawing) it is recommended that new stocks be prepared regularly.

Ecology

Habitat

According to the original description of the family *Halomonadaceae* (Franzmann et al. 1988), its members typically occurr in "temperate and Antarctic saline lakes, solar salt facilities, saline soils and marine environments". This is still true for the majority of the current species (➋ *Table 17.5*). Indeed the only exceptions to the above definition are *Carnimonas nigrificans*, *Chromohalobacter beijerinckii*, *Chr. canadensis*, *Chr. japonicus*, *Cobetia crustatorum*, *Halomonas alimentaria*, *H. cibimaris*, *H. daqingensis*, *H. desiderata*, *H. halodenitrificans*, *H. hamiltonii*, *H. jeotgali*, *H. johnsoniae*, *H. muralis*, *H. olivaria*, *H. stevensii*, *Halotalea alkalilenta*, *Kushneria sinocarnis*, *Modicisalibacter tunisiensis*, and *Zymobacter palmae*. Of these 20 organisms, only *Zymobacter palmae* is neither halophilic nor halotolerant (however, it is very tolerant to ethanol –up to 6 %).

So, the halomonads can be found in any saline environment, regardless of its geographical location. This includes oceans and seas (even at considerable depths), saline soils, salty foods, naturally occurring saline lakes, solar pans, etc. Since some of its members are also alkaliphilic, they are found in soda lakes and alkaline soils. Additionally, three species have been isolated from blood patients and from dialysis machines of a renal care centre.

On the genus level, not surprisingly, *Halomonas* is found to be the most ubiquitous genus with the largest number of species, and these are very heterogeneous.

As for the interactions of halomonads with other microorganims, Ivanova et al. (2002) characterized a heterotrophic microbial enrichment community established during the degradation of brown algae *Fucus evanescens*, and consisting of two species, *Pseudoalteromonas* sp. and *C. marina*. While the first was highly metabolically active (14 hydrolytic activities could be detected) and likely plays the main role in the initial stages of algal degradation, the second, *C. marina*, produced only caseinase and DNase but was resistant to the bacteriolytic activity of the former and utilized the degradation products of polysaccharides.

In a study about the temporal stability and biodiversity of two complex antilisterial cheese-ripening microbial consortia (Maoz et al. 2003), out of 400 isolates, three were identified as *H. venusta*, two as *H. variabilis*, and two as *Halomonas* sp. by Fourier-transform infrared spectroscopy and 16S ribosomal RNA sequence analysis.

Table 17.5
Habitats of members of the family *Halomonadaceae* from which the original strains were isolated

Species	Isolation place
Aidingimonas	
A. halophila	Salt lake located in in Xinjiang province (China)
Carnimonas	
Car. nigrificans	Raw cured-meat products (Spain)
Chromohalobacter	
Chr. beijerinckii	Salted beans and herrings
Chr. canadensis	Contaminant on medium containing 25 % NaCl
Chr. israelensis	Dead Sea
Chr. japonicus	Japanese salty food
Chr. marismortui	Dead Sea and solar salterns
Chr. nigrandesensis	Hypersaline sediment of Lake Tebenquiche (Chile)
Chr. salexigens	Solar salterns
Chr. sarecensis	Saline soil around a hypersaline lake (Bolivia)
Cobetia	
Cob. amphilecti	Internal tissue of the sponge *Amphilectus digitatus*
Cob. crustatorum	Traditional fermented seafood (Korea)
Cob. marina	Marine
Cob. litoralis	Sediment collected from the shore of the Sea of Japan, Russia
Cob. pacifica	Sediment collected from the shore of the Sea of Japan, Russia
Halomonas	
H. alimentaria	Jeotgal (traditional Korean fermented seafood)
H. alkaliantarctica	Saline lake Cape Russell in Antarctica
H. alkaliphila	Salt pool in Campania (Italy)
H. almeriensis	Solar saltern (Spain)
H. andesensis	Saline lake Laguna Colorada in Bolivia
H. anticariensis	Saline soil (Spain)
H. aquamarina	Marine
H. arcis	Hypersaline environments (China)
H. axialensis	Deep-sea hydrothermal-vent environments
H. beimenensis	Abandoned saltern
H. boliviensis	Soil around a hypersaline lake (Bolivia)
H. campaniensis	Mineral pool (Italy)
H. campisalis	Soil below a crystalline salt surface
H. caseinilytica	Saline lake on the Qinghai-Tibet Plateau (China)
H. cerina	Saline soils (Spain)
H. cibimaris	Traditional Korean fermented seafood
H. cupida	Marine
H. daqiaonensis	Littoral saltern
H. daqingensis	Oilfield soil
H. denitrificans	Saline water (Korea)
H. desiderata	Municipal sewage
H. elongata	Solar salterns
H. eurihalina	Hypersaline habitats (soils, salt ponds) and seawater
H. flava	Salt lake
H. fontilapidosi	Saline soil at Fuente de Piedra (Spain)
H. gomseomensis	Saline water (Korea)

Table 17.5 (continued)

Species	Isolation place
H. gudaonensis	Saline soil contaminated by crude oil (China)
H. halmophila	Dead Sea
H. halocynthiae	Gill tissues of the ascidian *Halocynthia aurantium*
H. halodenitrificans	Meat-curing brines
H. halophila	Saline soils
H. hamiltonii	Renal care centre
H. hydrothermalis	Deep-sea hydrothermal-vent environments
H. ilicicola	Solar saltern (Spain)
H. janggokensis	Saline water (Korea)
H. jeotgali	Traditional fermented seafood
H. johnsoniae	Renal care centre
H. kenyensis	Soda lake (Kenya)
H. koreensis	Solar saltern (Korea)
H. korlensis	Saline and alkaline soil
H. kribbensis	Solar saltern (Korea)
H. lutea	Salt lake
H. magadiensis	Littoral sediments of haloalkaline East African lakes
H. maura	Solar saltern (Morocco)
H. meridiana	Antarctic saline lakes
H. mongoliensis	Soda lake (Mongolia)
H. muralis	Biofilm covering a wall and a mural (Austria)
H. nanhaiensis	Sediment sample from the South China Sea
H. neptunia	Deep-sea hydrothermal-vent environments
H. nitroreducens	Solar saltern in Cahuil (Chile)
H. olivaria	Olive-processing effluents
H. organivorans	Saline soils (Spain)
H. pacifica	Marine
H. pantelleriensis	Hard sand from Pantelleria island (Italy)
H. qijiaojingensis	Salt lake
H. ramblicola	Hypersaline rambla (Spain)
H. rifensis	Solar saltern
H. sabkhae	Algerian sabkha
H. saccharevitans	Hypersaline environments (China)
H. salifodinae	Salt mine (China)
H. salina	Hypersaline soils, salt ponds, salt lakes, seawater
H. shengliensis	Crude-oil-contaminated saline soil (China)
H. sinaiensis	Salt lake (Egypt)
H. smyrnensis	Saltern area (Turkey)
H. stenophila	Saline soil
H. stevensii	Renal care centre
H. subglaciescola	Antarctic saline lake (Organic Lake)
H. subterranea	Hypersaline environments (China)
H. sulfidaeris	Deep-sea hydrothermal-vent environments
H. taeanensis	Solar saltern (Korea)
H. titanicae	Rusticles from the RMS Titanic
H. variabilis	North arm of the Great Salt Lake (USA)
H. ventosae	Saline soils (Spain)

Table 17.5 (continued)

Species	Isolation place
H. venusta	Marine
H. vilamensis	High-altitude Andean lakes
H. xianhensis	Saline soil contaminated by crude oil
H. xinjiangensis	Salt lake
H. zhanjiangensis	Sea urchin
H. zincidurans	Deep-sea environment
Halotalea	
Halot. alkalilenta	Alkaline olive mill wastes
Kushneria	
K. aurantia	Salty leaves of *Avicennia germinans* (Puerto Rico)
K. avicenniae	Salty leaves of *Avicennia germinans* (Puerto Rico)
K. indalinina	Solar saltern (Spain)
K. marisflavi	Marine
K. sinocarnis	Chinese traditional cured meat
Modicisalibacter	
M. tunisiensis	Oilfield-water injection sample
Salinicola	
S. halophilus	Solar saltern (Spain)
S. peritrichatus	Deep-sea sediment
S. salarius	Saline water (Korea)
S. socius	Salt mines (Russia)
Zymobacter	
Z. palmae	Palm sap in Okinawa Prefecture (Japan)

A recent study based on the metagenomic analysis of two hypersaline saltern ponds (19 % and 37 % NaCl) from Santa Pola (Spain) has demonstrated that the genera *Halomonas* and *Chromohalobacter*, which are commonly obtained in pure culture from similar salinity samples, are almost not represented in those ponds according to the metagenomic reads obtained (Ghai et al. 2011). This means that members of *Halomonadaceae* are less abundant in hypersaline environment than it was previously thought when using culture-dependent techniques.

The diversity and distribution of *Halomonas* populations in the hypersaline habitat Rambla Salada, situated in south-east Spain, have been studied using different molecular techniques (Oueriaghli et al. 2014). Denaturing gradient gel electrophoresis (DGGE) using specific primers for the 16S rRNA gene of *Halomonas* followed by a multivariate analysis of the results indicated that richness and evenness of the *Halomonas* populations were mainly influenced by the season, being the summer (the season with the highest salinity) the one with the highest value of diversity. Furthermore, canonical correspondence analysis (CCA) demonstrated that both salinity and pH significantly affected the structure of the *Halomonas* community. *Halomonas almeriensis* and two denitrifiers, *H. ilicicola* and *H. ventosae* were the predominant species. CARD-FISH showed that the percentage of *Halomonas* cells with respect to the total number of microorganisms in that habitat ranged from 4.4 % to 5.7 %. Finally, no significant differences between the types of samples studied, from either watery sediments or soil samples, were found (Oueriaghli et al. 2014). Classical cultivation methods have been also employed very recently to analyze the diversity of the halophilic bacterial community from Rambla Salada, being *Halomonas* the most abundant genus, representing 41.2 % of the 364 isolated strains (Luque, R., Béjar, V., Quesada, E., and Llamas, I., unpublished).

Pathogenicity, Clinical Relevance

Members of the *Halomonadaceae* were thought to be not pathogenic. There is one case report on the isolation of *H. venusta* from a human infection in a wound that originated from a fish bite (Von Graevenitz et al. 2000); however the identification of the organism alone does not prove its pathogenicity.

In 2007, Berger et al. (2007) reported an outbreak of "*Halomonas phocaeensis*" bacteraemia in a neonatal intensive care unit in Tunisia, attributed to contamination from a water bath used to warm fresh frozen plasma.

In a study performed seeking bacterial DNA signatures in unexplained deaths and critical illnesses, polymerase chain reaction of 16S RNA gene from culture-negative materials identified

an unspeciated *Halomonas* organism in one patient's blood (Nikkari et al. 2002). The phylogenetic analysis of the sequence data determined that this organism was part of the *H. variabilis–H. boliviensis–H. neptunia* cluster, but in an apparently distinct position (Stevens et al. 2009). Additionally, Stevens et al. (2009) isolated a total of 14 strains recognized as human pathogens causing infection and contamination in a dyalisis center. Exhaustive taxonomic characterization of these isolates led to the description of three new *Halomonas* species, *H. stevensii*, *H. hamiltonii* and *H. johnsoniae* (Kim et al. 2010a).

More recently, a patient developed a bacteremia caused by *Halomonas johnsoniae* (previously reported only as dialysis unit environmental contaminants) (Stevens et al. 2013). The medical community is alerted to the pathogenic potential of the genus in humans, but also in algae and animals (Kim et al. 2013).

Applications

The species of the *Halomonadaceae* can be used for several biotechnological purposes and, as in the case of other extremophilic microorganisms, many different applications have been suggested. The biotechnological potential and applications of moderately and halotolerant microorganisms have been reviewed in detail (Ventosa et al. 1998; Margesin and Schinner 2001; Mellado and Ventosa 2003; Quillaguamán et al. 2010; Oren 2010). Some of the most promising applications of members of *Halomonadaceae* include the production of compatible solutes and polyhydroxyalkanoates as well as extracellular compounds such as exopolysaccharides and enzymes, and their use in environmental bioremediation processes.

Compatible solutes are known for their stabilizing and protective effect on enzymes, nucleic acids, cell structures or whole cells subjected to low water activities, temperature stress, and other adverse conditions (Lippert and Galinski 1992; Galinski 1993; Knapp et al. 1999) and therefore could be useful for industrial and clinical purposes. A "bacterial milking" process to obtain ectoine from *Halomonas elongata* has been developed and patented (Sauer and Galinski 1998) and later industrially exploited by Bitop (Witten, Germany). The process is based on subjecting the bacteria repeatedly to osmotic shocks. An osmotic down-shock permits the excretion of the intracellular ectoine to the surrounding medium while subsequent exposure of the cells to a hyperosmotic shock quickly restores the original level of ectoine (Sauer and Galinski 1998). Very recently, an *ectD* (ectoine hydroxylase) deficient *H. elongata* mutant has been proved to produce ectoine from a variety of sugars derived from lignocellulosic biomass and thus has tremendous potential as a host for producing useful compounds from biomass resources (Tanimura et al. 2013). Concerning other halomonads, a process comprising two-step fed-batch cultivation has been investigated for the production of ectoine and hydroxyectoine using *Halomonas boliviensis* DSM 15516^T (Guzmán et al. 2009; Van-Thuoc et al. 2010a, b). The first cultivation was performed under optimal conditions for cell growth and resulted in a high cell mass concentration. During the second cultivation at higher salt concentration, accumulation of ectoines increased while cell mass decreased. Maximum productivity of total ectoines reached was 10 g l^{-1} d^{-1} at 18.5 % NaCl, which is among the highest reported so far. The accumulated ectoines were released by subjecting the cells to hypoosmotic shock and the cells were further recycled for the production process (Van-Thuoc et al. 2010a). A similar method in two stages has been employed for efficient ectoine production using *Halomonas salina* DSM 5928^T (Zhang et al. 2009; Lang et al. 2011). An ectoine absorption defective *H. salina* DSM 5928^T mutant (lacking the ectoine-specific transporter TeaABC), which compromised the negative feedback regulation of ectoine synthesis, was constructed to improve the efficiency of ectoine production up to 9.93 g l^{-1} d^{-1} (Xu and Zhang 2012). Apart from *Halomonas* species, only the species *Chromohalobacter salexigens* has been optimized for ectoine and hydroxyectoine production (Fallet et al. 2010; Rodríguez-Moya et al. 2013). Ectoine and its derivative hydroxyectoine are used in the cosmetic industry because of their moisturizing properties. The potential use of the *Chromohalobacter salexigens* and *Halomonas elongata ect* genes (responsible for the synthesis of ectoine) to obtain agriculturally important transgenic organisms tolerant to osmotic stress has already been proposed (Vargas et al. 2004). Nakayama et al. (2000) obtained transgenic cultured tobacco cells that accumulated the compatible solute ectoine from *H. elongata*, exhibiting a normal growth pattern under hyperosmotic conditions.

Poly-β-hydroxyalkanoate (PHA) is a polymer accumulated by many prokaryotes, that can be used for the production of biodegradable plastics ("biological polyesters") with properties resembling that of polypropylene. Several bacterial species of the family *Halomonadaceae* accumulate PHAs: *Cobetia amphilecti*, *Cob. crustatorum*, *Cob. litoralis*, *Cob. marina*, *Cob. pacifica*, *Chromohalobacter salexigens*, *Chr. sarecensis*, *Halomonas almeriensis*, *H. andesensis*, *H. anticariensis*, *H. alkaliphila*, *H. aquamarina*, *H. boliviensis*, *H. campaniensis*, *H. campisalis*, *H. caseinilytica*, *H. cerina*, *H. cibimaris*, *H. cupida*, *H. daqiaonensis*, *H. daqingensis*, *H. desiderata*, *H. elongata*, *H. eurihalina*, *H. fontilapidosi*, *H. halmophila*, *H. halodenitrificans*, *H. halophila*, *H. hamiltonii*, *H. jeotgali*, *H. johnsoniae*, *H. magadiensis*, *H. maura*, *H. meridiana*, *H. nitroreducens*, *H. olivaria*, *H. pacifica*, *H. pantelleriensis*, *H. ramblicola*, *H. rifensis*, *H. salina*, *H. sinaiensis*, *H. stevensii*, *H. subglaciescola*, *H. variabilis*, *H. ventosae*, *H. venusta*, *H. zhanjiangensis*, *Kushneria marisflavi*, *Salinicola salarius*, and *S. socius*. From those, the strain *Halomonas boliviensis* LC1^T reached PHA yields and volumetric productivities close to the highest reported so far, accumulating the compound to up to 88 % of its dry weight (Quillaguamán et al. 2006, 2007). Furthermore, *H. boliviensis* and other *Halomonas* species are able to co-produce PHA and osmolytes, i.e., ectoines and hydroxyectoine, in one process (Quillaguamán et al. 2010).

Strains from several species of *Halomonas* are of interest as producers of exopolysaccharides (EPS). These include *H. alkaliantarctica*, *H. alkaliphila*, *H. almeriensis*, *H. anticariensis*, *H. caseinilytica*, *H. cerina*, *H. daqiaonensis*, *H. daqingensis*, *H. eurihalina*, *H. fontilapidosi*, *H. halophila*,

H. maura, *H. nitroreducens*, *H. olivaria*, *H. ramblicola*, *H. rifensis*, *H. sabkhae*, *H. salina*, *H. sinaiensis*, *H. smyrnensis*, *H. stenophila*, and *H. ventosae*. EPS production has also been described in the species *Cobetia crustatorum*. The nutritional and environmental factors influencing the production of EPS have been thoroughly investigated (Quesada et al. 1993; Béjar et al. 1996; Béjar et al. 1998; Bouchotroch et al. 1999; Bouchotroch et al. 2000; Martinez-Checa et al. 2002; 2007; Arias et al. 2003; Mata et al. 2006; Llamas et al. 2012). Yield can reach 1–3 g of EPS per liter of medium after five days of cultivation. Glucose and sucrose are the most efficient carbon sources (Béjar et al. 1996) although many other nutrients can be used including end products such as molasses from sugar beet (Quesada et al. 2004). Production of the EPS is not inhibited by the presence of crude oil. Moreover, the composition of the biopolymer is different under such conditions showing a highly efficient emulsifying activity towards crude oil (Calvo et al. 2002). Interestingly, it has been observed that strains of *H. maura* and *H. eurihalina* produce more EPS at salt concentrations below 7.5 % (w/v), which are suboptimal for growth (Quesada et al. 1993; Bouchotroch et al. 2000). As for the chemical composition of the EPSs produced from *Halomonas* strains, they are anionic polymers composed mainly of carbohydrates and a minor fraction of proteins, uronic acids and acetyls. Sulfate substituents have also been detected (in some strains exceeding 20 % of the dry weight), and together with the uronic acids, make the EPS anionic. Depending on their composition EPSs have different functional properties and thus different applications, which include immunomodulation, biodetoxification of heavy-metal polluted environments, crude oil emulsification, viscosity enhancement in foods, protection against oxidative stress, among others (Quesada et al. 2004; Raveendran et al. 2013). Recently, it has been demonstrated that the EPS produced by *H. stenophila* strain B100 selectively induces apoptosis in human T leukaemia cells (Ruiz-Ruiz et al. 2011), suggesting that the search for new antineoplastic drugs should include the screening of other bacterial EPSs, particularly those isolated from halophiles. The properties of some of these EPSs such as mauran (from *H. maura*), H28 and V2-7 (from *H. eurihalina*) and those from strains Al12^T and Al16 (*H. ventosae*), from strains FP35^T and FP36 (*H. anticariensis*) and from strain M8^T (*H. almeriensis*) have been studied in detail highlighting in each case their biotechnological potentials (Pérez-Fernandez et al. 2000; Martinez-Checa et al. 2002, 2007; Arias et al. 2003; Mata et al. 2006; Llamas et al. 2012). Besides, genes involved in the biosynthesis of the exopolysaccharide mauran produced by *H. maura* have been identified, which form part of a gene cluster *epsABCDJ* (Arco et al. 2005). Furthermore, production of EPS in *H. anticariensis* was found to be influenced by a two-component regulatory system, GacS/GacA (Tahrioui et al. 2013b).

Another interesting field is the production of extracellular enzymes (i.e., amylases, proteases and nucleases) by moderately halophilic representatives of the family. Although few studies have been carried out, this is a promising subject that has been reviewed (Mellado et al. 2004). Sanchez-Porro et al. (2003) studied the diversity of moderately halophilic bacteria able to produce several extracellular hydrolytic enzymes (amylases, DNases, lipases, proteases and pullulanases) by screening different hypersaline locations in South Spain. In contrast to the scarce hydrolytic activity shown by culture collection strains, environmental isolates produce a variety of extracellular enzymes that could be of potential biotechnological interest. Identification at the genus level revealed that 25 strains and 2 out of 122 isolates belonged to *Halomonas* and *Chromohalobacter*, respectively. Another study with the aims to isolate and characterize the cultivable community of hydrolase producers inhabiting heavy-metal-contaminated soils in extreme conditions from the Atacama Desert showed that only 1 out of 25 isolates was closely related to the genus *Halomonas*, in particular to *H. organivorans*, possessing protease, lipase, amylase, DNase, and pullulanase activities (Moreno et al. 2012). Concerning the characterization of extracellular enzymes, an α-amylase produced by *Halomonas meridiana* has been studied at the biochemical and molecular level (Coronado et al. 2000a, b). The amylase showed optimal activity at 10 % NaCl, 37 °C and pH 7.0 (being relatively stable under alkaline conditions). The enzyme showed activity at high salt concentrations (up to 30 %). The main products resulting from the hydrolysis of starch were maltose and maltotriose. The gene encoding this α-amylase (*amyH*) has been cloned and expressed in the heterologous hosts *H. elongata* and the non-halophilic bacterium *E. coli* (Coronado et al. 2000b). It encodes a 457-residue protein with a molecular mass of 50 kDa. Besides, an extracellular α-amylase gene from the hyperthermophilic archaeon *Pyrococcus woesei* has been cloned and expressed in *H. elongata*, under the control of a native *H. elongata* promoter (Frillingos et al. 2000). More recently, a novel endoglucanase (Cel8H) from *Halomonas* sp. strain S66-4 has been biochemically characterized, cloned, expressed in *E. coli* and purified. The purified recombinant enzyme had an optimal activity of 4.9 U/mg at pH 5 and 45° C toward the substrate carboxymethylcellulose. It exhibited extraordinary properties which differed from endoglucanases reported previously at the point of high salt tolerance above 5 M, simultaneously with high stability at pH 4–12 and 40–60° C (Huang et al. 2010). Moreover, an α-glucosidase (HaG) with a estimated molecular mass of 58 kDa was isolated from *Halomonas* sp. strain H11 (Ojima et al. 2012a). HaG showed high hydrolytic activities toward maltose, sucrose, and p-nitrophenyl-α-D-glucoside but to almost no other disaccharides or malto-oligosaccharides higher than trisaccharides. HaG showed optimum activity to maltose at 30 °C and pH 6.5. Monovalent cations, such as K^+, Rb^+, Cs^+, and NH_4^+ increased the enzymatic activity to 2- to 9-fold of the original activity. This enzyme was tested to be useful for efficient synthesis of α-D-glucosylglycerol and α-glucosylated 6-gingerol (Ojima et al. 2012a, b).

Halomonads and other moderately halophilic bacteria can be also used for the degradation of toxic compounds both at high and intermediate salt concentrations, permitting the restoration of saline industrial residues and contaminated saline environments (Mellado and Ventosa 2003). However, few studies have been carried out. Some examples of halomonads with a potential for biodegradation and biotransformation of contaminants (such as aminomethane sulfonate, *p*-aminosalicylate,

Table 17.6

Organic compounds and chemicals that can be degraded or transformed by *Halomonadaceae* strains under hypersaline conditions

Compound	Organism	NaCl (%, w/v)	References
Benzoate	*Cobetia marina*	3–20	Rosenberg (1983)
Aminomethane sulfonate	*Chromohalobacter marismortui* VH1	5–15	Ternan and McMullan (2002)
Organophosphonates	*Chromohalobacter marismortui* VH1	5–15	Hayes et al. (2000)
Phenol	*Halomonas campisalis* A4[T]	0–15[a]	Alva and Peyton (2003)
p-aminosalicylate	*Halomonas organivorans* G-16.1[T]	10	García et al. (2004)
Benzoate	*Halomonas organivorans* G-16.1[T]	10	García et al. (2004); Moreno et al. (2011)
Cinnamate	*Halomonas organivorans* G-16.1[T]	10	García et al. (2004)
p-coumarate	*Halomonas organivorans* G-16.1[T]	10	García et al. (2004)
Ferulate	*Halomonas organivorans* G-16.1[T]	10	García et al. (2004)
p-hydroxybenzoate	*Halomonas organivorans* G-16.1[T]	10	García et al. (2004)
Phenol	*Halomonas organivorans* G-16.1[T]	10	García et al. (2004); Moreno et al. (2011); Bonfá et al. (2013)
Phenylacetate	*Halomonas organivorans* G-16.1[T]	10	García et al. (2004)
Phenylpropionate	*Halomonas organivorans* G-16.1[T]	10	García et al. (2004)
Salicylate	*Halomonas organivorans* G-16.1[T]	10	García et al. (2004)
Crude-oil	*Halomonas organivorans* PG-31	2.8	Hassanshahian et al. (2012)
Phenol	*Halomonas venusta*	8	Muñoz et al. (2001)
Phenol	*Halomonas* sp.	1–14	Hinteregger and Streichsbier (1997)
3-chlorobenzoate	*Halomonas* sp. EF43	1[c]	Kleinsteuber et al. (2001)
2,4-dichlorophenoxyacetate	*Halomonas* sp. EF43	1[c]	Kleinsteuber et al. (2001)
2,4-dichlorophenoxyacetate	*Halomonas* sp. I18	3.5–5.8[b]	Maltseva et al. (1996)
Formaldehyde	*Halomonas* sp. MA-C	0–20	Azachi et al. (1995)
Selenate	*Halomonas* sp. MPD-51 (and three more isolates)	0–32.5	De Souza et al. (2001)
Uranium compounds	*Halomonas* sp. WIPP1A	20	Francis et al. (2000)
Phenol	*Modicisalibacter tunisiensis* HU	10	Bonfá et al. (2013)

[a]At pH 8–11
[b]At pH 7.5–9.8
[c]At pH 10

organophosphonates, benzoate, 3-chlorobenzoate, cinnamate, *p*-coumarate, crude-oil, 2,4-dichlorophenoxyacetate, ferulate, formaldehyde, *p*-hydroxybenzoate, phenol, phenylacetate, phenylpropionate, salicylate, selenate and uranium compounds) are shown in *Table 17.6*. Maltseva et al. (1996) isolated halomonads able to use chloroaromatic compounds as the sole source of carbon and energy and studied in detail the degradation of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D). Kleinsteuber et al. (2001) reported the use of the alkaliphilic, moderately halophilic *Halomonas* sp. strain EF43 for the expression of the 2,4-D-degradative pathway by conjugation of the broad host range plasmid pJP4. This strain was able to degrade 2,4-D- and 3-chlorobenzoate under alkaline conditions in the presence of an additional carbon source. In 2004, García et al. (2004) described a novel species, *Halomonas organivorans*, which was able to use benzoic acid, *p*-hydroxybenzoic acid, cinnamic acid, salicylic acid, phenylacetic acid, phenylpropionic acid, phenol, *p*-coumaric acid, ferulic acid and *p*-aminosalicylic acid. Later, a screening of moderately halophilic bacteria able to use aromatic compounds permitted the isolation of a large number of members of the genus *Halomonas* able to degrade different compounds (García et al. 2005). Recently, *cat* and *ben* genes, involved in phenol and benzoate degradation, respectively, from *H. organivorans*, have been cloned, characterized and analyzed (Moreno et al. 2011), providing an ideal model system to investigate the potential use of this group of extremophiles in the decontamination of saline environments. Other recent publications also show the ability of *H. organivorans* to degradate contaminants, such as crude-oil (Hassanshahian et al. 2012) and phenol (Bonfá et al. 2013). The tolerance patterns of several *Halomonas* species and *Chromohalobacter marismortui* to ten heavy metals have been studied, as well as the influence of salinity and composition of culture media (Nieto et al. 1989). These studies may be interesting for future use of metal-tolerant halophilic strains as biological detoxicants.

Few studies have been carried out on the role of halomonads in the fermentation processes of foods and other products. *Cobetia crustatorum*, *Halomonas alimentaria*, *H. cibimaris*, and *H. jeotgali* were isolated from the traditional Korean fermented seafood (Kim et al. 2010b; Yoon et al. 2002; Jeong et al. 2013; Kim et al. 2011). The hydrolytic activity of *H. elongata* in bacon curing brines has been investigated (Hinrichsen et al. 1994).

Fuel ethanol production has been proposed using the pyruvate decarboxylase (PDC) of *Z. palmae* as biocatalyst (Raj et al. 2002). This enzyme is thermostable and showed the highest specific activity and lowest *Km* for pyruvate of all four known bacterial PDCs.

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