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Phylogeny of the family *Halomonadaceae* based on 23S and 16S rDNA sequence analyses

David R. Arahal,[1,2] Wolfgang Ludwig,[1] Karl H. Schleifer[1] and Antonio Ventosa[2]

[1] Lehrstuhl für Mikrobiologie, Technische Universität München, 85350 Freising, Germany

[2] Departamento de Microbiología y Parasitología, Facultad de Farmacia, Universidad de Sevilla, 41012 Seville, Spain

Author for correspondence: Antonio Ventosa. Tel: +34 954556765. Fax: +34 954628162. e-mail: ventosa@us.es

In this study, we have evaluated the phylogenetic status of the family *Halomonadaceae*, which consists of the genera *Halomonas*, *Chromohalobacter* and *Zymobacter*, by comparative 23S and 16S rDNA analyses. The genus *Halomonas* illustrates very well a situation that occurs often in bacterial taxonomy. The use of phylogenetic tools has permitted the grouping of several genera and species believed to be unrelated according to conventional taxonomic techniques. In addition, the number of species of the genus *Halomonas* has increased as a consequence of new descriptions, particularly during the last few years, but their features are too heterogeneous to justify their placement in the same genus and, therefore, a re-evaluation seems necessary. We have determined the complete sequences (about 2900 bases) of the 23S rDNA of 18 species of the genera *Halomonas* and *Chromohalobacter* and resequenced the complete 16S rDNA sequences of seven species of *Halomonas*. The results of our analysis show that two phylogenetic groups (respectively containing five and seven species) can be distinguished within the genus *Halomonas*. Six other species cannot be assigned to either of the above-mentioned groups. Furthermore, *Halomonas marina* forms a separate branch at a deeper level than the other species of the genus *Halomonas*, which suggests that it should be ascribed to a separate genus. The genus *Chromohalobacter* forms a monophyletic group constituted by *Chromohalobacter marismortui*, the recently reclassified species *Chromohalobacter canadensis* and *Chromohalobacter israelensis* and the recently proposed species *Chromohalobacter salexigens*. Finally, we propose to include the genus *Carnimonas*, with its single species *Carnimonas nigrificans*, in the family *Halomonadaceae*.

Keywords: *Halomonadaceae*, taxonomy, phylogeny, 23S and 16S rDNA sequences, moderately halophilic bacteria

INTRODUCTION

The family *Halomonadaceae* belongs to the γ-subclass of the *Proteobacteria*. It was proposed by Franzmann *et al*. (1988), according to results obtained with the 16S rDNA cataloguing technique, to accommodate the moderately halophilic and marine bacteria of the genera *Halomonas* and *Deleya*. More recently, a study based on comparison of 16S rDNA sequences from several moderately halophilic bacteria concluded that *Chromohalobacter marismortui* belongs to the family *Halomonadaceae* and that *Volcaniella eurihalina* should be reclassified as *Halomonas eurihalina* (Mellado *et al*., 1995). In addition, these authors stated the need for a polyphasic approach to determine the natural taxonomic position of the species belonging to the genera *Halomonas* and *Deleya*, as well as *Chromohalobacter marismortui*, *Halovibrio variabilis* and *Paracoccus halodenitrificans*. In addition, Dobson & Franzmann (1996) carried out a similar study and proposed that the genera *Halomonas* and *Deleya*, as well as the species *Halovibrio variabilis* and *Paracoccus halodenitrificans*, were unified into the single genus *Halomonas*. At the same time, the genus *Zymobacter* became the third genus to be included in the family

The EMBL accession numbers for the 23S and 16S rDNA gene sequences reported in this paper are AJ306870–AJ306894.

Table 1. Bacterial species used in this study, their sources and rDNA sequence information

Culture collections are abbreviated as: ACAM, Australian Collection of Antarctic Micro-organisms; ATCC, American Type Culture Collection; CCM, Czech Collection of Microorganisms; CECT, Colección Española de Cultivos Tipo; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen; IAM, Institute of Applied Microbiology; NCIMB, National Collections of Industrial Food and Marine Bacteria; UQM, University of Queensland Microbial Culture Collection.

Species	Type strain designation(s)	Original source	Sequence accession nos (bp)	
			16S rRNA	23S rRNA
Carnimonas nigrificans	CECT 4437^T; CTCBS1^T	Cured meat products	Y13299 (1519)	–
Chromohalobacter canadensis	ATCC 43984^T; NRCC 41227^T; DSM 6769^T	Medium contaminant (Canada)	AJ295143 (1532)	AJ306870 (2910)
Chromohalobacter israelensis	ATCC 43985^T; Ba$_1^T$; DSM 6768^T	Dead Sea	AJ295144 (1532)	AJ306871 (2909)
Chromohalobacter marismortui	ATCC 17056^T; CCM 3518^T; DSM 6770^T	Dead Sea	X87219 (1423)	AJ306872 (2998)
Chromohalobacter salexigens	DSM 3043^T; 1H11^T; CECT 5384^T	Solar saltern (Bonaire, Netherlands Antilles)	AJ295146 (1531)	AJ306873 (2908)
Halomonas aquamarina	IAM 12550^T; ATCC 14400^T; DSM 30161^T	Marine water (Hawaii, USA)	M93352 (1466); AJ306888 (1528)	AJ306874 (2914)
Halomonas campisalis	ATCC 700597^T; 4A^T	Alkali lake sediment (Washington, USA)	AF054286 (1400)	–
Halomonas cupida	ATCC 27124^T; 79^T; DSM 4740^T	Marine water (Hawaii, USA)	L42615 (1471)	AJ306875 (2910)
Halomonas desiderata	DSM 9502^T; FB2^T	Sewage treatment plant (Göttingen, Germany)	X92417 (1495)	AJ306876 (2910)
Halomonas elongata	ATCC 33173^T; 1H9^T; DSM 2581^T	Solar saltern (Bonaire, Netherlands Antilles)	M93355 (1479); X67023 (1470)	AJ306877 (2910)
Halomonas eurihalina	ATCC 49336^T; F9-6^T; DSM 5720^T	Saline soil (Alicante, Spain)	L42620 (1490); X87218 (1473)	AJ306878 (2911)
Halomonas halmophila	NCIMB 1971^T; DSM 5349^T; ATCC 19717^T	Dead Sea	M59153 (1540); AJ306889 (1530)	AJ306879 (2920)
Halomonas halodenitrificans	ATCC 13511^T; CCM 286^T; DSM 735^T	Meat-curing brines	L04942 (1531)	–
Halomonas halodurans	ATCC 29686^T; DSM 5160^T	Great Bay Estuary (New Hampshire, USA)	L42619 (1474)	–
Halomonas halophila	CCM 3662^T; F5-7^T; DSM 4770^T	Saline soil (Alicante, Spain)	M93353 (1478)	–
Halomonas magadiensis	NCIMB 13595^T; 21M1^T	East African alkaline lakes sediments	X92150 (1473)	–
Halomonas marina	ATCC 25374^T; 219^T; DSM 4741^T	Marine water (Hawaii, USA)	M93354 (1483); AJ306890 (1536)	AJ306880 (2910)
Halomonas meridiana	ACAM 246^T; UQM 3352^T; DSM 5425^T	Hypersaline lakes (Antarctica)	M93356 (1475); AJ306891 (1528)	AJ306881 (2914)
Halomonas pacifica	ATCC 27122^T; 62^T; DSM 4742^T	Marine water (Hawaii, USA)	L42616 (1480)	AJ306882 (2910)
Halomonas pantelleriensis	DSM 9661^T; AAP^T	Hard sand (Panterellia, Italy)	X93493 (1477)	AJ306883 (2912)
Halomonas salina	ATCC 49509^T; F8-11^T; DSM 5928^T	Saline soils (Alicante, Spain)	L42617 (1443); X87217 (1478); AJ243447 (1493); AJ243448 (1493); AJ295145 (1532)	AJ306884 (2908)

Table 1 (*cont.*)

Halomonas subglaciescola	UQM 2926^T; ACAM 12^T; DSM 4683^T	Organic Lake (Antarctica)	M93358 (1481); AJ306892 (1531)	AJ306885 (2910)
Halomonas variabilis	DSM 3051^T; isolate III^T	Great Salt Lake (Utah, USA)	M93357 (1470); AJ306893 (1528)	AJ306886 (2912)
Halomonas venusta	ATCC 27125^T; 86^T; DSM 4743^T	Marine water (Hawaii, USA)	L42618 (1479); AJ306894 (1530)	AJ306887 (2914)
Zymobacter palmae	IAM 14233^T; T109^T; DSM 10491^T	Palm sap (Okinawa Prefecture, Japan)	D14555 (1503); AF211871 (1509)	AF211871 (2560)*

* Sequence AF211871 starts at the beginning of the 16S rRNA gene and finishes at position 2560 of the 23S rRNA gene.

Halomonadaceae (Dobson & Franzmann, 1996). At the time of writing, there were 19 validly published species within the genus *Halomonas*, while *Chromohalobacter* and *Zymobacter* respectively contained four and one species. These species and their origins are listed in Table 1, in which a closely related organism, *Carnimonas nigrificans* (Garriga *et al*., 1998), that perhaps could be considered a member of the family *Halomonadaceae*, has been included. A full chronological record of the contributions to the taxonomy of this group of bacteria (Euzéby, 1997) reveals that more than half of the species have been reclassified at least once and their nomenclature was changed. In most cases, the experimental basis for these changes was comparison of the 16S rDNA sequences.

In our study, we have carried out a re-evaluation of the phylogeny of the species of *Halomonadaceae* using comparative sequence analysis of 23S and 16S rDNA. For this purpose, eight already available 16S rDNA sequences of type strains were resequenced to resolve undetermined positions and 18 new complete 23S rDNA sequences were obtained.

Over a period of only a few years, comparative sequence analysis of the small-subunit rRNA has become a major source for phylogenetic studies of micro-organisms. This is reflected in the literature and in the continually expanding number of freely accessible sequences, more than 16000 at the time of writing. In contrast, this number is much lower for the 23S rDNA (only about 500), despite it being more informative. In many cases, only partial sequences, alone or together with the 16S–23S rDNA intergenic spacer, are determined. Although partial sequences can be sufficient for bacterial identification, they should not be used for inferring phylogeny since incorrect conclusions may be drawn (Ludwig & Schleifer, 1995). Thus, there are not many examples of full 23S rDNA sequence-based phylogeny, such as those from Briones & Amils (2000), Ludwig *et al*. (1992, 1995), Martínez-Murcia *et al*. (1993) and Sallen *et al*. (1996). In our study, we have used this approach to determine in detail the phylogenetic relationship of species of the genera of the family *Halomonadaceae* and to clarify the classification of this heterogeneous bacterial group.

METHODS

Bacterial strains and cultivation conditions. The strains used in this study are listed in Table 1. The recommended media and growth conditions for each strain were used.

Extraction of total DNA. Two millilitres of a culture of exponentially growing cells was collected from broth cultures by centrifugation at 12000 ***g*** for 2 min. The pellets were washed with saline EDTA (0·15 M NaCl, 0·1 M EDTA, pH 8·0) and suspended in 500 µl saline EDTA. Proteinase K (2 µl, 20 mg ml^{-1}) was added and an incubation step was performed at 37 °C for 45 min. This was followed by a second incubation, after the addition of 40 µl 25% (w/v) SDS, this time at 60 °C for 10 min. Next, 180 µl 5 M

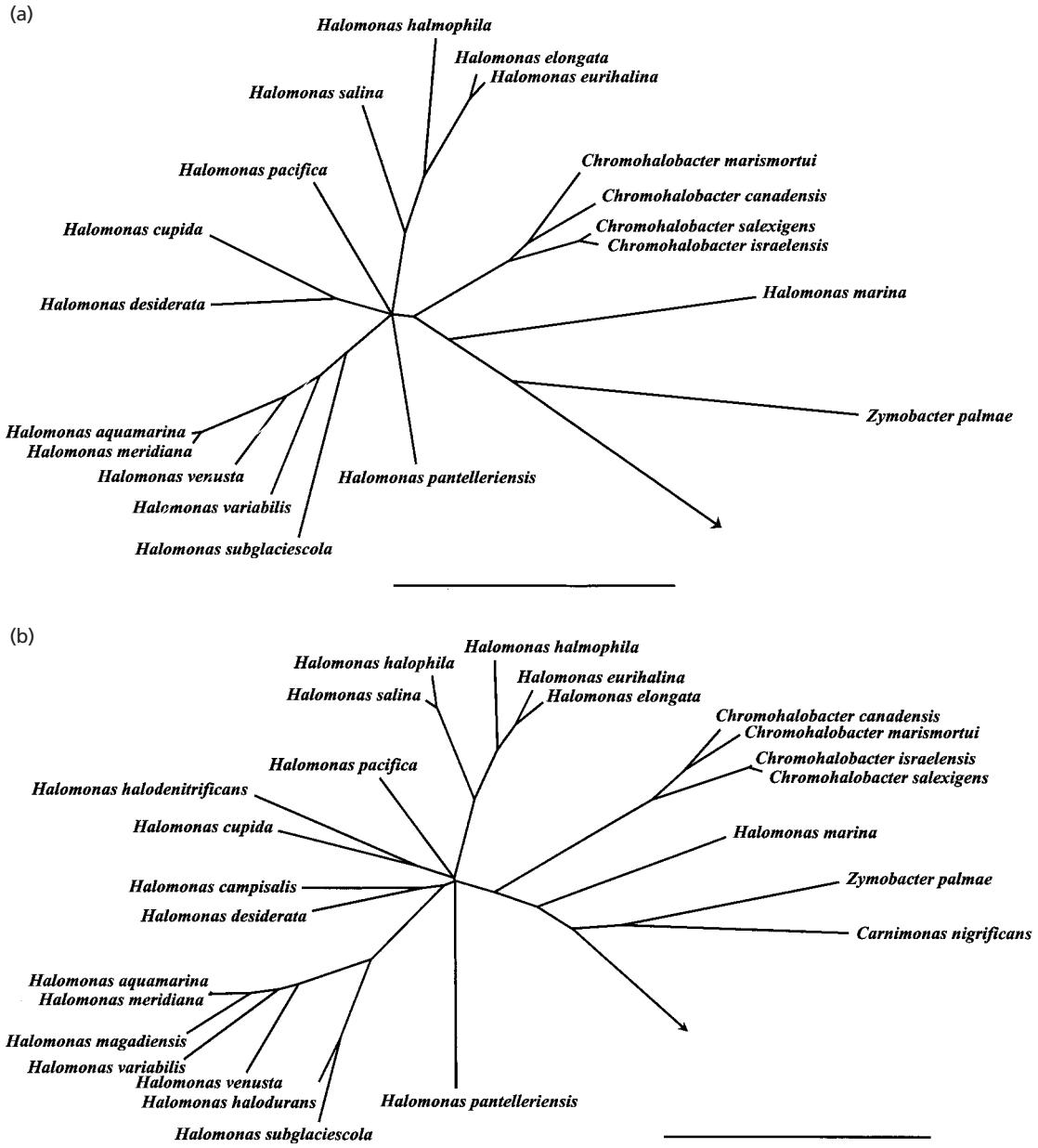

Fig. 1. Phylogenetic consensus trees of members of the genera *Halomonas*, *Chromohalobacter*, *Zymobacter* and *Carnimonas* constructed using 23S rRNA sequences (a) and 16S rDNA sequences (b). The arrows point to the outgroups, which have been removed to simplify the figure. Bars, 5% estimated sequence divergence.

sodium acetate and 745 µl chloroform/isoamyl alcohol (24:1) were added. The contents of the tubes were mixed gently and centrifuged for 2 min. After collecting the aqueous fraction, 2 vols cold ethanol was added to precipitate the DNA and the mixture was kept at −20 °C for at least 10 min. The precipitated DNA was then centrifuged and washed with 70% cold ethanol. Finally, the DNA was dissolved in redistilled water and checked by agarose gel electrophoresis.

23S and 16S rDNA gene amplification and sequencing. Approximately 10 ng total DNA was used for PCR amplification with the Taq PCR Core kit (Qiagen) following the recommendations of the manufacturer. Amplicons were checked by agarose gel electrophoresis and cleaned with the QIAquick PCR purification kit (Qiagen). For each microorganism, two fragments were amplified, one from position 8 of the 16S rDNA to position 2669 of the 23S rDNA (*Escherichia coli* numbering) and another from position 1091 of the 23S rDNA to the beginning of the 5S rDNA (Table 2). The first of these PCR products was used to sequence most of the 23S rDNA (5′ end) and, in some cases, also the full 16S rDNA. The second amplicon was used to reach the 3′ end of the 23S rDNA sequence. Sequencing was performed with a LICOR automated sequencer (MWG Biotech) using the Thermo Sequenase fluorescent-labelled primer sequencing kit (Amersham Pharmacia Biotech).

Phylogenetic analyses. 23S and 16S rDNA sequences analyses were performed separately, with the aid of the ARB

software package (Ludwig & Strunk, 1996). In the first case, about 500 complete or almost complete 23S rDNA sequences were available for the analysis, while this number was greater than 16000 in the case of the 16S rDNA sequences.

For the phylogenetic trees presented in Fig. 1, only sequences from the type strains of species whose names have been validly published were taken into account. When more than one sequence was available (Table 1), the most complete one was used. A distance matrix was obtained using a large number of outgroup sequences. The distance values were corrected for multiple base changes at single positions by the formula of Jukes & Cantor (1969) and a tree was reconstructed by applying the neighbour-joining method of Saitou & Nei (1987). The application of other treeing methods (maximum-parsimony and maximum-likelihood) allowed a collection of trees to be obtained that were compared to make an estimation of the confidence. Thus, collapsed branches in Fig. 1 indicate that the nodes affected showed more than one possible topology, whereas bifurcated branches are those that maintained their relative topology in all trees examined.

RESULTS AND DISCUSSION

In this study, we have determined the complete 23S rDNA sequences of 18 type strains and the complete 16S rDNA sequences of seven type strains (the previously available sequences of these strains contained several ambiguous positions) of members of the family *Halomonadaceae*. Following the recommendations of Ludwig *et al*. (1998), a combination of different treeing methods and filters was used for analysis of the 23S and 16S rDNA sequences. The trees presented in Fig. 1 synthesize the topologies obtained in each separate analysis. Only robust branches have been kept, while those affected by the methodology have been collapsed to form multifurcations.

As expected, there was good agreement between the 23S rDNA- and 16S rDNA-derived trees. Some minor differences can be detected, but it is also important to mention that the two datasets are not equivalent, since fewer 23S rDNA sequences are available. In contrast, for some species, more than one 16S rDNA sequence was available. Some of them were obtained in this study (Table 1). For the final analysis, the most complete sequences were used.

Several conclusions can be extracted from our phylogenetic analysis based on the 23S and 16S rDNA sequence comparison. Firstly, the genus *Halomonas* is not monophyletic and two phylogenetic groups are distinguishable. Group 1 comprises the type species, *Halomonas elongata*, and four other species, *Halomonas eurihalina*, *Halomonas halmophila*, *Halomonas halophila* and *Halomonas salina*. The mean 16S rDNA sequence similarity for this group was 98·2%. The same value was obtained with the 23S rDNA sequences. Group 2 is formed by the following species: *Halomonas aquamarina*, *Halomonas meridiana*, *Halomonas magadiensis*, *Halomonas variabilis*, *Halomonas venusta*, *Halomonas halodurans* and *Halomonas subglaciescola*. The mean 16S/23S rDNA sequence similarity for this group was 97·4 and 97·6%, respectively. The species *Halomonas pacifica*, *Halomonas halodenitrificans*, *Halomonas cupida*, *Halomonas desiderata*, *Halomonas campisalis* and *Halomonas pantelleriensis* did not fall clearly into either of the two groups mentioned above and did not form a group themselves. They shared relatively low values of sequence similarity with the strains included in groups 1 and 2 (91·7–96·4% for 16S rDNA sequences and 92·9–95·2% for 23S rDNA). Among themselves, the values are only slightly higher: 93·6–96·7% (16S rDNA) and 95·2–96·4% (23S rDNA). These results are in agreement with the phenotypic heterogeneity reported for the species of the genus *Halomonas*. Furthermore, there are other features that clearly support the need to clarify the current classification of the species of the genus *Halomonas*. The G+C content of the genus ranges from 52 to 68 mol%, a range too wide if we consider that it is generally accepted that G+C contents of members of the same genus should not differ by more than 10 mol% (Owen & Pitcher, 1985).

A phylogenetic group closely related to *Halomonas* is that of *Chromohalobacter marismortui*, which also includes *Halomonas canadensis* and *Halomonas israelensis*, recently proposed as members of the genus *Chromohalobacter* (Arahal *et al*., 2001a), and the newly proposed species *Chromohalobacter salexigens* (Arahal *et al*., 2001b). The mean 23S/16S rDNA similarity of this genus is 98·6/98·5%. In the consensus tree derived from the 23S rDNA, the node that clusters this genus is separate from that containing all *Halomonas* species, with the only exception of *Halomonas marina*, which always forms a deeper branching. The 23S/16S rDNA sequence similarities of this organism to any of the *Halomonas* or *Chromohalobacter* species respectively ranged from 91·8 to 94·9% and 90·9 to 93·0%, i.e. lower than 95% in all cases, which is generally accepted as a reference value for genus separation (Ludwig *et al*., 1998). Similarly, low values were obtained between the sequences of *Halomonas marina* and those of *Zymobacter palmae* and *Carnimonas nigrificans*. The affiliation of the marine organism *Halomonas marina* has changed since its description (Cobet *et al*., 1970) and it has been included in several genera (*Arthrobacter*, *Pseudomonas*, *Deleya* and *Halomonas*). Given these results, a reconsideration of the taxonomic status of *Halomonas marina* seems necessary.

Our phylogenetic study supports the inclusion of the genus *Carnimonas* in the family *Halomonadaceae*. In the original description of the single species *Carnimonas nigrificans* (Garriga *et al*., 1998), this possibility was rejected mainly because the 16S rDNA sequence signatures showed two differences from those described by Dobson & Franzmann (1996). However, we consider that there are several convincing reasons that support its inclusion. Firstly, it forms a very stable cluster with *Zymobacter palmae*. When the 16S rDNA

Table 2. Amplification and sequencing primers used in this study

Each forward amplification primer was used together with the reverse primer below it in the table. Sequencing primers were marked for infrared detection at the 5′ end. Sequences are listed according to the IUPAC code for nucleotide ambiguities. Primer positions are given according to the *E. coli* numbering.

Primer	Sequence	Position	Use
616Valt	AGAGTTTGATYMTGGCTCAG	8–27 (16S rRNA)	Amplification, Forward
985R	CCGGTCCTCTCGTACT	2654–2669 (23S rRNA)	Amplification, Reverse
1023V	GCGTAAYAGCTCACT	1091–1105 (23S rRNA)	Amplification, Forward
504R	SWGTTCGRVAWGGGA	35–49 (5S rRNA)	Amplification, Reverse
609RIII1	ACTACCVGGGTATCTAA	788–804	Sequencing, 16S rRNA
606RIII	CCCGRGAACGYATTCAC	1371–1378	Sequencing, 16S rRNA
609VIII2	AACAGGHTTAGATACCC	781–797	Sequencing, 16S rRNA
992IRry97	TTCCCTCACRGTACT	457–471	Sequencing, 23S rRNA
1020IRrmz98	TCTGGGYTGTTYCCCT	975–990	Sequencing, 23S rRNA
992IRvm97	AGTACCGTGAGGRAA	457–471	Sequencing, 23S rRNA
1019IRvm97	TAGCTGGTTCTYYCCGAA	803–820	Sequencing, 23S rRNA
1037IRrm97	CTTACCCGACAAGGAATTTCG	1934–1954	Sequencing, 23S rRNA
987IRry97	CTTAGATGCNTTCAG	2745–2759	Sequencing, 23S rRNA
1027VIR	AAACCGACACAGGTRG	1608–1623	Sequencing, 23S rRNA
328IRvm97	TCCTAAGGTAGCGAAATTCCTTG	1923–1945	Sequencing, 23S rRNA
1042GPHI	GTTTGGCACCTCGATGTCGRCTC	2490–2512	Sequencing, 23S rRNA

sequences of *Carnimonas nigrificans* and *Zymobacter palmae* are compared with those of the species of the genera *Halomonas* and *Chromohalobacter*, the similarity values are always quite high and, in most cases, even higher for the *Carnimonas nigrificans* 16S rDNA sequence.

The family *Halomonadaceae* was defined initially on the basis of 19 16S rDNA signatures (Dobson *et al*., 1993), but this was later reduced in four so that *Zymobacter palmae* could be included in the description of the family (Dobson & Franzmann, 1996). For the remaining 15 signatures, *Carnimonas nigrificans* differs in two residues, at positions 484 and 486, while *Zymobacter palmae* differs in none. But when the original 19 residues are checked, *Zymobacter palmae* shows four differences and *Carnimonas nigrificans* five, three of which (at positions 1424, 1439 and 1462) are identical in both species. Therefore, the requirement of having all 19 signatures is met only by the species of *Halomonas* (including *Halomonas marina*) and *Chromohalobacter*. *Zymobacter palmae* and *Carnimonas nigrificans* exhibit an almost equivalent number of mismatches (some of them coincident), reflecting, with some limitations, as we observe in Fig. 1, that the two genera are closely related and may have evolved from a common ancestor of *Halomonas–Chromohalobacter sensu lato*.

We therefore propose the inclusion of the genus *Carnimonas* in the family *Halomonadaceae*. A new description of the family is unnecessary, since the phenotypic traits of *Carnimonas nigrificans* are compatible with those reported for members of this family. *Carnimonas nigrificans* has been described as being able to grow with up to 8–10 % salts (Garriga *et al*., 1998), which is lower than the maximum salt concentration that allows growth of most species of the genera *Halomonas* and *Chromohalobacter*. The salinity range of *Zymobacter palmae* has not yet been reported.

The 16S rDNA sequence signatures of the family *Halomonadaceae* could be redefined to consist of 13 elements common to all members plus six residues with two possible bases. These are: 484 (A or G), 486 (C or U), 1424 (C or U), 1439 (C or U), 1462 (A or C) and 1464 (C or U).

The possibility of splitting the genus *Halomonas* into two or more genera is tempting, but has to be considered carefully to avoid excessive and unnecessary renaming. Besides, such a proposal should be accompanied by phenotypic or chemotaxonomic data. We have carried out a compilation of phenotypic features of the species included in our study for comparative purposes, but only a small fraction of these traits have been described for more than 80 % of the species (Table 3). In Table 3, the species have been grouped according to the results of the phylogenetic analysis. From these data, it can be concluded that there is not sufficient evidence to differentiate the phylogenetic groups within the genus *Halomonas*. Even for the species *Halomonas marina*, for which the phylogenetic evidence of separate generic status is strong, it is not possible to provide an unequivocal phenotypic description that differentiates it from the other members of the family *Halomonadaceae*.

Nevertheless, the recognition of the phylogenetic groups as presented here may help to understand this

Table 3. Differential features among the members of the family *Halomonadaceae*

Taxa are listed as: 1, *Halomonas elongata*; 2, *Halomonas eurihalina*; 3, *Halomonas halmophila*; 4, *Halomonas halophila*; 5, *Halomonas salina*; 6, *Halomonas aquamarina*; 7, *Halomonas meridiana*; 8, *Halomonas magadiensis*; 9, *Halomonas variabilis*; 10, *Halomonas venusta*; 11, *Halomonas halodurans*; 12, *Halomonas subglaciescola*; 13, *Halomonas pacifica*; 14, *Halomonas halodenitrificans*; 15, *Halomonas cupida*; 16, *Halomonas desiderata*; 17, *Halomonas campisalis*; 18, *Halomonas pantelleriensis*; 19, *Halomonas marina*; 20, *Chromohalobacter marismortui*; 21, *Chromohalobacter canadensis*; 22, *Chromohalobacter israelensis*; 23, *Chromohalobacter salexigens*; 24, *Zymobacter palmae*; 25, *Carnimonas nigrificans*. Data were taken from Akagawa & Yamasato (1989), Arahal *et al.* (2001a, b), Baumann *et al.* (1972), Berendes *et al.* (1996), Davis *et al.* (1969), Dobson *et al.* (1990), Duckworth *et al.* (2000), Fendrich (1988), Franzmann *et al.* (1987), Garriga *et al.* (1998), Hebert & Vreeland (1987), Huval *et al.* (1995), James *et al.* (1990), Mormile *et al.* (1999), Okamoto *et al.* (1993), Quesada *et al.* (1984, 1990), Romano *et al.* (1996), Rosenberg (1983), Valderrama *et al.* (1991), Ventosa *et al.* (1989), Vreeland *et al.* (1980) and this study. Characters are scored as: +, positive; −, negative; ND, not determined; d, differs among studies.

Characteristic	*Halomonas* rDNA group 1					*Halomonas* rDNA group 2							Ungrouped *Halomonas*							*Chromohalobacter*					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Morphology	Rods	Rods	Short rods	Short rods	Rods	Rods	Rods	Rods	Curved rods	Rods	Short rods	Rods	Rods	Short rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Short rods
Cell length (μm)	ND	2·0–2·5	0·9–1·3	1·5–2·0	2·0–2·5	4·0–6·0	1·9–4·5	4·0–6·0	1·0–3·0	ND	1·5–2·0	5–10	ND	0·9–1·2	ND	1·0–2·6	3–5	1·4–2·6	1·6–4·0	1·5–4·0	2·0–3·8	1·5–4·2	2·0–3·0	1·3–2·4	1·0–1·7
Cell width (μm)	ND	0·8–1·0	0·3–0·6	0·5–0·7	0·7–0·8	0·4–0·8	0·6–1·0	0·6–0·8	0·5–0·8	ND	0·4–0·6	0·5–1·1	ND	0·5–0·9	ND	0·4–0·6	1	0·4–0·7	0·8–1·2	0·6–1·0	0·6–1·2	0·6–0·9	0·7–1·0	0·7–0·9	0·5–0·6
Motility	+	−	+	+	−	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−
Flagellar arrangement*	L–P	–	L	Pe	–	Pe	L	ND	P	Pe	P	Pe	Pe	–	Pe	Pe	ND	ND	P	Pe	P	P	L–P	Pe	–
Pigmentation	None	Cream	White	Cream	Yellow–cream	None	White	Cream–beige	Cream	ND	None	Cream	ND	Cream	ND	None	ND	Cream–pink	Cream	Brown–yellow	White	Cream	White–cream	White	White
Facultative anaerobe	+	−	−	−	−	−	−	−	−	ND	−	−	ND	+	ND	+	+	−	ND	−	−	−	+	+	−
Salt range (%)	3·5–20	5–20	0·5–20	2–30	2·5–20	0–20	1–20	0–20	7–30	ND	3·5–20	0·5–20	ND	3–20	ND	0–18	1–26	1·2–15	2–12	2–30	3–25	3·5–20	0·9–25	ND	< 10
Temperature range (°C)	15–45	15–45	20–45	15–45	10–45	5–40	0–55	25–40	15–37	4–40	4–35	0–25	5–45	0–32	5–40	10–45	4–50	10–44	4–35	5–45	15–45	15–45	4–45	21–39	5–37
pH range	5–9	5–10	5–9	5–10	5–10	5–9	5–9	7–11	6·5–8·4	ND	5·5–8·5	5–9	ND	ND	ND	7–11	6–12	7·5–11	ND	5–10	5–9	5–9	5–9	3–10	ND
Hydrolysis of:																									
Casein	−	−	−	−	−	−	−	−	+	−	ND	+	−	ND	+	−	ND	−	+	−	−	−	d	ND	−
Aesculin	+	+	−	+	−	−	−	ND	+	−	+	−	−	−	+	ND	ND	ND	−	−	−	−	d	ND	+
Gelatin	+	+	−	−	−	−	ND	−	−	−	−	−	−	−	−	−	ND	−	−	−	−	−	d	−	−
Starch	−	−	−	−	−	d	+	−	−	−	−	−	−	−	−	−	ND	−	−	−	−	−	−	−	+
Tween 80	−	+	−	−	−	+	−	ND	ND	−	+	+	−	−	−	−	ND	ND	−	−	−	−	−	ND	−
Nitrate reduction	+	+	−	+	+	+	−	+	−	+	+	+	−	+	+	+	ND	+	−	−	+	+	+	−	−
Phenylalanine deaminase	−	−	ND	−	+	−	ND	−	−	−	−	−	−	ND	−	+	−	−	−	−	−	−	−	−	+
H_2S production	−	+	−	+	+	−	−	+	−	−	−	−	−	−	−	ND	ND	+	−	−	−	−	+	ND	ND
Urease	+	+	+	+	+	+	+	ND	+	+	d	−	+	ND	−	−	−		+	−	−	−	+	ND	−
Growth on:																									
Citrate	ND	+	−	+	+	−	+	+	−	+	+	−	+	ND	+	+	ND	+	+	−	+	ND	+	ND	+
Fructose	ND	−	+	+	+	+	+	+	−	+	+	−	+	ND	+	+	+	+	+	−	+	ND	+	ND	ND
Glucose	+	−	+	d	−	+	+	+	−	+	+	−	+	ND	+	+	+	+	+	+	+	ND	+	ND	ND
Glycerol	+	−	+	+	−	+	+	+	+	+	+	−	+	ND	+	+	+	+	+	ND	+	+	+	ND	ND
Mannose	+	+	+	+	−	+	+	+	−	−	+	−	−	ND	+	+	−	+	−	+	+	+	+	ND	+
Sucrose	+	+	+	+	−	−	+	+	−	+	ND	−	−	ND	+	+	+	+	−	+	−	+	+	ND	ND
G+C content (mol%)	60·5	58·8–59·1	63	66·7	60·4–64·2	57–58	58·2–59·9	62	61	52–55	63·2	60·9–62·9	67–68	65	60–63	66	66	65	62–64	62–65	62	65	62·4–66	55·8	56

*L, Lateral; P, polar; Pe, peritrichous.

heterogeneous group of halophilic micro-organisms and serve as a starting point for other studies. It is feasible that a comprehensive (and polyphasic) study may provide the data necessary for a more accurate classification of these organisms complementary to the phylogenetic view outlined in this study.

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