

Review

Taxonomy of the family *Halobacteriaceae*: a paradigm for changing concepts in prokaryote systematics

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The halophilic Archaea of the family *Halobacteriaceae* (36 genera with 129 species with standing in nomenclature as of November 2011) provide an excellent example of how changing concepts on prokaryote taxonomy and the development of new methods have influenced the way in which the taxonomy of a single group of prokaryotes is treated. This review gives an overview of the taxonomy of the family *Halobacteriaceae*, showing the impact that methods of phenotypic characterization, numerical taxonomy, chemotaxonomy and especially polar lipid analysis, 16S rRNA gene sequence comparisons, multilocus type analysis and comparative genomics have had on their classification.

Introduction

When in 1972 Helge Larsen (1922–2005) was honoured to deliver the A. J. Kluyver Memorial Lecture before the Netherlands Society for Microbiology, he chose as his title: ‘The halobacteria’s confusion to biology’. This intriguing title was a paraphrase on ‘The microbe’s contribution to biology’, the famous title of a series of lectures by Albert Jan Kluyver and Cornelis van Niel. At the time only two genera and three species were recognized within the family *Halobacteriaceae* (a name formally published two years later; Gibbons, 1974), and concepts such as 16S rRNA gene-based phylogeny and the special properties of the Archaea were still unknown. Still, it was already clear that halobacteria were fundamentally different from the other known prokaryotes as they lack a peptidoglycan cell wall, require high salt concentrations for growth and structural stability and have an unusual pigmentation (Larsen, 1973).

The first representatives of the family *Halobacteriaceae* were isolated more than a hundred years ago, and currently (November 2011) the family encompasses 36 genera with 129 species (Arahal *et al.*, 2011 and later updates). The complete genome sequences of at least 12 species have been published and more are expected to be released soon.

Abbreviations: In this paper three-letter abbreviations for genus names are used as recommended by the ICSP Subcommittee on the taxonomy of *Halobacteriaceae* (<http://www.the-icsp.org/taxa/halobacterlist.htm>): *Har.*, *Haloarcula*; *Hbt.*, *Halobacterium*; *Hcc.*, *Halococcus*; *Hfx.*, *Haloferax*; *Hla.*, *Halarchaeum*; *Hmc.*, *Halomicrobium*; *Hqr.*, *Haloquadratum*; *Hrd.*, *Halorhabdus*; *Hrr.*, *Halorubrum*; *Htg.*, *Haloterrigena*; *Nbt.*, *Natronobacterium*; *Ncc.*, *Natronococcus*; *Nmn.*, *Natronomonas*.

The family *Halobacteriaceae* are an excellent group to illustrate how concepts of prokaryote taxonomy, classification and nomenclature have changed since the genus name *Halobacterium* was formally introduced in 1957 (Elazari-Volcani, 1957). Methods of numerical taxonomy, chemotaxonomy, molecular phylogeny, multilocus sequence analysis (MLSA) and comparative genomics have all had an impact on the way we currently look at the taxonomy of this group. Not all classification problems have been solved yet, and ideas on the taxonomy of the family *Halobacteriaceae* are constantly changing. This review presents an overview of how our concepts on the taxonomy of these intriguing extremely halophilic Archaea have changed over the years.

Early descriptions of red extremely halophilic prokaryotes

The first descriptions of rod-shaped, red and extremely halophilic prokaryotes date from 1919 when Henrich Klebahn described ‘*Bacillus halobius ruber*’. Klebahn’s study of the ‘damaging agents of the klippfish’ was recently made available in an English translation prepared in collaboration with descendants of the author (DasSarma *et al.*, 2010). Soon after, Harrison & Kennedy (1922) isolated ‘*Pseudomonas salinaria*’ from the red coloration of cured codfish. The type strain of *Halococcus morrhuae* was isolated in 1880 by Farlow, and this species is found in the early literature as ‘*Sarcina litoralis*’ Poulsen 1879, ‘*Sarcina morrhuae*’ Farlow 1880, ‘*Micrococcus litoralis*’ (Poulsen 1879) Kellerman 1915, ‘*Micrococcus morrhuae*’ (Farlow 1880) Klebahn 1919, and ‘*Hcc. litoralis*’ (Poulsen 1879) Schoop 1935. Tindall (1992) presented an in-depth discussion on the history of the early isolates and the problems with strain identities.

and the family *Halobacteriaceae* are placed. Grant *et al.* (2001) proposed the name *Halobacteria* for a class consisting entirely of halophilic Archaea. This name, however, could not be validly published at the time, as it was a homotypic synonym of *Halomebacteria*, a class that encompasses the extreme halophiles and the mesophilic, often somewhat halophilic methanogens, with the *Halobacteriales* as the proposed type order of the class (Cavalier-Smith, 2002). The name *Halomebacteria* had priority over *Halobacteria* [Validation List No. 85, *Int J Syst Evol Microbiol* 52 (2002) 685–690]. In 2008, the Judicial Commission of the ICSP discussed the status of the names of classes proposed by Cavalier-Smith. It was unanimously agreed that all names listed by Cavalier-Smith (2002) should be rejected (Garrity *et al.*, 2011) and this opinion was adopted by the plenary meeting of the ICSP. The name *Halobacteria* thus regained status in the nomenclature as the name of the class that currently has one order, the *Halobacteriales*, with one family, the *Halobacteriaceae*.

In 2008, DasSarma & DasSarma proposed to rename the order, family and type genus of the *Halobacteriales* as *Haloarchaeales*, *Haloarchaeaceae* and *Haloarchaeum* (DasSarma & DasSarma, 2008). The changes were recommended to ‘modernize’ haloarchaeal taxonomy as, according to the opinion of the authors, the current order, family and genus designation had become obsolete. The newly proposed names were chosen to reflect membership in the archaeal rather than the bacterial domain. Indeed, the term ‘haloarchaea’ is extensively used to designate the aerobic halophilic Archaea, but this term has no status in the nomenclature. The change proposed by DasSarma & DasSarma is in violation of the General Considerations, Principles and Rules of the International Code of Nomenclature of Bacteria/Prokaryotes, and therefore the new names cannot be validly published. The aim of The Code is to bring stability of names; useless creation of names should be avoided and a legitimate name may not be replaced because it is inappropriate, disagreeable or another name is preferable (Principle 1(1), Principle 1(3) and Rule 55, respectively; Oren, 2008b). The name *Halararchaeum* was validly published in 2010 for a new genus within the family (Minegishi *et al.*, 2010). The proposed name was *Halararchaeum* rather than *Haloarchaeum*, in agreement with the orthography recommendations of the Code (Appendix 9), according to which a connecting vowel is dropped when the following word element starts with a vowel.

There is an interesting issue relating to the specific epithet of the type species of the type genus of the family *Halobacteriaceae*. The name originally proposed was *Halobacterium salinarium* (Elazari-Volcani, 1957), adopting the specific epithet from *Pseudomonas salinaria* (Harrison & Kennedy, 1922). The original spelling *Halobacterium salinarium* was corrected by Ventosa & Oren (1996) as supposedly the specific epithet is a grammatically incorrect form for the genitive of *salinae* (salt works, salt pan), the correct form being *salinarum*. However, in the original publication the etymology is L. adj. *salinarius* (-a, -um) of salt works, not

salinae, -arum (salt works). A renewed investigation of the issue yielded an unexpected result. Most Latin dictionaries do not list the adjective *salinarius*, of salt works, and the few that do contain such an entry all refer to Book 8 of ‘*De Architectura*’ by Marcus Vitruvius Pollio. However, nearly all editions of Vitruvius contain the text ‘... *ut etiam in areis salinarum videtur*’, where *salinarum* is a noun and not an adjective. Only in an Italian edition of 1567 and in a Dutch edition of 1649 of Vitruvius did I find a different version: ‘...*ut etiam in areis salinariis videtur*’, and this was undoubtedly the source of the adjective *salinarius* quoted by some dictionaries. I found no records that the adjective *salinarius* occurs elsewhere in the Latin writings from antiquity, and therefore it is not clear whether the adjective *salinarius* is correct classical Latin.

Among the above-mentioned 129 species names of the family *Halobacteriaceae*, one name is illegitimate: *Hfx. alexandrinus* (Asker & Ohta, 2002). As the genus name *Haloferax* is in the neuter gender, the epithet must also be in the neuter gender; i.e. *alexandrinum* (Rule 12c(1) of The Code).

Finally, there is a problem with the name *Hbt. piscisalsi* (Yachai *et al.*, 2008). This name should now be considered a junior synonym of *Hbt. salinarum*. *Hbt. piscisalsi* JCM 14661^T shares a high 16S rRNA gene sequence similarity and a high DNA–DNA relatedness with *Hbt. salinarum*, and this was confirmed by 16S rRNA gene sequencing of the type strain obtained from different culture collections. The authors of the species description acknowledged that their earlier published sequence was incorrect (Oren & Ventosa, 2010; Arahal *et al.*, 2011). A formal proposal to consider *Hbt. piscisalsi* as a junior synonym of *Hbt. salinarum* is now in press (Minegishi *et al.*, 2011).

Chemotaxonomy to guide classification of the family *Halobacteriaceae*: diversity of polar lipids

The Archaea were recognized as a separate branch of prokaryotes in the late 1970s. However, the unique lipids with ether bonds and isoprenoid hydrophobic side chains of the family *Halobacteriaceae*, later discovered to be a general feature of the Archaea, had already been known for a long time. In 1962, Morris Kates and coworkers had elucidated the structure of the lipids of *Hbt. salinarium* (*cutirubrum*) (Sehgal *et al.*, 1962), and soon after members of the genus *Halococcus* were found to possess similar ether lipids (Kates *et al.*, 1966). Although it was clear that the lipids of members of the genera *Halobacterium* and *Halococcus* differed considerably from those of other prokaryotes, including moderately halophilic ones, the potential impact of this finding on prokaryote taxonomy was not recognized at the time. Thanks to Kates’ work we know much about the diversity of the phospholipids and glycolipids among the representatives of the family *Halobacteriaceae* (Kates, 1978, 1996). Overviews of the structure of haloarchaeal lipids and their use in chemotaxonomy are found, for example, in papers by Kamekura (1993) and Oren (2006).

Polar lipid chemotaxonomy of the group is based on the presence or absence of the diether derivative of phosphatidylglycerol sulfate, the diversity of glycolipids or their absence, and the length of the isoprenoid hydrophobic chains. The glycolipid pattern, in combination with other properties examined in a numerical taxonomy study of a large number of isolates, was used to establish two new genera within the family: *Haloarcula* and *Haloferax*, renaming *Hbt. vallismortis* as *Har. vallismortis* and *Hbt. volcanii* as *Hfx. volcanii* (Torreblanca *et al.*, 1986). This was soon followed by the description of other species belonging to these new genera, *Har. hispanica* and *Hfx. gibbonsii* (Juez *et al.*, 1986) and transfer of *Hbt. denitrificans* to the genus *Haloferax* as *Hfx. denitrificans* on the basis of glycolipid analysis and other chemotaxonomic properties (Tindall *et al.*, 1989). Later 16S rRNA gene-based phylogenetic studies confirmed the status of these genera as distinct lineages.

Alkaliphilic members of the family *Halobacteriaceae* such as the genera *Natronomonas*, *Natronobacterium* and *Natronococcus* lack major amounts of glycolipids (Soliman & Trüper, 1982; Tindall *et al.*, 1984). Some alkaliphiles may, in addition to the major components phosphatidylglycerol and phosphatidylglycerolphosphate methyl ester, contain minor amounts of other phospholipids. Based on different patterns of three yet unidentified phospholipids it is possible to distinguish between the alkaliphiles *Nmn. pharaonis*, *Ncc. occultus*, *Nbt. gregoryi* and *Natrialba magadii* (Morth & Tindall, 1985a).

In the early 1980s it was reported that certain members of the family *Halobacteriaceae*, notably alkaliphilic representatives, have not only biphytanyl (C₂₀,C₂₀) diether lipids, but also contain C₂₀,C₂₅ diether (2-O-sesterterpanyl-3-O-phytanyl-*sn*-glycerol) lipids. A thin layer chromatography procedure was developed for the detection of such lipids (Ross *et al.*, 1981; De Rosa *et al.*, 1982). Some alkaliphiles also contain small amounts of C₂₅,C₂₅ (2,3-di-O-sesterterpanyl-*sn*-glycerol) diether lipids (De Rosa *et al.*, 1983). The ratios at which the different core lipids are produced depend to some extent on the growth conditions. In most haloalkaliphilic strains the relative amounts of diphytanyl diether lipids decrease with increasing salinity (Morth & Tindall, 1985b; Tindall, 1985).

There are a few reports of the occurrence of unsaturated isoprenoid chains in the lipids of some halophilic Archaea. Unsaturated phytanyl side chains were found in *Hrr. lacusprofundi* from Deep Lake, Antarctica, which grows at temperatures down to 4 °C (Franzmann *et al.*, 1988; Gibson *et al.*, 2005). The distribution of such 'phytenyl' lipids has been poorly explored and the property has not yet been considered in chemotaxonomic comparisons. However, unsaturated hydrophobic chains may be more common in the lipids of members of the family *Halobacteriaceae*: *Hrb. utahensis*, *Nmn. pharaonis*, *Hfx. sulfurifontis* and *Halobaculum gomorrense* were found to contain more unsaturated and polyunsaturated phytanyl and sesterterpanyl chains in their lipids when they were grown at the highest

possible salinities than at the lowest salt concentrations supporting growth (Dawson *et al.*, 2011).

Other chemotaxonomic properties used for the study of the family *Halobacteriaceae*

Except for polar lipid analysis, comparative chemotaxonomy has played only a minor role in taxonomic studies of the halophilic Archaea. Most members of the family *Halobacteriaceae* have a cell wall composed of glycoprotein subunits (Guan *et al.*, 2011), but members of the genus *Halococcus* have a complex polysaccharide wall (Schleifer *et al.*, 1982), and the cell wall of *Ncc. occultus* consists of repeating units of a poly(L-glutamine) glycoconjugate (Niemetz *et al.*, 1997). In-depth studies of the cell-wall structure, including the evaluation of the chemical structure of the sugar moieties in cell-wall glycolipids, have never been made in the framework of taxonomic comparisons. Whether such studies are relevant is doubtful in view of the recent finding that the type of S-layer glycoprotein N-glycosylation in *Hfx. volcanii* depends on the salt concentration at which the cells were grown (Guan *et al.*, 2011).

The chemical structures of the respiratory quinones and polyamines are seldom taken into account in taxonomic work (Oren, 2006). Polyamines are useful chemotaxonomic markers for many groups of prokaryotes, but this is not the case for the family *Halobacteriaceae*. The cells contain very low amounts of polyamines, near or below detection level. When polyamines have been detected at all, these have included putrescine, spermidine, spermine and agmatine (Cateni-Farina *et al.*, 1985; Hamana *et al.*, 1985, 1995; Kamekura *et al.*, 1986).

The most abundant isoprenoid quinones in the family *Halobacteriaceae* are MK-8 and MK-(8-VIII-H₂) (Collins *et al.*, 1981). The relative abundance of these two menaquinones varies according to the age of the culture and the salinity of the medium (Tindall *et al.*, 1991). In addition, methylated menaquinones have been detected in some species (Tindall & Collins, 1986; Collins & Tindall, 1987).

The impact of 16S rRNA gene sequence comparison on the taxonomy of the family *Halobacteriaceae*

The recognition in the late 1970s that *Hbt. halobium* (now renamed *Hbt. salinarum*) is a member of the Archaea was based on 16S rRNA oligonucleotide cataloguing, at the time the only method to obtain sequence information on rRNAs (Magrum *et al.*, 1978). Complete 16S rRNA gene sequences of *Hfx. volcanii* and *Hbt. cutirubrum* (*salinarum*) became available in 1983 and 1985, respectively (Gupta *et al.*, 1983; Hui & Dennis, 1985).

Phylogenetic studies of the family *Halobacteriaceae* based on 16S rRNA are complicated by the fact that some species contain more than one copy of the 16S rRNA gene, and these copies can be very different. Sequence heterogeneity

between the two genes encoding 16S rRNA from *Har. marismortui* was already known by the late 1980s (Mevarech *et al.*, 1989); these genes are divergent in 5% of the nucleotide positions (Mylvaganam & Dennis, 1992). All species of the genera *Haloarcula* and *Halomicrobium* have at least two different copies of the gene; in the genus *Haloarcula* the divergence is 4.8–5.6%, in the genus *Halomicrobium* this rises to 9% and all these genes are transcribed (Cui *et al.*, 2009). *Halosimplex carlsbergense* was also reported to have multiple divergent 16S rRNA genes (Vreeland *et al.*, 2002). Fluorescence *in situ* hybridization studies of *Har. marismortui* have shown that each single cell has ribosomes with type I and with type II 16S rRNA (Amann *et al.*, 2000), but the genes may be differentially expressed at different temperatures (López-López *et al.*, 2007).

16S rRNA gene comparisons have played a major role in the classification of prokaryotes as soon as the techniques for routine sequencing became available. In fact, our present-day ‘polyphasic’ taxonomy is to some extent dominated by 16S rRNA gene sequence comparisons, and this is also the case for the family Halobacteriaceae. Some taxonomic rearrangements and nomenclature changes were primarily based on 16S rRNA gene sequence information. A notable example is the establishment of the genus *Halorubrum*, now the genus with the largest number of species within the family, based on reclassification of four species earlier described as members of the genus *Halobacterium*: *Hrr. saccharovororum*, *Hrr. sodomense*, *Hrr. trapanicum* and *Hrr. lacusprofundi*. The new classification was supported by chemotaxonomy (polar lipid patterns) (McGenity & Grant, 1995).

Proposed minimal standards for description of new taxa in the order Halobacteriales

A document proposing minimal standards for description of new taxa in the order Halobacteriales, family Halobacteriaceae was prepared in 1997 on behalf of the ICSB/ICSP subcommittee on the taxonomy of Halobacteriaceae (Oren *et al.*, 1997). The polyphasic approach, which calls for determination of a range of phenotypic, chemotaxonomic and genotypic properties to be included in species descriptions, remains valid today, as confirmed in a recent meeting of the subcommittee (Arahal *et al.*, 2011). The required assessments include phenotypic tests such as the ability to grow on a range of single carbon sources, formation of acid from sugars, anaerobic growth on arginine or nitrate and a range of additional phenotypic properties, chemotaxonomic tests – primarily the determination of polar lipid composition, 16S rRNA gene sequencing, DNA–DNA hybridization tests comparing the new strain with its closest relatives (as based on 16S rRNA gene sequences), and the G+C content of the DNA. These proposed minimal standards have been followed in all or nearly all recent descriptions of novel species.

The case of *Hqr. walsbyi*, which has 46.9 mol% G+C in its DNA (Burns *et al.*, 2007), a value much lower than all other

described species (range: 59–70 mol%), shows that this parameter remains important. Anaerobic growth on arginine, originally documented in *Halobacterium* strains (Hartmann *et al.*, 1980), apparently occurs only in members of the genus *Halobacterium*. This led to the development of a selective enrichment and isolation method for *Halobacterium* spp. (Oren & Litchfield, 1999). Other modes of anaerobic growth have been documented in the family Halobacteriaceae, including photoheterotrophy based on energy conversion by the light-driven proton pump bacteriorhodopsin (Hartmann *et al.*, 1980), and fermentative growth as shown by *Hrd. tiamatea*, isolated from an anaerobic deep brine pool in the Red Sea (Antunes *et al.*, 2008). Genome analysis indicated that lactate dehydrogenase may participate in this fermentation (Antunes *et al.*, 2011).

Multilocus sequence analysis in Halobacteriaceae taxonomy

The use of multilocus sequence analysis (MLSA) to obtain more in-depth phylogenetic information than can be gained from 16S rRNA gene comparisons has been discussed in past meetings of the ICSP subcommittee on the taxonomy of Halobacteriaceae (Oren & Ventosa, 2010; Arahal *et al.*, 2011). Based on a comprehensive study involving 33 species belonging to 14 genera, Papke *et al.* (2011) recommended the following housekeeping genes as useful in such MLSA studies: *atpB* (ATPase subunit), *EF-2* (protein synthesis elongation factor), *radA* (DNA repair), *rpoB* (RNA polymerase subunit) and *secY* (protein export through the membrane). Analysis of phylogenetic trees based on these genes has largely confirmed the topology of 16S rRNA gene sequence-based trees.

A recent study by Minegishi *et al.* (2012) investigated the order of the genes found upstream of the 16S rRNA gene. Two clusters were recognized: one has the *pyrD* gene (coding for dihydroorotate oxidase), the other has the *lpdA* (dihydrolipoamide dehydrogenase) gene immediately upstream of the rRNA operon. It was suggested that this property may be used to split the family Halobacteriaceae, thus far the only family within the class Halobacteria and the order Halobacteriales, into two families. Discussions in the subcommittee strongly discouraged splitting of the family based solely on a single genotypic property as long as no phenotypic properties can be found that discriminate between the clusters (Arahal *et al.*, 2011).

The power of the MLSA approach to obtain information about the relationships between different isolates belonging to a single genus was shown in a study by Papke *et al.* (2007) in which a large number of *Halorubrum* strains isolated from a Spanish saltern and a salt lake in Algeria were compared using 16S rRNA, *atpB*, *bop*, *EF-2* and *radA* as marker genes. Although clusters could be defined by concatenation of multiple marker sequences, the barriers to exchange between them were found to be leaky. The authors suggested that no non-arbitrary way to circumscribe

'species' is likely to emerge for this group, or by extension, to apply generally across prokaryotes. Homologous recombination is very common and MLSA is an excellent approach to assess this.

Taxonomy of the family *Halobacteriaceae* in the genomics era

Complete genome sequences of members of the family *Halobacteriaceae* are becoming available rapidly. After the sequence of *Halobacterium* NRC-1 (a strain of *Hbt. salinarum*; Gruber *et al.*, 2004) was published in 2000 (Ng *et al.*, 2000), followed by *Har. marismortui* in 2004 (Baliga *et al.*, 2004) and *Nnm. pharaonis* in 2005 (Falb *et al.*, 2005), the genomes of least twelve further species have now been sequenced: *Hrr. lacusprofundi*, *Hmc. mukohataei*, *Halogeometricum borinquense*, *Htg. turkmenica* (Anderson *et al.*, 2011), *Hqr. walsbyi* (Bolhuis *et al.*, 2006), *Hfx. volcanii* (Hartman *et al.*, 2010), *Hrd. utahensis* (Bakke *et al.*, 2009), *Hrd. tiamatea* (Antunes *et al.*, 2011), *Har. hispanica* (an organism with two chromosomes and one megaplasmid; Liu *et al.*, 2011), *Halalkalicoccus jeotgali* (Roh *et al.*, 2010), *Natrialba magadii* and *Halopiger xanaduensis*. The genome size varies from 2.6 Mbp (*Halobacterium* NRC-1) to 5.4 Mbp (*Htg. turkmenica*). A second strain of *Hqr. walsbyi* isolated from a site geographically distant from the place of isolation of the type strain has also been sequenced; the genome sequences of the two strains are highly similar (Dyall-Smith *et al.*, 2011).

The range of organisms sequenced now encompasses organisms with an extremely high and with a moderate requirement for salt, neutrophiles as well as alkaliphiles, a facultative psychrophile, organisms with unusual cell morphologies, and a species with an anaerobe life style. More strains are currently being sequenced and it probably will not take more than a few years before the complete genome sequences of the type strains of all species of the family *Halobacteriaceae* will have been determined.

Interestingly, the list of genomes sequenced thus far does not include that of the type strain of *Hbt. salinarum* (ATCC 33171^T=CIP 104033^T=DSM 3754^T=JCM 8978^T=NBRC 102687^T=NCIMB 764^T=NRC 34002^T=VKM B-1769^T), the type species of the type genus of the family and the order. Two related strains have been sequenced: *Halobacterium* NRC-1 and *Hbt. salinarum* R1, and their genomes proved to be nearly identical. Still it is to be regretted that no genomic information is available for the nomenclatural type.

Comparison of the genome sequences available today has enabled some general conclusions about metabolic potentials of different members of the family to be made (Anderson *et al.*, 2011), but comparative genomics has not yet had a major impact on the taxonomy of the group.

Final comments

The above overview illustrates how our views on the taxonomy of the family *Halobacteriaceae* have changed since the

name *Halobacterium* was formally introduced more than half a century ago (Elazari-Volcani, 1957). The classification, first based on cell morphology, salt requirement, Gram staining reaction and pigmentation, was later modified on the basis of chemotaxonomy data, especially the polar lipid composition. In the 1980s, comparison of 16S rRNA gene sequences triggered many changes in the classification of the species. In recent years techniques such as MLSA and comparative genomics have yielded new information that will soon have an impact on the classification of the many new genera and species that have been described, especially during the past two decades. Similar developments have of course occurred in the taxonomy of other groups of prokaryotes, as taxonomic views constantly change when new techniques are introduced and new results become available.

Currently there are nearly 130 species of the family *Halobacteriaceae* with validly published names, and the results of recent metagenomics studies show that there are many more species to be isolated and characterized (Bodaker *et al.*, 2010; Narasingarao *et al.*, 2011; Ghai *et al.*, 2011). Still, there is no generally agreed-upon concept how to define a prokaryote species, and this is true also for the halophilic Archaea. Based on MLSA studies, it is even unlikely that a non-arbitrary way to circumscribe 'species' will emerge for this group, or by extension, to apply generally across prokaryotes. Papke *et al.* (2007) conclude the discussion section of their study on MLSA of a large number of *Halorubrum* isolates as follows: 'As Hanage *et al.* (2006) recently remarked of the claim that clusters we can call bacterial species exist, "In fact, there are almost no data that address this assertion, which in essence is a statement of belief. A more agnostic view is to ask whether populations of similar bacteria do invariably (or usually) form discrete, well-resolved genotypic clusters that merit the status of species and to consider which methods should be used to address this issue". We suggest that concatenation does not address the issue satisfactorily, because it will inevitably produce clusters as long as there is any degree of geographical or ecological structuring of bacterial populations. What we expect in terms of discreteness of such clusters before we will call them species remains to be negotiated. Until we have agreed on what we are looking for, we cannot tell whether we have found it'.

Thus, in spite of the great advances made in recent years, the picture is still far from clear. When in 1972 Helge Larsen delivered his lecture on 'The halobacteria's confusion to biology' (Larsen, 1973), he probably expected that modern science would soon solve the enigmas that existed at the time. Now, four decades later, considerable progress has been made, but some confusion still remains.

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