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REVIEW

Masahiro Kamekura

Diversity of extremely halophilic bacteria

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Abstract In this review, the history of the classification of the family *Halobacteriaceae*, the extremely halophilic aerobic Archaea, is reviewed with some emphasis on the recently described new genera *Halobaculum*, *Halorubrum*, *Natrialba*, *Natronomonas*, and "*Haloterrigena*." Speculation is made about the evolutionary relationship between members of the *Halobacteriaceae* and the extremely halophilic, anaerobic methanogens of the genera *Methanohalobium* and *Methanohalophilus.* Efforts to find missing links between the two groups are also reviewed.

Key words *Halobacterium* · *Haloarcula* · *Halococcus* · *Halorubrum* · *Natrialba* · *Halobaculum* · *Natronomonas* · *Natronobacterium* · *Natronococcus* · 16S rRNA genes · Halophilic Archaea

Introduction

The extremely halophilic bacteria that have been defined as microorganisms which grow best in media containing 2.5–5.2 M (saturated) NaCl (Kushner and Kamekura 1988) constitute the following three groups:

1. Members of the aerobic halophilic Archaea that require at least 12% (2 M) NaCl for growth are classified within the family *Halobacteriaceae*, which currently (as of January 1998) contains ten valid genera, namely, *Halobacterium, Halococcus, Haloarcula, Haloferax, Halorubrum, Halobaculum, Natrialba, Natronomonas, Natronobacterium*, and *Natronococcus* (Grant and Larsen 1989; Oren et al. 1995; McGenity and Grant 1995; Kamekura and Dyall-Smith 1995; Kamekura et al. 1997).
2. Members of the anaerobic, extremely halophilic methanogenic Archaea are found in the genera *Methanohalophilus* and *Methanohalobium* within the family *Methanosarcinaceae.*
3. Halophilic Eubacteria are known such as *Actinopolyspora halophila* or *Ectothiorhodospira halochloris*. The extremely halophilic actinomycete *A. halophila* is extraordinary in that it requires a minimum salt concentration of 12% for growth in liquid media, exactly the same as for the halophilic Archaea (Johnson et al. 1986). Details on the halophilic Eubacteria are summarized by Kushner and Kamekura (1988).

In this review, focus is placed on the halophilic Archaea, particularly on the family *Halobacteriaceae*.

History and the present status of the classification of the family *Halobacteriaceae*

Halobacteria are a group of microorganisms forming a part of the domain Archaea that require high salt concentrations for growth. For many years the taxonomy of halobacteria has been confused, partly because the classification system was not based on ribosomal DNA sequence data and partly because many of the deposited strains retained their original classification in the oldest genus, *Halobacterium.*

January 1, 1980, was a starting date for new bacterial nomenclature in accordance with the Bacteriological Code. The history of the classification of halobacteria after 1980 is summarized here (next) by tracing the validation of isolates of halobacteria. After the listing of each genus and species name, the original reference is cited. In cases in which the names were published outside the *International Journal of Systematic Bacteriology* (IJSB), numbers of the validation lists in which the new name was validated in IJSB are also indicated. Species names written in bold letters are presently accommodated in the genus, and (T) indicates the

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M. Kamekura
Noda Institute for Scientific Research, 399 Noda, Noda-shi, Chiba-ken 278-0037, Japan
Tel. +81-471-23-5573; Fax +81-471-23-5959
e-mail: mkamekur@supernig.nig.ac.jp

type species. The history before 1980 has been reviewed by many halobacteriologists, particularly by Tindall (1992).

Halobacterium Approved list (IJSB 30:225–420, 1980)
The genus *Halobacterium* was the first genus described to accommodate the extremely halophilic bacteria isolated from salted fish or hides. Seven species were listed in the Approved list (IJSB 30:225–420, 1980). They are shown below, with the generic names to which they were later transferred in parentheses:

Halobacterium cutirubrum, *Halobacterium halobium*, *Halobacterium salinarium* (→***Halobacterium salinarum***; Ventosa and Oren 1996)
Halobacterium saccharovorum (→*Halorubrum*)
Halobacterium trapanicum (→*Halorubrum*)
Halobacterium vallismortis (→*Haloarcula*)
Halobacterium volcanii (→*Haloferax*)

The following species, described after 1980, were once accommodated in *Halobacterium* and validated, but at present all of them have been transferred to other new genera as described below.

Halobacterium sodomense Oren 1983 (→*Halorubrum*)
Halobacterium pharaonis Soliman and Trüper 1983 (validation list No. 10) (→*Natronobacterium* →*Natronomonas*)
Halobacterium mediterranei Rodriguez-Valera et al. 1983 (validation list No. 12) (→*Haloferax*)
Halobacterium denitrificans Tomlinson et al. 1986 (→*Haloferax*)
Halobacterium lacusprofundi Franzmann et al. 1988 (validation list No. 29) (→*Halorubrum*)
Halobacterium distributum Zvyagintseva and Tarasov 1987 (validation list No. 31) (→*Halorubrum*)

At present, ***Halobacterium salinarum*** (T) is the only species that has been approved to belong to this genus. *Halobacterium cutirubrum* and *H. halobium* are now included in *H. salinarum* as belonging to the same species (Ventosa and Oren 1996). *Halobacterium salinarum* contains two major membrane glycolipids, S-TGD-1 and S-TeGD (Kates 1993).

Halococcus Approved list (IJSB 30:225–420, 1980)
Halococcus was the second genus after *Halobacterium* to be classified within the family *Halobacteriaceae*. Up to the present, the following four species have been validated:

Halococcus morrhuae (T) Approved list (IJSB 30:225–420, 1980)
Halococcus turkmenicus Zvyagintseva and Tarasov 1987 (validation list No. 31)
Halococcus saccharolyticus Montero et al. 1989 (validation list No. 32)
Halococcus salifodinae Denner et al. 1994

The 16S rRNA gene sequences of two strains of the type species, *Halococcus morrhuae*, ATCC 17082 and NRC 16008, had been reported, and comparative sequence analysis using available 16S sequences had clearly shown that *Halococcus* represents a distinct genus within the family *Halobacteriaceae*. However, the complete sequences of the three other validly described species (*Halococcus saccharolyticus*, *Halococcus turkmenicus*, and *Halococcus salifodinae*) had not been published, so phylogenetic support for their inclusion in this genus was lacking. To determine the phylogenetic relationships of these three species, Ventosa et al. (in manuscript) determined the 16S rRNA gene sequences of the type strains of the three species, and showed that *Halococcus saccharolyticus* and *Halococcus salifodinae* clustered nicely with the type species of *Halococcus morrhuae*. The three true *Halococcus* species do not lyse in distilled water or even with *N*-lauroylsarcosine, a detergent that causes the complete lysis of rod-shaped halobacteria. Halococci contain S-DGD-1 as the major membrane glycolipid (see Kates 1993 for the structure). However, *Halococcus turkmenicus*, which was lysed easily with *N*-lauroylsarcosine, was closely related to *Halobacterium trapanicum* JCM 9743 and strain GSL-11 (Ventosa et al. in manuscript). The phylogenetic position of *Halococcus turkmenicus* is discussed under "*Haloterrigens*."

Natronobacterium Tindall et al. 1984 (validation list No. 15)
The alkaliphilic members of *Halobacteriaceae* form a distinct physiological group because they not only require high NaCl concentrations but also high pH and low Mg^{2+} concentrations for growth. They have been isolated from a variety of alkaline, hypersaline lakes and soils. Microscopically, the initial isolates consisted of rods and cocci and were accordingly separated into two genera, *Natronobacterium* and *Natronococcus*, by Tindall et al. (1984). The genus *Natronobacterium* contained the following four recognized species, consisting of rod-shaped haloalkaliphiles:

Natronobacterium gregoryi (T) Tindall et al. 1984 (validation list No. 15)
Natronobacterium magadii Tindall et al. 1984 (validation list No. 15)
Natronobacterium pharaonis (←*Halobacterium pharaonis*) Tindall et al. 1984 (validation list No. 15)
Natronobacterium vacuolatum Mwatha and Grant 1993

The classification of these isolates was based largely on chemotaxonomic criteria, particularly phenotypic properties. Glycolipid analysis, which had been useful in the taxonomy of the neutrophilic halobacteria, had little impact in the classification of the natronobacteria, as these lack major amounts of glycolipids in their membranes. The complete sequence of the 16S rRNA gene of only one species, *Natronobacterium magadii*, had been reported, and comparisons with other halobacteria had clearly shown that it represented a distinct genus. However, the sequences of the other three species of *Natronobacterium* had not been published, so phylogenetic support for their inclusion in this genus was lacking. DNA-DNA hybridization of the four species indicated that they shared very little sequence homology (31%–38%) (Mwatha and Grant 1993).

To determine the phylogenetic relationships among the natronobacteria, and in particular to ascertain whether the four members of the genus *Natronobacterium* were indeed close relatives, Kamekura et al. (1997) determined the 16S rRNA sequences of the three unsequenced species of *Natronobacterium* and of one unclassified haloalkaliphilic strain SSL1. Surprisingly, when the similarity values of the four recognized members of the genus *Natronobacterium* were examined, all were less than 92% (the highest similarity was observed between *N. gregoryi* and *N. magadii*, 91.9%). Devereux et al. (1990) and Fry et al. (1991) have proposed that a similarity of less than 98% in 16S rRNA sequences was considered evidence for separate species, and a similarity less than 93%–95% indicates that they should be in different genera. These data clearly indicated that the four species may be sufficiently different to warrant classification in different genera. *Natronobacterium pharaonis* and *N. vacuolatum* showed particularly weak similarities to each other (88.3%), to *N. gregoryi* (88.5% and 88.1%, respectively), and to *N. magadii* (88.8% and 87.1%, respectively). *Natronobacterium magadii* was most similar to the strain SSL1 (95.9%) and similarity to *N. asiatica* 172P1 was 93.3%. The similarity of strain SSL1 to *N. asiatica* was even greater (96.6%).

These sequences were then incorporated into phylogenetic tree reconstructions of the family *Halobacteriaceae*. A variety of algorithms were utilized (maximum likelihood, maximum parsimony, distance matrix), which gave very similar topologies. As anticipated from the similarity values, the four recognized *Natronobacterium* species and strain SSL1 did not form a monophyletic group but were distributed throughout the tree. *Natronobacterium gregoryi* remains the type species and branches just before the natronococci, whereas *N. magadii* and strain SSL1 branched before *Natrialba*. The exact branching order of *N. magadii* and SSL1 was not clear in parsimony or distance matrix trees, but the bootstrap values indicated a clear separation of *N. magadii* and *N. gregoryi*. It has therefore been proposed to rename *Natronobacterium magadii* as *Natrialba magadii.* On the other hand, *Natronobacterium vacuolatum* was most closely related to the genus *Halorubrum* (see following; *Halorubrum*).

Natronobacterium pharaonis turned out to be most distantly related to the other genera. Even the most similar sequence to that of *N. pharaonis*, i.e., *Haloarcula marismortui* rRNA gene A, showed a low similarity value of 89.9%. The lack of bootstrap values at the branch point indicates that parsimony and distance methods could not confidently identify the deepest branching order in the tree (bootstrap values, 54%–55%). However, the maximum likelihood method gave strongly supported branch lengths in this region. The evidence from sequence similarity and tree reconstructions appears to present a clear case for this isolate as representing a new genus and to be redesignated as *Natronomonas pharaonis* gen. nov., comb. nov. (Kamekura et al. 1997) (see following; *Natronomonas*).

Duckworth et al. (1996) have recently isolated a variety of alkaliphilic Archaea from several soda lakes in the Kenyan section of the East African Rift Valley. Detailed taxonomic examination of these isolates might alter the classification of the alkaliphilic halophiles.

Natronococcus Tindall et al. 1984 (validation list No. 15)

Natronococcus occultus (T) Tindall et al. 1984 (validation list No. 15)

Natronococcus amylolyticus Kanai et al. 1995

The two species have been firmly retained in the genus *Natronococcus.*

Haloarcula Torreblanca et al. 1986 (validation list No. 22)

At present, the following six species have been validated:

Haloarcula vallismortis (T) (←*Halobacterium vallismortis*) Torreblanca et al. 1986 (validation list No. 22)

Haloarcula hispanica Juez et al. 1986 (validation list No. 22)

Haloarcula marismortui Oren et al. 1990

Haloarcula japonica Takashina et al. 1990 (validation list No. 36)

Haloarcula argentinensis Ihara et al. 1997

Haloarcula mukohataei Ihara et al. 1997

"*Haloarcula sinaiiensis*," isolated by Javor et al. (1982), and "*Haloarcula aidinensis*," isolated by Xu et al. (1995), have long been used in the phylogenetic tree reconstruction. "*Haloarcula californiae*" strain GN (Javor et al. 1982) has also not been validated.

The major membrane glycolipid of these species is TGD-2 (see Kates 1993), except *H. mukohataei*, which has S-DGD-1. Details on the difference of this species from other members of *Haloarcula* are discussed by Kamekura (in press).

Members of the genus *Haloarcula* are known to possess considerably heterogeneous 16S rRNA genes; *Haloarcula marismortui* A and B (94.4% similarity), "*Haloarcula sinaiiensis*" major and minor (97.3 %), two genes for *Haloarcula vallismortis* (96.2%), etc. We also have detected at least two heterogeneous genes from *Haloarcula hispanica*. The strain XA3-1, an isolate from northern China, is even more interesting in that its two genes exhibited just 92% similarity (Kamekura, unpublished data).

Square gas-vacuolated flat halophilic Archaea were first found by Walsby (1980) in a coastal brine pool in the Sinai peninsula, Egypt. Their presence has since been documented in a variety of hypersaline environments, but no pure culture has been available. The fact that members of the genus *Haloarcula* sometimes produce flat square cells is not sufficient as a proof for the phylogenetic positioning of the Walsby's square bacteria. Recently Oren's group noticed that a saltern crystallizer pond in Eilat, Israel, was dominated, more than 55%, by similar kinds of bacteria. They analyzed the glycolipids of cells collected from 10 l brine and found that they contained S-DGD-1 (Oren et al. 1996). Halophile strain 801030/1 isolated from Sinai, which sometimes produces square cells in culture (Alam et al. 1984), was recently found to belong to the genus *Haloarcula* based on 16S rRNA gene sequences and the polar lipid pattern (Oren, personal communication).

Haloferax Torreblanca et al. 1986 (validation list No. 22)

This genus now contains four valid species. The characteristic glycolipid is S-DGD-1, the same as those of the members of *Halococcus.*

Haloferax volcanii (T) (←*Halobacterium volcanii*) Torreblanca et al. 1986 (validation list No. 22)

Haloferax gibbonsii Juez et al. 1986 (validation list No. 22)

Haloferax mediterranei (←*Halobacterium mediterranei*) Torreblanca et al. 1986 (validation list No. 23)

Haloferax denitrificans (←*Halobacterium denitrificans*) Tindall et al. 1989

Taxonomic investigation of "*Haloferax* alicantei" is now in progress (Dyall-Smith, personal communication).

Halobaculum Oren et al. 1995

Halobaculum gomorrense (T) Oren et al. 1995

A mass bloom of halophilic archaea developed in the Dead Sea in the summer of 1992. In the past, enrichment cultures in which Dead Sea water or sediment was used as inocula have yielded three novel halophiles, *Haloferax volcanii*, *Haloarcula marismortui*, and *Halorubrum sodomense.* The main polar lipid of harvested biomass in 1992 was S-DGD-1, suggesting that the dominant organisms were relatives of the genus *Haloferax* (see above). One isolate, a pleomorphic rod-shaped bacterium, was further analyzed extensively by Oren's group. On the basis of 16S rRNA gene sequence data, they concluded that the isolate was sufficiently different from the previously described members of *Halobacteriaceae*, and named it as *Halobaculum gomorrense* gen. nov., sp. nov. (Oren et al. 1995).

Halorubrum McGenity and Grant 1995 (validation list No. 56)

Two articles were published in 1995 concerning reclassification of the genus *Halobacterium.* Based on the phylogenetic tree reconstructed by sequences of 16S rRNA genes, McGenity and Grant (1995) proposed to transfer four species, *Halobacterium saccharovorum, Halobacterium sodomense, Halobacterium lacusprofundi*, and *Halobacterium trapanicum*, to a novel genus, *Halorubrum*. The proposal of the new generic name was accepted and appeared in IJSB validation list No. 56.

In a paper published almost at the same time, Kamekura and Dyall-Smith (1995) proposed to transfer *Halobacterium saccharovorum*, *Halobacterium sodomense*, *Halobacterium lacusprofundi,* and *Halobacterium distributum* to a novel genus, *Halorubrobacterium*, and proposed a novel species, *Halorubrobacterium coriense*. In this article they briefly discussed the confusion regarding the identities of different strains of *Halobacterium trapanicum*, and refrained from including *Halobacterium trapanicum* in the new genus. The new genus name *Halorubrobacterium* was validated in list No. 57, but treated as a later synonym of *Halorubrum* (see also Oren and Ventosa 1996). Later, Kamekura et al. (1997) proposed to transfer the alkaliphilic halophile *Natronobacterium vacuolatum* to the genus *Halorubrum*, which now comprises seven valid species.

Halorubrum saccharovorum (T) (←*Halobacterium saccharovorum*) McGenity and Grant 1995 (validation list No. 56)

Halorubrum lacusprofundi (←*Halobacterium lacusprofundi*) McGenity and Grant 1995 (validation list No. 56)

Halorubrum sodomense (←*Halobacterium sodomense*) McGenity and Grant 1995 (validation list No. 56)

Halorubrum trapanicum (←*Halobacterium trapanicum*) McGenity and Grant 1995 (validation list No. 56)

Halorubrum distributum (←*Halorubrobacterium distributum* ← *Halobacterium distributum*) Oren and Ventosa 1996

Halorubrum coriense (←*Halorubrobacterium coriense*) Oren and Ventosa 1996

Halorubrum vacuolatum (←*Natronobacterium vacuolatum*) Kamekura et al. 1997

As described under *Natronobacterium*, *Natronobacterium vacuolatum* showed a 16S rRNA sequence similarity of 95.6% to *Halorubrum saccharovorum*, the type species of the genus *Halorubrum*, indicating that they may belong to the same genus. In addition, a close examination of the 37 aligned sequences, used in reconstructing the tree, has revealed many sequence signatures (Winker and Woese 1991). Sequences of the six species of *Halorubrum* possess 19 genus-specific signature bases. The *Natronobacterium vacuolatum* sequence shares 16 of these 19 bases. Sequences of spacer regions (regions between the 16S rRNA encoding gene and the 23S rRNA encoding gene) from the five recognized species of *Halorubrum* were easily aligned with that of *Natronobacterium vacuolatum*, and the alignment showed long stretches of conserved sequence. If sequences from members of the other genera were introduced, the alignment was severely disrupted. Altogether, these data seem to support the view that *Natronobacterium vacuolatum* is related to members of the genus *Halorubrum. Natronobacterium vacuolatum* was transferred to the genus *Halorubrum*, and the strain called *Halorubrum vacuolatum* comb. nov. (Kamekura et al. 1997).

No glycolipids have been detected in *Natronobacterium vacuolatum* (Mwatha and Grant 1993), whereas most of the species of the genus *Halorubrum* are characterized by the presence of the glycolipid S-DGD-3 (Kamekura and Dyall-Smith 1995; see Trincone et al. 1990 for the structure), but an exception has been reported, i.e., S-DGD-5 for *Halorubrum trapanicum* NRC 34021 (McGenity and Grant 1995). We cannot exclude the possibility that a trace amount of glycolipid is present in *Halorubrum vacuolatum*.

The two strains of *Halorubrum trapanicum* NRC 34021 and NRC 2856, at NRC, Ottawa, have died during storage (R. Latta, personal communication, 1995). The only culture of the type strain (NRC 34021) survives in the laboratory of W.D. Grant of University Leicester. Recently, Grant has redeposited his strain to The National Collection of Industrial and Marine Bacteria as NCIMB 13488. Also, they have submitted a short paper to the *International Journal of Systematic Bacteriology*, including a request for an opinion to the Judicial Commission, proposing NCIMB 13488 as the neotype strain of *Halorubrum trapanicum*.

Natrialba Kamekura and Dyall-Smith 1995 (validation list No. 57)
Natrialba asiatica (T) Kamekura and Dyall-Smith 1995 (validation list No. 57)
Natrialba magadii (←*Natronobacterium magadii*) Kamekura et al. 1997

The strains 172P1 and B1T have long been shown to contain a novel glycolipid, and the structure was finally determined as S2-DGD-1 (2,3-di-*O*-phytanyl-or phytanyl sesterterpenyl-1-*O*-[2,6-$(HSO_3)_2$-α-mannopyranosyl-(1→2)-α-glucopyranosyl]-*sn*-glycerol (Matsubara et al. 1994). The group consisting of 172P1 and B1T forms a distinct group that is highly supported by bootstrap analysis in the reconstruction of phylogenetic trees based on 16S rRNA gene sequences. The two strains possess C_{20}, C_{25} core diether lipids as shown by chemical analyses and also by the double spots of PG and PGP-Me (phosphatidyglycerophosphate methyl ester) on thin-layer chromatography (TLC). Both strains are not red pigmented, unlike other members of *Halobacteriaceae*, with a content of bacterioruberins less than 0.1% of that of *Halobacterium salinarum* NRC 34001 as estimated by the absorption of neutral lipid fractions at 500nm. Cultivation under illumination does not cause pigmentation, whereas intensities of the pigmentation of the strain GSL11 and *Halobacterium trapanicum* JCM 9743 (now "*Haloterrigens turkmenicus*"; see following) were enhanced upon illumination during cultivation on agar slopes.

Natronobacterium magadii showed a high sequence similarity of 16S rRNA gene to that of strain SSL1 (95.9%) and to those of *Natrialba asiatica* 172P1 (type strain) and *Natrialba asiatica* B1T (93.3%, and 93.7%, respectively). These data support the view that *Natronobacterium magadii* and the strain SSL1 are members of the genus *Natrialba*, and we have proposed the transfer of *Natronobacterium magadii* to the genus *Natrialba* as *Natrialba magadii* and regard the strain SSL1 as a *Natrialba* species.

Lipid analyses, however, do not support the close 16S rRNA sequence similarities. No glycolipid has been detected in *Natrialba magadii*, but the strain SSL1 has a minor amount of glycolipid DGD-4 (glucose-(1→6)-glucose glycerol diether; Kates 1993; Upasani et al. 1994). *Natrialba asiatica* has a glycolipid S2-DGD (mannose-2,6-disulfate-(1→2)-glucose glycerol diether).

A variety of extreme halophiles recently were isolated from hypersaline ponds in Northern China. Some strains, OF8 and QX1, have been suggested to belong to the genus *Natrialba* judged from the glycolipid compositions and 16S rRNA gene sequences (Kamekura et al., unpublished data).

Natronomonas Kamekura et al. 1997
Natronomonas pharaonis (T) (←*Natronobacterium pharaonis* ← *Halobacterium pharaonis*) Kamekura et al. 1997

As described under *Natronobacterium*, *Natronobacterium pharaonis* was only distantly related to any species of the family *Halobacteriaceae*, with similarities less than 89.9%. Thus, *Natronobacterium pharaonis* was transferred to the genus *Natronomonas*, gen. nov. (Kamekura et al. 1997).

"***Haloterrigena***" (submitted to IJSB (Ventosa et al., in manuscript)

The new species of the new genus, "*Haloterrigena turkmenicus*," has been proposed to accommodate the following species: *Halococcus turkmenicus*, *Halobacterium trapanicum* JCM 9743, and *Halobacterium trapanicum* NCIMB 784.

As described under *Halococcus*, *Halococcus turkmenicus* turned out not to belong to this genus, but was closely related to *Halobacterium trapanicum* JCM 9743 and strain GSL-11 from the phylogenetic tree reconstrution based on 16S rRNA sequences. *Halobacterium trapanicum* NCIMB 784 also clustered with the *Halobacterium trapanicum* JCM 9743/strain GSL-11 group. Moreover, DNA-DNA hybridization studies have shown that the three strains, *Halococcus turkmenicus*, *Halobacterium trapanicum* JCM 9743, and strain GSL-11 are considered to belong to the same species because the DNA-DNA homology values among them are greater than 75%.

The type strain of the new species should be the oldest validated strain. Reviewing the histories of all three isolates showed that *Halobacterium trapanicum* would appear to be the earliest described species. The history of *Halobacterium trapanicum* has been reviewed extensively by Tindall (1992). There had been serious discrepancies between different laboratories when reporting the morphology and glycolipids of the type strains (Kamekura and Dyall-Smith 1995; Kamekura [in press]). The only culture of the type strain (NRC 34021) surviving in the laboratory of W.D. Grant was recently deposited to NCIMB as 13488, and the confusion and dispute over *H. trapanicum* were finally settled, as described under *Halorubrum*. Accordingly, *Halobacterium trapanicum* JCM 9743 cannot be used as the type strain, leaving *Halococcus turkmenicus* as the next isolate to be described and validated. Isolation and characterization of *Halococcus turkmenicus* was reported in 1987 by Zvyagintseva and Tarasov, and validated in the validation list No. 31 in 1989. Ventosa et al. therefore proposed to transfer *Halococcus turkmenicus* to "*Haloterrigena*" gen. nov. as "*Haloterrigena turkmenicus*" gen. nov., comb. nov., and to accommodate *Halobacterium trapanicum* JCM 9743, strain GSL-11, and *Halobacterium trapanicum* NCIMB 784 in the same species (Ventosa et al., in manuscript).

The strain GSL-11 (initially designated as L-11) was isolated from the Great Salt Lake by F.J. Post of Utah State University (Post and Al-Harjan 1988) and provided to M. Kamekura in 1984. Later, it was incorporated into taxonomic studies as L-11.

The relationship to extremely halophilic methanogens

The kingdom Euryarchaeota comprises all types of Archaea: five orders of methanogens (*Methanobacteriales*, *Methanomicrobiales*, *Methanococcales*, *Methanosarcinales*, and *Methanopyrales*), *Halobacteriales*, *Archaeoglobales*, *Thermoplasmales*, and *Thermococcales*. It is of interest that the *Methanomicrobiales*/*Methanosarcinales* lineage has spawned the extreme halophiles, the sulfate reducers, and the cell-wall-less *Thermoplasma*.

The Archaea of the two orders *Halobacteriales* and *Thermococcales* (hyperthermophiles) are unique in that they are devoid of any tetraether lipids. It is however noteworthy that some mesophilic methanogens form both diether and tetraether lipids while others form only diethers, and some thermophilic methanogens, including some hyperthermophiles (e.g., *Methanopyrus kandleri*) completely lack tetraethers. The relatively close relationship of *Methanomicrobiales/Methanosarcinales* and *Halobacteriales* has also been confirmed by analyses of 23S rRNA sequences (De Rijk et al. 1995). The fact that the former are strict anaerobes while the latter are aerobes poses a question on the nature of the precursors of these Archaea during evolution.

It is also of great interest that *Methanosarcinales* include alkaliphilic or halophilic methanogens; some extremely halophilic methanogens have been isolated from hypersaline environments, such as *Methanohalophilus mahii*, *Methanohalophilus halophilus*, and *Methanohalobium evestigatum*. Kamekura and Kates (1997) have speculated on the evolutionary relationship between members of the *Halobacteriaceae* and halophilic methanogens. However, a close relationship between members of the *Halobacteriaceae* and these halophilic methanogens is not supported by 16S RNA encoding gene sequences.

Future prospects for halobacteria

In recent years, microbial diversity in nature has been studied by analysis of 16S ribosomal RNA genes amplified by polymerase chain reaction (PCR) with DNA extracted directly from environmental samples. Molecular phylogenetic studies indicate a much greater phylogenetic and probably physiological diversity of Archaea than previously assumed. Crenarchaeotal phylotypes, which were originally thought to consist solely of hyperthermophilic organisms, have been detected in nonthermophilic environments, such as subsurface ocean waters, polar seas, soybean fields, and freshwater lake sediments etc. (Schleper et al. 1997).

Jurgens et al. (1997) have sequenced some cloned 16S rRNA genes amplified by PCR with archaea-specific primers from mixed-population DNA extracted from forest soil in northern Finland. Quite extraordinary are the 869-bp sequences of two clones, FFSB12 and FFSB 9, which were almost identical to that of *Halobacterium salinarum*, with only 6-bp differences. Jurgens' group has obtained more of this type of sequence (Jurgens, personal communication), an extraordinary finding in view of the nonhypersaline nature of the environment.

As indicated in this review, aerobic archaeal halophiles are restricted in diversity compared with the other branches of Archaea, such as methanogens and sulfur-dependent thermophiles. However, novel phylotypes of halobacteria have been isolated from hypersaline environments (Benlloch et al. 1995). Two clones, HAC1 and HAC4, obtained from a sample of a crystallization pond in Alicante, Spain, were found to be significantly different from 16S rRNA genes from all other isolated species reported so far, with 22 bases distinct from consensus sequences compiled from 41 strains to date, the differences being scattered throughout the whole sequences. Comparison with the consensus sequences of methanogens, including those of extremely halophilic methanogens *Methanohalophilus* and *Methanohalobium* spp. (see foregoing), suggested that HAC1 and HAC4 were not methanogens, and might represent members of a novel family within the order *Halobacteriales*. Such organisms might shed light on the evolutionary relationship between extreme halophiles and methanogens within the kingdom Euryarchaeota. The author of this minireview is also trying to isolate such organisms from saline environments.

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