Diversity of *Haloquadratum* and other haloarchaea in three, geographically distant, Australian saltern crystallizer ponds

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Abstract *Haloquadratum walsbyi* is frequently a dominant member of the microbial communities in hypersaline waters. 16S rRNA gene sequences indicate that divergence within this species is very low but relatively few sites have been examined, particularly in the southern hemisphere. The diversity of *Haloquadratum* was examined in three coastal, but geographically distant saltern crystallizer ponds in Australia, using both culture-independent and culture-dependent methods. Two 97%-OTU, comprising *Haloquadratum-* and *Halorubrum-*related sequences, were shared by all three sites, with the former OTU representing about 40% of the sequences recovered at each site. Sequences 99.5% identical to that of *Hqr. walsbyi* C23T were present at all three sites and, overall, 98% of the *Haloquadratum*-related sequences displayed ≤2% divergence from that of the type strain. While haloarchaeal diversity at each site was relatively low (9–16 OTUs), seven phylogroups (clones and/or isolates) and 4 different clones showed ≤90% sequence identity to classified taxa, and appear to represent novel genera. Six of these branched together in phylogenetic tree reconstructions, forming a clade (MSP8-clade) whose members were only distantly related to classified taxa. Such sequences have only rarely been previously detected but were found at all three Australian crystallizers.

Keywords Archaea · Halobacteria · Halobacteriaceae · Hypersaline · Cultivation · Biodiversity

Introduction

The microbial flora of thalassohaline, hypersaline waters, with salinities close to saturation, have been studied for many years, but only relatively recently has it been possible to identify and to culture many of the dominant groups (Antón et al. 2002; Burns et al. 2004a, b). The majority are extremely halophilic Archaea belonging to the family *Halobacteriaceae* (Oren 2002), although halophilic Bacteria, such as *Salinibacter*, may also be present (Antón et al. 2002). A peculiar organism which often dominates these microbial communities is the square haloarchaeon of Walsby (*Haloarchaeum walsbyi*), an extremely thin, square-shaped organism that was first cultivated in the laboratory in 2004 (Bolhuis et al. 2004; Burns et al. 2004b). Formally described in 2007, it has been named *Haloquadratum walsbyi*, with the type strain being C23T (Burns et al. 2007).
The cells of *Hqr. walsbyi* are easily recognized under light microscopy not only by their shape, thin squares of about 2 µm per edge, but also because they contain phase-bright gas vesicles and phase-dark granules of PHB (Burns et al. 2007; Walsby 1980). They often make up 40–80% of total cells in neutral, thalassohaline, hypersaline waters, such as salt lakes, sabkhas and solar saltern crystalliser ponds (Antón et al. 1999; Burns et al. 2004a). The genome sequence of a Spanish isolate (HBSQ001) has been determined (Bolhuis et al. 2006), and was later compared to environmental DNA sequences recovered from the same saltern crystalliser pond from which this isolate was recovered (Cuadros-Orellana et al. 2007). Compared to other halochoreae, the genome of *Hqr. walsbyi* has an unusually low G+C% (=49); its gene density is much lower (mainly due to the presence of many pseudogenes); and it forms a remarkably coherent species, with 16S rRNA gene sequences from environmental DNA that do not diverge by more than 1.6% from that of the isolate HBSQ001 (Legault et al. 2006). Indeed, the two described members of this genus, one from Australia and the other from Spain, show only 2 nt differences in their 16S rRNA genes (strains C23T and HBSQ001, respectively) (Burns et al. 2007), yet they were isolated from ponds 16,950 km apart.

Clone library and FISH studies of individual saltern crystallizers in Israel, Spain, Tunisia and Turkey (Antón et al. 1999; Baati et al. 2008; Mutlu et al. 2008; Oren et al. 2006), also support the view that *Hqr. walsbyi* is widespread, often dominant within the microbial community, and forms a tightly coherent species. In hypersaline waters where *Haloquadratum* is not dominant (and perhaps not present), other halochoreaeal groups dominate, such as *Halorubrum*, *Halococcus*, or *Halobacterium* (Bidle et al. 2005; Bowman et al. 2000; Pašić et al. 2007), but the reasons for this are unknown.

While the divergence of *Haloquadratum* appears to be low, other, common halochoreae that co-exist in these and similar waters, such as *Halorubrum* and *Halococcus*, display a more usual level of speciation. *Halorubrum* has 24 recognized species, and *Halococcus* has seven (http://www.the-icsp.org/taxa/halobacteriaceae.html). 16S rRNA gene sequence studies indicate that many more species exist in nature (Burns et al. 2004a; Ochsenreiter et al. 2002). Why is it that *Haloquadratum* appears to be far more homogeneous than similar organisms that compete with it for space and nutrients? To determine if this difference reflects the small number of sites that have so far been studied, mostly in the northern hemisphere, we examined the diversity of halochoreae in the crystallizer ponds of three, widely separated, Australian solar salterns. Using cultivation-independent and culture-dependent methods, two major groups of halochoreae were found to be present in similar proportions at the three locations (*Haloquadratum* and *Halorubrum*), but each site also possessed a significant fraction of unique organisms representing novel genera within the Halobacteriaceae. The great majority of *Haloquadratum*-related sequences were found to be very closely related showing ≤2% divergence, but there was some evidence of speciation.

### Materials and methods

#### Sample sites, collection, and water analyses

The sites, water chemistry and microbial counts are summarized in Table 1 and supplementary Table 1. Water samples (0.8–1 L) were collected from each saltern crystalliser in a sterile glass Schott bottle submerged 25 cm beneath the surface. These were sent by overnight courier to the laboratory (at ambient temperature) and cell samples were removed and processed for DNA extraction the next day. The three Australian sites were the Cheetham Salt Ltd crystallizer ponds at: Dry Creek, crystallizer pond no. 7, Price, South Australia; Bajool crystallizer pond no. 4, Bajool, Queensland; and Lara storage pond no. 1, Corio, Victoria. Chemical analyses were performed at a private laboratory. Total cell counts were performed as described previously (Burns et al. 2004a; Dyall-Smith 2001).

#### Media and cultivation

**DBCM2 medium** and modified growth medium (MGM) were prepared as described previously (Dyall-Smith 2001). Solid media used 1.5% agar (Difco-Bacto). Cultivation from water samples used direct plating on MGM and by liquid extinction cultures using DBCM2 medium, following the protocols described previously (Burns et al. 2004a, b).

#### DNA extraction, clone libraries, sequence analysis and phylogenetic tree reconstructions

DNA extraction and the construction of clone libraries of 16S rRNA genes were performed as described previously (Burns et al. 2004a). Briefly, the cells from 10 ml of water sample were pelleted, broken open using a bead-beater, and genomic DNA extracted using chloroform/isoamyl alcohol extraction, then precipitated from the aqueous phase with 0.54 vol of isopropanol. For each DNA preparation, five replicate PCRs (each 50 µl in volume) were prepared, and 16S rRNA genes amplified using PCR primers that selectively amplified archaeal genes [archF1, ATTCCGGTT GATCCTGC (Ihara et al. 1997); 1492ra, ACGHHTACK TTGTACGACTT (Grant et al. 1999)]. Replicate PCRs were pooled, separated on agarose gels, and the ~1.5 kb DNA band cloned using the Invitrogen TOPO TA cloning
Clone sequences were screened for chimeric PCR products using the online program Bellerophon ver.3 (http://greengenes.lbl.gov/) (Huber et al. 2004). Sequences were then used to reconstruct phylogenetic trees using the ARB phylogeny software package by Ludwig et al. (1998) (http://www.arb-home.de). Sequences were imported into ARB, aligned with the full-length 16S rRNA gene sequences of the Greengenes database (http://greengenes.lbl.gov), checked for consistency and, where necessary, manually adjusted and added to the main backbone tree using the parsimony addition methods within ARB. The maximum likelihood algorithm was used for full tree reconstruction, as implemented within the ARB package (AXML). Bootstrap values were assigned based on 1000 replicate tree reconstructions using distance (NJ/Felsenstein correction) or parsimony (PHYLIP/DNAPARS) algorithms. Diversity indices, OTU clustering, and multiple sample statistics were computed using the MOTHUR suite of programs, version 1.4, available at http://schloss.micro.umass.edu/.

Sequences

The 16S rRNA gene sequences retrieved in this study have been deposited at Genbank (www.ncbi.nlm.nih.gov), and have accession numbers GQ374922–GQ375042.

Results

Sample sites, water chemistry and microscopy

Three coastal, solar salterns, at widely differing locations in Australia, were selected. These were in South Australia (Dry Creek), Victoria (Lara), and Queensland (Bajool). The Bajool site is about 1700 km from either the Lara or Dry Creek sites, and it is about 640 km between the latter two sites (Fig. 1). Crystallizer pond samples were collected prior to harvesting of salt in May 2007, and were at salt saturation (~34% w/v). Sample locations, climatic data, water analyses and cell counts are presented in Table 1, and the detailed water chemistry is given in supplementary Table 1. The salt compositions were similar to those reported for other marine solar salterns (Burns et al. 2004a), and all had total cell counts above \(10^7\) cells/ml, with high proportions (>40%) of cells displaying the typical morphological characteristics of Hqr. walsbyi (Burns et al. 2007; Walsby 1980).

Archaeal diversity

To assess the diversity of Archaea present in each water sample, cells were collected by centrifugation, their DNA
extracted, and 16S rRNA genes PCR amplified using haloarchaeal-specific primers. The products were cloned into TOPO plasmids and sequenced (see Methods), and about 40, near-complete 16S rRNA gene sequences (average of 1,440 nt) were determined for each sample. Table 1 summarizes the numbers of clones sequenced, the number of 97% sequence similarity OTUs, indices of community variation and estimates of species richness. Sequences were imported into the ARB phylogeny package, aligned to the Greengenes pre-aligned dataset (http://greengenes.lbl.gov), and compared by percentage similarity (Fig. 2) and phylogenetic tree reconstructions (Fig. 3).

As expected from the initial microscopy, sequences related to Haloquadratum dominated the clone libraries of all three samples, representing just over 40% of the sequences at each site (Fig. 2). Halorubrum-related sequences were also significant at all sites (8–20%), while Halonotius-related sequences were present at two sites (10–15%). The latter group was identified in a previous study (Burns et al. 2004a), and has recently been formally described (Burns et al. 2009a). The number of 97%-similarity groups (97%-OTUs) varied between 9 and 16, a relatively low number compared to the microbial communities found in non-extreme environments (Janssen et al. 2002), but typical for crystallizer ponds (Benlloch et al. 2002; Pašić et al. 2007). Each site possessed sequences representing novel taxa, and these were highly variable between the sample sites, with many showing very low levels of similarity to classified taxa of the Halobacteriaceae.

The relationships between the archaeal sequences recovered from the three sites are more clearly seen when...
presented as a phylogenetic tree reconstruction, as shown in Fig. 3, which includes all the clone sequences as well as some isolate sequences from this study (see below) along with representatives of 27 genera of the Halobacteriaceae. For convenience, closely branching sequences have been collapsed into numbered phylogroups, most of which consist of sequences belonging to the same 97%-OTU. Where a phylogroup contains more than one OTU, this is indicated under the name. Single sequences that branch separately from the others are named according to site and clone number (e.g., LDS clone 35). 51 clone sequences were closely similar to each other and to the rRNA gene sequence of *Hqr. walsbyi* C23T (analyzed in more detail below), and they branched tightly together forming *Haliquadratum*-related phylogroup 1. By comparison, the next most dominant phylogroup, *Halarubrum*-related phylogroup 4, was considerably more divergent, as denoted in Fig. 3 by differences in their rightwards-extending peaks, and the number of 97%-OTUs. Several minor groups are scattered throughout the tree, and show varying affinities to currently classified taxa. A particularly deeply branching group of related sequences (bottom of Fig. 3) was designated the MSP-clade (discussed further below), and contained sequences from all three sites.

In parallel to the culture-independent assessment of archaeal diversity, cultivation was also used in order to confirm, and possibly extend, the diversity captured by the PCR-generated clone library sequences. Using the methods described in our previous studies, water samples were plated onto solid media (MGM), and also titrated in DBCM2 liquid medium (extinction cultures), and incubated for at least 12 weeks (plates) or 8 weeks (liquid). A total of 201 pure isolates of haloarchaeaa were recovered, and a random selection of 132 of these had near-complete 16S rRNA gene sequences determined. Due to the biases involved in cultivation, and that many isolates had nearly identical 16S rRNA gene sequences, only a few of these sequences were included in the tree reconstruction shown in Fig. 3 (see legend). Cultivation results are summarized at the right side of the tree, where asterisks indicate the phylogroups for which isolates were recovered. Representatives of five phylogroups were cultivated, including the major common clades related to *Haliquadratum*, *Halarubrum*, and *Halonotius*. The sequences of isolates closely matched (≥99% identity) clone library sequences of their corresponding phylogroup, excepting phylogroup 9, for which only isolates were recovered. The closest full-length matches in Genbank to the two phylotype 9 sequences showed only 95% identity, e.g., accession GQ281682 (isolate TNN28, saltern crystallizer, China). For many of the phylogroups and deeply branching clone sequences, isolates were not recovered, including members of the MSP8-clade. Seven phylogroups and four clones showed only ≤90% sequence identity to classified taxa, and so appear to represent novel genera.

A Venn diagram shown in Fig. 4 summarizes the shared and site-specific diversity of 97% OTUs for the three sites, using all the sequence data from both clones and isolates. Apart from the two clades found at all sites, there are 4–10 97%-OTUs that are found only at a single site, and 1–3 that are shared between two of the three sites.

Looking more closely at the *Haliquadratum*-related sequences of phylogroup 1 (Fig. 3), the 52 sequences determined in this study differed from each other and from the type strain, *Hqr. walsbyi* C23T, by ≤2%. A more closely related subgroup of these sequences that differed from each other by ≤0.5% included members originating from all three sites, while even more similar sequences (99.7% identity) were shared between the Bajool and Dry Creek sites, and between the Bajool and Lara sites. Since the *Hqr. walsbyi* genome contains two 16S rRNA genes that differ by 3 positions (0.2% divergence, strain C23T, unpublished data), at least some of the divergence observed between very similar clone sequences could derive from differences in paralogous genes. Indeed, in an alignment of these clone sequences, the same 3 positions were hypervariable, and two displayed the same base variation as seen between the C23T copies, i.e., C→T at *E. coli* positions 198 and 845.

To compare the sequences derived from this study with those from other sites, *Hqr. walsbyi*-related 16S rRNA gene sequences were retrieved from Genbank by a BLASTN search using the C23T sequence. 28 sequences of at least 1400 nt, of good quality and non-chimeric (Bellerophon checked), and that were deposited as part of a published study, were retained and aligned with C23T.
sequence (Table 2). Within this group of sequences were members that differed from \textit{Hqr. walsbyi} C23\textsuperscript{T} by only 2–5 nt, and had been recovered from saltern crystallizer ponds in Spain (Bolhuis et al. 2006) (Legault et al. 2006), Israel (Sorensen et al. 2005), Turkey (Mutlu et al. 2008), Tunisia (Baati et al. 2008) and Peru (Maturrano et al. 2006) (see Fig. 1 for locations). Except for the high-altitude Peruvian saltern, all of the \textit{Hqr. walsbyi}-related sequences recovered at the other sites differed by less than 1% from that of the (Australian) type strain. Data from the studies cited in Table 2, as well as other, previous published studies, indicate that \textit{Hqr. walsbyi} was numerically significant at all of these sites (e.g., Oren et al. 2006), and at most sites it was the dominant archaeal clade.

By comparison, the \textit{Halorubrum}-related sequences (phylogroup 4) displayed 7% sequence divergence, and this value was unchanged if the sequences of the 78 cultured isolates that branched with this phylogroup were included (data not shown). Similar divergence values have been reported in previous studies of natural isolates of \textit{Halorubrum} spp. (Legault et al. 2006), and even within the classified species of this genus, the 16S rRNA gene sequence divergence is 7–8%.

Phylogroup 2 is a tight cluster of 4 OTUs, each consisting of a single clone sequence. The sequences branched closely to each other and to phylogroup 1. Two sequences shared 97% sequence similarity to the 16S rRNA gene of \textit{Hqr. walsbyi} C23\textsuperscript{T}, and may represent novel species of \textit{Haloquadratum}. The other two sequences were 96.4% similar to each other but only 93% similar to \textit{Hqr. walsbyi} C23\textsuperscript{T}, and so probably represent different species of a separate, as yet uncultivated, genus (Fry et al. 1991).

The closest matching sequences in Genbank to the other phylogroups and phylotypes are given in supplementary Table 2. Phylogroup 3 was represented by two clone sequences, and these were 97% identical to an isolate (CSW 2.24.4) recovered previously in this laboratory from the Moolap saltern crystallizer, 10 km from the Lara site (Burns et al. 2004a). Phylogroup 5 includes clones and isolates, and represents a new genus within the Halobacteriaceae, with the type species being \textit{Halonotius pteroides} strain 1.15.5 (Burns et al. 2009a). The latter strain was also isolated from the Moolap site. Phylogroup 8 sequences are closely related to \textit{Natronomonas pharaonis}, a haloalkali-phile isolated from an African soda lake with a pH around 10. \textit{Nmn. pharaonis} has an optimum pH of 8.5 but the sites examined in the current study were all around pH 7. A new species of this genus has recently been proposed to accommodate such neutrophilic members (Burns et al. 2009b).

MSP8-clade of related phylogroups

19 clone sequences, forming six separate 97%-OTUs branched together in phylogenetic tree reconstructions, and deeply away from the other taxa and phylogroups (Fig. 3). The level of sequence identity of members of this clade to recognized taxa was around 74% but chimera-checking programs indicated that they were valid rRNA sequences. The clade was named after one of two related clone sequence that were recovered from an alkaline saltern pond in east Africa in 1996 (Grant et al. 1999), and that branched

### Table 2 Haloquadratum sequences reported by other published studies

| Countries (site) | Sequence origin | Accessions | Similarity to C23\textsuperscript{T} (%) | References |
|------------------|-----------------|------------|---------------------------------|------------|
| Israel (Eilat)   | 1 PCR-clone/ArcA11 | DQ103682 | 99.6                            | Sorensen et al. (2005) |
| Peru (Maras)     | Clone 2A5\textsuperscript{a} | AY987831 | 98.9                            | Maturrano et al. (2006) |
| Spain (Alicante) | Cosmid clone 7B05 | 85372643 | 99.6                            | Legault et al. (2006) |
|                  | Cosmid clone 2B08 | 85680316 | 99.7                            |            |
|                  | \textit{Hqr. walsbyi} HBSQ001 | 110666976 | 99.9                            | Bolhuis et al. (2006) |
| Tunisia (Sfax)   | 12 PCR-clones/SFE1D061 | CU467224\textsuperscript{b} | 99.2–99.8                      | Baati et al. (2008) |
|                  | 9 PCR-clones/SFE1A101 | CU467209\textsuperscript{c} | 99.3–99.7                      |            |
| Turkey (Tuz Lake)| DGG\textsuperscript{d} band 4A | – | 99                               | Maturrano et al. (2008) |
| Turkey (coastal)| DGG\textsuperscript{d} band 8A | – | 100                              |            |
| Turkey (Tuz Lake)| PCR-clones/July | EF459726–9 | 99.3–99.7                      |            |

\textsuperscript{a} The Haloquadratum sequences from this study contained ambiguous bases, so only one representative sequence was used here.

\textsuperscript{b} Representative clone of 12 clones, forming the dominant archaeal OTU in crystallizer pond S5.

\textsuperscript{c} Representative clone of nine clones, forming the third most dominant archaeal OTU in crystallizer pond S5.

\textsuperscript{d} DGGE Denaturing gradient gel electrophoresis. These were sequences obtained from PCR amplified environmental DNA that was separated by DGGE.
specifically with the Australian sequences. The MSP8 sequence shares only 86–89% similarity to the Australian sequences, and across the entire MSP-clade there was a similar level of divergence (86–87% similarity).

Discussion

The dominant haloarchaeal group at three, geographically distant, Australian crystallizer ponds, was *Hqr. walsbyi*, and the great majority of the recovered 16S rRNA gene sequences related to this taxon diverged by less than 2% from each other, and from the type strain of this genus (C23T). Sequences 99.5% identical to the type strain were present at all sites, and previous studies of crystallizer ponds in Spain (Antón et al. 1999; Bolhuis et al. 2006; Legault et al. 2006), Tunisia (Baati et al. 2008), Turkey (Mutlu et al. 2008) and Israel (Sorensen et al. 2005) have recovered sequences that show similarly close relationships to the type strain, and to each other. The type strain was isolated in 2004 from the Cheetham saltern in Moolap, Australia, only 10 km away from the Lara site examined in the present study. In our study of the Moolap site in 2004, twenty 16S rDNA gene sequences were recovered, and while most were <600 nt, they also indicated ≥99% identity to *Hqr. walsbyi* C23T (Burns et al. 2004a). Preliminary data from the genome sequencing of the C23T strain indicate that 85% of its genome sequence is closely related to that of strain HBSQ001, and that these shared sequences are, on average, 98.6% identical (manuscript in preparation). This comparison supports the close relationship of these strains inferred from 16S rRNA gene sequence data. The combined evidence points to a globally distributed and strongly coherent species that has remained at all sites, and previous studies of crystallizer ponds in Spain (Antón et al. 1999; Bolhuis et al. 2006; Legault et al. 2006), Tunisia (Baati et al. 2008), Turkey (Mutlu et al. 2008) and Israel (Sorensen et al. 2005) have recovered sequences that show similarly close relationships to the type strain, and to each other. The type strain was isolated in 2004 from the Cheetham saltern in Moolap, Australia, only 10 km away from the Lara site examined in the present study. In our study of the Moolap site in 2004, twenty 16S rDNA gene sequences were recovered, and while most were <600 nt, they also indicated ≥99% identity to *Hqr. walsbyi* C23T (Burns et al. 2004a). Preliminary data from the genome sequencing of the C23T strain indicate that 85% of its genome sequence is closely related to that of strain HBSQ001, and that these shared sequences are, on average, 98.6% identical (manuscript in preparation). This comparison supports the close relationship of these strains inferred from 16S rRNA gene sequence data. The combined evidence points to a globally distributed and strongly coherent species that has remained dominant for at least a decade (Antón et al. 1999; Burns et al. 2004a, b; Legault et al. 2006; this study). It coexists and competes with other haloarchaea, such as *Halorubrum* that, as judged by 16S rRNA gene sequence data, display significantly greater genetic diversity.

Divergence within and between species of prokaryotes has become much easier to quantitate with the availability of many complete genome sequences. Within the Bacteria, some species show high levels of strain variation, such as those of *Pseudomonas* and *Bacillus*, while others show extremely limited divergence, particularly pathogens and obligate endosymbionts (Delager et al. 2009). Even freeliving species that usually dominate the microbial flora of specific environments show differing levels of variation. For example, the marine bacterium “Pelagibacter” (Wilhelm et al. 2007) shows considerably higher variation compared to the very limited diversity among strains of *Candidatus Accumulibacter phosphatis*, a bacterium observed in sludge bioreactors in the US and Australia (Kunin et al. 2008). Clearly, hypersaline environments also possess dominant groups that differ in their level of genetic variation, i.e., *Haloquadratum* versus *Halorubrum*. Genomic and metagenomic studies of *Haloquadratum* should help pinpoint the factors underlying these differences.

How the world-wide homogeneity of *Haloquadratum* maintained is yet to be understood but there are some grounds for proposing a combination of factors that could be important, including a global dispersal system, natural reservoirs, and strong selection pressures. Natural salt lakes often dry out completely, and the crystallizers of marine solar salterns are drained and refilled annually. Dispersal of *Haloquadratum* via the oceans is unlikely, as this organism cannot form spores, does not grow at salt concentrations less than about 14% w/v and lyzes at low salt (2% w/v). However, survival inside the fluid inclusions of salt crystals has been shown for other haloarchaea (e.g., see Fendrihan et al. 2009 and the references cited), and this could allow dispersal by wind or by the migratory birds that frequent hypersaline waters. Recently, haloarchaea have been found as normal flora of the salt glands of a species of migratory bird (Brito-Echaverría et al. 2009). The ability to cope with cyclical, extreme conditions may also apply strong selective pressures that limit intraspecies divergence, and *Haloquadratum* may be better adapted to these different extremes. For example, in addition to growing well in saturating solutions of sodium chloride, *Haloquadratum* tolerates high magnesium concentrations that occur in crystallizer ponds after the precipitation of sodium chloride (Bolhuis et al. 2006). Indeed, the C23T strain achieves higher cell densities above 1 M MgCl2 (Burns et al. 2007).

A surprising feature of the archaeal diversity seen in this study was the presence and relatively high frequency of sequences forming the MSP8 clade, that branched very deeply from the other haloarchaea (86–87% nt similarity). The first reported examples of such sequences (MSP8, MSP41) were recovered from a study of Lake Magadi, an East African soda lake, in 2000 (Grant et al. 1999). Similar sequences have been rarely detected, but a 2008 study of saltern ponds in Tunisia (Baati et al. 2008) recovered one clone sequence (accession FN391274) that was most closely related to phylogroups 12 and 13 (97% similarity) in the current study. The deep branching of the MSP8 clade makes it an important target for cultivation studies, to determine if the metabolic capabilities of these species differ appreciably from those of the classified haloarchaea.

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