

# Intraspecific polymorphism of 16S rRNA genes in two halophilic archaeal genera, *Haloarcula* and *Halomicrobium*

Heng-Lin Cui · Pei-Jin Zhou · Aharon Oren ·  
Shuang-Jiang Liu

Received: 9 July 2008 / Accepted: 16 September 2008 / Published online: 3 October 2008  
© Springer 2008

**Abstract** All members of the genera *Haloarcula* and *Halomicrobium* whose names have been validly published were surveyed for 16S rRNA gene polymorphism, and the transcription of the genes from two species was investigated during growth at different NaCl concentrations. The species of *Haloarcula* and *Halomicrobium* harbour at least two different 16S rRNA gene copies, and 18 new sequences of 16S rRNA genes were obtained. The type I and type II 16S rRNA genes of *Haloarcula* are divergent at 4.8–5.6% of their nucleotide positions. The type III and type IV 16S rRNA genes from *Halomicrobium mukohataei* JCM 9738<sup>T</sup> are 9.0% divergent, which represents the highest intraspecific divergent 16S rRNA genes so far seen. Phylogenetic analysis based on 16S rRNA genes indicated that all type I 16S rRNA genes were clustered, and the

same was true for the type II 16S rRNA genes of *Haloarcula* species. The two clusters, respectively generated from type I and type II 16S rRNA genes, were sharply separated and their divergences (4.8–5.6%) are in the range of various divergence usually found between genera in the order *Halobacteriales* (about 5–10%). Results from reverse transcription-PCR showed that the type I and type II copies of *Har. amylolytica* BD-3<sup>T</sup> and type III and type IV copies of *Hmc. mukohataei* JCM 9738<sup>T</sup> were all transcribed to 16S rRNA molecules under different salt concentrations (15–28% NaCl).

**Keywords** Halophilic archaea · *Haloarcula* · *Halomicrobium* · 16S rRNA gene polymorphism

Communicated by A. Driessen.

H.-L. Cui · P.-J. Zhou · S.-J. Liu  
State Key Laboratory of Microbial Resources,  
Institute of Microbiology, Chinese Academy of Sciences,  
100080 Beijing, People's Republic of China

H.-L. Cui  
School of Food and Biological Engineering,  
Jiangsu University, 212013 Zhenjiang,  
People's Republic of China

A. Oren  
The Institute of Life Sciences and the Moshe Shilo Minerva  
Center for Marine Biogeochemistry,  
The Hebrew University of Jerusalem,  
91904 Jerusalem, Israel

S.-J. Liu (✉)  
Institute of Microbiology, Chinese Academy of Sciences,  
Datun Road Jia 3#, Chaoyang District,  
100101 Beijing, People's Republic of China  
e-mail: liusj@sun.im.ac.cn

## Introduction

The gene coding for the small subunit ribosomal RNA (SSU) has been an important molecular chronometer for identification and classification of prokaryotes (Woese et al. 1990) and is now being extensively used to evaluate prokaryotic diversity in natural environments (Case et al. 2007). However, some prokaryotic species harbour several divergent 16S rRNA genes in their genomes, which cause concern about the reliability of 16S rRNA gene analysis in the classification and identification of prokaryotes, as well as in the evaluation of prokaryotic diversity (Clayton et al. 1995; Wang et al. 1997; Yap et al. 1999; Marchandin et al. 2003). Within the domain *Archaea*, several methanogenic species such as *Methanocaldococcus jannaschii*, *Methanospirillum hungatei* and *Methanothermobacter thermautotrophicus* exhibit 16S rRNA gene polymorphisms displaying 0.1% divergence, while some halophilic archaea harbour more divergent 16S rRNA gene copies

with 0.1–6.8% divergence. These include species of *Haloarcula*, *Halosimplex carlsbadense* (Vreeland et al. 2002), *Natrinema* sp. strain XA3-1 (Boucher et al. 2004), *Haloquadratum walsbyi* (Burns et al. 2007) and *Haladaptatus paucihalophilus* (Savage et al. 2007). Boucher et al. (2004) proposed that 16S rRNA gene polymorphisms are an evolutionarily stable characteristic of halophilic archaea.

The occurrence of different copies of 16S rRNA gene has been known for several members of *Haloarcula*, and transcription of each copy of the 16S rRNA genes is probably related to environmental fluctuations (Dennis 1999). In *Haloarcula marismortui*, the two types of 16S rRNA genes are present in approximately equal amounts in ribosomes, and both rRNA operons of *Har. marismortui* have been shown to be expressed during cultivation under standard laboratory growth conditions (Mylvaganam and Dennis 1992; Amann et al. 2000). Salinity fluctuations in hypersaline environments may have a significant influence on the physiology of halophiles (Dennis 1999; Dennis and Shimmin 1997), and the presence of two distinct ribosome populations may therefore allow *Har. marismortui* to maintain essential protein synthesis during the periods of environmental stress. In fact, three rRNA operons were found in the genome of *Har. marismortui* (Baliga et al. 2004), and all the three copies of 16S rRNA genes are transcribed in the wild-type *Har. marismortui*. However, a single rRNA operon mutant (*rrnA*,  $\Delta$ *rrnB*,  $\Delta$ *rrnC*) grows on rich media similar to the wild-type (Tu et al. 2005). Other members of *Haloarcula* such as *Har. quadrata* and *Har. amylolytica* have two copies of 16S rRNA genes which differ at 5.0% of their nucleotide positions, while *Har. hispanica* and *Har. vallismortis* harbour two types of 16S rRNA gene with 2.4 and 2.9% divergence, respectively. It was reported that *Har. japonica* possessed four copies of the 16S rRNA gene (Gemmell et al. 1998), whereas multicopy genes have not been reported for *Har. argentinensis* and *Halomicrobium mukohataei* (Ihara et al. 1997; Oren et al. 2002).

To better understand the 16S rRNA gene polymorphism in *Haloarcula* and the related genus *Halomicrobium*, we designed specific-primer-based PCR towards identifying specific gene copies in each species, and investigated the transcription of different types of 16S rRNA genes at different salinities in representative species of *Haloarcula* and *Halomicrobium*.

## Materials and methods

### Strains and growth conditions

*Har. argentinensis* JCM 9737<sup>T</sup>, *Har. quadrata* JCM 11048<sup>T</sup>, *Har. japonica* JCM 7785<sup>T</sup> and *Hmc. mukohataei*

JCM 9738<sup>T</sup> were purchased from JCM (Japan Collection of Microorganisms, Wako, Japan). *Har. hispanica* CGMCC 1.2049<sup>T</sup>, *Har. marismortui* CGMCC 1.1784<sup>T</sup> and *Har. vallismortis* CGMCC 1.2048<sup>T</sup> were from CGMCC (China General Microbiological Culture Collection, Beijing, China). *Har. amylolytica* BD-3<sup>T</sup> was recently described (Yang et al. 2007).

Unless stated otherwise, cultures were grown aerobically with vigorous shaking at 37°C in a Complete Medium (CM medium) (Sehgal and Gibbons 1960), while for reverse transcription (RT)-PCR experiments, *Har. amylolytica* BD-3<sup>T</sup> and *Hmc. mukohataei* JCM 9738<sup>T</sup> were cultivated in CM media with different NaCl concentrations (15, 20, 25 and 28%, w/v).

### Design of specific primers

All three 16S rRNA gene sequences of *Har. marismortui* ATCC 43049<sup>T</sup> were retrieved from its genome (NC\_006396; NC\_006397) and were used to design specific primers targeting the different 16S rRNA gene copies of *Haloarcula*, while the two 16S rRNA gene sequences of *Hmc. mukohataei* JCM 9738<sup>T</sup> (cloned and sequenced in this study) were used to design specific primers for *Halomicrobium*. These sequences were aligned using the ClustalW program in MEGA3.1 software (Kumar et al. 2004) and the most divergent regions from each were used for primers design (Table 1).

### Identification of 16S rRNA gene polymorphism in *Haloarcula* and *Halomicrobium*

Genomic DNAs from all halophilic archaeal strains were prepared as described by Ng et al. (1995). Different copies of 16S rRNA genes in each species were surveyed by PCR amplification of individual copies of the 16S rRNA gene with the above-designed specific primers. PCR was performed in a thermal cycler (MJ Research PTC-200, USA) for 30 cycles (5 min denaturing step at 95°C in the first cycle; 0.5 min denaturing at 95°C, 0.5 min annealing at 55°C and 0.5 min elongation at 72°C, with a final extension step at 72°C for 5 min). The PCR products were examined on a 1.5% agarose gel.

### Amplification of 16S rRNA genes with universal primers and phylogenetic analysis

The universal oligonucleotide primers designed for amplification of the haloarchaeal 16S rRNA genes were the forward primer 0018F (5'-ATTCCGGTTGATCCTGCC-3') and the reverse primer 1518R (5'-AGGAGGTGATCAGCCGC-3'), designed based on the upstream and downstream sequences of the 16S rRNA genes of all

**Table 1** Specific primers for detection of two types of 16S rRNA genes in *Haloarcula* and *Halomicrobium*

Genera	16S gene types	Primers	Nucleotide positions <sup>a</sup>	Primer sequences	Product sizes (bp)
<i>Haloarcula</i>	Type I	H1F	569–586	5'-CGTCCAGCGGAAACTGTC-3'	223
		H1R	776–792	5'-GCAGGCTCGTAGCCTAC-3'	
	Type II	H2F	569–586	5'-CGTCCGGTGGAAGACTACA-3'	
		H2R	776–792	5'-ACGTGCTCATGGCGCGT-3'	
<i>Halomicrobium</i>	Type III	HM1F	145–162	5'-TAATCCCCGATACAGCTC-3'	545
		HM1R	675–691	5'-GGGTCCGCTTTTCTGAG-3'	
	Type IV	HM2F	144–162	5'-CTAATCGTGGATATCGCAT-3'	
		HM2R	675–689	5'-GTCCGGCTTCTCAGC-3'	

F forward, R reverse

<sup>a</sup> Nucleotide positions according to the *Har. marismortui* ATCC 43049<sup>T</sup> rRNA operon

species of *Halobacteriaceae*. The amplified products were recovered from the agarose gel by using a gel extraction kit (Tiangen, China), and were cloned into the pGEM-T vector (Promega, USA) and transformed into *Escherichia coli* TOP 10. Positive clones harbouring cloned 16S rRNA gene fragments were selected by PCR using specific primers (type I–type IV, Table 1) and were sequenced by Sino-GenoMax Company Limited (Beijing, China). All sequences were checked for possible chimeric artefacts by the Chimera Detection program in the Ribosomal Database Project II (Cole et al. 2003). The nucleotide sequences determined in this study have been deposited in the GenBank database under accession numbers EF645680–EF645694. Multiple sequence alignments were performed using the ClustalW program integrated in MEGA3.1 software. Phylogenetic trees were reconstructed using the Neighbour-Joining and Maximum-Parsimony methods (Saitou and Nei 1987) with the Kimura 2-parameter calculation model (Kimura 1980) in MEGA3.1 software. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches (Felsenstein 1985).

#### RT-PCR and identification of different 16S rRNAs

Mid-exponential phase cultures of *Har. amylolytica* BD-3<sup>T</sup> and *Hmc. mukohataei* JCM 9738<sup>T</sup> cultivated in CM medium with different NaCl concentrations were harvested and total RNAs were extracted as previously described (Nieuwlandt et al. 1995).

For reverse transcription (RT), the reverse primer 1518R was used to prime the synthesis of cDNA. One microgram portion of total RNAs was used as template, and RT was carried out according to the instructions of the supplier (MMLV-RT, Epicentre Biotechnologies). The mock cDNA reaction where RT was not carried out, excluding the possibility of PCR amplification from residual genomic DNA, was used as the control. One microliter of the RT

mixture was used directly as the template in subsequent PCRs. A 20- $\mu$ l PCR mixture contained 10 pmol of specific primers, 200 mM of each dATP, dGTP, dCTP and dTTP, 1 U of *Taq* DNA polymerase (Promega), and 1 $\times$  buffer provided with the *Taq* polymerase.

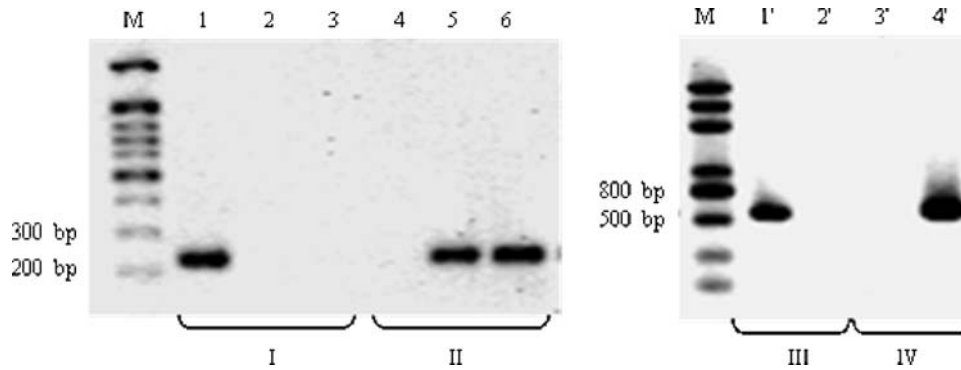
## Results

### Specificity of the newly designed primers

Four sets of primers that target the variable region of the 16S rRNA gene were designed. The sequences, target positions at the 16S rRNA gene, and the expected sizes of PCR products are listed in Table 1. The specificity of each primer pair was experimentally tested by using clones with a single 16S rRNA gene from either *Har. amylolytica* BD-3<sup>T</sup> or *Hmc. mukohataei* JCM 9738<sup>T</sup> (Fig. 1). When 16S rRNA gene-specific amplification was performed with the newly designed primers, PCR products of the expected size were obtained. Each pair of primers was shown to amplify a fragment of the expected size only from its corresponding 16S rRNA gene and did not cross-react with any of the non-targeted 16S rRNA genes.

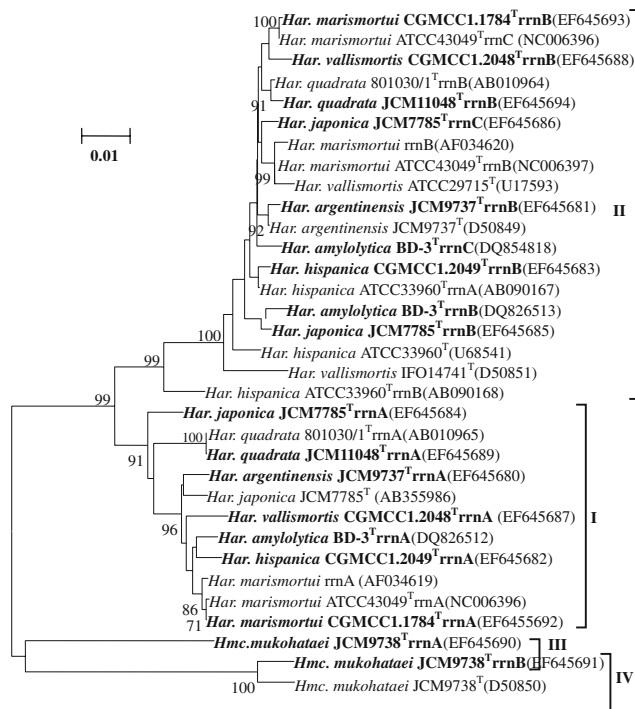
### Polymorphism of 16S rRNA genes and their influence on phylogenetic analysis of the species of *Haloarcula* and *Halomicrobium*

By using the designed 16S rRNA gene-specific primers and extracted genomic DNAs as templates, the polymorphism of 16S rRNA genes in species of the genera *Haloarcula* and *Halomicrobium* were studied. At least two different 16S rRNA genes were detected in seven *Haloarcula* species and also in the sole *Halomicrobium* species, *Hmc. mukohataei*. Each of the different types of 16S rRNA gene copies was sequenced, and 18 new sequences of 16S rRNA genes were obtained (GenBank Acc. Nos. EF645680–



**Fig. 1** Specificity of primers for detection of two types of 16S rRNA genes in *Har. amylolytica* BD-3<sup>T</sup> (left) and *Hmc. mukohataei* JCM 9738<sup>T</sup> (right). Lanes M-DNA marker, lanes 1 and 4: *Har. amylolytica*

BD-3<sup>T</sup> *rrnA*, lanes 2 and 5; *Har. amylolytica* BD-3<sup>T</sup> *rrnB*, lanes 3 and 6; *Har. amylolytica* BD-3<sup>T</sup> *rrnC*, lanes 1' and 2'; *Hmc. mukohataei* JCM 9738<sup>T</sup> *rrnA*, lanes 2' and 4'; *Hmc. mukohataei* JCM 9738<sup>T</sup> *rrnB*



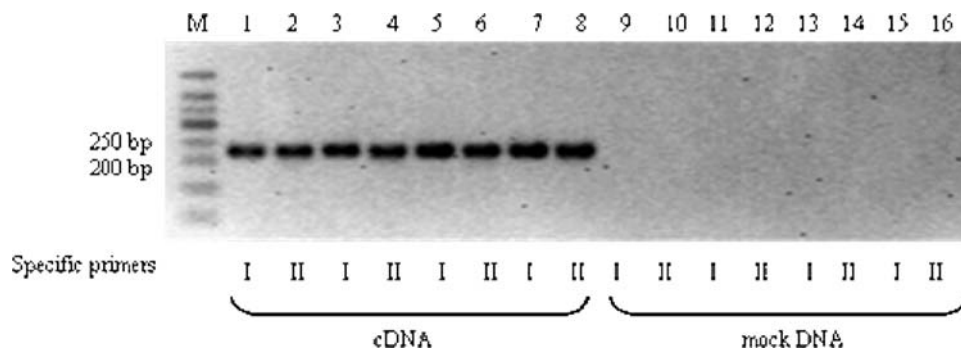
**Fig. 2** Neighbour-Joining phylogenetic tree based on 16S rRNA gene sequences of all members of *Haloarcula*, *Halomicrobium*. Bootstrap values (%) are based on 1,000 replicates and are shown for branches with more than 70% bootstrap support. Bar represents 0.01 expected changes per site. The boldfaced species were newly sequenced in this study

EF645694, DQ826512–DQ826513, DQ854818). The overall size of the amplified fragments of 16S rRNA genes of *Haloarcula* species was 1472 bp, while the two 16S rRNA gene fragments of *Hmc. mukohataei* JCM 9738<sup>T</sup> were 1473 bp (*rrnA*) and 1472 bp (*rrnB*), respectively. Sequence alignments and analyses of the 16S rRNA genes of *Haloarcula* species showed that these genes formed two stable clusters (Fig. 1). The genes that clustered with *rrnA* and *rrnB* or *rrnC* of *Har. marismortui* ATCC 43049<sup>T</sup> were,

respectively, designated as type I and type II 16S rRNA genes. Alignment of the type I and type II 16S rRNA gene sequences of all *Haloarcula* species revealed 4.8–5.6% nucleotide substitutions. The *rrnA* and *rrnB* of *Hmc. mukohataei* JCM 9738<sup>T</sup> were, respectively, designated as type III and type IV 16S rRNA genes, and they showed 9.0% divergence, representing the highest intraspecific divergent 16S rRNA genes so far known.

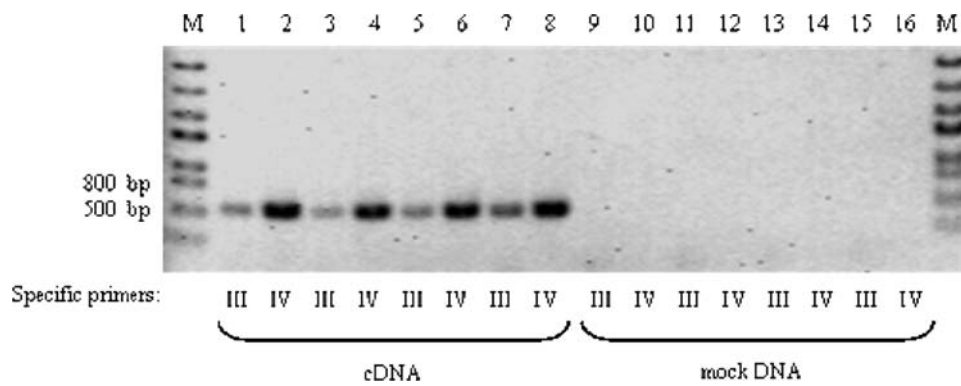
#### Detection of various types of 16S rRNA molecules by RT-PCR

To examine whether all the 16S rRNA genes from *Haloarcula* and *Halomicrobium* species were transcribed and whether their transcription was regulated by the NaCl concentration, total RNAs were prepared from cultures grown in CM medium containing different concentrations of NaCl. The cDNAs were first synthesized from the total RNAs by RT, using the universal primer 1518R that was complementary to the 3' end of both type I and type II, or type III and type IV 16S rRNAs. Second, the specific PCR primers (Table 1) were used, so that either type I, type II, type III, or type IV cDNAs of 16S rRNA fragments were amplified. The results are shown in Figs. 3 and 4. When the cDNAs were used as the template in PCR, all pairs of primers (I–IV, Table 1) amplified fragments of the correct size. None of the primers amplified products from the mock cDNA reaction where RT was omitted, which excluded the possibility of PCR amplification from genomic DNA contaminants. The RT-PCR experiment clearly demonstrated that both type I and type II 16S rRNA genes were transcribed in *Har. amylolytica*. Although these experiments did not quantify the transcription of each type of 16S rRNA gene, the amounts of the type I and type II 16S rRNAs from cells cultivated at different NaCl concentrations were comparable as judged by the intensities of the bands of PCR-amplified fragments, indicating that the type



**Fig. 3** Detection of type I and type II 16S rRNAs in *Har. amylolytica* BD-3<sup>T</sup> cultivated in CM medium at different concentrations of NaCl by RT-PCR. Lanes M-DNA marker, lanes 1, 2, 9 & 10: cultivated in CM medium with 15% NaCl, lanes 3, 4, 11 and 12: cultivated in

CM medium with 20% NaCl, lanes 5, 6, 13 and 14: cultivated in CM medium with 25% NaCl, lanes 7, 8, 15 and 16: cultivated in CM medium with 28% of NaCl



**Fig. 4** Detection of type III and type IV 16S rRNAs in *Hmc. mukohataei* JCM 9738<sup>T</sup> cultivated in CM medium at different concentrations of NaCl by RT-PCR. Lanes M-DNA marker, lanes 1, 2, 9 and 10: cultivated in CM medium with 15% NaCl, lanes 3, 4, 11

and 12: cultivated in CM medium with 20% NaCl, lanes 5, 6, 13 and 14: cultivated in CM medium with 25% NaCl, lanes 7, 8, 15 and 16: cultivated in CM medium with 28% of NaCl

I or type II genes were transcribed at all tested NaCl concentrations. The type III and type IV 16S rRNA genes of *Hmc. mukohataei* were also transcribed at all NaCl concentrations (Fig. 4).

## Discussion

In the domains *Eukarya* and *Bacteria*, the presence of two distinct types of SSU genes in a single genome has been reported to be an isolated event that affects only a few species such as two thermophilic actinomycetes, *Thermomonospora chromogena* and *Thermobispora biopsora* (Wang et al. 1997; Yap et al. 1999), *Vibrio* species (Harth et al. 2007), the apicomplexan *Plasmodium berghei* (Gunderson et al. 1987) and the metazoan *Dugesia mediterranea* (Carranza et al. 1996). As to halophilic archaea, 16S rRNA gene polymorphism may be a widespread feature. Here, we present data on the extent of 16S rRNA gene polymorphism for all species of *Haloarcula* and

*Halomicrobium* whose names have been validly published. We have confirmed that four types (type I–type IV) of 16S rRNA genes occur in *Haloarcula* and *Halomicrobium*. Type I and type II were detected in *Haloarcula* and they were actively transcribed at NaCl concentrations of 15–28%. Type III and type IV were found in *Halomicrobium* and they were both transcribed at different NaCl concentrations. Differential transcription of the two genes of *Har. marismortui* has been shown to be temperature-dependent (López-López et al. 2007).

The type I and type II 16S rRNA genes of *Haloarcula* fall into two monophyletic clusters (cluster I and cluster II in Fig. 2), suggesting two distinct evolutionary lineages in each species. The clustering of these two related monophyletic groups indicates that all species of *Haloarcula* might have speciated from a common ancestral organism in which the divergent copies of 16S rRNA genes had already existed in the genome. *Hmc. mukohataei* JCM 9738<sup>T</sup> also harbours two different 16S rRNA genes (type III and type IV). Of the two 16S rRNA genes of *Hmc. mukohataei*, type

III (16S *rrnA*) is more related to *Haloarcula* groups based on the sequence similarity, while the IV (16S *rrnB*) is more distant in the tree. The type III and type IV genes of *Hmc. mukohataei* showed 9% divergence. This is the highest value so far known.

The degree of intraspecies divergences observed for the 16S rRNA genes of *Haloarcula* (more than 5.0%) and *Halomicrobium* (9.0%) is far greater than the average interspecies divergence (~2.0%) and is more in the range of the divergence usually found between different genera in the family *Halobacteriaceae* (about 5–10%) (Boucher et al. 2004). This large divergence may cause concerns on the identification of haloarchaeal species based on 16S rRNA gene sequence. Historically, one example of taxonomic confusion possibly caused by intragenomic SSU gene heterogeneity is the status of the genera *Haloterrigena* and *Natrinema* (Boucher et al. 2004). This current study clearly demonstrated that two types of 16S rRNA genes occur in *Haloarcula* species and that all *Haloarcula* species fall into two monophyletic clusters corresponding to the type I and type II 16S rRNA genes. In-depth phylogenetic analysis would avoid potential confusion on identification of potential *Haloarcula* species only when a single 16S rRNA gene sequence is available. Ideally, all copies of 16S rRNA genes need to be sequenced, and the specific primers designed in this study could help obtaining different types of 16S rRNA genes from a single genome. When considering the effects of this heterogeneity on the accuracy of species diversity estimation by 16S rRNA-based methods, it is conceivable that analysis of environmentally originated 16S rRNA gene pools may lead to an overestimation of the halophilic archaeal diversity. Therefore, the 16S rRNA genes should be used with caution as an indicator in studies of environmental haloarchaeal biodiversity (Crosby and Criddle 2003).

**Acknowledgments** This study was supported by the National Basic Research Program from the Ministry of Science and Technology (MOST) of China (Grant No. 2004CB719601) and a start-up grant from Jiangsu University (Grant No. 08JDG016).

## References

- Amann G, Stetter KO, Llobet-Brossa E, Amann R, Antón J (2000) Direct proof for the presence and expression of two 5% different 16S rRNA genes in individual cells of *Haloarcula marismortui*. *Extremophiles* 4:373–376
- Baliga NS, Bonneau R, Facciotti MT, Pan M, Glusman G, Deutsch EW, Shannon P, Chiu Y, Weng RS, Gan RR, Hung P, Date SV, Marcotte E, Hood L, Ng WV (2004) Genome sequence of *Haloarcula marismortui*: a halophilic archaeon from the Dead Sea. *Genome Res* 14:2221–2234
- Boucher Y, Douady CJ, Sharma AK, Kamekura M, Doolittle WF (2004) Intra-genomic heterogeneity and inter-genomic recombination among haloarchaeal rRNA genes. *J Bacteriol* 186:3980–3990
- Burns DG, Janssen PH, Itoh T, Kamekura M, Li Z, Jensen G, Rodríguez-Valera F, Bolhuis H, Dyall-Smith ML (2007) *Haloquadratum walsbyi* gen. nov., sp. nov., the square haloarchaeon of Walsby, isolated from saltern crystallizers in Australia and Spain. *Int J Syst Evol Microbiol* 57:387–392
- Carranza S, Giribet G, Riberat C, Baguna J, Riutort M (1996) Evidence that two types of 18S rDNA coexist in the genome of *Dugesia (Schmidtea) mediterranea* (Platyhelminthes, Turbellaria, Tricladida). *Mol Biol Evol* 13:824–832
- Case RJ, Boucher Y, Dahllöf I, Holmström C, Doolittle WF, Kjelleberg S (2007) Use of 16S rRNA and *rpoB* genes as molecular markers for microbial ecology studies. *Appl Environ Microbiol* 73:278–288
- Clayton RA, Sutton G, Hinkle PS Jr, Bult C, Fields C (1995) Intraspecific variation in small-subunit rRNA sequences in GenBank: why single sequences may not adequately represent prokaryotic taxa. *Int J Syst Bacteriol* 45:595–599
- Cole JR, Chai B, Marsh TL, Farris RJ, Wang Q, Kulam SA, Chandra S, McGarrell DM, Schmidt TM, Garrity GM, Tiedje JM (2003) The Ribosomal Database Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. *Nucleic Acids Res* 31:442–443
- Crosby LD, Criddle CS (2003) Understanding bias in microbial community analysis techniques due to *rrn* operon copy number heterogeneity. *Biotechniques* 34:790–794
- Dennis PP (1999) Expression of ribosomal RNA operons in halophilic archaea. In: Oren A (ed) *Microbiology and biogeochemistry of hypersaline environments*. CRC Press, Boca Raton, pp 319–329
- Dennis PP, Shimmin LC (1997) Evolutionary divergence and salinity-mediated selection in halophilic archaea. *Microbiol Mol Biol Rev* 61:90–104
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791
- Gemmell RT, McGenity TJ, Grant WD (1998) Use of molecular techniques to investigate possible long-term dormancy of halobacteria in ancient halite deposits. *Ancient Biomol* 2:125–133
- Gunderson JH, Sogin ML, Wollett G, Hollingdale M, de la Cruz VF, Waters AP, McCutchan TF (1987) Structurally distinct, stage-specific ribosomes occur in *Plasmodium*. *Science* 238:933–937
- Harth E, Romero J, Torres R, Espejo RT (2007) Intra-genomic heterogeneity and inter-genomic recombination among *Vibrio parahaemolyticus* 16S rRNA genes. *Microbiology* 153:2640–2647
- Ihara K, Watanabe S, Tamura T (1997) *Haloarcula argentinensis* sp. nov. and *Haloarcula mukohataei* sp. nov., two new extremely halophilic archaea collected in Argentina. *Int J Syst Bacteriol* 47:73–77
- Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16:111–120
- Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* 5:150–163
- López-López A, Benlloch S, Bonfá M, Rodríguez-Valera F, Mira A (2007) Intra-genomic 16S rDNA divergence in *Haloarcula marismortui* is an adaptation to different temperatures. *J Mol Evol* 65:687–696
- Marchandin H, Teyssier C, Simeon De Buochberg M, Jean-Pierre H, Carriere C, Jumas-Bilak E (2003) Intra-chromosomal heterogeneity between the four 16S rRNA gene copies in the genus *Veillonella*: implications for phylogeny and taxonomy. *Microbiology* 149:1493–1501
- Mylvaganam S, Dennis PP (1992) Sequence heterogeneity between the two genes encoding 16S rRNA from the halophilic archaeobacterium *Haloarcula marismortui*. *Genetics* 130:399–410

- Ng WL, Yang CF, Halladay JT, Arora P, DasSarma S (1995) Protocol 25: isolation of genomic and plasmid DNAs from *Halobacterium halobium*. In: DasSarma S, Fleischmann EM (eds) Archaea: a laboratory manual: halophiles. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 179–180
- Nieuwlandt DT, Palmer JR, Armbruster DT, Kuo YP, Oda W, Daniels CJ (1995) Protocol 23: a rapid procedure for the isolation of RNA from *Haloferax volcanii*. In: DasSarma S, Fleischmann EM (eds) Archaea: a laboratory manual: halophiles. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 161–162
- Oren A, Eleri R, Watanabe S, Ihara K, Corcelli A (2002) *Halomicrobium mukohataei* gen. nov., comb. nov., and emended description of *Halomicrobium mukohataei*. Int J Syst Evol Microbiol 52:1831–1835
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
- Savage KNL, Krumholz LR, Oren A, Elshahed MS (2007) *Haladaptatus paucihalophilus* gen. nov., sp. nov., a halophilic archaeon isolated from a low-salt, sulfide-rich spring. Int J Syst Evol Microbiol 57:19–24
- Sehgal SN, Gibbons NE (1960) Effect of some metal ions on the growth of halobacterium cutirubrum. Can J Microbiol 6:165–169
- Tu D, Blaha G, Moore PB, Steitz TA (2005) Gene replacement in *Haloarcula marismortui*: construction of a strain with two of its three chromosomal rRNA operons deleted. Extremophiles 9:427–435
- Vreeland RH, Straight S, Krammes J, Dougherty K, Rosenzweig WD, Kamekura M (2002) *Halosimplex carlsbadense* gen. nov., sp. nov., a unique halophilic archaeon, with three 16S rRNA genes, that grows only in defined medium with glycerol and acetate or pyruvate. Extremophiles 6:445–452
- Wang Y, Zhang ZS, Ramanan N (1997) The actinomycete *Thermobispora bispora* contains two distinct types of transcriptionally active 16S rRNA genes. J Bacteriol 179:3270–3276
- Woese CR, Kandler O, Wheelis ML (1990) Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci USA 87:4576–4579
- Yang Y, Cui HL, Zhou PJ, Liu SJ (2007) *Haloarcula amylolytica* sp. nov., an extremely halophilic archaeon isolated from Aibi salt lake in Xin-Jiang, China. Int J Syst Evol Microbiol 57:103–106
- Yap WH, Zhang Z, Wang Y (1999) Distinct types of rRNA operons exist in the genome of the actinomycete *Thermomonospora chromogena* and evidence for horizontal transfer of an entire rRNA operon. J Bacteriol 181:5201–5209