Phylogenetic relationships within the family *Halobacteriaceae* inferred from *rpoB'* gene and protein sequences

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In order to clarify the current phylogeny of the haloarchaea, particularly the closely related genera that have been difficult to sort out using 16S rRNA gene sequences, the DNA-dependent RNA polymerase subunit B' gene (*rpoB'*) was used as a complementary molecular marker. Partial sequences of the gene were determined from 16 strains of the family *Halobacteriaceae*. Comparisons of phylogenetic trees inferred from the gene and protein sequences as well as from corresponding 16S rRNA gene sequences suggested that species of the genera *Natrialba*, *Natronococcus*, *Halobiforma*, *Natronobacterium*, *Natronorubrum*, *Natrinema*/*Haloterrigena* and *Natronolimnobius* formed a monophyletic group in all trees. In the *RpoB'* protein tree, the alkaliphilic species *Natrialba chahannaoensis*, *Natrialba hulunbeirensis* and *Natrialba magadii* formed a tight group, while the neutrophilic species *Natrialba asiatica* formed a separate group with species of the genera *Natronorubrum* and *Natronolimnobius*. Species of the genus *Natronorubrum* were split into two groups in both the *rpoB'* gene and protein trees. The most important advantage of the use of the *rpoB'* gene over the 16S rRNA gene is that sequences of the former are highly conserved amongst species of the family *Halobacteriaceae*. All sequences determined so far can be aligned unambiguously without any gaps. On the other hand, gaps are necessary at 49 positions in the inner part of the alignment of 16S rRNA gene sequences. The *rpoB'* gene and protein sequences can be used as an excellent alternative molecular marker in phylogenetic analysis of the *Halobacteriaceae*.

Haloarchaea, members of the family *Halobacteriaceae*, are a group of extremely halophilic micro-organisms that form part of the domain *Archaea*. At the time of writing, haloarchaeal strains are classified in 80 species of 24 genera (Burns *et al.*, 2007; Castillo *et al.*, 2006). Although the majority are neutrophilic, 16 alkaliphilic species have been isolated from soda lakes in Kenya, China, India, Egypt, etc. (Horikoshi, 1999; Rees *et al.*, 2004). Strains isolated in the early 1980s are classified in the genera *Natronobacterium*,

Natronococcus and *Natronomonas* (Kamekura *et al.*, 1997), and some recent isolates are accommodated in *Halalkalicoccus* (Xue *et al.*, 2005) and *Natronolimnobius* (Itoh *et al.*, 2005). These five genera are so far composed exclusively of alkaliphilic strains. On the other hand, the genera *Halobiforma*, *Halorubrum*, *Natrialba* and *Natronorubrum* consist of both neutrophilic and alkaliphilic species. Since the haloalkaliphilic members lack detectable amounts of glycolipids (Grant *et al.*, 2001), analysis of the 16S rRNA genes has been a major criterion for their classification at the genus level. Thus, there is a need for other targets for chemotaxonomy and complementary molecular markers for the phylogeny of the *Halobacteriaceae* (Wright, 2006).

The DNA-dependent RNA polymerase subunit β (in bacteria) or B (in archaea) gene (*rpoB*) has become a

The GenBank/EMBL/DDBJ accession numbers for the *rpoB'* gene sequences determined in this study are AB295622–AB295637, as detailed in Table 1. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Haloferax* sp. BV2 is AB258304.

Alignments of deduced RpoB amino acid sequences are available as supplementary material with the online version of this paper.

popular phylogenetic marker in recent years (Adékambi *et al.*, 2003; Case *et al.*, 2007; Dahllöf *et al.*, 2000; Korczak *et al.*, 2004). *rpoB* fulfils the following criteria to be useful in the phylogenetic analyses of archaea (Klenk & Zillig, 1994): (i) it is present in all archaea, and is a single-copy conserved gene, (ii) it is one of the important components of the transcription apparatus and therefore highly constrained to evolve at a reasonably slow rate and (iii) subunit B is the largest component of the RNA polymerase, approximately 1100 amino acids, in archaea; it is therefore large enough to guarantee high confidence in the phylogenetic analysis. The order of subunits in decreasing size is B, A', A'', D, E, F, G, H, I, K, L, M and N (Langer *et al.*, 1995). The fact that only a single copy of each subunit is present in all bacteria and archaea is a tremendous advantage over the 16S rRNA gene for phylogenetic analyses (Acinas *et al.*, 2004; Cilia *et al.*, 1996). In the case of the *Halobacteriaceae*, species of some genera possess multiple copies of the 16S rRNA gene that exhibit very large divergence, which may lead to paralogous phylogeny. For example, *Haloarcula marismortui* possesses three copies that show 5% divergence (Dennis *et al.*, 1998; Baliga *et al.*, 2004), while two copies in *Haloarcula japonica* (K. Nakasone, unpublished data) differ by 5.2%. Two copies in *Halosimplex carlsbadense* display 6.7% divergence (Vreeland *et al.* 2002), and one of the four genes in *Natrinema* sp. XA3-1 differs by 5% from the other three copies (Boucher *et al.*, 2004).

Substantial studies on the polymerase of haloarchaea were initiated by the group of Wolfram Zillig (Zillig *et al.*, 1978). In *Halobacterium halobium* (presently regarded as a synonym of *Halobacterium salinarum*) and three methanogenic archaea, subunit B was shown to be split into smaller subunits, B' and B'' (Gropp *et al.*, 1986), and complete sequences of the genes of subunits B'', B', A' and A'' (original nomenclature was B'', B', A and C; Leffers *et al.*, 1989) of *Hbt. halobium* were determined. At present, it is known that the *rpoB* genes of all haloarchaea and methanogenic archaea whose genomes have been sequenced and that of *Archaeoglobus fulgidus* are fragmented into *rpoB'* (5' side) and *rpoB''* (3' side). Recently, Walsh *et al.* (2004) have proposed that *rpoB'* can be used as an alternative to the 16S rRNA gene in the taxonomic investigation of haloarchaea. They demonstrated that the gene provided a similar degree of phylogenetic resolution to the 16S rRNA gene, yet does not bear the problem of paralogy, by showing that compositional bias of the nucleotides and amino acids of each sequence did not affect their phylogenetic analyses. A disadvantage in using the subunit B' is that the amplification of genes by PCR and subsequent sequencing are relatively difficult. In some strains, primer sets were not able to amplify the *rpoB'* gene or primers for sequencing the amplified genes did not work (Walsh *et al.*, 2004). This drawback is, however, compensated for by the fact that the sequences are highly conserved in archaea, particularly in members of the *Halobacteriaceae*. In this study, we ascertained that the complete *rpoB'*

sequences of the following five haloarchaea can be aligned without any gaps or deletions: *Hbt. salinarum* NRC-1 (1827 bp), *Har. marismortui* ATCC 43049^T (1827 bp), *Haloquadratum walsbyi* DSM 16790 (1836 bp) (Bolhuis *et al.*, 2006), *Natronomonas pharaonis* DSM 2160^T (1833 bp) (Falb *et al.*, 2005) and *Haloferax volcanii* DS2 (1830 bp). The first four gene sequences were downloaded from the Kyoto Encyclopedia of Genes and Genomes (KEGG; <http://www.genome.jp/kegg/>). The sequence of *Hfx. volcanii* DS2 was taken from the UCSC Archaeal Genome Browser (<http://archaea.ucsc.edu/>). The three to nine base differences observed amongst the five sequences are found in the 5' termini. Partial sequences of *rpoB'* determined by Walsh *et al.* (2004) (GenBank accession numbers AJ809508–AJ809527; 1305 bp, with two exceptions of 1291 and 1299 bp) were also aligned completely with the five sequences without any gaps or deletions. On the other hand, the lengths of *rpoB''* of the five haloarchaea mentioned above were 1566–1578 bp. They also aligned quite well, and the 12 base differences were concentrated in 3'-terminal positions, where *rpoB* was split into *rpoB'* and *rpoB''*.

In the present study, we sequenced PCR-amplified *rpoB'* subunit genes of 11 alkaliphilic strains of the genera *Halobiforma*, *Halorubrum*, *Natrialba*, *Natronococcus*, *Natronolimnobius* and *Natronorubrum*, as well as six allied neutrophilic strains of the genera *Halobiforma*, *Haloferax* and *Natrinema*. The gene sequences and their deduced amino acid sequences were aligned with the 23 sequences mentioned above. Phylogenetic analysis demonstrated that *rpoB'*- and *RpoB'*-based phylogenies are mostly congruent with the 16S rRNA gene-based phylogeny, but some incongruence was also observed. This study may be helpful for the accurate classification of haloalkaliphilic archaea, complementing the 16S rRNA gene-based phylogeny.

The haloarchaeal strains used in this study and accession numbers for their 16S rRNA and *rpoB'* gene sequences are listed in Table 1. The DNA was extracted using the method of Tamaoka (1994) and a segment of *rpoB'* (approx. 1300 bp) was amplified by PCR using the following 50 µl mixture: 5 µl 10× ExTaq PCR buffer, 5 µl dNTPs (2.5 mM each), 2.5 µl forward primer, 2.5 µl reverse primer (1.6 µM each), 34 µl distilled water, 0.5 µl *Taq* DNA polymerase (TaKaRa) and 0.5 µl purified DNA. In some experiments, Platinum *Taq* DNA polymerase High Fidelity (Invitrogen) was also used. Primers 444F (5'-TCCCGTACCCNGARCAYAAAY-3'; Walsh *et al.*, 2004) and 1743R (5'-TTGAAYGCGTAGSWSATCTC-3', designed in this work) were used for the amplification of *rpoB'* genes from *Hbf. nitratireducens* JCM 10879^T, *Hbf. laciisalsi* JCM 12983^T, *Haloferax* sp. BV2, *Nab. chahannaensis* JCM 10990^T, *Nab. magadii* NCMB 2190^T, *Nnm. pellirubrum* JCM 10476^T, *Natrinema* sp. XA3-1, *Ncc. occultus* NCMB 2192^T, *Nln. baerhuensis* JCM 12253^T, *Nlm. innermongolicus* JCM 12255^T and *Nrr. bangense* AS 1.1984^T. Another primer set, 398F (5'-ACCCGCGAGCTCATCTTCGGYAT-3') and 1752R (5'-AGTAGAAGYTTTRAANGCRTA-3'; Walsh *et al.*,

Table 1. Strain details and accession numbers of 16S rRNA and *rpoB'* gene sequences used in this study

Strain	16S rRNA gene	<i>rpoB'</i> gene
Halobacterium		
<i>Hbt. salinarum</i> NRC-1 (=ATCC 700922)	AE005128	AE005138
Haloarcula		
' <i>Har. aidinensis</i> ' JCM 10024	AB000563*	AJ809508
<i>Har. japonica</i> JCM 7785 ^T	Unpublished	AJ809509
<i>Har. marismortui</i> ATCC 43049 ^T	AF034620	AY596297
Halobaculum		
<i>Hbl. gomorrense</i> DSM 9297 ^T	L37444	AJ809510
Halobiforma		
<i>Hbf. haloterrestris</i> JCM 11627 ^T	AF333760	AJ809511
<i>Hbf. lacisalsi</i> JCM 12983 ^T	AY277582	AB295626
<i>Hbf. nitratireducens</i> JCM 10879 ^T	AB045012	AB295625
Halococcus		
<i>Hcc. saccharolyticus</i> ATCC 49257 ^T	AB004876	AJ809512
Haloferax		
<i>Hfx. volcanii</i> NCMB 2012 ^T	K00421	Contig-3152†
<i>Hfx. denitrificans</i> ATCC 35960 ^T	D14128	AJ809513
<i>Hfx. mediterranei</i> ATCC 33500 ^T	D11107	AJ809514
<i>Haloferax</i> sp. BV2 (=JCM 13923)	AB258304	AB295629
Haloquadratum		
<i>Hqm. borinquense</i> ATCC 700274 ^T	AF002984	AJ809515
Haloquadratum		
<i>Hqr. walsbyi</i> JCM 12895	AY676200‡	AM180088
Halorhabdus		
<i>Hrd. utahensis</i> DSM 12940 ^T	AF071880	AJ809516
Halorubrum		
<i>Hrr. coriense</i> ACM 3911 ^T	L00922	AJ809517
<i>Hrr. distributum</i> VKM B-1733 ^T	D63572	AJ809518
<i>Hrr. vacuolatum</i> NCIMB 13189 ^T	D87972	AB295630
Halosimplex		
<i>Hsx. carlsbadense</i> JCM 11222 ^T	AJ586107	AJ809519
Haloterrigena		
<i>Haloterrigena</i> sp. GSL-11 (F. J. Post)	D14126	AJ809520
Natrialba		
<i>Nab. asiatica</i> JCM 9576 ^T	D14123	AJ809522
<i>Nab. chahannaensis</i> JCM 10990 ^T	AJ004806	AB295631
<i>Nab. hulunbeirensis</i> JCM 10989 ^T	AF262026	AB295632
<i>Nab. magadii</i> NCMB 2190 ^T	X72495	AB295633
Natrinema		
<i>Nnm. pallidum</i> NCIMB 784	AJ002948	AJ809523
<i>Nnm. pellirubrum</i> JCM 10476 ^T	AJ002947	AB295623
<i>Nnm. versiforme</i> JCM 10478 ^T	AB023426	AB295624
<i>Natrinema</i> sp. XA3-1 (P. Zhou)	AJ586109–AJ586112	AB295622
Natronobacterium		
<i>Nbt. gregoryi</i> NCMB 2189 ^T	D87970	AJ809524
<i>Natronobacterium</i> sp. SSL6 (=ATCC 43987)	ND	AJ809525
Natronococcus		
<i>Ncc. occultus</i> NCMB 2192 ^T	Z28378	AB295635
<i>Ncc. amylolyticus</i> JCM 9655 ^T	D43628	AB295634
Natronolimnobi		
<i>Nln. innermongolicus</i> JCM 12255 ^T	AB125108	AB295636

Strain	16S rRNA gene	<i>rpoB'</i> gene
<i>Nln. baerhuensis</i> JCM 12253 ^T	AB125106	AB295637
Natronomonas		
<i>Nmn. pharaonis</i> DSM 2160 ^T	CR936257	AJ809526
Natronorubrum		
<i>Nrr. bangense</i> AS 1.1984 ^T	Y14028	AB295627
<i>Nrr. tibetense</i> AS 1.2123 ^T	AB005656	AB295628
<i>Natronorubrum</i> sp. Tenzan-10 (=JCM 10938)	AB046927	AJ809527

ND, No data available.

*Corresponds to the 16S rRNA gene of strain B₂ (=JCM 10025).

†Obtained from the *Haloferax volcanii* Genome Project (http://www.ornl.gov/sci/techresources/Human_Genome/publicat/00santa/118.html).

‡Corresponds to the 16S rRNA gene of the type strain C23^T (=JCM 12705^T).

2004), was used for *Nrr. tibetense* AS 1.2123^T, while the third primer set 462F (5'-GCCWCSCCCCGNATTAC-GAT-3') and 1743R was used for *Hrr. vacuolatum* NCIMB 13189^T, *Nab. hulunbeirensis* JCM 10989^T, *Nnm. versiforme* JCM 10478^T and *Ncc. amylolyticus* JCM 9655^T.

The thermal cycle for amplification began with an initial denaturation step of 5 min at 94 °C followed by 35 cycles of denaturation (45 s, 94 °C), annealing (45 s, 48 °C) and extension (80 s, 72 °C). Thermal cycling was followed by a final terminal extension step of 5 min at 72 °C. Aliquots (5 µl) from the PCR were analysed by agarose gel (1%) electrophoresis and the remaining 45 µl was subjected to electrophoresis and the desired DNA fragment was eluted using a GenElute spin column (Sigma). The DNA was recovered in 50 µl TE buffer, precipitated by 95% ethanol, washed with 70 and 95% ethanol and finally dissolved in 30 µl distilled water. The purified DNA fragments were cloned into pT7Blue T-vector using competent cells of *Escherichia coli* JM109. Positive clones (two to five) were selected for plasmid preparation using a Macherey-Nagel kit. The inserts were sequenced using the BigDye Terminator cycle sequencing kit (Pharmacia Biotech) and an Applied Biosystems ABI Prism DNA sequencer. The sequencing primers were 863F (5'-GACGGCCTCGTCAACCCCGA-3'), 1092R (5'-CGGCTCGCRAAYTTTCNCC-3') (Walsh *et al.*, 2004) and vector-specific primers T7 (5'-TAATACGACTCACTATAGGG-3') and U19 (5'-GTTTTCCTCAGTCACGACGT-3'). In this study, three additional primers were used in sequencing of the gene in some strains: 841F (5'-CACCTCGACGAGGATGGCCTCGTCAA-3'), 1175R (5'-GTGAASGGCATGTCTCTCGTG-3') and 1466R (5'-ACCATGTGGTAGAGCTTSTGGTAGAA-3'). The 16S rRNA gene sequence of *Haloferax* sp. strain BV2 isolated from Green Bath Lake in Romania was determined as described previously (Enache *et al.*, 2007).

Multiple alignments of the nucleotide and deduced amino acid sequences were generated using CLUSTAL W and

phylogenetic trees were reconstructed by the neighbour-joining method (Saitou & Nei, 1987) and evaluated by 1000 bootstrap samplings.

The alignment of 34 *rpoB'* gene sequences contained 1305 sites, of which 672 (51.5 %) were constant across all taxa. A phylogenetic tree reconstruction (Fig. 1; left side) showed that the species of the genera with multiple sequences individually formed coherent clusters except for *Natronorubrum* and *Haloterrigena/Natrinema*. It was noted that the genera *Natrialba*, *Natronococcus*, *Halobiforma*, *Natronobacterium*, *Natronolimnobi*, *Natronorubrum*, *Natrinema* and *Haloterrigena* formed a monophyletic group. The same group was also observed in the tree reconstructed from amino acid sequences translated from the corresponding *rpoB'* gene sequences (Fig. 1; right side). The alignment contained 435 sites, of which 281 (64.5 %) were constant across all taxa. Furthermore, the same group was supported in the tree reconstructed in this study from 16S rRNA gene sequences (Fig. 2). This group observed in the three trees seems equivalent to clade I detected by Walsh *et al.* (2004), composed of *Natrialba*, *Halobiforma*,

Natronobacterium, *Natronorubrum*, *Natrinema* and *Haloterrigena*. The presence of this group was also detected in the phylogenetic tree presented by Wright (2006) based on 16S rRNA gene sequences. Taken together, the trees shown in Figs 1 and 2 suggested strongly that the genera *Natronococcus* and *Natronolimnobi* are also components of clade I. A separate, rather loose group, clade II, composed of *Haloferax*, *Halogeometricum*, *Halobaculum* and *Halorubrum* (Walsh *et al.*, 2004), was also observed in the *rpoB'* gene and protein trees inferred in this study. Clade II, however, was not reproduced in the phylogenetic tree of Wright (2006) or a tree composed of sequences of the type strains of all species of the *Halobacteriaceae* that have validly published names (M. Kamekura, unpublished data).

A closer look at the topology inside clade I in the three trees showed that all species of genera with multiple species formed individual coherent clusters in the 16S rRNA gene tree (Fig. 2). *Nab. asiatica*, a neutrophilic species, and three alkaliphilic species, *Nab. chahannaensis*, *Nab. hulunbeirensis* and *Nab. magadii*, formed a single cluster in the *rpoB'*

Fig. 1. Neighbour-joining phylogenetic trees inferred from *rpoB'* gene sequences (left) and deduced RpoB' protein sequences (right) of strains of the family *Halobacteriaceae*. Support for nodes corresponds to bootstrap values for 1000 replicates; only values greater than 700 are displayed. Bars, 0.1 substitutions per site. Details of strain names and sequence accession numbers are given in Table 1.

Fig. 2. Neighbour-joining phylogenetic tree inferred from 16S rRNA gene sequences (left) in comparison with that derived from *rpoB'* gene sequences (right). Bars, 0.01 (16S rRNA gene) or 0.1 (*rpoB'* gene) substitutions per site. See the legend to Fig. 1 for other details.

gene tree. In the *RpoB'* protein tree, however, the three alkaliphilic species formed a tight group, and the neutrophilic species *Nab. asiatica* formed a group with species of *Natronorubrum* and *Natronolimnobius*. In order to make this situation clearer, we tried to amplify the gene from a strain of another neutrophilic species, *Natrialba aegyptiaca* JCM 11194^T, but none of the *rpoB'* gene primers were able to amplify a product. The *RpoB'* protein tree reminded us of the suggestion that the three alkaliphilic species could be placed in a different genus (Xu *et al.*, 2001). Further investigations of *rpoB'* gene and protein sequences of many strains that have been suggested to belong to the genus *Natrialba* should clarify the taxonomy of this genus.

The genus *Natronorubrum* is composed of three species at the time of writing (Cui *et al.*, 2006). The 16S rRNA gene sequences of two species and isolate *Natronorubrum* sp. Tenzan-10 formed a tight group in the 16S rRNA gene tree (Fig. 2). In the work of Walsh *et al.* (2004), only isolate *Natronorubrum* sp. Tenzan-10 (=JCM 10938) was sequenced as a representative. In this study, we sequenced the *rpoB'* genes from the type strains of two species and noticed that the three sequences did not form a coherent

cluster; two clustered with *Natronolimnobius* species and one with *Haloterrigena* and *Natrinema* species in the gene tree, whereas *Nrr. tibetense* clustered with *Natronolimnobius* species and *Nab. asiatica* in the protein tree (Fig. 1). More study is needed to clarify this situation.

Halorubrum is another genus composed of alkaliphilic and neutrophilic species. Although *rpoB'* genes have not been sequenced from the recently described alkaliphilic species *Hrr. tibetense* (Fan *et al.*, 2004) and *Hrr. alkaliphilum* (Feng *et al.*, 2005), the alkaliphilic species *Hrr. vacuolatum* clustered with the two neutrophilic species in all trees. Other genera with multiple species, *Haloferax*, *Haloarcula*, *Halobiforma*, *Natronococcus* and *Natronolimnobius*, were also supported as individual coherent groups in all trees.

It has been pointed out that the taxonomy of the genera *Haloterrigena* and *Natrinema* has problems (Tindall, 2003), and one of the aims of this work was to solve this problem based on *rpoB'* gene and protein sequences. We determined sequences from *Nnm. pellirubrum* JCM 10476^T, *Nnm. versiforme* JCM 10478^T and *Natrinema* sp. XA3-1 in this work. Amplification products of the expected size were obtained from *Htg. saccharovitans* JCM 12889^T and *Htg.*

thermotolerans DSM 11552^T, but the sequences of five clones were not those of *rpoB'*, suggesting that unrelated portions had been amplified. More effort will be needed for the design of better primers for amplification of *rpoB'* from these strains. The phylogenetic position of *Natrinema* sp. XA3-1 inferred from *rpoB'* gene and protein sequences was similar to that inferred from three of its 16S rRNA genes, SSU-B, -C and -D, rather than SSU-A (Fig. 2) (see also Fig. 5b of Boucher *et al.*, 2004). Although we need more data from *Haloterrigena*, the available sequences formed coherent groups in the three trees, suggesting that the species of the two genera are very closely related. According to DNA G+C content, however, two lineages can be distinguished. *Nm. pellirubrum*, *Natrinema* sp. XA3-1, *Nm. pallidum* and *Haloterrigena* sp. GSL-11 are characterized by G+C contents greater than 65 mol%. Another lineage consisting of *Nm. versiforme* and *Htg. turkmenica* is characterized by G+C contents lower than 65 mol%.

Despite difficulties in amplification and sequencing encountered by Walsh *et al.* (2004), Case *et al.* (2007) and in this study, the most important advantage of the use of the *rpoB'* gene is that the sequences are highly conserved amongst species of the family *Halobacteriaceae*. The 16 sequences determined in this study and those determined by Walsh *et al.* (2004) could be aligned unambiguously without any gaps. Thus, if gaps or insertions are detected in alignment of newly determined sequences, they are likely to come from misreading of sequencing or from PCR mutations. Furthermore, a set of a gap and an insertion in a short stretch of a sequence caused by two mistakes in reading (for example, within 10 to 15 nucleotides), but not apparent in the alignment of the nucleotides, will be detected in the alignment of the translated amino acid sequences (see an alignment of the translated amino acid sequences shown in Supplementary Fig. S1). On the other hand, gaps at 11 positions were required to obtain a reasonable alignment of the five 16S rRNA gene sequences from strains of *Hbt. salinarum* (1473 bp), *Har. marismortui* (1472 bp), *Hfx. volcanii* (1472 bp), *Hqr. walsbyi* (1472 bp; HQR01) and *Nmn. pharaonis* (1467 bp). In the alignment used in the reconstruction of Fig. 2, gaps were necessary in 49 positions in the inner part of the alignment and at 19 and 33 positions at the 5' and 3' termini, respectively.

We would like to conclude that, in spite of some incongruities, the *rpoB'* gene can be used as an excellent alternative molecular marker in phylogenetic analysis of the *Halobacteriaceae*.

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